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Development and application of DNA analytical methods for the detection of GMOs in food

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Abstract

The principle of direct detection of recombinant DNA in food by the polymerase chain reaction (PCR) is discussed following the three main steps: DNA-extraction, amplification by PCR and verification of PCR products.

Suitable methods for genomic DNA isolation from homogenous, heterogeneous, low DNA containing matrices (e.g. lecithin), gelatinising material (e.g. starch), derivatives and finished products based on classical protocols and/or a combination with commercially available extraction kits are discussed. Various factors contribute to the degradation of DNA such as hydrolysis due to prolonged heat-treatment, nuclease activity and increased depurination and hydrolysis at low pH. The term ''DNA quality'' is defined as the degree of degradation of DNA (fragment size less than 400 bp in highly processed food) and by the presence or absence of potent inhibitors of the PCR and is, therefore, a key criterion. In general, no DNA is detectable in highly heat-treated food products, hydrolysed plant proteins (e.g. soya sauce), purified lecithin, starch derivatives (e.g. maltodextrins, glucose syrup) and defined chemical substances such as refined soya oil.

If the nucleotide sequence of a target gene or stretch of transgenic DNA is already known specific primers can be synthesised and the segment of rDNA amplified. Detection limits are in the range 20 pg–10 ng target DNA and 0.0001–1% mass fraction of GMO. Amplification products are then separated by agarose gel electrophoresis and the expected fragment size estimated by comparison with a DNA molecular weight marker.

Several methods are used to verify PCR results and they vary in reliability, precision and cost. They include specific cleavage of the amplification products by restriction endonucleases or the more time-consuming, but also more specific, transfer of separated PCR-products onto membranes (Southern Blot) followed by hybridisation with a DNA probe specific for the target sequence. Alternatively, PCR products may be verified by direct sequencing. Nested-PCR assays combines high specificity and sensitivity.

Methods for the screening of 35S-promoter, NOS-terminator and other marker genes used in a wide range of GMOs, the specific detection of approved products such as FlavrSavr™ tomatoes, Roundup Ready™ Soya, Bt-maize 176 and official validated methods for potatoes and genetically modified micro-organisms, that have a model character, are available. Methods to analyse new GMO products are being validated by interlaboratory tests and new techniques are in development (e.g. EC project: DMIF-GEN). However, these efforts may be hampered by the lack of availability of GMO reference material as well as specific sequence information which so far can only be obtained from the suppliers. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: PCR assays; GMO; Detection methods

1. Introduction

National regulatory authorities, retailers and consumer groups in Europe require labelling of food products in which genetically modified organisms (GMOs) are still detectable. Genetically modified (GM) crops such as Roundup Ready™ Soybeans from Monsanto and the Bt176-corn from Novartis arrived in Switzerland and in the European Union (EU) before they were approved (Butler, 1996). No detection methods were available at that time to check incoming raw material. The national regulatory authorities and the food industry were, therefore, forced to develop and validate reliable methods to identify foods derived from genetically engineered crops that, in general, were based on detection of recombinant DNA (rDNA), the additional protein or fatty acid. Legislation in the EU requires modified ingredients which are ''no longer equivalent to their conventional counterparts'' to be labelled according to the Novel Food Regulation 258/97 (Regulation EC, 1997). The council

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Regulation 1139/98/EC of 26 May 1998 (Regulation EC, 1998) provides the basis for the labelling of foodstuffs derived from Bt-maize and genetically modified soybeans. In its Art. 2(2), the 1139/98 regulations states that ≪ foodstuffs in which neither protein nor DNA resulting from genetic modification is present shall not be subject to labelling ≫. Switzerland requires labelling of GMO-derived products on the basis of detectability using DNA-analytical methods (Swiss Food Ordinance, 1995). In the EC a maximum limit for unavoidable presence of GMO material in each ingredient is being discussed as a basis for labelling. In Norway and Hungary a labelling limit of 2% GMOs, in Switzerland 1% GMOs has already been set. Newly introduced traits or marker genes from distinct species can be detected directly by DNA amplification using the polymerase chain reaction (PCR) which is the method of choice for the identification of GMO-derived products as described in recent reviews (Hammes & Hertel, 1995; Meyer, 1995c; Niederhauser, Gilgen & Meyer, 1996; Hemmer, 1997; Hemmer & Pauli, 1998). PCR methods have a superior range of applicability compared to protein-based and other methods in particular for processed food.

In this paper a short description of the PCR principle, the methods available today and their ability to identify GM crops is given. Individual sample preparations are discussed and limitations of the methods, such as heat-treatment of food samples, sensitivity, specificity and false results, pointed out.

2. DNA analysis: a tool for authenticity testing

2.1. History

DNA-analytical methods based on PCR (Saiki et al., 1985, 1988) have become more and more important in quality and safety assurance of food due to their simplicity, specificity and sensitivity for monitoring micro-organisms (Allmann et al.,1995; Candrian, 1995) and for the detection of food constituents (Meyer & Candrian, 1996a), such as wheat (Allmann, Candrian & Lüthy, 1993), soya (Meyer, Chardonnens, Hübner & Lüthy, 1996b), celery (Jankiewicz et al., 1997), as well as the identification of meat (Meyer, Candrian & Lüthy, 1994; Meyer, Höfelein, Lüthy & Candrian, 1995a) and fish species (Hübner, Burgener & Lüthy, 1997). The first detection method specifically developed for the identification of a commercialised genetically engineered plant was demonstrated for detection of the FlavrSavr™ tomato (Meyer, 1995b,c).

2.2. Principle of the PCR

Segments of genomic DNA can be amplified in vitro under conditions in which short oligonucleotides primers (having the complementary sequence to the target DNA) synthesise that DNA by a thermostable enzyme (*Taq* DNA polymerase) and the addition of deoxynucleotides. Repeated cycling of this reaction enables amplification of specific sequences from very few target DNA molecules. (Figs. 1 and 2). At least two types of primers may be considered for use. If the nucleotide sequence of a target gene or stretch of DNA is already known specific primers may be synthesised (Ehrlich, 1989). DNA can be isolated from meat and plants, raw materials and derivatives, or food products using suitable methods for extraction. A few μl are then subjected to the amplification procedure (PCR) and the amplification products are analysed by agarose gel electrophoresis (Meyer & Candrian, 1996a).

PCR products are then verified by specific cleavage of the amplification products by restriction endonucleases or by transfer of separated PCR-products onto membranes (Southern Blot) followed by hybridisation with a DNA probe specific for the target sequence. Alternatively, PCR products may be verified by direct sequencing or by the use of nested-PCR assays (Fig. 3).

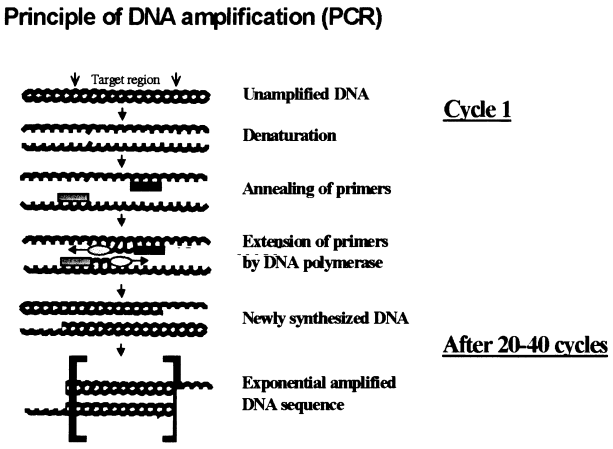

Fig. 1. Schematic presentation of DNA amplification by PCR (Swiss Food Manual, 1998).

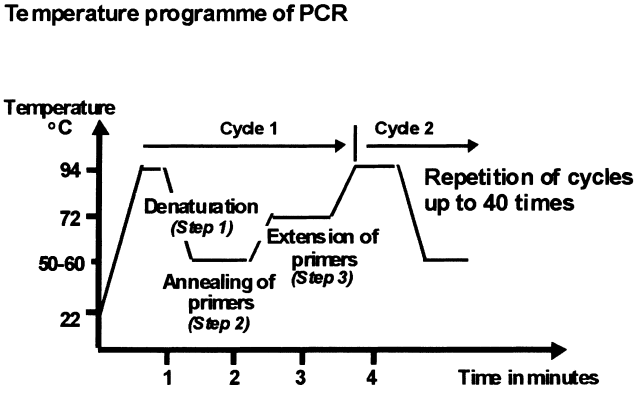

Fig. 2. Schematic presentation of PCR temperature/time diagram (Swiss Food Manual, 1998).

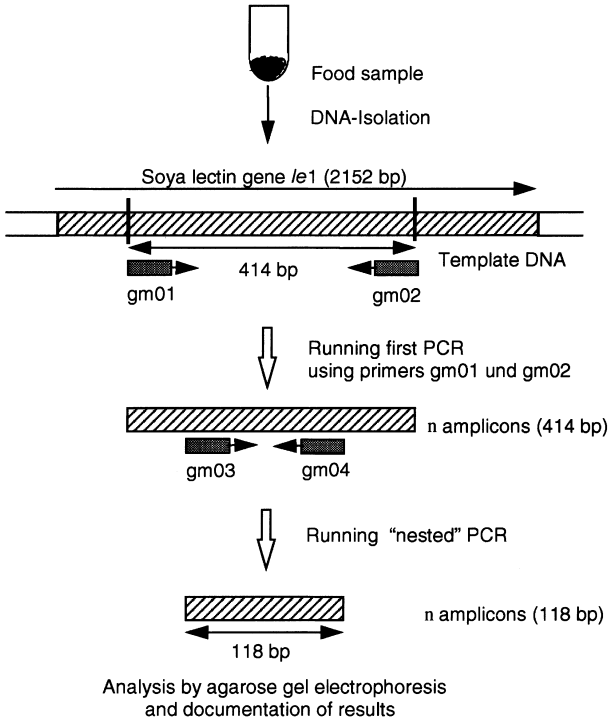

Fig. 3. Schematic presentation of the soya DNA detection strategy in food. DNA is isolated from food samples using suitable methods for extraction and a few µl are subjected to the first PCR assay. Running this PCR with a pair of specific primers directed against the soya *le* 1 gene amplifies a fragment with the defined size of 414 bp. This first amplicon is used as template DNA in the second PCR which amplifies a 118 bp fragment with a pair of internal primers. Thus, this so-called nested PCR is highly specific and extremely sensitive (Meyer et al., 1996b).

3. Methods and their application

3.1. Sample preparation

Homogeneity of samples is one of the critical points in DNA analysis. Only 100–300 mg of material will be analysed (L 24.01-1, 1997; L 23.01.22-1, 1998; Swiss Food Manual, 1998) and, therefore, this must be representative for the whole batch (ranging from kg to tons). Therefore, during sampling it must be considered that only a small proportion of, e.g. soybeans of GMO origin, could be distributed throughout the whole lot and that a low degree of contamination of less than 1:10 000 should still be detectable. In addition cross-fertilisation (maize-pollen) in the fields and cross-contamination of crops and dust during harvest, transportation, milling and food processing cannot be avoided. Reasonable and practicable sampling plans for raw materials and processed products are, therefore, needed. Sampling plans to test raw shelled peanuts for aflatoxins (Whitaker, Springer, Defize, deKoe & Coker, 1995) demonstrate the immense expense of this approach.

Methods for homogenisation of samples are described in the Swiss Food Manual using soya products as example. Fifty g of soy beans (kernels or grits) or 30 g of dry samples (flakes, flour) should be incubated with 100, or 60 ml, respectively, of sterile water for up to 20 h and then homogenised in a blender. Thirty g of wet samples (tofu, sausages) should be homogenised directly and liquid samples should be shaken thoroughly before weighing. In order to avoid cross-contamination between samples disposable material and decontamination solutions (e.g. hypochlorite solution and HCl or DNA-Away™, Promega) are recommended. Cross-contamination by dust should be avoided by physical separation of the sample preparation aeras.

3.2. DNA isolation methods

Currently two DNA isolation methods are favoured and applied to a wide range of raw material such as soya beans, corn and derivatives including lecithin and crude oil and in food products:

- The "CTAB" method (L 23.01.22-1, 1998) is based on a "classical" protocol for plant tissues. Food samples are incubated in the presence of the detergent CTAB (hexadecyltrimethyl-ammonium bromide), then extracted with chloroform and the DNA is precipitated with isopropanol.
- The other method (Swiss Food Manual, 1998) uses a DNA-binding silica resin (Wizard™, Promega) to purify DNA directly from a solution obtained after enzymatic (proteinase K) and chemical (SDS) treatment of the food sample. In order to increase the yield of DNA isolated from very low DNA-containing matrices, such as lecithin, the extraction of five times 100 mg of sample is recommended. The extracted DNA is then pooled together and purified again.
- In addition official validated DNA-isolation methods for bacterial DNA from raw sausages (L 08.00-44, 1997) and yoghurt (L 02.02-4, 1997) are available.

The current methods have been improved so that amplifiable DNA can be prepared in a shorter time. Commercially available kits, modified "CTAB"-protocols or a combination of both have been reported based on experience gathered in different laboratories as briefly discussed below:

- Nine different extraction methods for nucleic acids in soybean samples (tofu, soy flour and lecithin) have been evaluated (Zimmermann, Lüthy & Pauli, 1998). It was demonstrated that extraction methods like Wizard™ (Promega), DNeasy™ (QIAGEN® AG, Switzerland), Nucleon® Phytopure (Scotlab GmBH, Germany) using DNA-binding resins and "CTAB", resulted in comparatively low yields of high quality of DNA for amplification. Simpler, faster and cheaper methods such as ROSE, Alkali or

Chelex®-100 (Bio Rad) resulted in relatively high yields of relatively poor quality of DNA.
- The Nucleon® Phytopure kit was sucessfully applied to many vegetables and processed food (Jankiewicz et al., 1997) and especially removes polysaccharides from the sample.
- A modified method for lecithin was described (Wurz, Rüggeberg, Brodmann, Waiblinger & Pietsch, 1998). Ten ml hexane and 1 ml guanidine thiocyanate containing buffer were added to 2 g of lecithin and mixed thoroughly. After centrifugation the aqueous phase was mixed with chloroform and centrifuged again. The upper phase was mixed with glycogen and the DNA was precipated with isopropanol. The dried pellet was dissolved and purified by gel filtration (Microspin S300, Pharmacia Biotech Sweden).
- A modification of the "CTAB" method was described (Meyer & Jaccaud, 1997) where the final precipitation step with isopropanol was replaced by QIAquick™ purification (QIAGEN® AG) of DNA. For heterogeneous samples the test portion was increased to 2 g. For matrices containing low levels of DNA, such as lecithin, QIAamp Maxi columns (QIAGEN® AG) were used to increase the yield of DNA. Gelatinising samples (starch) were incubated with α-amylase (Termamyl 120L, Novo Nordisk, Denmark) to obtain a fluid solution before extraction.
- The amount of DNA in highly purified lecithin and refined vegetable oil is too low to be detected by current extraction methods (Parkes, Sawyer & Martin, 1998; Pauli, Liniger & Zimmermann, 1998). The presence of DNA in crude and refined rapeseed oil was demonstrated but could not be identified unequivocally in the refined oil (Hellebrand, Nagy & Mörsel, 1998).

Purity and yield of isolated DNA are two critical points in DNA preparation for PCR analysis. DNA isolated from processed food is highly degraded (fragment size less than 400 bp in highly processed food) and must be purified from protein, fat and polysaccharides. Polysaccharides, polyphenolics (tannins) and other secondary compounds pose a major problem in some food matrices, such as chocolate, because they are difficult to separate from DNA and can irreversibly interact with proteins and nucleic acids (e.g. polysaccharides have been reported to inhibit DNA polymerase).

The quantity of DNA extracted can be estimated by different techniques: UV spectroscopy at 260 nm; measurement of fluorescence induced by reagents such as PicoGreen (Molecular Probes, OR, USA); or densitometry on a gel stained with ethidium bromide or fluorescent nucleic acid gel stains. DNA may be contaminated with RNA and nucleotides leading in many cases to underestimation of the DNA content if no RNase treatment is performed. The DNA content and composition (genomic, mitochondrial and chloroplast DNA) depends on tissue type. Normally, the extracted DNA must be diluted prior to PCR (5–50 ng are used per reaction).

3.3. PCR assays

Methods today available for the detection of GMOs are summarised in Table 1 and are briefly discussed below. Primers are designed to target introduced DNA sequences that span over the boundary of two adjacent genetic elements (e.g. promoters, structural genes, terminators) or to specifically detect the altered gene sequence, certain regulatory elements or marker genes. The use of a combination of different primer sets for different genetic elements and control experiments for the presence of amplifiable DNA (Table 2) are prerequisites.

The specific sequence of the target gene or introduced DNA must be known for the design of specific primers and the corresponding GMO and non-GMO reference (negative control) material must be available. The specificity of the PCR assay is based on the choice of primers and optimisation of PCR parameters. Optimal PCR primers can be chosen using programs such as "*OLIGO 5.0 Primer Analysis Software*" (NBI, Plymounth, MN, USA).
- *Model systems* (*Table 1*). Three methods have been developed and validated by the German BgVV-working group ("Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin", Berlin) and have been included in the list of official methods ("Amtliche Sammlung von Untersuchungsverfahren") according to Section 35 of the German Food Act (LMBG, Lebensmittel- und Bedarfsgegenständegesetz). One method describes identification of a genetically engineered potato, the second detection of a genetically modified microorganism (*Lactobacillus curvatus*) in fermented raw sausages (Schulze, Hertel, Bögl & Schreiber, 1996) and the third identification of a genetically engineered strain of *Streptococcus thermophilus* used as a starter culture in yoghurt (Lick, Keller, Bockelmann & Heller, 1996).
- *Specific systems* (*Table 1*). Specific detection of the FlavrSavr™ tomato (Calgene), the first approved genetically engineered crop, was demonstrated by a PCR-assay targeting the combination of the sequence of the 35S-promoter from the Cauliflower Mosaic Virus (CaMV35S) followed by the antisense gene from the polygalacturonase. In addition the presence of the *npt*II (neomycin phosphotransferase II) marker gene was determined.

Several product-specific PCR methods have been described for detection of Roundup Ready™ Soybeans (Monsanto). The principle of these strategies is shown in

Table 1
Detection of genetically engineered food by PCR[a]

GMO product (Company), method	Reference	Target sequences (gene)
Potato B33-INV	L 24.01-01 (1996)	*aph*IV (hygromycin phosphotransferase)
Potato B33 (alterd starch composition)	Hassan-Hauser, Mayer & Hörtner, 1998	*npt*II, gbss-as; B33, T-DNA
Lactobacillus curvatus in raw sausages	L 08.00-44 (1997)	*kat*A/cat
Streptococcus thermophilus in yoghurt	L 02.02-04 (1997)	*cat*/*lac*Z
FlavrSavr™ tomato (Calgene)	Meyer, 1995b	*npt*II, d-P-35S/polygalacturonase
Tomato (Zeneca)	"EU-project"	Polygalacturonase/nos 3′
Screening method (applicable to most GMO crops approved for food use)	Pietsch, Waiblinger, Brodman & Wurz, 1997	*npt*II, P-35S, nos 3′
Validation	Brodmann et al., 1997	P-35S, nos 3′
EC-ringtest	Lipp, EC Joint Research Center, Italy	P-35S, nos 3′
Roundup Ready™ Soybeans (Monsanto)	Wurz & Willmund, 1997	P-35S/CTP-EPSPS
Validation	L 23.01.22-1 (1998)	P-35S/CTP-EPSPS
	Meyer & Jaccaud, 1997	P-35S/CP4 EPSPS, P-35S/ CTP-EPSPS
	Köppel, Stadler, Lüthy & Hübner, 1997	P-35S/CP4 EPSPS, CTP-EPSPS/ CP4 EPSPS
	Van Duijn, Hessing, & Van Der Kamp, 1997a; Van Duijn, van Biert, Bleeker-Marcelis, Vlooswijk & Hessing	P-35S, nos 3′, CP4 EPSPS/nos 3′
	Hörtner, 1997	CP4 EPSPS/nos 3′
"Maximizer" maize, Bt-176 (Novartis)	Ehlers et al., 1997	Synth., truncated cryIA(b), *bla, bar,* P-35S/ *bar*
	Studer, Dahinden, Lüthy & Hübner, 1997	Synth., truncated CryIA(b)
	Hupfer, Hotzel, Sachse, & Engel, 1997	Synth., truncated CryIA(b)
	Hupfer et al., 1998	P-CDPK/CryIA(b)
	Brodmann, 1998	P-PEP-C/CryIA(b)
"Bt-11" maize (Novartis)	Ahl Goy, 1998	Synth., truncated CryIA(b)
"Liberty Link" maize, T25	Höchst, Schering, & AgrEvo, 1998	Synthetic PAT, P-35S/bla
"Yield Gard" maize	(Monsanto)	Synthetic PAT, Synth., truncated CryIA(b), P-35S

[a] *npt*II = neomycin phosphotransferase II gene (kanamycin resistance), P-35S = cauliflower mosaic virus (CaMV) 35S promoter, nos 3′ = 3′ nontranslated region of the nopaline synthase gene, *bar* = phosphinothricin acetyltransferase gene (PAT), *gbss*-as = granule bound starch synthase gene-antisense, *bla* = β-lactamase gene (ampicillin resistance).

Table 2
PCR-based detection methods used as control systems for amplifiable DNA

Target organisms, specificity	Reference	Target sequence (gene)
Eukaryotes	Allmann et al., 1993	18S rRNA
Plants	Taberlet, Gielly, Pautou & Bouvet, 1991	cpDNA (tRNA gene)
Vertebrates	Meyer et al., 1995a	mtDNA (*cyt*b gene)
Soya	Meyer et al., 1996b	Lectin gene, *le*1
Soya	Wurz et al., 1998	Lectin gene, *le*1
Soya	Van Duijn et al., 1997b	Soy heat-shock protein gene, HSP
Maize	Studer et al., 1997	Zein (methionine-rich storage protein)
Maize	Ehlers et al., 1997	Invertase, *ivr*1
Wheat	Allmann et al., 1993	25S-18S rRNA

Fig. 4, targeting the introduced DNA sequences that span across the boundary of two or three genetic elements, such as the CaMV35S promoter, the EPSPS petunia CTP gene, the CP4 EPSPS gene of *Agrobacterium sp.* strain CP4 or the NOS-terminator. Two methods (A and D) are performed as nested-PCRs, the amplification product of method B is verified by hybridisation.

Several product-specific PCR methods have been described for detection of the Bt-176 maize (Novartis). Various detection strategies have been applied, such as simple and nested-PCRs, targeting for the marker genes (*bar* and *bla* gene) and the synthetic truncated CryIA(b) gene, conferring European corn borer tolerance, or targeting the introduced DNA sequence that spans across two genetic elements, as shown in Fig. 5.

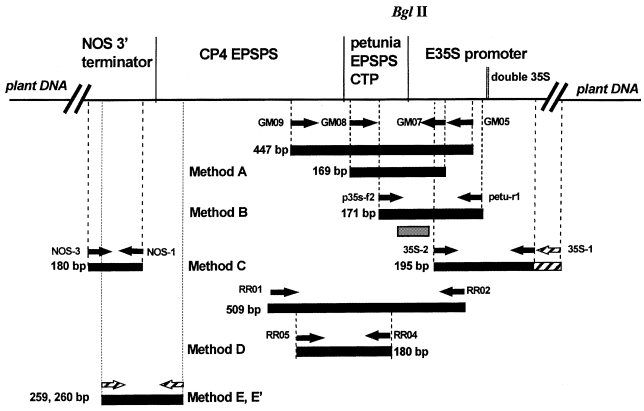

Fig. 4. Detection strategies of Roundup Ready™ Soybeans (Pagette et al., 1995). Method A (Meyer & Jaccaud, 1997); method B (Wurz & Willmund, 1997); screening method C (Pietsch et al., 1997); nested-PCR method D (Köppel et al., 1997); method E and E′ (Hörtner, 1997; Van Duijn et al., 1997b, respectively).

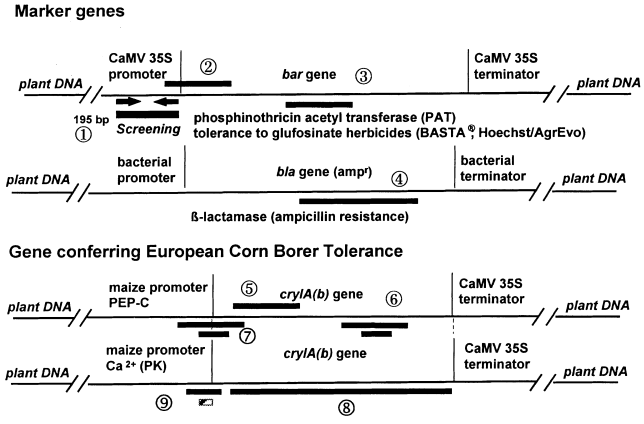

Fig. 5. Detection strategies of Bt-176 maize from Novartis (Koziel et al., 1993). ①, (Pietsch et al., 1997); ②, ③, ④, ⑤, (Ehlers et al., 1997); ⑥, (Studer et al., 1997); ⑦, (Brodmann, 1998); ⑧, (Hupfer et al., 1997); ⑨, (Hupfer et al., 1998).

Product-specific PCR methods for Bt-11 maize (Novartis), YieldGard maize (Monsanto) and T25 maize (AgrEvo) are available on request from the companies or are still in the process of being developed and validated.

If no product-specific tests are available, or in addition to them, detection of at least three genetic elements (P-35S, nos 3′ and *npt*II) may be performed. This screening PCR method is applicable to most GMO crops approved for food use and has become an official method in Switzerland. This method (nos 3′ and P-35S) was tested in national and international interlaboratory comparisons of GM soya and maize materials.

- *Control systems for amplifiable DNA* (*Table 2*). Prior to a GMO-product-specific or screening PCR-assay, the presence of amplifiable DNA in a food matrix can be determined using universal or species-specific PCR assays.
- *Verification*. Several methods are used to verify PCR results and they vary in reliability, precision and cost. Specific cleavage of the amplification products by restriction endonuclease is the simplest method to identify the amplification products. The presence of the 35S-promoter, for example, is confirmed if the 195 bp fragment is cleaved by the restriction endonuclease *Xmn*I yielding two fragments of 115 and 80 bp (Swiss Food Manual, 1998).

The more time-consuming, but also more specific, transfer of separated PCR-products onto membranes (Southern Blot) followed by hybridisation with a DNA probe specific for the target sequence is used in all official methods listed in the German Food Act.

Alternatively, PCR products may be verified by direct sequencing. This is the most accurate proof of amplified DNA. However, this opportunity is not available in all laboratories and is not the method of choice for routine analysis.

Nested-PCR assays combine high specificity and sensitivity. Use of two pairs of primers spanning the boundary of two or three genetic elements is regarded as sufficiently specific for a GMO-product. In general, the increased sensitivity of nested-PCR systems allows low levels of GMOs to be detected in raw material and finished products.

4. Limitations

The official Swiss method uses detection of the cauliflower mosaic virus promoter (P-35S) and the nopaline synthase terminator (nos 3′) which are present in most GM crops approved today (Hemmer, 1997; Hemmer & Pauli, 1998). However, this screening method has limited sensitivity and specificity (Brodmann et al., 1997). Therefore, positive results must be confirmed by a combination of a control PCR and product-specific methods, including verification of amplified fragments.

Mixtures of approved and non-approved GM crops (e.g. cross-contamination of soya and maize), as well as, complex food products cannot be determined unequivocally by the currently available methods.

The presence of false-positive fragments of almost the same size as the expected 195 bp-fragment from the P-35S-PCR, but not digestable with the restriction endonuclease *Xmn*I, has been reported (Hübner, University of Berne, personal communication). It must be taken into account that false-positive results from screening methods based on P-35S, nos 3′ and *npt*II sequences may occur due to natural contamination of food plants by, e.g. cauliflower mosaic virus (CaMV) in *Cruciferae* (e.g. rapeseed, broccoli), microorganisms containing the *nos*-target sequence such as *Agrobacterium* or *npt*II-containing bacteria from the soil.

Another limitation of PCR-based methods, encountered in routine diagnostic analytical applications, is false-positive results due to accidental contamination of the sample or reagents. Sources include cross-contamination between samples or carry-over from previous PCRs. Physical separation of the individual steps of the PCR, i.e. sample preparation, pre-PCR, and post-PCR, is necessary to prevent carry-over contamination of extracted DNA and PCR products (Heinrich, 1991; Yap, Lo, Fleming & McGee, 1994). Avoiding dust and the use of UV germicidal lamps in the sample preparation (DNA isolation) and pre-PCR (mastermix preparation) aeras reduces the possibility of contamination. The use of separate sets of supplies and pipetting devices dedicated to sample preparation, setting up reactions and post-PCR analysis (agarose gel electrophoresis) is also necessary. All reagents must be divided into aliquots to minimise the number of repeated samplings necessary. All reagents used in the PCR must be prepared, divided and stored in an area free of PCR-amplified products. Nevertheless, if contamination occurs the lots of reagents should be easily replaceable. To prevent cross-contamination of the barrels of pipetting devices aerosol resistant tips must be used. A separate pipette at the post-PCR location is recommended for adding previous PCR products to the amplification mixture for nested-PCR.

A combination of pre- and post-PCR sterilisation involves incorporating dUTP in all PCR products by substituting dUTP for TTP (Yap et al., 1994). Before commencing any PCR, fully pre-assembled reactions are treated with uracil DNA glycosylase, which cleaves the uracil base from the phosphodiester backbone of uracil-containing DNA, but has no effect on naturally occurring DNA templates containing thymidine bases (Longo, Berninger & Hartley, 1990; Pang, Modlin & Yolken, 1992).

False-negative results are less common in PCR due to the extremely high sensitivity of this reaction. However, control experiments (control-PCR using universal or species-specific primers, spiking of food sample with target-DNA) are necessary to indicate the presence of amplifiable DNA or inhibitors in a sample. The efficiency and sensitivity of such control PCRs should not be lower than the GMO specific assays applied.

Standardisation of PCR assays is difficult because variations in parameters, such as sample weight, extraction procedure, DNA quality, primer and DNA-polymerase, thermocycler and temperature profile, cycle number and verification of amplicons, all affect sensitivity.

Finally, the applicability of PCR methods to detect GMOs in derivatives (starch, oil, lecithin) and processed products is limited by the quality of the DNA present. Any physical or chemical treatment of food samples, such as heat, pH or shear forces results in a decrease in the average DNA fragment size due to random cleavage of these macromolecules and could make detection impossible (Meyer & Candrian, 1996a; Hemmer, 1997; Meyer & Jaccaud, 1997; Hupfer, Hotzel, Sachse & Engel, 1998; Parkes et al., 1998; Pauli et al., 1998). Based on our experience DNA is no longer detectable in derivatives of starch (glucose sirup, maltodextrin, etc.), purified lecithin, refined vegetable oil, soya sauce and powder and highly heat-treated (certain extruded or sterilized) finished products. The presence of DNA, originating from the microbiologial host organism, in an insufficiently purified enzymatic preparation has been reported (Wolf, 1996).

5. Methods in development and future needs

Specific PCR methods to distinguish between natural contamination by CaMV or *Agrobacterium* and the presence of these sequences in GMOs and more specific methods for newly approved GMOs are required. Interpretation of results will become more complex because of the increased number of different methods applied. Therefore, frame conditions and decision matrices (e.g. duplicate analysis, repetition, positive and negative controls, confirmation, control experiments, etc.) must be standardised.

New methods and techniques are being developed within the framework of the European Research Project ''Development of Methods to Identify Foods Produced by Means of Genetic Engineering'', DMIF-GEN (project no. SMT4-CT96-2072). In the scope of this project DNA-extraction methods have been compared (Zimmermann et al., 1998), new primers and probes have been defined, ring tests with tomato, processed maize and soya are being performed and a database will be set up recording detailed information about GMO containing food on the market, sequences, primers and detection methods (Schreiber, 1997).

How many tests are necessary to identify GMOs in food unequivocally and how can the efficiency of routine analyses be improved? These are the key questions for the future. Therefore, development and standardisation of multiplex-PCRs, semi-quantitative methods (Studer, Rhyner, Lüthy & Hübner, 1998), as well as the automation and application of new techniques are required for fast and specific routine screening of GMOs.

Beside specific primers and probes reference materials of approved and processed GMOs are required. Certified reference materials (CRMs) such as dried soya bean powder containing different mass fractions (0%, 0.1%, 0.5%, 2.0%) of genetically modified Roundup Ready™ soybeans produced by the Institute for Reference Materials and Measurements (IRMM) on behalf of Fluka Chemie AG are very helpful as external standards for quantification and for interlaboratory comparison regarding sensitivity and specificity.

References

Ahl Goy, P., Novartis Seeds, AG., Basel, Switzerland (1998). PCR method for the identification of the genetically modified (Bt-11-) maize. Personal communication.

Allmann, M., Candrian, U., & Lüthy, J. (1993). Polymerase chain reaction (PCR): a possible alternative to immunological methods assuring safety and quality of food: Detection of wheat-contamination in non-wheat food products *Z. Lebensm. Unters. Forsch.*., *196*, 248–251.

Allmann, M., Höfelein, Ch., Köppel, E., Lüthy, J., Meyer, R., Niederhauser, C., Wegmüller, B., & Candrian, U. (1995). Polymerase chain reaction (PCR) for the detection of pathogenic microorganisms in bacteriological monitoring of dairy products *Res. Microbiol.*., *146*, 85–97.

Brodmann, P. (1998). Personal communication.

Brodmann, P., Eugster, A., Hübner, P., Meyer, R., Pauli, U., Vögeli, U., & Lüthy, J. (1997). Nachweis gentechnisch veränderter Roundup Ready™ Sojabohnen mittels der Polymerase Kettenreaktion (PCR). Methodenvorprüfung im Rahmen der Subkommission 29a des Schweizerischen Lebensmittelbuches *Mitt. Gebiete Lebensm. Hyg.*., *88*, 722–731.

Butler, D. (1996). EU urges national action to police imports of genetically altered maize *Nature*., *384*, 502–503.

Candrian, U. (1995). Polymerase chain reaction in food microbiology *J. Microbiol. Methods*., *23*, 89–103.

Ehlers, B., Strauch, E., Goltz, M., Kubsch, D., Wagner, H., Maidhof, H., Bendiek, B., Appel, B., & Buhk, H. -J. (1997). Identification of genetically modified maize by PCR *Bundesgesundheitsblatt*., *4/97*, 118–121.

Ehrlich, H.A. (Ed.), (1989). *PCR technology. Principles and applications for DNA amplification*. New York: Stockton Press.

Hammes, W. P., & Hertel, Ch. (1995). Mit Hilfe der Gentechnik erzeugte Lebensmittel: Novel Foods und die Problematik ihres Nachweises *Biologie unserer Zeit*., *25*, 246–255.

Hassan-Hauser, Ch., Mayer, W., & Hörtner, H. (1998). Detection of the starch modifying gbss-antisense construct in transgenic potatoes *Z. Lebensm. Unters. Forsch. A*., *206*, 83–87.

Heinrich, M. (1991). PCR carry-over. Biotech Forum Europe *Int. J. Biotechnol. Heft*., *10*, 594–597.

Hellebrand, M., Nagy, M., & Mörsel, J. -T. (1998). Determination of DNA traces in rapeseed oil *Z. Lebensm. Unters. Forsch. A*., *206*, 237–242.

Hemmer, W. (1997). BATS Report 2/97: Foods derived from genetically modified organisms and detection methods. Basel, ISSN 1420-228X.

Hemmer, W., & Pauli, U. (1998). Labelling of food products derived from genetically engineered crops. A survey on detection methods. *European Food Law Rev. 1/98*, *8*, 27–38.

Höchst, Schering, & AgrEvo GmbH, Germany (1998). PCR primers for the identification of the genetically modified T25 maize plants. Personal communication.

Hörtner, H. (1997). Nachweismöglichkeiten gentechnisch hergestellter Lebensmittel *Ernährung/Nutrition*., *21*, 443–446.

Hübner, P., Burgener, M., & Lüthy, J. (1997). Application of molecular biology for the identification of fish. In R. Amadò, R. Battaglia (Eds.), *Proceedings of the ninth european conference on food chemistry* (Vol. 1), *Authenticity and adulteration of food – the analytical approach, 24–26 September 1997, Interlaken* (pp. 49–54). ISBN: 3-9521414-0-2.

Hupfer, Ch., Hotzel, H., Sachse, K., & Engel, K. -H. (1997). Detection of genetically modified insect-resistant Bt maize by means of polymerase chain reaction *Z. Lebensm. Unters. Forsch. A*., *205*, 442–445.

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

Jankiewicz, A., Hübner, P., Bögl, K. W., Dehne, L. I., Vieths, S., Baltes, W., & Lüthy, J. (1997). Celery allergy: PCR as a tool for the detection of trace amounts of celery in processed foods. In R. Amadò, R. Battaglia (Eds.), *Proceedings of the ninth european conference on food chemistry* (Vol. 1), *Authenticity and adulteration of food – the analytical approach, 24–26 September 1997, Interlaken* (pp. 131–136). ISBN: 3-9521414-0-2.

Köppel, E., Stadler, M., Lüthy, J., & Hübner, P. (1997). Sensitive method for the detection of the genetically engineered soybean Roundup Ready™ *Mitt. Gebiete Lebensm. Hyg.*., *88*, 164–175.

Koziel, M. G., Beland, G. L., Bowmann, C., Carozzi, N. B., Crenshaw, R. Crossland, L. et al. (1995). Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis. Bio/Technology 11*, 194–200.

L 02.02-4 (1997). Untersuchung von Lebensmitteln: Nachweis einer gentechnischen Veränderung von *Streptococcus thermophilus* in Joghurt durch Amplifizierung der veränderten DNA-Sequenz mit Hilfe der PCR (Polymerase Chain Reaction) und Hybridisierung des PCR-Produktes mit einer DNA-Sonde. In *Amtliche Sammlung von Untersuchungsverfahren nach §35 LMBG; Verfahren zur Probenahme und Untersuchung von Lebensmitteln, Tabakerzeugnissen, Kosmetischen Mitteln und Bedarfsgegenständen/BgVV. Loseblattausgabe, Stand September 1997.* Bd. 1, Beuth Verlag GmbH, Berlin, Köln.

L 08.00-44 (1997). Untersuchung von Lebensmitteln: Nachweis einer gentechnischen Veränderung von *Lactobacillus curvatus* in Rohwurst durch Amplifizierung der veränderten DNA-Sequenz mit Hilfe der PCR (Polymerase Chain Reaction) und Hybridisierung des PCR-Produktes mit einer DNA-Sonde. In *Amtliche Sammlung von Untersuchungsverfahren nach §35 LMBG; Verfahren zur Probenahme und Untersuchung von Lebensmitteln, Tabakerzeugnissen, Kosmetischen Mitteln und Bedarfsgegenständen/BgVV. Loseblattausgabe, Stand February 1996.* Bd. 1, Beuth Verlag GmbH, Berlin, Köln.

L 23.01.22-1 (1998). Untersuchung von Lebensmitteln: Nachweis einer gentechnischen Veränderung von Sojabohnen durch Amplifizierung der veränderten DNA-Sequenz mit Hilfe der PCR (Polymerase Chain Reaction) und Hybridisierung des PCR-Produktes mit einer DNA-Sonde. In *Amtliche Sammlung von Untersuchungsverfahren nach §35 LMBG; Verfahren zur Probenahme und Untersuchung von Lebensmitteln, Tabakerzeugnissen, kosmetischen Mitteln und Bedarfsgegenständen/BgVV. Loseblattausgabe, Stand February 1998.* Bd. 1, Beuth Verlag GmbH, Berlin, Köln.

L 24.01-1 (1997). Untersuchung von Lebensmitteln: Nachweis einer gentechnischen Veränderung von Kartoffeln durch Amplifizierung der veränderten DNA-Sequenz mit Hilfe der PCR (Polymerase Chain Reaction) und Hybridisierung des PCR-Produktes mit einer DNA-Sonde. In *Amtliche Sammlung von Untersuchungsverfahren nach §35 LMBG; Verfahren zur Probenahme und Untersuchung von Lebensmitteln, Tabakerzeugnissen, kosmetischen Mitteln und Bedarfsgegenständen/BgVV. Loseblattausgabe, Stand February 1997.* Bd. 1, Beuth Verlag GmbH, Berlin, Köln.

Lick, S., Keller, M., Bockelmann, W., & Heller, K. J. (1996). Rapid amplification of *Streptococcus thermophilus* by primer-specific PCR amplification based on its *lacZ* gene *System. Appl. Microbiol.*., *19*, 74–77.

Longo, M. C., Berninger, M. S., & Hartley, J. L. (1990). Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions *Gene*., *93*, 125–128.

Meyer, R., & Jaccaud, E. (1997). Detection of genetically modified soya in processed food products: Development and validation of a PCR assay for the specific detection of glyphosate-tolerant soybeans. In R. Amadò, R. Battaglia (Eds.), *Proceedings of the ninth European conference on food chemistry* (Vol. 1), *Authenticity*

and adulteration of food – the analytical approach, 24–26 September 1997, Interlaken (pp. 23–28). ISBN: 3-9521414-0-2.

Meyer, R. (1995b). Detection of genetically engineered plants by polymerase chain reaction PCR using the Flavr Savr™ tomato as an example *Z. Lebensm. Unters. Forsch.*., *201*, 583–586.

Meyer, R. (1995c). Detection of genetically engineered food by the polymerase chain reaction (PCR) *Mitt. Gebiete Lebensm. Hyg.*., *86*, 648–656.

Meyer, R., & Candrian, U. (1996a). PCR-based analysis for the identification and characterization of food components *Lebensm.-Wiss. u.-Technol.*., *29*, 1–9.

Meyer, R., Candrian, U., & Lüthy, J. (1994). Detection of pork in heated meat products by the polymerase chain reaction *J. AOAC Int.*., *77*, 617–622.

Meyer, R., Chardonnens, F., Hübner, P., & Lüthy, J. (1996b). Polymerase chain reaction (PCR) in the quality and safety assurance of food. Detection of soya in processed meat products *Z. Lebensm. Unters. Forsch.*., *203*, 339–344.

Meyer, R., Höfelein, Ch., Lüthy, J., & Candrian, U. (1995a). Polymerase chain reaction-restriction fragment length polymorphism analysis: A simple method for species identification in food *J. AOAC Int.*., *78*, 1542–1551.

Niederhauser, Ch., Gilgen, M., & Meyer, R. (1996). Genetically engineered food plants: research and detection with DNA-analytical methods *Mitt. Gebiete Lebensm. Hyg.*., *87*, 307–367.

Padgette, S. R., Kolacz, K. H., Delanny, X., Re, D. B., LaVallee, B. J., Tinius, C. N., Rhodes, W. K., Otero, Y. I., Barry, G. F., Eichholtz, D. A., Peschke, V. M., Nida, D. L., Taylor, N. B., & Kishore, G. M. (1995). Development, identification, and characterization of a glyphosate tolerant soybean line *Crop Sci.*., *35*, 1451–1461.

Pang, J., Modlin, J., & Yolken, R. (1992). Use of modified nucleotides and uracil-DNA glycosylase (UNG) for the control of contamination in the PCR-based amplification of RNA *Mol. Cell. Probes.*, *6*, 251–256.

Parkes, H. (Ed.), Sawyer, J., & Martin, B. (1998). *An investigation into extraction of DNA and protein from vegetable oils and starches.* Laboratory of the Government Chemist, Teddington, UK, Project report LGC/FA/1998/002.

Pauli, U., Liniger, M., & Zimmermann, A. (1998). Detection of DNA in soybean oil *Z. Lebensm. Unters. Forsch. A*., *207*, 264–267.

Pietsch, K., Waiblinger, H. U., Brodmann, P., & Wurz, A. (1997). Screeningverfahren zur Identifizierung gentechnisch veränderter pflanzlicher Lebensmittel *Deutsche Lebensm.-Rundsch.*., *93*, 35–38.

Regulation (EC), (1997). No. 258/97 of the European Parliament and of the Council of 27 January 1997 concerning Novel Foods and Novel Food Ingredients. *Official J. European Communities*, *No. L 43*, 1–5.

Regulation (EC), (1998) No. 1139/98 of the European Parliament and of the Council of 26 May 1998 concerning the compulsory indication of the labelling of certain foodstuffs produced from genetically modified organisms of particulars other than those provided for the Directive 79/112/EEC. *Official Journal of the European Communities*, No. L 159/4, 4–7.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., & Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, *239*, 481–487.

Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., & Arnheim, N. (1985). Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia *Science*., *230*, 1350–1354.

Schreiber, G.A. (1997). The European Commission Research Project: Development of methods to identify foods produced by means of genetic engineering. In G. A. Schreiber,K. W. Bögl (Eds.), *Food produced by means of genetic engeneering, 2nd status report.* Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, BgVV, Berlin, 01/1997.

Schulze, M., Hertel, C., Bögl, K. W., & Schreiber, G. A. (1996). Nachweis mit Hilfe gentechnischer Verfahren hergestellter Lebensmittel. Erarbeitung und Validierung von Methoden in Rahmen des §35 LMBG. *Bundesgesundheitsblatt,* Sonderheft, *Dez./96*, 31–36.

Studer, E., Dahinden, I., Lüthy, J., & Hübner, P. (1997). Detection of the genetically engineered ''maximizer'' maize using the polymerase chain reaction (PCR) *Mitt. Gebiete Lebensm. Hyg.*., *88*, 515–524.

Studer, E., Rhyner, C., Lüthy, J., & Hübner, P. (1998). Quantitative competitive PCR for the detection of genetically modified soybean and maize. *Z. Lebensm. Unters. Forsch. A, 207*, 207–213.

Swiss Food Manual (1998). *Molecular biological methods,* Chapter 52B, Eidgenössische Drucksachen und Materialzentrale Bern.

Swiss Food Ordinance of March 1, (1995). SR 817.02. Eidgenössische Drucksachen und Materialzentrale Bern, Switzerland.

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three noncoding regions of chloroplast DNA *Plant Mol. Biol.*., *17*, 1105–1109.

Van Duijn, G., Hessing, M., & Van Der Kamp, J. W. (1997a). Identification of transgenic plants in foods *C. R. Acad. Agric. Fr.*., *83*, 153–157.

Van Duijn, G., van Biert, R., Bleeker-Marcelis, H., Vlooswijk, R., & Hessing, M. (1997b). The detection of genetically modified crops for the food industry. In *Understanding the commercial and regulatory issues for genetically modified and novel foods and food ingredients, 24–25 September 1997.* Conrad International Hotel, Brussels, Belgium.

Whitaker, T. B., Springer, J., Defize, P. R., deKoe, W. J., & Coker, R. (1995). Evaluation of sampling plans used in the United States, United Kingdom and the Nederlands to test raw shelled peanuts for aflatoxin *J. AOAC Int.*., *78*, 1010–1018.

Wolf, Ch. (1996). Entwicklung eines Nachweisverfahrens für Enzympräparate aus gentechnisch veränderten Organismen. Diplomawork, University of Kaiserslautern, Germany.

Wurz, A., & Willmund, R. (1997). Identification of transgenic glyphosate-resistant soybeans. In G. A. Schreiber, K. W. Bögl (Eds.), *Food produced by means of genetic engeneering, 2nd status report.* Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, BgVV, Berlin, 01/1997.

Wurz, A., Rüggeberg, H., Brodmann, P., Waiblinger, H. U., & Pietsch, K. (1998). DNA-Extraktionsmethode für den Nachweis gentechnisch veränderter soja in sojalecithin. *Deutsche Lebensmittel Rundschau*, *94*, 159–161.

Yap, E. P. H., Lo, Y.-M. O., Fleming, K. A., & McGee, J. O'D. (1994). False-positives and contamination in PCR. In H. G. Griffin, A. M. Griffin (Eds.), *PCR technology: current innovations*, Boca Raton, USA: CRC Press.

Zimmermann, A., Lüthy, J., & Pauli, U. (1998). Quantitative and qualitative evaluation of nine different extraction methods for nucleic acids on soybean food samples. *Z. Lebensm. Unters. Forsch. A*, *207*, 81–90.