Wizard® Magnetic DNA Purification System for Food: Part I. DNA Isolation and Analysis of GMO Foods by PCR

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Genetically modified organisms (GMO) are being used in the production of food for human consumption. GMO technology can be used to introduce resistance of crops to pathogens and herbicides as well as changing the nutritional content of food. Isolation of DNA from various foods presents a unique set of challenges. Here we introduce and demonstrate the robust and easy-to-use Wizard® Magnetic DNA Purification System for Food (Cat.# FF3750).

Introduction

A variety of genetically modified crops have been approved for use in foodstuffs* around the world (1). Foodstuffs that consist of or have been derived from genetically modified organisms (GMO) must be labeled as such according to the European Regulation on Novel Foods and Novel Food Ingredients (EC/258/97) (2). The regulation stipulates that all GMO technologies be approved by an expert committee and that GMO products be distinguished from their non-GMO counterparts by labeling.

A generally accepted method for determining GMO content in foodstuffs is the detection of DNA sequences used in the genetic modification. As an example, the genetic modification of a commercially available soybean involves the introduction of a glyphosate-resistant 5-enol-pyruvyl-shikimate-3 phosphate synthase (EPSPS) gene and the elements used for EPSPS gene expression (3): the cauliflower mosaic virus (CaMV)-derived 35S promoter and the nopaline synthase (*NOS*) transcription terminator sequence. Detection of the 35S and the *NOS* sequences by PCR methods is widely used for the identification of these GMO soybeans.

Some currently available GMO foodstuffs are listed in Table 1. When this diversity of items is combined with the diversity of processed foods, it is clear that a versatile DNA purification system is needed for the testing of DNA for specific GMO sequences.

We designed the Wizard® Magnetic DNA Purification System for Food[a] to be used in the evaluation of GMO sequences by standard and nested PCR[b]. The use of MagneSil™ Paramagnetic Particles[c] (PMPs) in the Wizard® Magnetic DNA Purification System for Food adds scalability for varying sample sizes and flexibility, as well as automation of sample handling. Part II of this article, beginning on page 19, reports on the use of our Wizard® Magnetic DNA System for Food, the BioSmart Allin1.0 GMO Detection Kit (distributed by Promega) and Labsystems' KingFisher™ automated lab station for processing 24 samples simultaneously.

**Foodstuffs are defined as substances that can be used or prepared for use as food.*

E-mail *techserv@promega.com* for technical questions

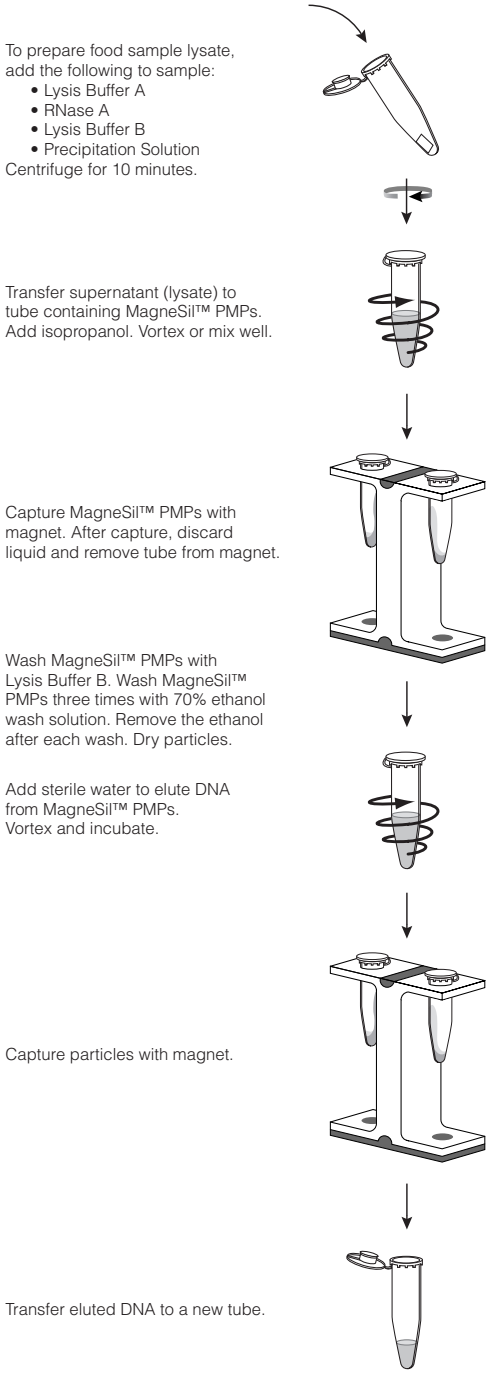

Figure 1. Schematic of DNA isolation using the Wizard® Magnetic DNA Purification System for Food.

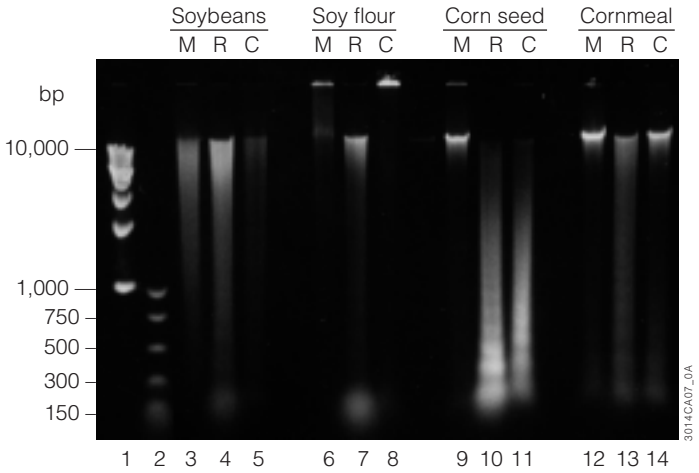

Figure 2. DNA isolation from foodstuffs products by three methods. DNA was isolated from 200mg samples of ground soybeans, soy flour, ground sweet corn seed (Trinity variety) and cornmeal using Wizard® Magnetic DNA Purification System for Food (M), Wizard® Resin (R) (4) and the CTAB (C) method (5). Dried soybeans and corn seeds were pulverized before processing. Samples of 10µl of the 100µl elutions were loaded onto a 1% agarose gel stained with ethidium bromide. Lanes 1 and 2, 1kb DNA Step Ladder (Cat.# G6941) and PCR Markers (Cat.# G3161), respectively.

Results of DNA isolation from food

Figure 1 outlines the procedure for purification of DNA using Wizard® DNA Purification System for Food. The procedure is adaptable to many foodstuffs, such as those listed in Table 1. The Wizard® Magnetic DNA System for Food includes protocols for 200mg and 1g sample amounts, as well as protocol modifications for corn and canola oils, which prove more difficult to process. Using MagneSil™ PMPs offers convenient, scalable sample handling to include samples outside the 200mg–1g range.

Figure 2 shows a comparison of DNA extractions using CTAB, Promega's previously described Wizard® Resin(d) procedure (4), and the Wizard® Magnetic DNA Purification System for Food on 200mg samples from soybeans, soy flour, corn seeds and cornmeal. CTAB is a detergent/chloroform-based DNA purification method. The Wizard® Magnetic DNA System for Food does not require the use of organic solvents such as chloroform. As shown in Figure 2, DNA yield by the three methods is comparable. Typically the particular DNA extracted for these food types is about 10–20kb with a 1–10kb smear seen in some samples. Soy flour DNA tends to be of higher molecular weight when purified with the CTAB method; however, this particular DNA is more difficult to amplify. The soy flour sample isolated with the Wizard® Magnetic DNA System for Food contained a mixture of high and low molecular weights, which amplified easily by PCR (Figure 3).

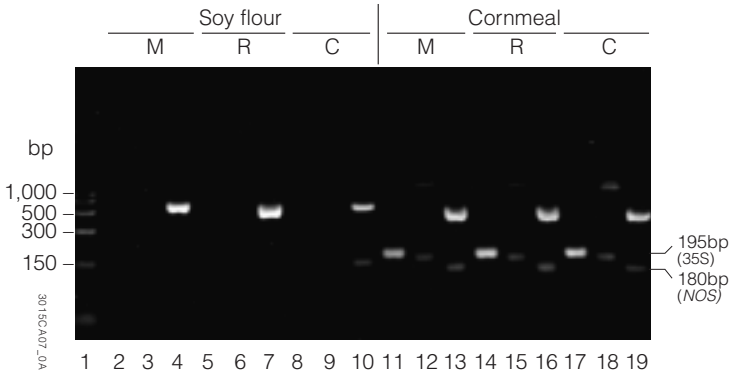

Figure 3. PCR was set up using 1µl of 100µl DNA elutions from soy flour and cornmeal samples processed using the three methods listed in Figure 2. The following amplification method was modified for the Perkin Elmer Thermocycler from the DG JRC Environment Institute method (5). Three reactions were set up for each sample and are shown on the gel in the order: 35S promoter, *NOS* terminator and chloroplast control (to assess the presence of inhibitors). The chloroplast control amplifies the intron of the *trn*L (UAA) gene in chloroplast DNA (6). AmpliTaq Gold® DNA polymerase was used with a Perkin Elmer 480 thermocycler and the following cycling parameters: 95°C for 1 minute, then 40 cycles of 94°C, 1 minute; 54°C , 1 minute; 72°C , 1 minute, followed by a 7-minute extension at 72°C. A 10µl aliquot of each amplification reaction was resolved on a 4% Reliant® NuSieve® 3:1 agarose precast minigel (BioWhittaker Molecular Applications Cat.# 54929). The 35S amplification product is 195bp; the *NOS* terminator, 180bp. The chloroplast control primers amplify one or more bands of varying size depending on plant species and variety. The cornmeal samples all amplified equally well with strong positive 35S, *NOS* terminator and chloroplast control bands. Lane 1, PCR Markers (Cat.# G3161).

Table 1. Some Currently Available GMO Foods.

Whole Foods	Processed Foods
Soybeans	Cornmeal
Corn	Corn flakes
Rapeseed	Corn chips
Tomato	Chocolate
Squash	Soy flour
Potato	Soy meal
Papaya	Soy milk
Canola	Tofu
Melon	
Sugarbeet	
Flax	

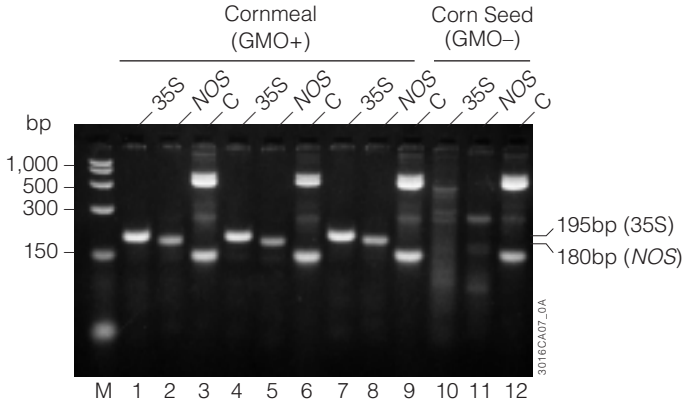

Figure 4. DNA was isolated from cornmeal and ground corn seed using the Wizard® Magnetic DNA Purification System for Food. Detection of GMO sequences by PCR was performed using 1µl of DNA samples as in Figure 3. The first three replicate sample sets (lanes1–9) are from GMO+ cornmeal. Samples represented in lanes 10–12 are from GMO– corn seed; nonspecific bands are seen in these 35S and *NOS* lanes. Lane M, PCR Markers (Cat.# G3161). Lanes labeled C: chloroplast PCR positive controls; lane 10, corn seed sample, negative for 35S promoter; lane 11, corn seed sample, negative for *NOS*.

Figure 3 shows the PCR amplification products from soy flour and cornmeal DNA samples isolated using the methods described in Figure 2. For the food samples tested here, the Wizard® DNA System for Food provided DNA sufficient to perform at least 100 standard PCR amplificatipons. The soy flour samples isolated with Wizard® products (Magnetic or Resin) show strong chloroplast products, a weak positive 35S band and no *NOS* products. The soy flour DNA prepared by the CTAB method showed weak chloroplast bands and was negative for 35S and *NOS* products.

Detection and Confirmation of 35S and NOS Sequences

To further test the quality of DNA for amplification, GMO-positive and -negative samples were analyzed. Figure 4 provides examples of both positive and negative amplifications from GMO and control samples using the Wizard® Magnetic DNA System for Food. Inclusion of a positive control is essential to insure that inhibitors of the amplification are not responsible for false negative results (6).

A second confirmation of the identity of the 35S PCR product is restriction analysis (Figure 5). Amplification primers listed in the DG JRC Environmental Institute method (5) were used, and restriction anaylysis of the 35S PCR product with *Xmn* I was expected to yield two fragments of 115 and 80bp. Food samples, especially processed food, and ingredients, vary greatly in the quantity of DNA as well as ease of purification.

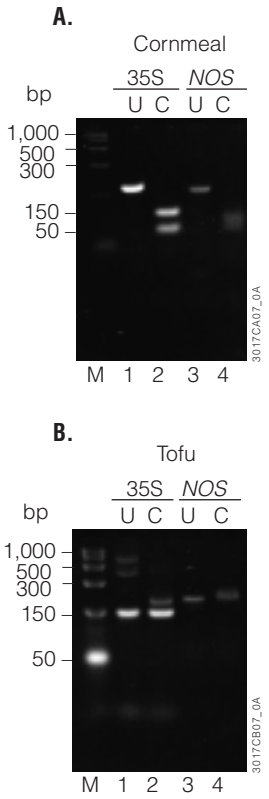

Figure 5. Confirmation of GMO sequences by restriction digestion. Panel A: The presence of 115 and 80bp fragments, by digestion with *Xmn* I, confirm presence of exogenous 35S DNA. The presence of 84 and 96bp fragments, by digestion with *Nsi* I, confirm presence of exogenous *NOS* DNA. Nonspecific PCR products will not show correct restriction fragments as demonstrated by DNA isolated from tofu (**Panel B**) using this test. A strong band was seen for the 35S PCR product as well as for *NOS*, but these bands were nonspecific and did not show the correct fragment sizes following restriction digestion as seen with the cornmeal sample. Lanes M, PCR Marker (Cat.# G3161); lanes 1 and 3 are undigested (U) PCR products; lanes 2 and 4 are digested (C) PCR products.

To demonstrate the flexibility of the Wizard® Magnetic DNA System for Food, DNA was purified from two kinds of corn chips as well as soy milk and soy flour (Figure 6). Other samples, such as soy lecithin, chocolate, corn flakes and vegetable oil either contain a very small amount of DNA due to their composition, or they occur in highly processed foods (i.e., processed by extensive heating), so that larger quantities of sample are required to obtain sufficient amounts of DNA for GMO testing.

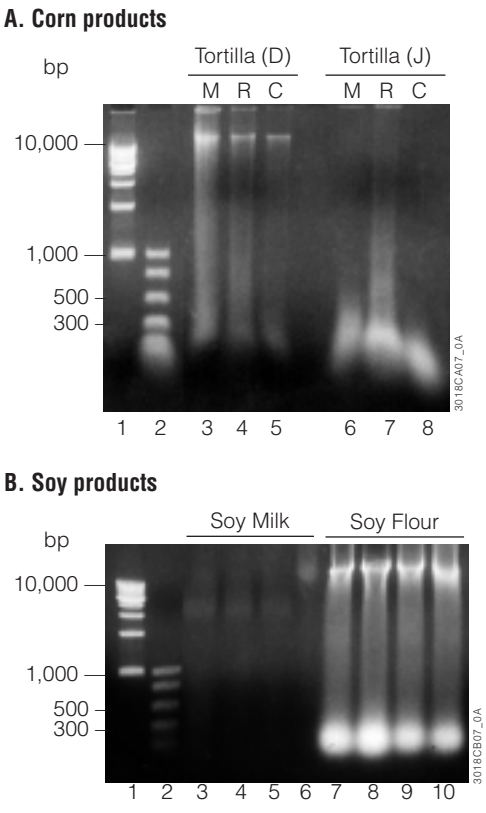

Figure 6. DNA samples isolated from tortilla chips, soy milk and soy flour. **Panel A:** DNA was isolated from 300mg of corn chips using Wizard® Magnetic DNA Purification System for Food (M), Wizard® Resin (R) and CTAB (C). A sample of 15µl of a 40µl elution was resolved on a 1% agarose gel. All methods gave good yields of DNA, with the corn chips from manufacturer J showing mostly low molecular weight fragments. Lanes 1 and 2, 1kb DNA Step Ladder (Cat.# G6941) and PCR Markers (Cat.# G3161), respectively. **Panel B:** DNA isolated from 4gm of soy milk or 1gm of soy flour using method M. Ten percent of a 100µl elution of soy milk DNA was loaded on a 1% agarose gel. High molecular weight DNA is seen, as well as a 3–10 kb smear of lower molecular weight DNA. A 10µl sample of a 500µl eluate of soy flour DNA was resolved on the same gel. The soy flour samples were not treated with RNase A. RNA is evident as low molecular weight fragments on the gel along with the genomic DNA ranging from 1–20kb. Markers are as in Panel A.

As the quality of DNA inherently is different from the many different commercially available stuffs that we tested, the reproducibility of the DNA purification was assessed using well-characterized food standards. Figure 7 shows the DNA purified from GMO standards obtained from Fluka. The Fluka reference set used contains 0, 0.1, 0.5 and 2% GMO maize. The Wizard® DNA System for Food provides DNA suitable and sufficient for 100 PCR amplifications from a 200mg food sample eluted in 100µl and allows the detection of GMO content as low as 0.1% in maize samples (cornmeal) (Figure 8).

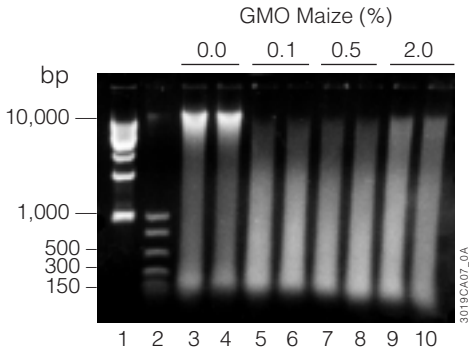

Figure 7. Duplicate Fluka maize reference standard maize samples containing up to 2% GMO maize were processed with the Wizard® Magnetic DNA Purification System for Food. Ten percent of 100µl eluates of DNA in duplicate were loaded on a 1% agarose gel. The 0% standard shows a greater yield and higher molecular weight DNA because non-GMO maize undergoes minimal processing in the preparation of the reference standard. Lanes 1 and 2, 1kb DNA Step Ladder (Cat.# G6941) and PCR Markers (Cat.# G3161), respectively.

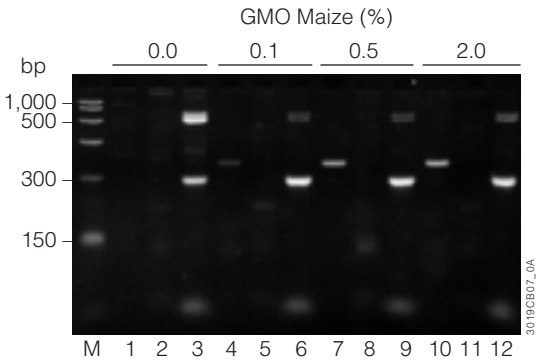

Figure 8. Fluka reference DNA used in PCR analysis. PCR products from the 35S promoter, *NOS* terminator and chloroplast positive control were generated, as for Figure 4, for each level of GMO content. Ten percent of 100µl reactions were resolved on a 4% NuSieve® 3:1 agarose gel. The 0% standard shows no amplification of 35S or *NOS* sequences as expected; the chloroplast control is positive. The 0.1% to 2% standard samples show weak to strong amplification of 35S sequences and negative *NOS* sequences. This assay shows that GMO sequences are detectable in the 0.1% Fluka maize reference standards. Lane 1, PCR Markers (Cat.# G3161).

Summary

The Wizard® Magnetic DNA Purification System for Food provides a versatile method of DNA purification from many foods and foodstuffs. The magnetic separation format with MagneSil™ PMPs allows scalability over a broad range of sample sizes. GMO content can be reliably detected to as low as 0.1%. The Wizard® DNA System for Food is easy-to-use and can be readily adapted for use in automated systems, such as Labsystems' Kingfisher™ (see Part II, page 19).

References

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Ordering Information

Product	Size	Cat.#
Wizard® Magnetic DNA Purification System for Food	200 assays	FF3750
	400 assays	FF3751

[a]U.S. Pat. No. 6,027,945.

[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.

[c]Patent Pending.

[d]U.S. Pat. No. 5,658,548, Australian Pat. No. 689815 and other patents.