JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Development of Sensitive Crop-Specific Polymerase Chain Reaction Assays Using 5S DNA: Applications in Food Traceability

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The 5S intergenic spacers were amplified using a common pair of primers and sequenced from four species (*Brassica napus*, *Zea mays*, *Helianthus annuus*, and *Glycine max*). Crop-specific assays were developed from primers designed from the spacers and tested to amplify corresponding DNAs in both conventional end-point and real-time polymerase chain reactions (PCRs). The high copy numbers of the 5S DNA in plants make it possible to detect very small amounts of DNA using this marker. This sensitivity made it possible to compare different DNA extraction methods for highly processed food products using 5S spacers, even allowing dilution of templates to overcome PCR inhibition.

KEYWORDS: DNA detection; soy; sunflower; maize; oilseed rape

INTRODUCTION

The authenticity of food products is an important issue in the European Community, as judged by the projects in this area, funded within EC Frameworks IV, V, and VI. Correct labeling and traceability, the ability to trace and follow food, feed, and ingredients through all stages of production, processing, and distribution, safeguard the consumer against fraud and life-threatening allergenic reactions and empower the consumer to choose whether to eat genetically modified crops. Although proteins and other chemicals have been used to identify certain products, DNA markers are the best-suited for identification of biological samples. This is because of the remarkable durability of DNA, even in hostile environments that are encountered during many of the processing steps (*1*).

The use of DNA markers as diagnostic tools for food authenticity, provenance, and traceability of variety/type composition of complex food matrices has been investigated in an increasing number of projects worldwide (*2*−*5*). However, some processed food contains highly degraded DNA and/or polymerase chain reaction (PCR) inhibitors, both of which may affect the subsequent PCRs used for the amplification of diagnostic DNA sequences. These effects may be overcome by modification of the DNA extraction process and PCR assay design and conditions. It is sometimes possible to overcome these inhibitory effects by extensive dilution of the DNA extract; however, this may not be an option when the amount of DNA in the sample is limited. In these cases, a sensitive method for the detection of small amounts of highly degraded DNA is necessary. Therefore, it is important to be able to optimize the DNA extraction method as a means of assessing the extract.

Markers from the chloroplast genome are often used for testing plant samples (*1*) because of their relatively high copy numbers (*6*). However, these markers are often generic and therefore not suitable for analyzing a food product containing many plant species. In addition, the number of chloroplasts, and hence markers, is variable in different tissues (*7*). An alternative plant sequence in high copy numbers is the 5S DNA. These genes are present in many thousands of copies per haploid genome and arranged in tracts of tandem repeats in a few locations (*8*−*10*). The repeats are several hundreds of base pairs in length containing the 5S rRNA gene (approximately 120 bp) and the nontranscribed spacer. The 5S rRNA gene sequence is very well-conserved between plant species while the spacer is species-specific and the sequence has been used for phylogenetic studies (*11*−*14*) and species identification (*15*−*17*).

Here, we aim to exploit the conserved nature of these highly repetitive sequences and their tandem arrangement to amplify the 5S DNA spacers from four diverse crop species—oilseed rape (*Brassica napus* L.), maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), and soy (*Glycine max* L. Merrill)—using a "common" primer pair. We show that the 5S spacer from these four crop species can be used to develop crop-specific assays, which are highly sensitive as a consequence of their copy numbers, permitting evaluation of different DNA extraction procedures even from highly processed foods such as oils, where recovered DNA is expected to be very low in number and quality.

MATERIALS AND METHODS

Development of a Sensitive PCR Assay for the Detection of Plant DNA Using 5S DNA. Sequences of 5S rRNA gene of sunflower (http://www.man.poznan.pl/5SData/), maize (AF242644-7), oilseed rape

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10.1021/jf063259v CCC: $37.00
Published on Web 05/19/2007

Table 1. Species-Specific Primers for Each Crop Based on the Alignment of 5S Gene Sequences

name	sequence (5′–3′)	amplicon length (bp)	amplicon T_m (°C)
Sun5S-F	GTGAAAGGAAGGCTTTGACG	108	92.1
Sun5S-R	CTTTCCACGCCTTGATCC		
OSR5S-F	GGCTGCGGAAAGTTATGG	90	81.2
OSR5S-R	GTGCATCGAGGTTAACGG		
Soya5S-F	CTTTTTGCCCTTATTCTGAG	122	79.6
Soya5S-R	CTACACCGAACGAGCCAC		
Maize5S-F	AATGGGTGACCGTTCTCG	75	78.1
Maize5S-R	CCTCCGCACAAAGTACCC		

Table 2. PCR Settings in the Lightcycler To Test Primer Specificity[a]

step	temp (°C)	hold time	acquisition mode	slope (°C/s)	program
denaturation	95	10 min	none	20	none
amplification	95	3 s	none	20	quanti
(35 cycles)	60	5 s	none	20	
	72	0 s	single	20	
melting curve	99	0 s	none	20	melt
	70	5 s	none	20	
	99	10 s	cont	0.1	
cooling	40	30 s	none	20	none

[a] Channel setting F1/1.

(ACX05060), and soy (ACX15199) were acquired through GenBank at the National Centre for Biotechnology Information website (http://www.ncbi.nlm.nih.gov). The sequences were aligned using Staden Package software (*18*) and primers (Sigma-Genosys Ltd., Haverhill, United Kingdom) designed in the conserved coding region using Oligo 6.45 (National Biosciences Inc., Plymouth, MN) to amplify the 5S DNA spacer. DNAs from sunflower, maize, soy, and oilseed rape were amplified using the primers 5SDNAF (5′-CTGGGAAGTCCTCGTGTTG-3′) and 5SDNAR (5′-TTAGTGCTGGTATGATCGCA-3′). All PCRs were performed in 20 μL volume containing 20 ng of DNA, 1 U of FastStart *Taq* DNA Polymerase (Roche Diagnostics Ltd., Lewes, United Kingdom), 1× buffer (Roche), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, and 0.5 μM each forward and reverse primer. PCRs were carried out in a thermocycler Perkin-Elmer 9700 (Applied Biosystems, Warrington, United Kingdom) with the following profile: 95 °C for 5 min, 35 cycles at 95 °C for 20 s, 60 °C for 30 s, and 72 °C for 30 s; final extension at 72 °C for 3 min. The bands were excised from the gel and purified with QIAquick gel extraction kit (Qiagen, Crawley, United Kingdom) following the manufacturer's instructions. The PCR products were sequenced using both forward and reverse PCR primers with BigDye Terminator cycle sequencing in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Primers to amplify the 5S spacer from each of the four crop species were designed using the sequences obtained from the amplification with 5SDNA and 5SDNAR and were tested for specificity and cross-reactions (**Table 1**). The primers were designed to amplify amplicons of different lengths and T_m values in order to be distinguishable using agarose gels and in a real-time PCR platform. DNAs from sunflower, maize, soy, and oilseed rape were amplified with the four primer pairs using the same reaction conditions as above but a different cycling profile: 95 °C for 5 min, 35 cycles at 95 °C for 30 s, and 60 °C for 10 s. The primers were also tested using a Lightcycler v 1.0 (Roche); reactions were carried out in a 10 μL volume including 5 ng of DNA, 1× Fast Start DNA Master SYBR Green I Lightcycler (Roche), 2.5 mM $MgCl_2$, and 0.5 μM each forward and reverse primer as described in **Table 2**.

DNA Extracts from Highly Processed Foods. Different food products (soy milk, soy single cream, powdered soy milk for infant, lecithin granules, and soy sauce) and sunflower and maize oils (**Table 3**) were purchased from local shops. Commercial kits and in-house methods were applied for the extraction of DNA from these products.

Table 3. Products Tested for the DNA Extraction

code	base	supplier/source	product
A	soy	Alpro	soy cream
B	soy	Tesco	soy milk
C	soy	So good	soy milk
D	soy	Cow & Gate	soy infant formula
E	soy	Optima	100% lecithin granules
F	sunflower	Sainsbury's	pure sunflower oil
G	maize	Mazola	pure maize oil
H	soy	Amoy	light soy sauce
I	soy	Amoy	dark soy sauce

Method 1: Wizard Magnetic for Food Protocol (Promega, Southampton, United Kingdom). The food products were extracted according to the manufacturer's instructions, except that *n*-heptane was used in place of *n*-hexane.

Method 2: NIAB Protocol A. One milliliter of each sample (0.4 g for samples D and E) was mixed with 500 μL of *n*-heptane and vortexed vigorously for 1 min, and 400 μL of digestion buffer (50 mM Tris, pH 8.5, 1 mM EDTA, and 0.5% Tween 20) and 20 μg of proteinase K were added and incubated for 30 min at 48 °C. The aqueous phase was recovered by centrifugation for 10 min at 11500*g* and transferred to a new tube. Ice-cold isopropanol (500 μL) was added and mixed, and the reaction was left at 4 °C for 1 h. After centrifugation (20 min at 11500*g*), the supernatant was pipetted off and the DNA was resuspended with 100 μL of water and purified by using the QIAquick gel extraction kit (Qiagen) according to the manufacturer's instructions.

Method 3: NIAB Protocol B. This method was as above except that the precipitated DNA was washed twice with 500 μL of 70% ethanol, air-dried, dissolved in 100 μL of water, and then purified by using the Gene Clean III kit, Bio 101, according to the manufacturer's instructions.

Method 4: Hurst A. The samples were extracted according to Hurst et al. (*3*) except that MagneSil PMPs (Wizard Magnetic for Food, Promega) was used in place of Magyx suspension.

Method 5: Hurst B. The samples were extracted according to Hurst et al. (*3*) except that glassmilk (Gene Clean III kit, MP Biomedicals, Cambridge, United Kingdom) was used in place of Magyx suspension.

Method 6: Official Swiss Method for Lecithin and Oil DNA Extraction A. The samples were extracted according to *Swiss Food Manual* (*19*).

Method 7. This method was as above with a further purification using Gene Clean III kit.

The specific primers were used to detect the presence of the corresponding DNA in the extracted samples following the conditions mentioned previously and by using 1 μL of the elution as a template.

Furthermore, five samples (B, D, E, H, and I; **Table 3**) were chosen and dilutions (10-, 100-, and 1000-fold) of the DNAs extracted with the different protocols were amplified with the primers.

RESULTS AND DISCUSSION

Development of a Sensitive PCR Assay for the Detection of Plant DNA Using 5S DNA. Primers designed on the conserved region of the 5S gene were used to amplify sunflower, maize, oilseed rape, and soy DNAs. Sequences of the DNA fragments produced showed homology to sequences in the NCBI database using the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST/) for the oilseed rape (95% against X60723), maize (98% against AF242644), and soy (98% against X15199) spacers. No corresponding sequence was found for the sunflower sequence. The sequences of 5S DNA spacers from the sunflower and oilseed rape were submitted to the Genbank database (accession numbers DQ865267−DQ865268, respectively).

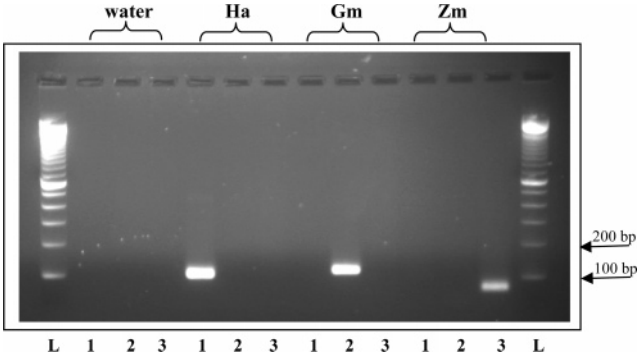

Figure 1. Agarose gel electrophoresis of PCR products obtained using species-specific primers. The amplification of each specific product using their respective primers is shown. No cross-reactions are detected between the different species. Lane 1 contains PCR with sunflower (Ha)-specific primers; lane 2 contains soy (Gm)-specific primers, and lane 3 contains maize (Zm)-specific primers. L denotes marker (100 bp ladder; Invitrogen, Paisley, United Kingdom).

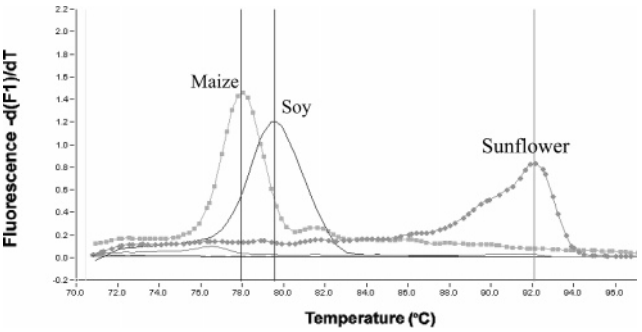

Figure 2. Amplification and differentiation of three specific 5S spacer amplicons using Lightcycler real-time PCR.

Four specific primer pairs were designed to amplify short fragments to reflect the highly degraded templates expected from processed material. The products ranged between 75 and 108 bp in lengths and were sufficiently different to be distinguishable by using agarose gels (**Figure 1**) and also by putative melting temperatures of the products for real-time PCR detection (**Figure 2**). The quality of the DNA present in the samples is of particular significance in food diagnostics. Because recovered DNA is expected to be highly degraded, short amplicons are preferable for successful detection (*20*). The primers amplified the expected sizes for each crop, and specificity for each species was determined (**Figure 1**). The test for oilseed rape showed the presence of DNA, even in the water controls (not shown). Failure to control contamination, even after all components of the reactions were completely changed, resulted in no more work on this crop. The reason for contamination can be explained by the extensive areas of oilseed rape grown nearby, resulting in pervasive pollen during the flowering period. If this is correct, "DNA testing" of oilseed rape is a major concern where the crop is grown.

The amplifications, including cross-reactions, were conducted in parallel in a real-time platform (Lightcycler version 1.0) by using SYBR green detection (**Figure 2**). Three well-distinguished peaks were obtained for the three crops: 78 °C for maize, 79.6 °C for soy, and 92 °C for sunflower.

In PCR reactions containing all six primers, species-specific products for each species are amplified when each DNA is added singly (**Figure 3A**) or when individual primer pairs are used to amplify mixed samples (**Figure 3B**).

DNA Extracts from Highly Processed Foods. Soy-, maize-, and sunflower-derived products were purchased from commercial sources and used in this study (**Table 3**). Various factors may contribute to the degradation of DNA in food such as hydrolysis of the DNA due to prolonged heat treatment, enzymatic degradation by nucleases, and depurination and hydrolysis of DNA at low pH. The degree of PCR inhibition is to a great extent dependent on the food type. For example, heat treatment continuously degrades DNA resulting in a strongly reduced average fragment length (*21*). For instance, lecithin undergoes heating and chemical treatments, soy sauce fermentation, and soy milk heating during their production. Furthermore, PCR can be inhibited by various compounds present in foods: Substances such as carbohydrates, phenols, fatty acids, or oils contained in the food products can be carried forward in the eluted DNA and would impair PCR (*22*).

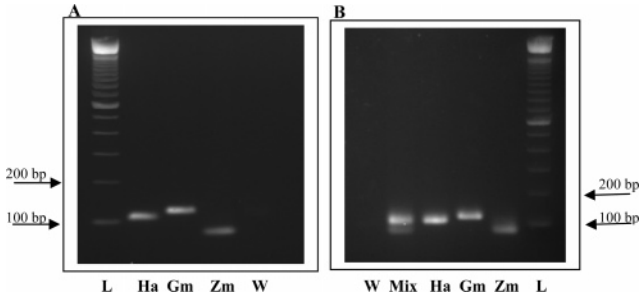

Figure 3. Agarose gel electrophoresis of (**A**) multiplexed primers with sunflower (Ha), soy (Gm), and Zm (maize) DNA and (**B**) species-specific primers with all three DNAs (mix) and each alone. Water control (W) and 100 bp ladder (L; Invitrogen) were loaded along the samples.

The choice and optimization of the DNA extraction procedures that eliminate potential inhibitory components is of primary importance for the success of a given PCR method, especially when dealing with processed material (*23*−*25*): the purification step in DNA extraction protocols seems to be a key step. For this purpose, different protocols and purification alternatives including commercial kits and published methods were applied to the tested products.

The extracted DNAs were not visible when loaded in an agorose gel; this is consistent with previous studies carried out in processed food products (*3*−*5*). The presence and amplifiability of the DNAs were verified by using the species-specific primers developed. Two protocols, one and three, were able to amplify species−specific amplicons in all of the samples analyzed (summarized in **Table 4**).

The Wizard Magnetic for food has already been applied for the extraction of DNA from fatty matrix such as olive oil (*4*) and maize- and soy-derived products (*5*). The latter compared four different extraction protocols (Wizard, Promega; DNeasy Plant Minikit and QIAamp DNA Stool Minikit, Qiagen; and Nucleospin Food, Macherey-Nagel) from processed food such as crackers, polenta, tacos, and tofu by evaluating the integrity of the DNA based on amplification product length. Short fragments (less than 200 bp) could be amplified in all samples, while longer amplicons were dependent on product and extraction method.

Methods 4 and 5 were used for the DNA extraction from maize- and soy-derived products such as lecithin, extruded defatted soy, corn puff snacks, dried soybeans, maize grits, and maize kernels (*3*). The authors found that reasonable DNA could only be obtained from soybeans and maize kernels and that overall the yields were low. Nevertheless, they were able to amplify short DNA fragments (less than 200 bp), therefore confirming the presence of DNA.

In general, soy milk products, soy cream, and lecithin-extracted samples gave stronger amplification (more products

Table 4. DNA Extraction for Processed Food

extraction protocols	methodology	result
1. Wizard Magnetic for Food (Promega)	commercial product	good for all products
2. NIAB and QIAquick	Tris-based buffer containing SDS and proteinase K, hexane extraction and isopropanol precipitation, and column clean up product	OK for soy milk but not good for oils and low amounts of DNA products
3. NIAB & Gene Clean	same as above but silica matrix-based clean up product	good for all products
4. Modified Hurst et al. (*3*) and magnetic beads	Tris-based buffer containing SDS and RNase, isopropanol precipitation, and magnetic bead purification	OK for soy milk but not good for oils and low amounts of DNA products
5. Modified Hurst et al. (*3*) and Gene Clean	same as above but silica matrix-based clean up product	OK for soy milk but not good for oils and low amounts of DNA products
6. Official Swiss method for lecithin and oil DNA extraction	guanidine thiocyanate buffer with hexane extraction, chloroform extraction, and isopropanol precipitation	OK for soy milk but not good for oils and low amounts of DNA products
7. Official Swiss method for lecithin and oil DNA extraction and Gene Clean	same as above but silica matrix-based clean up product	OK for soy milk but not good for oils and low amounts of DNA products

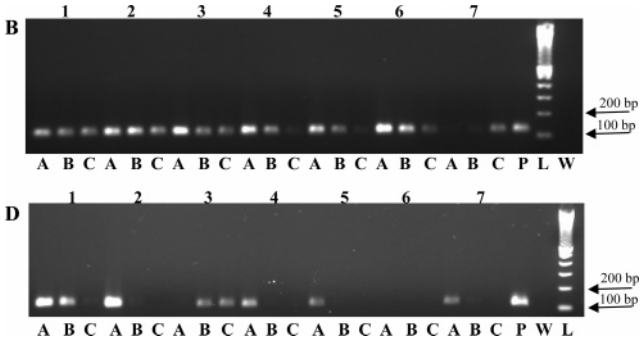

Figure 4. Amplification of 10- (**A**), 100- (**B**), and 1000-fold (**C**) dilutions of DNAs from food samples B and D (see **Table 3**) for the different extraction methods used in this study. No template, water control (W), and 100 bp ladder (L; Invitrogen) were loaded along the samples.

per unit of sample of template in PCRs) as compared to the oils and soy sauce samples. This reflects the quality and amount of DNA contained in the starting material. In some cases, the simplest and possibly most effective way to avoid inhibition of PCR is the dilution of the sample. To ensure that inhibition was not responsible for failure of amplification, PCRs were performed using three 10-fold dilutions of each sample. The amplification of the more diluted DNAs leads to a decrease in intensity of the amplification products consistent with less target DNA (**Figure 4**). Nevertheless, in some cases, the failure to amplify in the higher DNA concentrations is consistent with PCR inhibition: some samples showed amplification only when diluted DNA was used as the template; for example, sample B, extraction method 7, and sample D, extraction method 3 (**Figure 4**). In fact, most DNA samples extracted from oils and soy sauces did not give amplification with undiluted DNA. It is only with the use of high-copy number markers that allows inhibition to be assessed since single-copy targets will amplify once diluted when starting with very small amounts of template.

The use of a sensitive test is of special importance when verifying the presence of DNA in samples with a little amount of template (especially extracted from foodstuffs). Quality control measures include carrying out appropriate control PCR reactions using DNA primers designed to recognize DNA from the specific crop. Furthermore, from the study, it was evident that the choice of the method can have a great influence in the recovery of the DNA as highlighted also by Peano et al. (*5*). In this work, the 5S DNA was successfully used to detect the presence of DNA from soy-derived samples and vegetable oils. The specificity and sensitivity of the DNA tests may make it possible to identify adulteration of more expensive oils with cheap oils.

ACKNOWLEDGMENT

We thank Andy Greenland for critical reading of the manuscript.

LITERATURE CITED

(1) Martellossi, C.; Taylor, E.; Lee, D.; Graziosi, G.; Donini, P. DNA extraction and analysis from processed coffee beans. *J. Agric. Food Chem.* **2005**, *53*, 8432−8436.

(2) Pafundo, S.; Agrimonti, C.; Marmiroli, N. Traceability of plant contribute in olive oil by AFLPs. *J. Agric. Food Chem.* **2005**, *53*, 6995−7002.

(3) Hurst, C. D.; Knight, A.; Bruce, I. J. PCR detection of genetically modified soya and maize in foodstuffs. *Mol. Breed.* **1999**, *5*, 579−586.

(4) Breton, C.; Claux, D.; Metton, I.; Skorski, G.; Berville, A. Comparative study of methods for DNA preparation from olive oil samples to identify cultivar SSR alleles in commercial oil samples: possible forensic applications. *J. Agric. Food Chem.* **2004**, *52*, 531−537.

(5) Peano, C.; Samson, M. C.; Palmieri, L.; Gulli, M.; Marmiroli, N. Qualitative and quantitative evaluation of the genomic DNA extracted from GMO and non-GMO foodstuffs with four different extraction methods. *J. Agric. Food Chem.* **2004**, *52*, 6962−6968.

(6) Bendich, A. Why do chloroplasts and mitochondria contain so many copies of their genome? *BioEssays* **1987**, *6*, 279−282.

(7) Lamppa, G. K.; Bendich, A. J. Changes in chloroplast DNA levels during development of pea (*Pisum sativum*). *Plant Physiol.* **1979**, *64*, 126−130.

(8) Goldsbrough, P. B.; Ellis, T. H.; Cullis, C. A. Organisation of the 5S RNA genes in flax. *Nucleic Acids Res.* **1981**, *9*, 5895−5904.

(9) Ellis, T. H. N.; Lee, D.; Thomas, C. M.; Simpson, P. R.; Cleary, W. G.; Newman, M. A.; Burcham, K. W. G. 5S rRNA genes in *Pisum*: Sequence, long range mapping and chromosomal organization. *Mol. Gen. Genet.* **1988**, *214*, 333−342.

(10) Sastri, D. C.; Hilt, K.; Appels, R.; Lagudah, E. S.; Playford, J.; Baum, B. R. An overview of evolution in plant 5S DNA. *Plant Syst. Evol.* **1992**, *183*, 169−181.

(11) Barciszewska, M. Z.; Mashkova, T. D.; Kisselev, L. L.; Barciszewska, J. The nucleotide sequences of 5S ribosomal RNAs from two plants: rape and white beet. *Nucleic Acids Res.* **1987**, *15*, 363.

(12) Yang, Y. W.; Tseng, P. F.; Tai, P. Y.; Chang, C. J. Phylogenetic position of *Raphanus* in relation to *Brassica* species based on 5S rRNA spacer sequence data. *Bot. Bull. Acad. Sin.* **1998**, *39*, 153−160.

(13) Matyášek, R.; Fulneöek, J.; Lim, K. Y.; Leitch, A. R.; Kovaõík, A. Evolution of 5S rDNA unit arrays in the plant genus *Nicotiana* (Solanaceae). *Genome* **2002**, *45*, 556−562.

(14) Liu, Z. L.; Zhang, D.; Wang, X. Q.; Ma, X. F.; Wang, X. R. Intragenomic and interspecific 5s rDNA sequence variation in five Asian pines. *Am. J. Bot.* **2003**, *90*, 17−24.

(15) Cai, Z. H.; Li, P.; Dong, T. T.; Tsim, K. W. Molecular diversity of 5S-rRNA spacer domain in *Fritillaria* species revealed by PCR analysis. *Planta Med.* **1999**, *65*, 360−364.

(16) Ma, X. Q.; Duan, J. A.; Zhu, D. Y.; Dong, T. T.; Tsim, K. W. Species identification of *Radix Astragali* (Huangqi) by DNA sequence of its 5S-rRNA spacer domain. *Phytochemistry* **2000**, *54*, 363−368.

(17) Bertea, C. M.; Luciano, P.; Bossi, S.; Leoni, F.; Baiocchi, C.; Medana, C.; Azzolin, C. M. M.; Temporale, G.; Lombardozzi, M. A.; Maffei, M. E. PCR and PCR-RFLP of the 5S-rRNA-NTS region and salvinorin A analyses for the rapid and unequivocal determination of *Salvia divinorum*. *Phytochemistry* **2006**, *67*, 371−378.

(18) Staden, R.; Beal, K. F.; Bonfield, J. K. The Staden package. *Methods Mol. Biol.* **2000**, *132*, 115−130.

(19) *Swiss Food Manual*; 2004. Chapter 52B: Méthodes de biologie moléculaire.

(20) Hupfer, C.; Hotzel, H.; Sachse, K.; Engel, K. H. Detection of the genetic modification in heat-treated products of Bt maize by polymerase chain reaction. *Z. Lebensm. Unters. Forsch.* **1998**, *206*, 203−207.

(21) Moreano, F.; Busch, U.; Engel, K. H. Distortion of genetically modified organism quantification in processed foods: influence of particle size compositions and heat-induced DNA degradation. *J. Agric. Food Chem.* **2005**, *53*, 9971−9979.

(22) Wilson, I. G. Inhibition and facilitation of nucleic acid amplification. *Appl. Environ. Microbiol.* **1997**, *63*, 3741−3751.

(23) Di Pinto, A.; Forte, V. T.; Guastadisegni, M. C.; Martino, C.; Schena, F. P.; Tantillo, G. A comparison of DNA extraction methods for food analysis. *Food Control* **2007**, *18*, 76−80.

(24) Smith, D. S.; Maxwell, P. W.; De Boer, S. H. Comparison of several methods for the extraction of DNA from potatoes and potato-derived products. *J. Agric. Food Chem.* **2005**, *53*, 9848−9859.

(25) Meyer, R.; Candrian, U. PCR-based DNA analysis for the identification and characterization of food components. *Lebensm. Wiss. Technol.* **1996**, *29*, 1−9.

Received for review November 10, 2006. Revised manuscript received February 27, 2007. Accepted March 7, 2007. This work was carried out with the financial support of the Co-Extra project: GM and non-GM supply chains, their CO-EXistence and TRAceability (Contract 007158), funded by the European Commission through the Sixth Framework Programme under the Food Quality and Safety Priority.

JF063259V