Automated DNA Isolation from Genetically Modified Soybeans and Soybean Derived Food Material with the MagNA Pure LC System

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Introduction

Worldwide, the production of genetically modified organisms (GMO) is increasing rapidly. In Japan, a country that imports many crops from all over the world, the use of GMO is a social concern. Therefore, the construction of a fast and reliable inspection system is demanded. For this purpose, we have developed a new high throughput method for DNA isolation of GMOs from food. The method contains two steps:

- manual pretreatment using an extraction buffer
- automated DNA isolation performed with the MagNA Pure LC DNA Isolation Kit

The method was tested with the raw soybean material and a variety of food products containing soybeans. The tests included criteria like DNA integrity, yield, purity, reproducibility and scalability.

Materials and Methods

Materials

0.5 %, 1.0 %, 2.0 % and 5.0 % GMO standard material from Roundup Ready soybeans (Soya Bean Powder SB-Set) was obtained from Fluka. Food products (bean-curd, fried tofu, tofu, yuba, soymilk, dried tofu, extracted protein) were bought in regular food shops. The MagNA Pure LC DNA Isolation Kit I and the LightCycler FastStart DNA Master Hybridization Probes were obtained from Roche Applied Science. PCR primers and probes for cauliflower mosaic virus (CaMV) 35 S and for lectin were synthesized at the Nihon Gene Research Lab (NGRL).

Table 1 : Yield, purity and amplification of DNA isolated from the GMO standards (%) (n = 5). Samples were analyzed in 5-fold replicates

%	Yield (µg)		Purity (OD_{260}/OD_{280})		Crossing point[1]	
	Mean value ± S.D.	CV (%)	Mean value ± S.D.	CV (%)	Mean value ± S.D.	CV (%)
0.5	34.10 ± 1.19	3.50	1.85 ± 0.026	1.41	33.24 ± 0.22	0.66
1.0	34.00 ± 2.57	7.56	1.82 ± 0.026	1.43	32.62 ± 0.16	0.49
2.0	35.40 ± 3.49	9.86	1.80 ± 0.025	1.39	32.38 ± 0.096	0.30
5.0	35.80 ± 1.78	4.97	1.79 ± 0.013	0.73	31.73 ± 0.15	0.47
100	40.35 ± 2.41	5.97	1.80 ± 0.012	0.67	28.46 ± 0.15	0.53

[1]crossing points reflect sample concentration

Pretreatment of samples

50 mg of powdered GMO standard material containing 100 %, 5 %, 2 %, 1 % and 0.5 % (content rate) Roundup Ready Soybeans or 50 mg (wet weight) of each of the soybean products, were incubated with 800 µl extraction buffer (10 mM Tris pH 8.0, 100 mM NaCl, 2 mM EDTA, 1 % w/v sodiumdodecylsulfate) and 100 µl 5 M guanidine thiocyanate at 60 °C for 10 minutes. After incubation, 1 ml chloroform was added and shaken vigorously. After centrifugation at 12,000 g for 5 minutes at room temperature, the upper phase was transferred to a new tube. 200 µl of the upper phase was applied to the sample cartridge of the MagNA Pure LC Instrument.

DNA isolation on the MagNA Pure LC

After pretreatment, the upper phase (200 µl) was processed in the MagNA Pure LC and subsequently tested in LightCycler assays. Genomic DNA was prepared using the MagNA Pure LC DNA Isolation Kit I according to the manufacturer's guidelines. The High Performance DNA Isolation Protocol was applied and DNA was eluted with 100 µl elution buffer.

Analysis of the isolated DNA

The integrity of the isolated DNA was checked on a 1% agarose gel. DNA yield and purity were assessed by OD measurements. For the analysis of the GMO standard soybean powder, 100 ng Roundup Ready DNA was used for the LightCycler assay with CaMV 35 S primers and probes. To check the amplificability of the DNA isolated from the food material, LightCycler PCRs were performed using lectin-specific primers and hybridization probes.

The PCR for CaMV 35 S and lectin was performed using 2 µl master mix (LightCycler FastStart DNA Master Hybridization Probes), 3 mM $MgCl_2$, 0.2 µM fluorescein

probe, 0.4 µM LC Red 640 probe, 0.5 µM of each primer, 100 ng DNA and water to a final volume of 20 µl. The PCR protocol was: predenaturation 95 °C/10 minutes; 40 PCR cycles: denaturation 95 °C/15 seconds, annealing 60 °C/15 seconds (detection), extension 72 °C/15 seconds; cooling 40 °C/30 seconds.

Results and Discussion

DNA integrity

Agarose gel analysis of the DNA from GMO standard material and powdered soybeans showed that the DNA was of high integrity. The molecular weight range of the isolated DNA was usually about 20 kb (data not shown).

Yield and purity

OD analysis revealed a yield of up to 34 µg genomic DNA from 200 µl upper phase (sample material after pretreatment). As the total upper phase was at least 600 µl, the total yield was up to 102 µg genomic DNA from 50 mg starting material. The OD_{260}/OD_{280} ratio was 1.8 ± 0.05, indicating DNA of high purity (Table 1).

Reproducibility and scalability

Isolation of DNA from samples containing various amounts of GMO in 5-fold replicates showed excellent reproducibility. The coefficient of variation (CV) for yield was < 10 % and CV for purity was < 1.5 % (Table 1). With regard to LightCycler crossing points using the CaMV 35 S promoter specific primers and hybridization probes, the CV was < 1 % (Table 1). Analysis of LightCycler crossing points showed good scalability (Figures 1, 2).

Usefulness of MagNA Pure LC DNA isolation for final food products from soybeans

DNA was isolated from seven final food products of soybeans. OD analysis revealed yields of up to 35 µg genomic DNA/50 mg starting material. The DNA isolated from final food products was of lower molecular weight (data not shown). The OD_{260}/OD_{280} ratio was 1.85 ± 0.15, indicating DNA of high purity. To confirm the amplificability of the DNA, the LightCycler PCR was performed for all food samples using lectin specific primers and hybridization probes and showed no PCR inhibition (Figure 3).

Summary

The GMO and food DNA isolation method using the MagNA Pure LC System is a valuable method for efficient automated isolation of DNA from various samples. The isolated DNA is of high quality and shows no PCR inhibition. The reproducibility is excellent and no cross contamination was found (data not shown). We also established the isolation of genomic DNA from maize (unpublished data). Thus, the MagNA Pure LC and LightCycler System allow high throughput detection of GMO DNA.

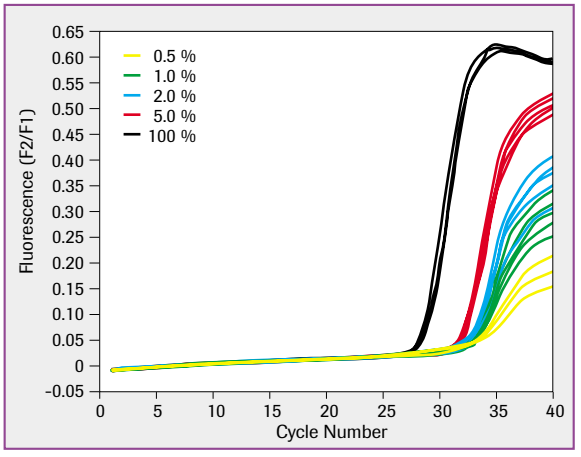

Figure 1: LightCycler PCR with DNA isolated from various GMO standard materials. All samples were analyzed in 5-fold replicates; 100 ng DNA was used

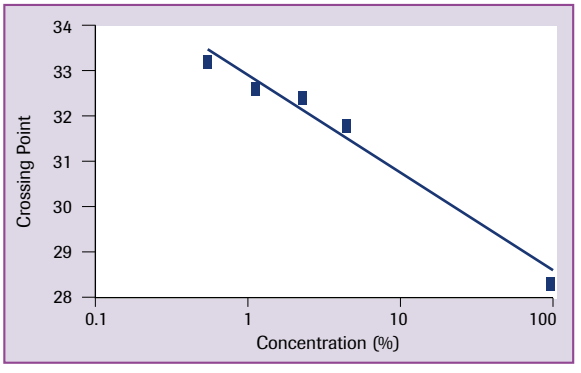

Figure 2: Scalability of sample concentration (%). DNA was isolated from 0.5 %, 1 %, 2 %, 5 % and 100 % samples and analyzed by LightCycler PCR

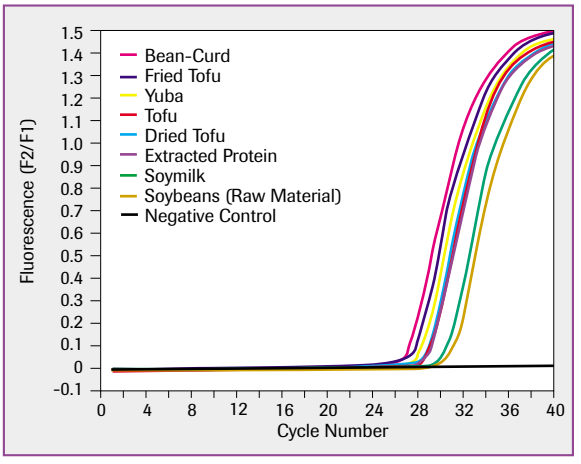

Figure 3: LightCycler PCR with DNA isolated from soybeans (raw material) and various final food products of soybeans. 100 ng DNA was used

Order INFO

Product	Pack Size	Cat. No.
MagNA Pure LC DNA Isolation Kit I	1 kit (192 isolations)	3 003 990
MagNA Pure LC Instrument	1 instrument plus accessories	2 236 931
LightCycler FastStart DNA Master Hybridization Probes	1 kit (96 reactions)	3 003 248