

Screening for Genetically Modified Organisms in Food Using Promega's Wizard® Resin



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Genetically modified organisms (GMOs), primarily in the form of plant material, have become available in the world food markets with ever increasing abundance. Because of public concern over the safety of GMOs, individual countries and coalitions such as the European Union (EU) have instituted labeling requirements for all foods containing any amount of GMO products. The method presented here allows detection of small amounts of GMOs present in food shipments.

INTRODUCTION

The first commercially available genetically modified crop was the FLAVR-SAVR tomato marketed by Calgene. Today, the two largest GMO crops are corn (maize) and soybean. Differences in labeling requirements of various countries, hesitation by many producers to claim GMO content due to public perception and combining of crops from many growers has made it difficult to police and track the source of the crop. Corn and soybeans bound for the EU are typically collected from growers in centralized elevators, loaded onto train cars for transport to a port, then loaded in huge batches onto ships for delivery to the recipient country. This procedure creates a random mixture of the crop from a vast number of farms, any of which could be growing GMOs. Detection of potentially small amounts of GMOs (0.1%) present in these mixtures is the goal of the new screening method endorsed by the EU (1).

THE BASIS OF DETECTION

Transgenic plants usually contain three essential elements: a transgene (the new genetic element that has been introduced to give the plant the desired new characteristics), a promoter (which controls when the transgene is expressed in the transgenic plant) and a terminator (which makes sure that the transgene ends properly and does not create chimeric proteins with those from the plant). Although many potential promoter and terminator elements have been identified, the most commonly used are the CaMV 35S promoter, from the 35S RNA of the phytopathogenic cauliflower mosaic virus, and the NOS terminator, derived from the nopaline synthase gene from the *Agrobacterium tumefaciens* Ti plasmid.

Almost all commercially developed transgenic crop plants contain either the CaMV 35S promoter or the NOS terminator. The ability to detect these elements will allow detection of the vast majority of GMO plant material. In fact, a study conducted in 1997 concluded that either the NOS terminator or the CaMV 35S promoter sequences were present in 27 of 28 EU-approved genetically modified crops (2). It should be noted that the CaMV 35S promoter or the NOS terminator may be present naturally in CaMV or *A. tumefaciens*-infected plants, respectively. A positive result therefore does not conclusively indicate the presence of transgenic material. The DG JRC Environment Institute Consumer Protection & Food Unit in collaboration with 28 different laboratories has developed and validated two protocols for DNA extraction methods and a PCR-based amplification method to identify genetically modified maize and soy products based on these sequences.

The procedure reported here uses Promega's Wizard® Minipreps (Cat.# A7141) or Midipreps Resin^(a) (Cat.# A7701) and PCR Core System I^(b) (Cat.# M7660). The PCR Core System I contains all necessary reagents to perform PCR other than the DNA template and target-specific primers, which must be provided by the user. A Vac-Man® Laboratory Vacuum Manifold (Cat.# A7231) can be used with this procedure, allowing up to 20 samples to be processed at one time.

DNA ISOLATION

Sample packs of soybean powder and maize powder (Fluka) were used as Institute for Reference Materials and Measurements (IRMM)-certified reference standards. Roasted animal feed soybeans were generously donated by a local farm, while soy flour and corn meal, devoid of any GMO labeling, were purchased from local retailers in Madison, Wisconsin. Samples other than soybeans (0.3g) were placed in disposable microcentrifuge tubes containing 600µl of NANOpure® water and homogenized. The soybeans were soaked overnight in water and crushed in a minimal volume prior to the assay. Extreme care was taken to ensure no cross-contamination of samples, and pre- and post-amplification work areas were established in the laboratory. All dispensing was performed using ART® Aerosol Resistant Pipet Tips. The DNA isolation procedure, illustrated in Figure 1, is as follows:

1. Transfer 0.3g of each homogenate to a new tube containing 860µl of extraction buffer (10mM Tris-HCl [pH 7.5], 150mM NaCl, 2mM EDTA, 1% SDS), 100µl of 5M Guanidine-HCl (Cat.# H5381) and 40µl of 20mg/ml Proteinase K (Cat.# V3021).
2. Incubate at 55–60°C for 3 hours with intermittent mixing.
3. Allow samples to cool at room temperature for 10 minutes.
4. Centrifuge 10 minutes at 14,000 × g in a tabletop microcentrifuge.
5. For each preparation, attach one 3ml syringe barrel to the Luer-Lok® extension of a Wizard® Minicolumn and attach this minicolumn/syringe barrel assembly to the Vac-Man® Laboratory Vacuum Manifold.
6. Check to ensure all stopcocks are closed on the Vac-Man® Laboratory Vacuum Manifold before proceeding.

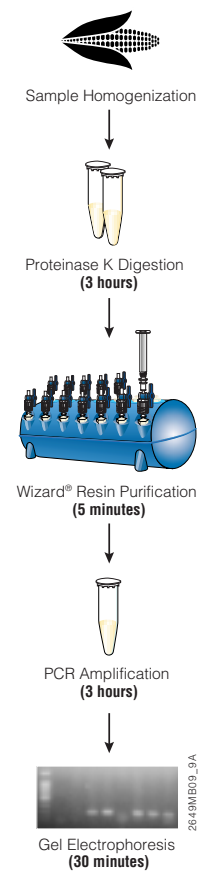


Figure 1. Schematic diagram of the DNA isolation procedure.

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7. Add 1ml of Wizard® Resin to each minicolumn/syringe assembly.
8. Carefully remove 300µl of the cleared supernatant from each sample and transfer it to the barrel of the minicolumn/syringe assembly containing the Wizard® Resin.
9. Open the stopcocks and apply a vacuum to pull the resin/supernatant mix into the minicolumn. When the entire sample has completely passed through the column, close the stopcock and turn off the vacuum at its source.
10. Add 2ml of 80% isopropanol to each minicolumn/syringe assembly and reapply the vacuum to draw the solution through the minicolumn.
11. Remove the syringe barrel and transfer the minicolumn to a 1.5ml microcentrifuge tube. Centrifuge the minicolumn at 10,000 × g in a microcentrifuge for 2 minutes to remove any residual alcohol.
12. Transfer the minicolumn to a new microcentrifuge tube, add 50µl of 70°C water to the column and allow it to interact with the resin for 1 minute.
13. Elute the DNA by centrifugation at 10,000 × g for 1 minute in a microcentrifuge.

This procedure was used for all samples studied.

AMPLIFICATION REACTION

PCR primers for the 35S promoter and NOS terminator were manufactured at Promega Corporation according to the EU recommendations. Table 1 shows the sequences for the sense and antisense oligonucleotide primers.

Reaction components (from Promega's PCR Core System I, Cat.# M7660) were thawed, mixed thoroughly and placed on ice. For sample consistency, a master mix was assembled containing all reagents for the amplification reaction with the exception of the template DNA. The master mix was dispensed into 0.5ml reaction tubes on ice.

Table 1. PCR Primers for the 35S Promoter and NOS Terminator.

35S Promoter Oligonucleotide Primers	
Sense:	5' GCT CCT ACA AAT GCC ATC A 3'
Antisense:	5' GAT AGT GGG ATT GTG CGT CA 3'
NOS Terminator Oligonucleotide Primers	
Sense:	5' GAA TCC TGT TGC CGG TCT TG 3'
Antisense:	5' TTA TCC TAG TTT GCG CGC TA 3'

Sample templates were added to the chilled reaction mixtures and covered with Mineral Oil (Cat.# DY1151). The assembled reactions were then placed in a Perkin-Elmer Model 480 Thermal Cycler preheated to 94°C, and PCR was performed using the profiles for the Perkin-Elmer GeneAmp® 2400 in accordance with the EU recommendations (Table 2).

ANALYSIS

Following completion of the cycling reaction, 10µl of each reaction was analyzed by agarose gel electrophoresis. Results are shown in Figure 2. Detection of fragments with specific sizes of 195bp or 180bp indicates that the sample contains 35S promoter or NOS terminator sequences, respectively. Samples used in Figure 2, Panel A, contain amplification products for the 35S promoter sequence primers. The 195bp product is seen in all samples with the exception of the negative control (no template DNA) and the zero standards for maize and soy. The NOS terminator primers (Panel B) yielded similar results with the exception of the 2% maize standard. The Bt-176 maize strain used for the GMO standards does not contain the NOS terminator sequences. It is interesting to note that the corn meal used in these studies does contain both the 35S and NOS sequences. None of the food products used for these studies contained any notification of GMO content on their packaging. GMO labeling is not required within the United States.

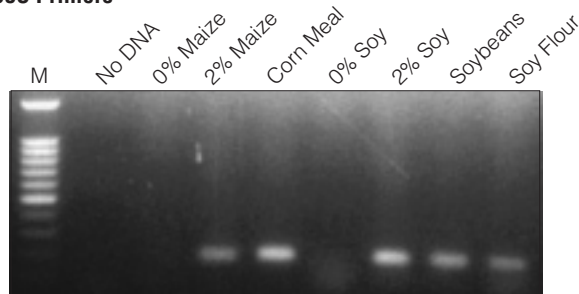
Table 2. Thermal Cycler Profiles for PCR Using 35S and NOS Primers.

	35S Promoter Primers				
	Crocodile II Appligene	GeneAmp® 2400 Perkin-Elmer	Progene Techne	Robocycler®	Trio Biometra
Denaturation	2min/98°C	3min/94°C	3min/95°C	3.5min/98°C	2min/98°C
Amplification	8sec/95°C	20sec/94°C	36sec/95°C	50sec/95°C	30sec/95°C
	30sec/54°C	40sec/54°C	72sec/54°C	95sec/54°C	40sec/54°C
	40sec/72°C	60sec/72°C	84sec/72°C	115sec/72°C	40sec/72°C
Number of cycles	40	40	40	40	40
Final extension	3min/72°C	3min/72°C	3min/72°C	4.25min/72°C	3min/72°C
Cooling phase	1min/30°C	1min/4°C	1min/4°C	1min/6°C	1min/4°C
Heating rate	1°C/sec	0.77°C/sec	1.5°C/sec	–	1°C/sec
Cooling rate	Fast	3.15°C/sec	1.5°C/sec	–	1°C/sec
	NOS Terminator Primers				
	Crocodile II Appligene	GeneAmp® 2400 Perkin-Elmer	Progene Techne	Robocycler®	Trio Biometra
Denaturation	3min/95°C	3min/94°C	3min/95°C	4.5min/98°C	2min/98°C
Amplification	20sec/95°C	20sec/94°C	36sec/95°C	65sec/95°C	30sec/95°C
	40sec/54°C	40sec/54°C	72sec/54°C	105sec/54°C	40sec/54°C
	40sec/72°C	60sec/72°C	84sec/72°C	115sec/72°C	40sec/72°C
Number of cycles	40	40	40	40	40
Final extension	3min/72°C	3min/72°C	3min/72°C	4.25min/72°C	3min/72°C
Cooling phase	1min/30°C	1min/4°C	1min/4°C	1min/6°C	1min/4°C
Heating rate	1°C/sec	0.77°C/sec	1.5°C/sec	–	1°C/sec
Cooling rate	Fast	3.15°C/sec	1.5°C/sec	–	1°C/sec



	Single PCR (μl)	10 Reactions Master Mix (μl)
DNA template (5–10ng/μl)	5	--
Primer 1 (50pmol/μl)	1	10
Primer 2 (50pmol/μl)	1	10
10X Reaction Buffer	10	100
MgCl ₂ (25mM Solution)	6	60
PCR Nucleotide Mix (10mM)	2	20
<i>Taq</i> DNA Polymerase ^(b) (5u/μl)	0.5	5
Nuclease-Free Water	74.5	745
Total Volume	100	950

A. 35S Primers



B. NOS Primers

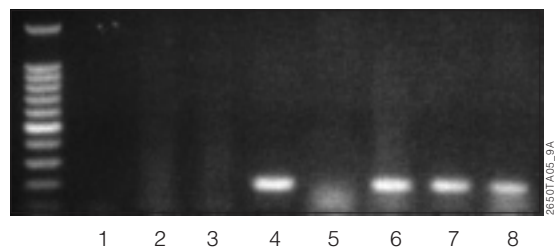


Figure 2. PCR amplification products from GMO samples. A 10μl sample from each amplification reaction was mixed with 2μl of Blue/Orange Loading Dye, 6X (Cat.# G1881) and was subjected to electrophoresis on a 1% agarose 1X TAE gel. The 100bp DNA Ladder (Cat.# G2101) was run as a size marker (lane M). **Panel A:** Results of amplification using the 35S promoter sequence primers. **Panel B:** Results of amplification using the NOS terminator sequence primers. Lane 1, negative control reactions; lane 2, 0% GMO maize standard; lane 3, 2% GMO maize standard; lane 4, corn meal; lane 5, 0% GMO soy standard; lane 6, 2% GMO soy standard; lane 7, soybeans; lane 8, soy flour.

SUMMARY

The procedure and cycling parameters used here are those recommended by the EU and not developed by Promega Corporation. Although the DNA purification procedure is time-consuming, it requires little manipulation. The detection of positive and negative results was straightforward and did not present any problems with the samples tested. Using this procedure, EU reference labs have obtained a sensitivity of 0.1% GMO content. This procedure provides an efficient process for isolation of DNA suitable for amplification from a variety of food sources.

REFERENCES

1. Screening method for the identification of genetically modified organisms (GMO) in food. DG JRC, Environment Institute, Consumer Protection & Food Unit.
2. Hemmer, W. (1997) Foods derived from genetically modified organisms and detection methods. BATS. Available on the Internet at: www.bats.ch/abstr/297intro.htm



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Ordering Information

Product	Size	Cat.#	Price (\$)
Wizard® Minicolumns	250 each	A7211	
Wizard® Minipreps DNA Purification Resin ^(a)	250ml	A7181	
Wizard® Midipreps DNA Purification Resin ^(a)	1,000ml	A7701	
PCR Core System I ^(b)	200 reactions	M7660	
Guanidine-HCl	100g	H5381	
Proteinase K	100mg	V3021	
Mineral Oil	12ml	DY1151	
100bp DNA Ladder	250ml (50 lanes)	G2101	
Blue/Orange Loading Dye, 6X	(3 x 1ml) 3ml	G1881	
Vac-Man® Laboratory Vacuum Manifold		A7231	
ART® 10, Ultramicro Pipet Tip, 0.5–10μl	960/pk	DY1051	
ART® 20P, Pipet Tip, 20μl	960/pk	DY1071	
ART® 200, Pipet Tip, 200μl	960/pk	DY1121	
ART® 1000, Pipet Tip, 1,000μl	768/pk	DY1131	

Note: 3ml syringe tubes can be purchased from most laboratory supply companies.

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^(a)U.S. Pat. Nos. 5,658,548 and 5,808,041, and Australian Pat. No. 689815 have been issued to Promega Corporation for nucleic acid purification on silica gel and glass mixtures.

^(b)The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.