Wizard® Magnetic DNA Purification System for Food: Part II. Semi-Automated DNA Isolation and Analysis of GMO Foods

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The widespread demand for labeling of foodstuffs containing genetically modified organisms (GMO) has created a need for accurate GMO testing methods. One recommended method requires the rapid isolation of DNA from many different sources. Promega has developed the Wizard® Magnetic DNA Purification System for Food, which allows isolation of DNA from foodstuffs. The DNA is then suitable for subsequent genetic analysis. This article demonstrates the use of a semi-automated method for isolation of genomic DNA from soy flour and cornmeal. The purified DNA was analyzed using the Biosmart Allin 1.0 GMO Screening System that is now available through Promega. This system identifies GMO through PCR amplification and is provided with controls for soy and corn products.

Introduction

While the nucleic acid content of food ingredients is of minor nutritional interest, the isolation of DNA and RNA and subsequent analysis using amplification-based techniques is critical to many research and food analysis applications. Although the introduction of foreign genetic material into commercially important crops has led to the introduction of many favorable agronomic traits, public concern has resulted in discussions regarding the labeling and selling of food that contains GMO (1,2). Currently, the labeling of transgenic foodstuffs is required in some countries (e.g., European Union, Australia, New Zealand and Japan), while other countries, including the United States and Canada, have not yet adopted requirements for GMO labeling.

A common method to determine whether foodstuffs contain genetically modified ingredients involves purification of genomic DNA from the material in question and subsequent analysis by PCR[a] amplification. One of the most important factors directing transgene expression in plants is the promoter and terminator elements that are used to control transcription of the foreign gene. Two of the most common genetic elements used to drive transgene expression are the constitutive 35S promoter from the cauliflower mosaic virus (CaMV) and the 3´ terminator isolated from the nopaline synthase gene (*NOS*) of *Agrobacterium tumefaciens*. These two genetic elements are often targeted in PCR-based GMO tests, as they do not naturally occur in agricultural crop sources, are widely present in commercial GMO-containing foods and can be detected with great sensitivity (3,4).

Promega has developed a system designed for the rapid isolation and subsequent PCR-based GMO analysis of DNA from agricultural and food materials. The Wizard® Magnetic DNA Purification System for Food[b] utilizes MagneSil™ Paramagnetic Particles[c] (PMPs) to rapidly isolate DNA from many different starting sources (see Part I of this article, beginning on page 14). We used this system to isolate genomic DNA from soy flour and cornmeal. Once isolated, we analyzed the DNA by gel electrophoresis, quantitated it by reading sample absorbance at 260nm and analyzed the DNA for GMO content using the Biosmart Allin 1.0 GMO Screening System distributed by Promega. We demonstrate the excellent performance of these coupled technologies and discuss how these systems can be used for GMO testing of other agricultural and food materials.

Semi-Automated Isolation of Genomic DNA from Foodstuffs

A semi-automated protocol for the Wizard® Magnetic DNA Purification System for Food is described here using the KingFisher™ instrument (Labsystems). Following a brief lysis procedure (Table 1), this instrument allows the "hands-off" isolation of genomic DNA from up to 24 lysed samples. Genomic DNA was isolated from soy flour and cornmeal purchased at a local supermarket (Madison, WI). Two-hundred milligram and 100mg amounts of each food ingredient were measured and placed in 1.5ml microcentrifuge tubes. Using this semi-automated format for the Wizard® Magnetic DNA Purification System for Food, genomic DNA isolation occurs in two parts. The first part of the procedure involves the preparation of a sample lysate using the provided Lysis Buffers as outlined in Table 1.

The second part of the procedure uses Promega's MagneSil™ Paramagnetic Particles (PMPs) to rapidly capture the genomic DNA present in the lysate. Table 2 lists the 96 well plate setup and solutions required to perform the assay using the KingFisher™ instrument. Figure 1 shows the steps of the automated procedure using the KingFisher™ instrument. The purified DNA samples are eluted in water and are ready for PCR analysis.

Table 1. Preparation of Lysate.

1. Add 125µl of Lysis Buffer A per 50mg of starting material.
2. Add 5µl of RNAse A Solution and vortex vigorously.
3. Add 62.5µl of Lysis Buffer B per 50mg of starting material and vortex vigorously.
4. Incubate at room temperature for 10 minutes, vortexing periodically.
5. Add 187.5µl of Precipitation Solution per 50mg of starting material and vortex vigorously.
6. Spin the samples in a microcentrifuge on high speed for 10 minutes.
7. Remove the supernatant (lysate) to a new 1.5ml microcentrifuge tube.

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Table 2. Setup by Row for 96 Well Plate.

Row	Contents
A	50µl Sample Lysate + 45µl isopropanol + MagneSil™ PMPs
B	100µl Lysis Buffer B
C	100µl 70% ethanol
D	100µl 70% ethanol
E	100µl 70% ethanol
F	100µl 70% ethanol
G	50µl Nuclease-Free Water

Analysis of Isolated DNA

The effects of increasing sample size and concentration of MagneSil™ PMPs on DNA yield and quality were investigated. Genomic DNA was isolated from either 100 or 200mg (dry weight) of starting material, and increasing concentrations of MagneSil™ PMPs were used in the semi-automated protocol. Aliquots of each isolated genomic DNA preparation were analyzed by agarose gel electrophoresis and ethidium bromide visualization (Figure 2). DNA yield was estimated using spectrophotometric measurements at 260nm, and the quality of the isolated genomic DNA was evaluated by calculating an A_{260}/A_{280} ratio. There appears to be an increase in yield of DNA with an increasing amount of MagneSil™ PMPs (Figure 2). The DNA purified is of high quality and contains little detectable RNA.

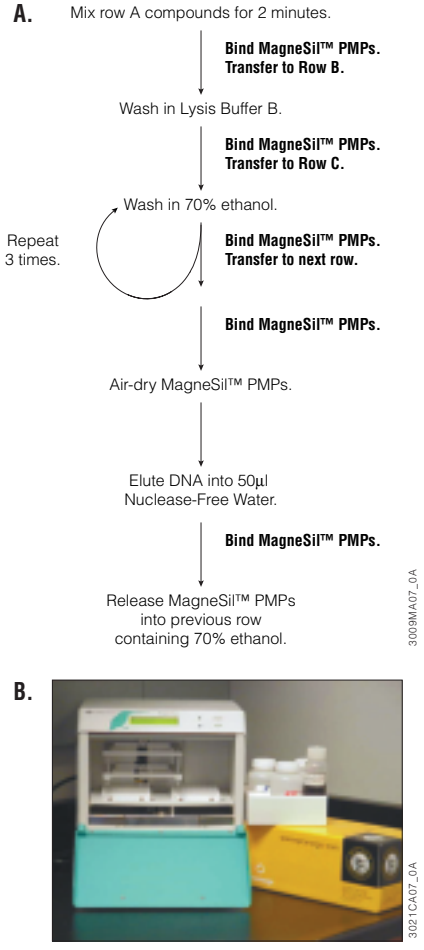

Figure 1. Isolation of DNA using the KingFisher™ instrument. Panel A: MagneSil™ Paramagnetic Particle (PMPs)-capture of genomic DNA. **Panel B:** KingFisher™ instrument.

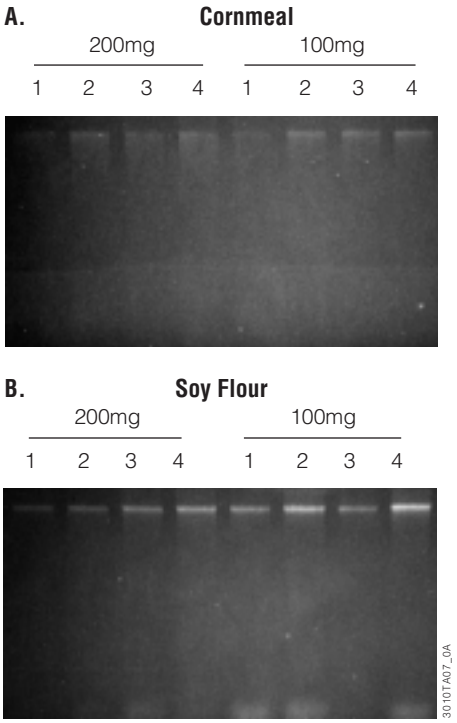

Figure 2. Agarose gel analysis of genomic DNA isolated from cornmeal and soy flour using increasing amounts of MagneSil™ PMPs. Aliquots (20µl) of each DNA preparation were resolved by 1% agarose gel electrophoresis, and the DNA was visualized by staining with ethidium bromide. **Panel A**: Genomic DNA isolated from the amount of cornmeal indicated. **Panel B**: Genomic DNA isolated from the amount of soy flour indicated. For both panels, lanes 1 represents use of 1mg of MagneSil™ PMPs; lane 2, 1.5mg; lane 3, 2mg; lane 4, 2.5mg.

The purified genomic DNA was analyzed for GMO content using the Biosmart Allin 1.0 GMO Screening System. This system uses a series of two nested PCR amplifications to detect the presence of GMO materials by amplification of the 35S CaMV promoter sequence. For the first round of PCR, 5µl of isolated genomic DNA was added to a reaction mixture (containing 40µl of the Allin Mix 1 component, 5µl of internal control template and 1 unit of *Taq* DNA polymerase (AmpliTaq®, Perkin Elmer). The reactions were overlaid with mineral oil and placed in a thermocycler. The samples were amplified as recommended in the *Biosmart Allin 1.0* technical manual (5) using the following cycling parameters: 3 minutes at 95°C, followed by 40 cycles of 30 seconds at 95°C and 40 seconds at 55°C, ending with one cycle of 3 minutes at 72°C. One microliter of the first-round PCR product was used as the template in the second PCR amplification containing 49µl of the Allin Mix 2 component and 1 unit of *Taq* DNA polymerase. The nested reaction was overlaid with mineral oil and placed in a thermocycler. The samples were amplified as directed using the following parameters: 1 minute at 95°C, followed with 45 cycles of 40 seconds at 95°C, 40 seconds at 50°C and 30 seconds at 72°C, ending with one cycle of 3 minutes at 72°C. Aliquots of the PCR products were separated on a 2.5% agarose gel and visualized with ethidium bromide staining (Figure 3).

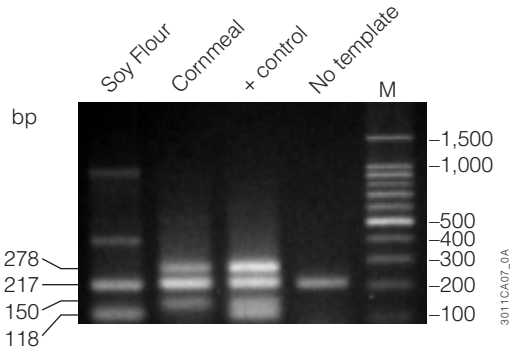

Figure 3. GMO analysis of genomic DNA using the Biosmart Allin 1.0 GMO Screening System. Genomic DNA isolated from cornmeal and soy flour was amplified using the Allin 1.0 GMO Screening System. Twenty microliters of the nested PCR products were resolved by 2.5% agarose gel electrophoresis. The PCR products were visualized by ethidium bromide staining. Lane M, 100bp DNA Ladder (Cat.# G2101). All reactions were 'spiked' with the internal control DNA template and should contain the 217bp internal control band. The PCR products present are the 278bp maize zein, the150bp 35S promoter and the 118bp soy lectin.

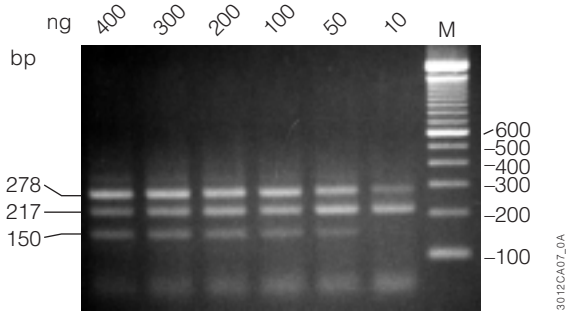

Figure 4. Limit of detection for the Biosmart Allin 1.0 GMO Screening System. DNA isolated from cornmeal was amplified using the Allin 1.0 System with decreasing amounts of starting template as indicated. Twenty microliters of the nested PCR products were resolved by 2.5% agarose gel electrophoresis. The PCR products were visualized by ethidium bromide staining. The PCR products present are the 278bp maize zein; 217bp internal control and the 150bp 35S promoter. Lane M, 100bp DNA Ladder (Life Technologies). The GMO detection limit for this cornmeal sample was 50ng of genomic DNA as estimated by A_{260} measurement.

For the samples analyzed, only the cornmeal tested positive for GMO content as indicated by the presence of a 35S PCR product. No GMO content was detected in the soy flour. Internal control PCR products indicated that there were no PCR inhibitors in the samples. In addition, sample identity controls are provided with the Biosmart Allin 1.0 System. Corn samples will produce a 278bp control band for maize zein; soy samples will produce a 118bp control band for soya lectin.

It is recommended to use 400ng of template for the initial PCR with the Biosmart Allin 1.0 System (5). We found the limit of detection to be 50ng of starting material (Figure 4). However, this detection limit will be sample-dependent. Failure to amplify using the corn- and soy-specific PCR primers provided in the Biosmart Allin 1.0 System indicates that there is too little template in the reaction or the presence of PCR inhibitors in the DNA sample preparation. Therefore, detection limits must be determined for each sample type.

Critical Aspects of Sample Preparation for GMO Assays

Due to the diversity of samples sources, some may require additional processing steps prior to lysate preparation. This additional processing will depend upon variables such as the size, physical state and composition of the starting materials (see Part I of this article, page 14). Whenever possible, samples should be processed to achieve a fine, dry powder. When grinding is not pssible, mincing the starting material may also increase DNA yields. It is essential to mix samples and reagents well. Thus, the vortex steps in the procedure are critical: *Failure to vortex and mix the samples vigorously will result in little to no purified DNA.* For some foodstuffs, it is helpful to heat to melting. For example, when processing chocolate, DNA yield is improved when the chocolate is melted compared to chocolate that is crushed (data not shown).

In the experiments described here, cornmeal and soy flour were compared. The average yield of DNA between samples was similar, as determined by spectrophotometric measurement. However, this method will not discriminate between single-stranded and double-stranded DNA species and only estimates the amount of total DNA present. Different food products vary in the amount of additives, preservatives and cell material present as well as degree of processing. As a result, the yield and purity of the genomic DNA can vary significantly with different starting material. Therefore, it is important to titrate each product sample to determine the starting quantity necessary to yield the amount of DNA needed for downstream applications.

The Biosmart Allin 1.0 GMO Screening System detected the 35S promoter in the cornmeal sample, indicating GMO content. The 35S promoter was not detected in the soy flour, indicating the GMO content was below the detectable threshold (0.1% GMO) (Figure 3). However, it is important to note that the Allin 1.0 GMO System is qualitative, not quantitative, for GMO.

When using purified DNA in a GMO detection system, such as the Allin 1.0 GMO Screening System, it is important to use the internal control provided with the system. This control provides information as to whether the sample contains PCR inhibitors. The internal control is simply a DNA template 'spiked' into the GMO detection reactions. Failure to amplify the internal control indicates that the sample contains PCR inhibitors, and further purification of the DNA is required before GMO content can be assayed.

Conclusions

In this report, we demonstrated the integration of a semi-automated protocol for the Wizard® Magnetic DNA Purification System for Food with the BioSmart Allin 1.0 GMO Screening System for rapid and robust GMO testing of foodstuffs. The Wizard® Magnetic DNA Purification System for Food was used to isolate DNA from soy flour and cornmeal. The system's MagneSil™ PMP-based isolation procedure is scalable and can be adapted easily to many sample types. Automation of the procedure allows the user to rapidly adopt a common DNA isolation method to virtually any scale and throughput level. For these experiments, we used a KingFisher™ instrument to rapidly isolate PCR-ready DNA from up to 24 food lysates at once. This same method could be adapted to a variety of robotic and liquid handling platforms.

We tested the isolated genomic DNA using the Allin 1.0 GMO Screening System. This system detects the 35S promoter element that is widely used in commercial crops to drive transgene expression and co-amplifies a confirmatory internal control sequence. This method is extremely sensitive and has been widely accepted as a technique for GMO detection. Together, Promega's Wizard® Magnetic DNA Purification System for Food and the Allin 1.0 GMO Screening System (offered through Promega) provide a set of GMO screening products for target samples such as food ingredients and processed food products.

References

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2. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official Journal L 043, 14/02/1997, 1–7.
3. Pietsch K. *et al.* (1997) *Deutsche Lebensmittel RundschauHeft* **2**, 35.
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5. *Biosmart Manual for Allin 1.0 Part# GE024*, Biosmart molecular analytical laboratories.

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Ordering Information

Product	Size	Cat.#
Wizard® Magnetic DNA Purification System for Food	200 assays	FF3750
	400 assays	FF3751
Biosmart Allin 1.0 GMO Screening System	3 × 20 assays	FF3730
	6 × 20 assays	FF3740

Related Products

Product	Size	Cat.#
100bp DNA Ladder	250µl (50 lanes)	G2101
ART® 20P, Pipet Tip, 20µl	960/pack	DY1071
ART® 200, Pipet Tip, 200µl	960/pack	DY1121
ART® 1000, Pipet Tip, 1,000µl	960/pack	DY1131
Mineral Oil	12ml	DY1151
Nuclease-Free Water	50ml (2 × 25ml)	P1193