Applied Biosystems

Isolation, Detection, and Quantitation of Genetically Modified DNA in Food Materials

Introduction

In many parts of the world, the isolation, detection, and quantitation of genetically modified (GM) DNA content in soybeans, maize, and other foods is becoming increasingly important. Currently, the only fail-safe method of distinguishing GM foods is by molecular-level analysis of the changes in DNA that result when new gene sequences are inserted.

The most widely known example involves a bacterial gene that encodes resistance to glyphosate, the active ingredient in the herbicide known commercially as Roundup®. The method involves incorporating the 5-enolpyruvyl shikimate-3-phosphatesynthase (EPSPS) genes from *Agrobacterium tumefaciens* into maize or soybean plant seeds, which results in glyphosate-resistant Roundup Ready® plant strains. Maize from such plants is processed into flour and incorporated into corn chips, taco shells, and other processed foods. Similarly resistant strains of soybeans are used to produce animal feed, protein, oil, and lecithin.

This Application Note discusses the use of the ABI PRISM™ 6100 Nucleic Acid PrepStation and TransPrep chemistry for the isolation of genomic DNA from genetically modified soybeans, and from a soy flour containing a standard level of GM content. The standard is defined by the European Union's Institute of Reference Materials and Measurement (IRMM). Analysis of genetically modified organisms (GMO) in isolated DNA, using real-time quantitative PCR, the ABI PRISM® 7000 or 7900HT Sequence Detection System (SDS), and the TaqMan® GMO Detection and Quantitation System will also be discussed.

ABI PRISM™ 6100 Nucleic Acid PrepStation

GMO Detection Kit

The TaqMan GMO Detection kit uses the 5' nuclease method with fluorescent dye-labeled probes for multiplex detection and quantitation. It targets the Cauliflower Mosaic Virus 35S promoter, a GMO-specific sequence present in all GM soy and maize events approved for use in food by the European Union, and in the vast majority of GMO events approved by other countries.

TransPrep Chemistry

TransPrep chemistry isolates DNA directly from tissue or food materials. It can also be used to isolate DNA from the RNA-depleted filtrates that

Step 1
Homogenization of sample with 1X lysis buffer using a rotor-stator or bead-beater type homogenizer.

Step 2
Incubation of 50-100 mg of sample in 1X lysis buffer at 100°C for 15 minutes with constant shaking or vortex mixing every 3 minutes.

Step 3
Centrifugation of the sample at 14,000 rpm for 5 minutes to pellet solid debris.

Step 4 (optional)
Pre-filtration of the centrifuged lysate to remove remaining particulates.

Step 5
Removal of 200 µL of supernatant liquid (from Step 3 or 4) and addition of 100 µL of DNA Precipitation Solution 1 and 300 µL of DNA Precipitation Solution 2.

Step 6
Purification of DNA on the 6100 PrepStation or the 6700 Workstation using the TransPrep chemistry protocol.

DNA Isolation Protocol Overview

are obtained after RNA isolation of homogenized tissue samples, *i.e.,* DNA and total RNA isolated from a single homogenized tissue sample.

Isolating DNA from Food Products

Isolating DNA from food products is difficult because of sample complexity and the potential carryover of materials that severely inhibit downstream PCR-based assays into the final DNA eluate. The 6100 PrepStation and associated TransPrep DNA isolation chemistry achieve fast (~1.5 hours), high-quality isolation of DNA in a 96-well format, even from complex materials including animal feed and soy flours, tofu, taco shells, and other substances with GM content. The isolated material is free of inhibitors and useful for any downstream application.

Isolation Protocol

1. Homogenization (optional)

a. Dry food materials including flour, animal feed, and potato or corn chips (crisps) are powdered with a pestle and mortar or in a motorized (e.g., a Waring®) blender. They are then homogenized in the blender or in a rotor-stator (e.g., a Polytron® PT 1200) hand-held homogenizer, or in a bead-beater type (e.g., a Kleco) pulverizer.

b. Typically, 50 mg of powdered material is then homogenized in 1 mL of 1X Lysis Buffer (a 1:1 mixture of 2X RNA Lysis Reagent and Ca/Mg-free PBS) for 30–60 seconds per sample or until the sample is finely dispersed and has no major particulates.

c. Oily or fatty material (e.g., vegetable or animal-derived oils) is homogenized in 1X Lysis Buffer for 30–60 seconds.

d. Liquid materials are directly homogenized with an equal volume of 2X Lysis Reagent for 30–60 seconds.

2. Incubation at elevated temperature

The 50-mg sample of dispersed food material is incubated in 1X Lysis Buffer for 15 minutes at 100°C with constant shaking or vortex mixing every 3 minutes to lyse samples and release nucleic acids into solution.

3. Centrifugation

Samples are centrifuged for 5 minutes at 14,000 rpm to remove solid debris.

4. Filtration (optional)

The supernatant liquid obtained after centrifugation is removed and pre-filtered using Tissue Pre-Filter Tray 1 to ensure that all particulates are removed from the sample.

5. Reagents

A 200-µL aliquot of the filtered homogenate or supernatant liquid, obtained after centrifugation or centrifugation and filtration, is removed and DNA precipitation reagents are added.

6. Purification

The DNA is then purified on either the 6100 PrepStation or the ABI PRISM™ 6700 Automated Nucleic Acid Workstation using a 96-well purification tray and the TransPrep chemistry protocol.

Results

In the following examples, DNA was extracted from complex food products and flours containing GM soy, and detected using the Applied Biosystems TaqMan® GMO Soy 35S Detection Kit and the 7900HT or the 7000 system. The level of GM content in the isolated DNA was compared to known standards by means of a real-time PCR assay targeting the 35S promoter sequence and the lectin gene. An assay measuring the level of inhibition in the isolated DNA was also performed using real-time PCR. It showed that the samples contained no PCR inhibitors.

Method One

Isolation of DNA and Detection of GM Content in Miscellaneous Feed Materials

Using the following procedure, DNA was isolated from granular/oily animal feed containing GM soy, and from a 100% transgenic, powdered soy flour obtained by homogenizing transgenic soybean seeds. The isolated DNA was then assayed by real-time PCR, and the TaqMan GMO Detection Kit established the level of GM content in the animal feed.

Isolation Protocol

1) 5 g of each feed and flour sample were dry-homogenized in a Moulinex® homogenizer for 30–60 seconds to yield a fine powder.

2) 50 mg of the dry powder was placed in 1 mL of 1X Lysis Buffer in a 2-mL Eppendorf® tube. Each sample was isolated in duplicate.

3) Each sample was incubated for 15 minutes at 100°C, with constant shaking or vortex mixing every 3 minutes.

4) Samples were then centrifuged at 14,000 rpm for 5 minutes.

5) 200 µL of the supernatant liquid was removed and placed in a clean Eppendorf tube or 96-well deep-well plate.

6) 100 µL of TransPrep DNA Precipitation Solution 1 and 300 µL of TransPrep DNA Precipitation Solution 2 were added to the samples and mixed.

Table 1. Lysate DNA Purification on the 6100 PrepStation

Step	Description	Volume (µL)	Position	Time (sec)	Vacuum (%)	Action
–	Pre-wet wells with DNA Wash Solution 1 or tape over unused wells	40	Waste	–	–	–
1	**Load samples**	600*	Waste	120	20	Press **F1** (Start)
2	Add **DNA Wash Solution 1**	650	Waste	90	20	Press **F1** (Start)
3	Add **DNA Wash Solution 2**	650	Waste	90	20	Press **F1** (Start)
4	Pre-Elution Vacuum	–	Waste	30	30	Press **F1** (Start)
5	Touch Off at Waste	–	Touch Off	–	–	Press **F1** (Start)
6	Add DNA Elution Solution and Incubate	150	Collection	120	0	Press **F1** (Start)
7	Final Elution Step	–	Collection	120	20	Press **F1** (Start)
8	Touch Off at Collection	–	Touch Off	–	–	Press **F1** (Start)

***200 µL of lysate and 400 µL of a 1:3 mixture of DNA Precipitation Solution 1 and DNA Precipitation Solution 2. See the following protocol for details: "TransPrep Chemistry Purification of gDNA from Filtrates Obtained After the Isolation of RNA from Homogenized Animal or Plant Tissue Samples." The protocol can be downloaded from www.appliedbiosystems.com.**

7) Varying volumes of the lysate were then purified on the 6100 PrepStation, using the protocol shown in Table 1.

Real-Time PCR Analysis of GM Content

Five microliters of starting DNA template obtained from the isolations (Table 2) was added to 45 µL of TaqMan® Universal PCR Master Mix containing probe and primer sequences from the GMO test kit for detecting the 35S promoter sequence of the Cauliflower Mosaic Virus-inserted transgene. The assay was run on a 7900HT system in triplicate.

Inhibition of PCR in Isolated DNA Samples

The inhibition of PCR by materials carried from the purification process into the isolated DNA sample is one of the most common problems associated with molecular detection of GM content. Poor-quality isolations yield highly impure preparations of DNA ($A_{260/280}$ <1.5), which may be heavily contaminated with protein, glycoprotein, or other materials inherent in the sample tested. These inhibitors cause the results of the downstream real-time PCR assay to be less sensitive and more variable. Inhibition effects can cause dramatic shifts in threshold cycles; in extreme cases, no amplification is observed even after 40 PCR cycles.

DNA samples isolated from both the 100% transgenic soy flour and food products made from raw materials with GM content were analyzed for inhibition of PCR (Figure 3). This assay was performed by creating a 1:4 dilution series (0 dilution, 1:4, 1:16, 1:64, 1:256, and 1:1024) of the purified DNA, with each dilution point analyzed in triplicate. A plot of the logarithm of dilution against the threshold cycle, as measured by the 5' nuclease assay, will be a straight line if no PCR inhibitors are present. If inhibitors are present, a significant upward deflection of the line may occur at the 0, 1:4, and 1:16 dilution points.

Table 2. Yield and Purity of DNA Measured by UV Absorbance

Sample	Amount of Lysate Purified	Concentration of DNA Isolated Volume 150 µL	$A_{260/280}$ Ratio
1. Soy 100%*	100 µL	50.6 µg/mL	2.03
2. Soy 100%	200 µL	74.2 µg/mL	1.96
3. Soy 100%	300 µL	88.3 µg/mL	2.03
4. Soy 100%	600 µL	51.2 µg/mL	2.01
5. Feed Miscellaneous A**	100 µL	19.0 µg/mL	1.72
6. Feed Miscellaneous A	200 µL	26.1 µg/mL	1.84
7. Feed Miscellaneous A	300 µL	28.2 µg/mL	1.86
8. Feed Miscellaneous A	600 µL	32.6 µg/mL	1.96

***A 100% transgenic, powdered soy flour**
****A granular/oily animal feed, containing GM soy materials**

Method Two

DNA Isolation and GMO Detection/ Quantification in IRMM Reference Materials

DNA was isolated from a genetically modified IRMM soy standard. IRMM materials are prepared with defined levels of GM content (0.1%, 0.5%, 1.0%, 2.0%, and 5.0% by weight in a standard soy flour) and are used as the standard for quantifying unknown samples.

Samples were purified as in Method One—50 mg of reference material was incubated in 1 mL of 1X Lysis Buffer. Purification was performed on the 6100 PrepStation with TransPrep chemistry. The DNA was eluted in 150 µL of elution solution.

Isolation Protocol

1) 50 mg of the dry powder was placed in 1 mL of 1X Lysis Buffer in a 2-mL Eppendorf tube. Each sample was isolated in duplicate.

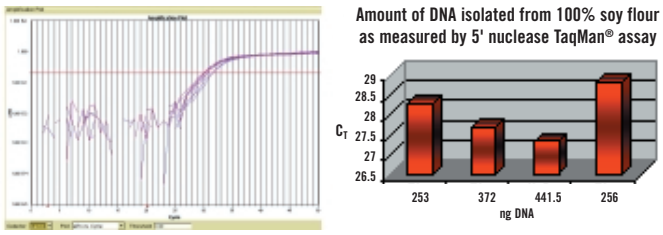
2) Next, each sample was incubated for 15 minutes at 100°C with constant shaking or vortex mixing every 3 minutes.

3) Samples were then centrifuged at 14,000 rpm for 5 minutes.

4) 200 µL of the supernatant liquid was removed and placed in a clean Eppendorf tube or 96-well deep-well plate.

5) 100 µL of DNA Precipitation Solution 1 and 300 µL of DNA Precipitation Solution 2 were added to the samples and thoroughly mixed.

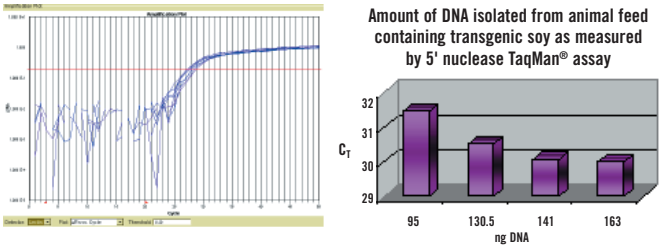
6) 200 µL of the lysate generated in step 5 was purified on a 6100 PrepStation, using the TransPrep protocol described in Method One.



Sample	Amount of Lysate Purified	Average C_T	Transgenic DNA*
1. Soy 100%	100 µL	28.30	253.0 ng
2. Soy 100%	200 µL	27.68	372.0 ng
3. Soy 100%	300 µL	27.33	441.5 ng
4. Soy 100%	600 µL	28.80	256.0 ng

*Amount detected by 5' nuclease assay from 5 µL of input template

Figure 1. Amplification of DNA isolated from 100% transgenic soy flour



Sample	Amount of Lysate Purified	Average C_T	Transgenic DNA*
1. Feed Miscellaneous A	100 µL	31.48	95.0 ng
2. Feed Miscellaneous A	200 µL	30.54	130.5 ng
3. Feed Miscellaneous A	300 µL	30.08	141.0 ng
4. Feed Miscellaneous A	600 µL	29.99	163.0 ng

*Amount detected by 5' nuclease assay from 5 µL of input template

Figure 2. Amplification of DNA isolated from GM containing animal feed

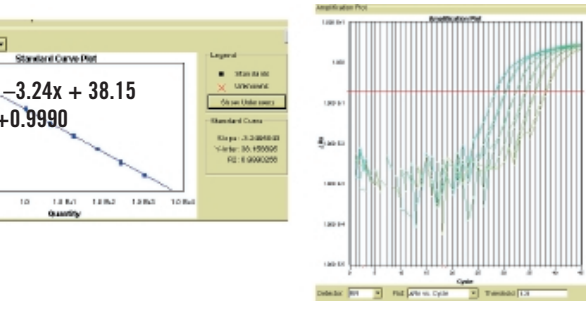
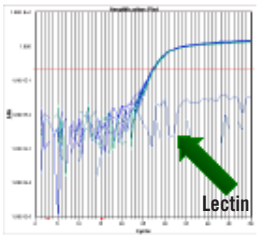
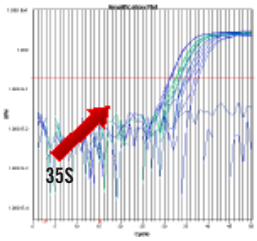


Figure 3. Amplification (using the 5' nuclease assay) of the 35S promoter sequence from a 1:4 dilution series of DNA, isolated from 100% transgenic soy flour (using TransPrep chemistry). The results show no inhibitors present in the isolated DNA.





Sample	C_T Lectin	C_T 35S
1. IRMM Soy 0.1%	27.24	36.89
2. IRMM Soy 0.5%	27.27	34.95
3. IRMM Soy 1.0%	27.06	33.54
4. IRMM Soy 2.0%	27.38	32.76
5. IRMM Soy 5.0%	27.10	31.40

Figure 4. Real-time PCR analysis (using a 7900HT system and the 5' nuclease assay) for the lectin and 35S genes in DNA, isolated from IRMM reference materials (using a 6100 PrepStation and TransPrep chemistry).

Results

Table 3 shows the yield and purity of DNA isolated from IRMM GMO reference materials on a 6100 PrepStation as analyzed by UV spectroscopy. Results indicate that high-quality DNA was isolated from the samples. Figure 4 shows amplification of DNA for lectin and 35S target sequences. The 35S amplification plot shows the expected decrease in C_T value with increasing GM content.

Summary

High-quality, high-throughput detection of GM content in complex food materials is possible using the Applied Biosystems "whole solution" set of products—the 6100 PrepStation, TransPrep chemistry, the 7900HT or 7000 system, and 5' nuclease TaqMan® GMO Detection Kits.

Acknowledgment

We would like to thank Marco Cappelletti and Nicola Cirenei of Applied Biosystems Italy for supplying the datasets that appear in this Application Note.

Table 3. Yield and Purity of DNA from IRMM GM Materials

Samples	Concentration of Isolated DNA	$A_{260/280}$ Ratio
1. IRMM Soy 0.1%	57.0 µg/mL	1.96
2. IRMM Soy 0.5%	100.1 µg/mL	1.89
3. IRMM Soy 1.0%	100.9 µg/mL	1.83
4. IRMM Soy 2.0%	59.2 µg/mL	1.83
5. IRMM Soy 5.0%	74.6 µg/mL	1.82

Ordering Information

Description	Quantity/Rxn	P/N
ABI PRISM® Instrument Systems		
ABI PRISM™ 6100 Nucleic Acid PrepStation		6100-01
ABI PRISM™ 6700 Automated Nucleic Acid Workstation		6700-01
ABI PRISM® 7000 Sequence Detection System		4330087
ABI PRISM® 7900HT Sequence Detection System		4329003
TransPrep Chemistry Reagents and Consumables		
Nucleic Acid Lysis Solution	250 mL	4305895
DNA Precipitation Solution 1	100 mL	4325962
DNA Precipitation Solution 2	250 mL	4325964
DNA Wash Solution 1	1 L	4325958
DNA Wash Solution 2	1 L	4325960
DNA Elution Solution 1	250 mL	4325956
gDNA Purification Tray 1	10/Box	4318641
Protocol	1	4326965a
TaqMan® GMO Detection Kits		
TaqMan® GMO Maize 35S Detection Kit	100 Rxn	4327690
TaqMan® GMO Maize 35S Detection Kit/Protocol	100 Rxn	4327693
TaqMan® GMO Soy 35S Detection Kit	100 Rxn	4327691
TaqMan® GMO Soy 35S Detection Kit/Protocol	100 Rxn	4327692

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