Chapter 14
The Detection of Genetically Modified Organisms: An Overview

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Introduction

Genetically modified organisms (GMOs) are those whose genetic material has been altered by the insertion of a new gene or by the deletion of an existing one(s). Modern biotechnology, in particular, the rise of genetic engineering, has supported the development of GMOs suitable for research purposes and practical applications (Gepts, 2002; Novoselova, Meuwissen, & Huirne, 2007; Sakakibara & Saito, 2006). For over 20 years GM bacteria and other GM organisms have been used in laboratories for the study of gene functions (Maliga & Small, 2007; Ratledge & Kristiansen, 2006). Agricultural plants were the first GMOs to be released into the environment and placed on the market. Farmers around the world use GM soybeans, GM corn and GM cotton that are herbicide tolerant, or insect resistant, or combine several traits that reduce the costs associated with crop production (Corinne, Fernandez-Cornejo, & Goodhue, 2004). Biotech crop coverage increased globally by 13% (12 million hectares) in 2005–06 (James, 2007), and, for example, in 2007 over 70% of all soybean-producing areas were covered by GM varieties.

Although transgenesis of livestock began around 20 years ago, GM farm animals, including fish, are still not as common as GM plants, the development of which began somewhat earlier. Transgenic plants are most often developed by the insertion of an alien (recombinant) gene using the soil bacteria, *Agrobacterium tumefaciens*, which is able to transfer a piece of its own genetic information into a plant cell. While GM plant development is at least partially based on naturally occurring mechanisms, the engineering of most transgenic livestock relies on highly technical approaches, such as pronuclear microinjection. However, newly developed techniques [sperm mediated gene transfer (SGMT), somatic cell nuclear transfer (SCNT)] have been recently introduced that enable transgenic animals to be produced more efficiently and more cheaply. These have been successfully applied to the development of several types of GM animals including cattle, sheep, pigs, chicken and fish. The potential benefits of GM animals include accelerated animal

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growth, enhanced resistance to disease, and better meat quality (Niemann, Kues, & Carnwath, 2005).

The biomedical and agricultural communities have called for more research into the use of viral vectors in the development of farmed GM animals in the hope that modified animals may prove more resistant to diseases (e.g. trypanosomiasis), as well as to the cold. GM animals are able to produce human proteins in their milk or eggs, for e.g. GM chickens may produce as much as 0.1 g of human protein in each egg. Apart from the potential pharmaceutical applications of such protein (e.g. US patent 20060185029), it is also expected that GM animal products will follow GM plants and GM foodstuffs onto the food market in the near future (Wheeler, 2007). With GM animal products likely to appear on the consumer's fork sometime soon, regulatory bodies have been set up to develop guidelines and discuss key issues (e.g. *Codex Alimentarius*) such as food safety, risk to the environment and ethical concerns. These discussions have resulted in regulations being developed for the protection of consumer rights.

However, the situation is proving complicated to manage because GM technologies and their derived products are perceived in different ways, both on a regional basis and by the various competing groups within a region. International organizations, such as WTO, OECD, FAO and *Codex Alimentarius*, are attempting to produce a harmonized approach, but there are differences between the legislative approaches followed in North America, Asia and Europe.

To protect consumer rights in the EU, the placing of GM foods on the market is subject to "the precautionary principle" in EC food law (Just, Alston & Zilberman, 2006). Based on this principle, all GM products have to be proven safe in accordance with the complex procedure prescribed by EC Regulation 1829/2003 on genetically modified food and feed. The safety of each GM product is assessed by the EFSA (European Food Safety Authority) GMO panel of experts, with information submitted by each applicant being subject to detailed risk analysis. The availability of validated detection and quantification methods is one of the prerequisites for the acceptance of GM products. Applicants are required to submit a specific method for the identification and quantification of the particular GMO they wish to market. The method supplied must be verified by the Community Reference Laboratory (CRL) at the Institute of Consumer Health and Protection in Ispra, Italy (http://ihcp.jrc.ec.europa.eu), and validated in collaborative trials conducted by laboratories affiliated to the European Network of GMO Laboratories (ENGL). Once the method has been validated it is published at http://gmocrl.jrc.it/statusofdoss.htm. Apart from the method, an applicant must also provide a sample of the control material to the CRL, together with, on a confidential basis, information about the DNA sequence inserted into the genome of the modified organism. Additional legislation covers GM handling procedures (Table 14.1); the whole system being based on the traceability and appropriate labeling of approved GMO products. (In the EU, all products containing more than 0.9% of GM material as 'unavoidable contamination of a constituent' have to be labeled.)

The need to be able to trace GMOs and their derived products on the market has generated a demand for analytical methods capable of reliably detecting, identifying

Table 14.1 Basic EU legislation driving GM handling

Directive 2001/18/EC	On the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC
Regulation (EC) 1829/2003	On genetically modified food and feed
Regulation (EC) 1946/2003	On the transboundary movements of genetically modified organisms (GMOs)
Regulation (EC) 1830/2003	On traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC
Regulation (EC) 65/2004	On establishing a systém for the development and assignment of unique identifiers for genetically modified organisms

and quantifying them; such methods provided the basis for the implementation of appropriate labeling rules.

Basic Approaches in GMO Analysis

When information about a modified sequence is available, unambiguous identification of the GMOs and transgenic elements used in a food product is, in most instances, easy to realize. At present, hundreds of GMOs around the world have been assessed with respect to food safety, some of which have also been approved by the EU.

The detection method selected is a reflection of the way in which a particular GMO was developed. The development of a GMO generally consists of the following steps: preparation of a suitable recombinant construct; delivery of the construct into the target cell(s); selection and further development of an entire transgenic organism. The following elements constitute the basis of a recombinant gene construct: a promoter that drives gene expression; a DNA sequence coding for the protein; and a terminator that halts gene expression. These elements (e.g. a virus promoter, followed by an animal/plant gene, and a bacterial terminator) may all originate from different species. Usually, the construct delivered into the cell also contains a selectable marker that enables identification of the transgenic cells. Other types of constructs, e.g. Cre/lox system (Sauer, 2002), allow the deletion of the gene of interest from the genetic equipment of a cell.

Selectable markers are used with antibiotic resistance [e.g. neomycin phosphotransferase II (NPTII)] or herbicide resistance genes, the latter currently only being used in the case of transgenic plants. In the case of animals either antibiotic resistance genes [e.g. chloramphenicol acetyl transferase (CAT), NPTII] or other appropriate markers, such as green fluorescent protein (GFP), β-galactosidase (beta-gal) or secreted alkaline phosphatase (AP) are used. Marker genes, used for the selection of transformed cell lines, are also often used for the detection of GMOs.

GMOs and their derivatives are identified by detecting the DNA molecules, RNA molecules, proteins or other metabolites associated with, or derived from, a specific genetic modification. As with any analytical procedure, a precise sequence must be followed, which involves a representative sample being screened for the presence of GM materials, and, in the case of a positive result, the appropriate identification and quantification method being applied (Fig. 14.1).

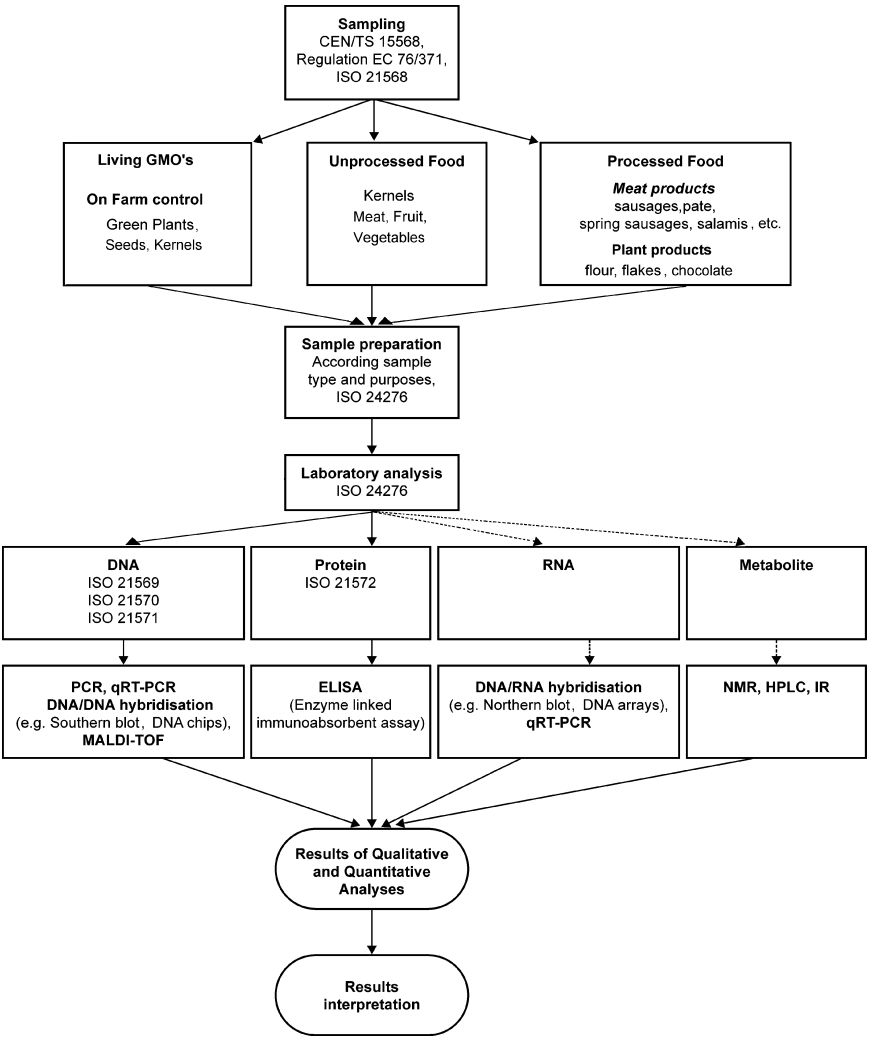

Fig. 14.1 Flowchart of DNA detection process. Detection of GMOs is based on EC regulation 1829/2004 and relevant ISO standards. Beginning with sampling, the process is then followed by the preparation of a test portion, by analyte extraction and by analysis. Results should be issued in accordance with ISO standards

Sampling

Sampling and sample preparation are crucial steps in the process of GMO detection (Terry, Harris & Parkes, 2002). While comprehensive analytical procedures exist to deliver precise results, the ability of a sampling procedure in general, and a sample size in particular, to erroneously influence final values should not be underestimated. The sampling procedure determines the representativeness of a result. In the case of GM material, because the distribution of GM particles in a lot is usually random and heterogeneous, a distribution-free statistical model has been suggested (Paoletti et al., 2006), and models have been developed that combine information about all stages of GMO event detection (Macarthur et al., 2007).

Being closely connected with statistical requirements, sample size has often been the subject of considerable discussion. It must be large enough to enable reliable GM detection at the desired sensitivity. A sample containing at least 3,500 particles is necessary to ensure that a 1% concentration of GMO in a batch will be reliably detected with a probability of $p \leq 0.05$. To obtain this probability, in the case of low level contamination, e.g. 0.1%, a test sample should contain 10 times more particles. Processed foods and complex matrices contain GM materials as one of several ingredients, so strongly stratified variance distribution can be expected. This is why it not always possible to use existing sampling strategies for the detection of other components in food commodities (Berger & von Holst, 2001).

General principles and rules for the sampling of GM foodstuffs are described in more detail in *ISO 21568, Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products -Sampling*. In accordance with this procedure, samples delivered to laboratories must be stored under appropriate conditions, and further test portions prepared. A test portion should reflect the concentration of GM material in the sample. A laboratory will analyze at least two test portions prepared from the identical sample. Analyte processing then follows.

Analysis of GM Material

The detection of GM materials is based on the identification of the DNA sequence, RNA sequence, protein sequence, or even metabolites, all of which result from genetic modification (Table 14.2). Methods differ with respect to their analytical parameters, feasibility and cost-effectiveness. Only DNA- and protein-based methods are currently used for control purposes.

Protein-Based Analysis

Typically, the production of specific proteins distinguishes GMOs from non-GMOs. Intact proteins are the required target for protein-based analysis; in the case of GMOs, by using immunoassays. Immunoassays are analytical measurement

Table 14.2 Basic overwiev of detection methods

Target	Method	Matrix (example)
DNA	PCR, qPCRm DNA/DNA hybridisation (e.g. Southern blot, DNA arrays), MALDI-TOF	Processed/unprocessed food_– Meat products as sausages, paté, spring sausages, salami
RNA	DNA/RNA hybridisation e.g. Northern blot), qRT-PCR	Living GMOs
Protein	ELISA (Enzyme Linked Immonoabsorbent Assay)	Unprocessed food
Metabolite	NMR, HPLC, NIRS	Containing lipids and fatty acid

systems that use antibodies as test reagents. Antibodies, produced in the serum of animals in response to foreign substances (antigens), are proteins that specifically bind to the substance that elicited their production. ELISA (Enzyme Linked Immunosorbent Assay) is the technique most commonly used for the specific binding of an antibody to a target protein. The principal advantage of ELISA lies in the high specificity of the ensuing immunological reaction, which allows antigenic substances to be accurately identified, even in the presence of interfering compounds. It also offers a high degree of automation and sample throughput. In addition, easy-to-use variations of this technique, such as lateral flow strips or dipstick kits, may offer semi-quantitative tests of considerable practical value, for example, in the detection of transgenic plants, such as 'Roundup Ready' soybeans. While such tests (described in detail in international standard ISO 21572, *Foodstuffs - Methods for the detection of genetically modified organisms and derived products - Protein based methods)* cannot be used to reliably analyze processed materials, they are well suited to the inspection of raw materials, and, therefore, might be of use in the identification of live transgenic animals. Because they offer speedy turnaround times, and require relatively small investment in both equipment and personnel, ELISA and protein strip tests are the methods of choice for differentiating between GM and non-GM entities, and for identifying the modification event.

DNA-Based Methods

Unlike protein, DNA is a stable molecule capable of being identified even when broken, degraded or denatured to some extent. In even highly-processed food matrices it is possible to detect, isolate and further analyze DNA. Polymerase Chain Reaction (PCR), which can be used for processed material in which DNA has been degraded, is currently the method of choice for such analysis.

Polymerase Chain Reaction

PCR (Mullis & Falloona, 1987) allows the multiplication of certain gene sequences. The whole procedure is based on the activity of *Taq* polymerase (DNA-dependent DNA polymerase), which was originally isolated from the bacterial species

Thermus aquaticus. This enzyme, which basically requires a DNA template (as short as 200 pb is sufficient) and pieces of single-stranded DNA (primers) surrounding the target sequence, is able to produce (amplify) millions of copies of the part of the genetic information that is to be analyzed. PCR is a cyclical process, each cycle consisting of three successive steps running at different temperatures. The first step in a cycle involves the separation of the two strands of the original DNA molecule. The second step involves the binding of the two primers to their complementary strands. Using the complementary strands as a template, the third step involves making two copies of the original double-stranded DNA molecule by adding the right nucleotides. Once a cycle is completed, it can be repeated, typically between 30–50 times. With the number of copies doubling in each cycle, the number of target sequences grows exponentially according to the number of cycles undergone (Holst Jensen, 2004).

If an analyte contains a target sequence, for e.g. part of the transgene- or species-specific internal gene, amplification products can be subsequently visualized and/or quantified. The whole procedure consists of: (1) DNA extraction and purification, (2) amplification of the target sequence, (3) visualization by electrophoresis or fluorogenes detection, (4) quantification and (5) interpretation of results. The application of PCR to GMO analysis has been described in the literature (Lipp et al., 2005; Miraglia et al., 2004).

DNA Isolation

DNA quality, in particular its purity, and DNA quantity are critical parameters for GMO analysis. DNA can be isolated either as high molecular weight DNA obtained from fresh material (e.g. blood, grains) or as fragmented DNA obtained from elderly or processed matrices (e.g. bone parts, flour, sausages, pâté).

The basic steps necessary to accomplish DNA isolation have been described by several authors (Somma 2004; Spoth & Strauss, 1999). DNA isolation begins with homogenization of the test portion, grinding and cell disruption. Cell disruption, which results from homogenization followed by the addition of a buffer consisting, amongst other reagents, of a detergent (e.g. SDS or CTAB) and DNase inhibitors (β-mercaptoethanol and EDTA), enables the transfer of DNA from a cell into the buffer. The mixture is further purified as cell debris, proteins and RNA from the extract are all removed. DNA is then precipitated. DNA can be further purified by appropriate procedures, such as the use of commercial kits based, for example, on gel filtration.

Several variations of this basic procedure exist. For example, PVP-based methods are particularly suitable for DNA extraction from raw or boiled meat, sausages, chopped meat and processed meat products containing soybean protein. CTAB-based methods have been shown to be efficient for the isolation of DNA from gravy, hamburgers, fatty salami and ham. All of these methods have been validated in collaborative trials, and are described in detail in *ISO 21 571 "Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Nucleic acid extraction"*.

The resulting DNA should meet the quality criteria. Typically, its absorbance is measured in UV light, which enables the presence of any contaminating proteins and/or phenolic compounds to be identified. Gel electrophoresis is used to detect the presence of contaminating RNAs that may interfere with upstream reactions.

PCR Analysis

In order to use PCR, the precise nucleotide sequences flanking both ends of the target DNA region must be known. Any PCR-based detection strategy is dependent on a detailed knowledge of the transgenic DNA sequence, which, in the case of authorized GMOs, can be found in the published databases (e.g. http://www.agbios.com/dbase.php). It also depends on the selection of the appropriate oligonucleotide primers. In addition to the well-documented issues concerning primer selection (Burpo, 2001), the choice of primers depends on the objectives of PCR analysis.

Once the DNA is isolated, its amplificability must be ascertained. Usually, a species-specific sequence is used. Species-specific genes (e.g. lectin from soybeans, zein from corn) should be represented by one copy per haploid genome. Protocols exist for the amplification of many species-specific sequences, and may be used for control purposes (Von Holst, Baeten, Berben, & Brambilla, 2004)

If the isolated DNA is amplifiable, screening methods are usually applied to exclude negative samples from subsequent analysis. Screening tests for the common transgenic are executed elements (e.g. promoters, terminators, selectable markers) present in some GMOs. In the case of GM plants, the 35S CaMV promoter (35S subunit of the cauliflower mosaic virus) and the T-NOS terminator (from the nopaline strain of *Agrobacterium tumefaciens*) are used for screening. However, this method is not GMO-specific, and contamination of the commodities by either of the two organisms (CaMV and *A. tumefaciens*) may lead to false positives. Therefore, in case of a positive result, further specific tests must be performed to verify the findings.

With the increasing number of GMOs in the market, not all kinds of GMOs can be reliably detected using existing screening methods. Some transgenic coding sequences might also occur across multiple GM varieties or species. If the primers are specific to a transgene occurring in multiple GMs it becomes difficult to locate the original source of the GM material in complex matrices. Construct-specific methods, involving, for example, one primer specific to the promoter and another specific to the coding sequence, may help to identify GMOs more accurately. However, event-specific methods, spanning transgene- and species-specific sequences, remain desirable for GM plant identification Fig. 14.2 (Yang et al., 2007).

Separation (either on agarose gel or by using PAGE) followed by visualization by UV light enable the amplification products to be easily identified and evaluated (Fig. 14.3). Because each amplification product is characterized by its specific size, it can be identified by checking it against a molecular ladder and by using positive controls. The most respected control materials are the certified reference materials produced by the Institute for Reference Material and Measurement (IRMM), an EU Joint Research Institution (JRC) located in Geel, Belgium. Also,

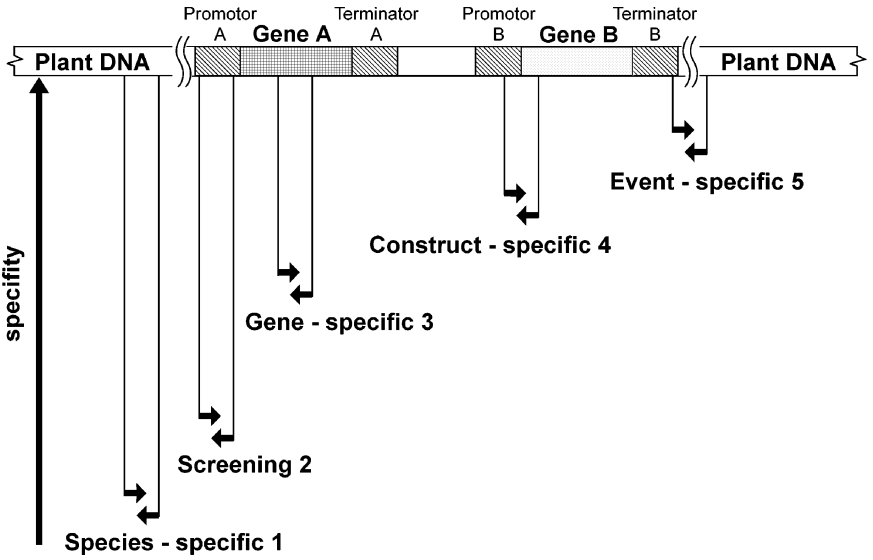

Fig. 14.2 Plots show possible accommodation of PCR primers within recombinant sequence. If common elements (e.g. 25s CaMV, T.NOS) are amplified, it can be described as screening. (a). Screening eliminates samples that probably do not contain GMOs. When transgenic elements are common across several GMOs (e.g. Bt gene) the assay is referred to as transgene-specific (b). When other primers, spanning two different elements (e.g. promoter, coding sequence), are common for several GMOs, the system/assay is referred to as construct-specific (c). Primers spanning specifies-specific and transgene-specific are unique for an event which is referred as event-specific (d).

CRL produces plasmid control materials containing GM-specific amplicons for the use of EU controlled laboratories. While the most reliable method for confirming the authenticity of a PCR product is still by its sequencing, only a few laboratories are equipped to carry out this approach routinely. Hence, few authors have reported on its use (Byrne, 2002; DMIF-GEN Final Report, 1999; Ehlers et al., 1997). Other methods used to confirm the authenticity of the amplicon include restriction analysis and Southern hybridization (Einspanier, 2006).

Several modifications/enhancements have been made to the PCR method, one of which, multiplex PCR, involves using several primers in the reaction mixture. However, multiplex PCR is not widely used as it has a higher risk of producing false results.

PCR is a highly sensitive method, theoretically able to efficiently amplify a single DNA molecule. In practice, reliable results are obtained when the reaction mixture contains 25–100 molecules of template DNA (Berdal & Holst-Jensen, 2001; Kay & van den Eede, 2001). Consequently, PCR is susceptible to external contamination. Therefore, several controls must be employed during each test to minimize false-positive results caused by contamination. As a minimum, these tests must include: positive control (tube containing reagents and DNA with target sequence, e.g. from positive standard); negative master mix control (control containing reagents and water, instead of analyzed DNA); and extraction control (control containing reagents

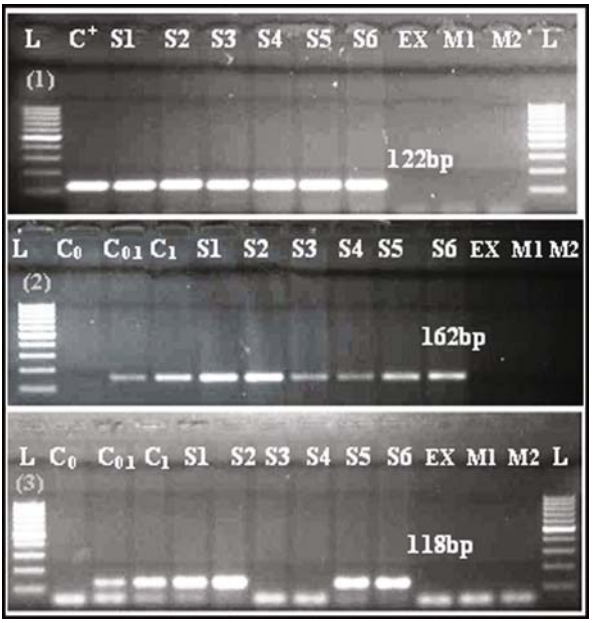

Fig. 14.3 DNA-based GMO analysis consists of several steps. (1) The quality of the isolated DNA is checked by amplification (PCR) of the internal gene, which is then followed by, (2) screening for common DNA promoters (e.g. 36S CaMV in the case of transgenic plants), and (3) by identification of the specific transgene in cases of positive screening. All reactions involve negative PCR controls (Neg), Negative extraction controls (Next), positive controls (P)

and solution resulting from extraction blank). Another often-used improvement is the running of PCR in real-time format, rather than using the more cost-effective, but less reliable, conventional PCR.

Quantification by PCR

Real-time PCR analysis, the most precise method of gene quantification currently available, requires special equipment able to trap and process fluorescent signals. This method is used to measure the amount of fluorescence in a PCR reaction, and from that, to estimate the amount of product synthesized during PCR. Internal probes, coupled to labels that are able to emit fluorescent light, are added to each reaction. As the probes hybridize to the amplification products (one probe per molecule), the intensity of the fluorescent light produced corresponds to the amount of newly synthesized DNA molecules. Thus, real-time PCR resolves the problem of establishing a relationship between the initial concentration of target DNA and the total amount of PCR product synthesized during the reaction. Several types of hybridization probes are available, with TaqMan probes currently being the most widely used in GMO diagnostics. Other types of probes exists (e.g. LUX, FRET, etc.) but are not currently validated for GMO detection (Bowyer, 2007).

The aim of quantification is to determine the ratio (expressed as a percentage) of two DNA targets. The first target is a species-specific single copy sequence (e.g. lectin in the case of soybeans) and the second is the transgene of the analyzed GMO. The comparative and standard curve methods are the two main approaches used to estimate the target molecule concentration from the fluorescence intensities recorded for the tested samples (Applied Biosystems, 2001). The standard curve method is more robust because it takes into account the amplification efficiency of different amplicons. Being dependent on reaction setup and DNA purity, the amplification efficiency of both targets may differ (Cankar, Štebih, Dreo, Žel, & Gruden, 2006). Technically, either one multiplex (duplex) reaction or two simplex reactions may be applied. Each reaction type has its advantages and disadvantages. For instance, multiplex reactions minimize pipetting errors, whereas simplex reactions reduce the problem of overlapping fluorescent signals coming from different internal probes.

For each target, a calibration curve is measured. After calibration curve and test sample data have been collected, the concentration of GM material is calculated (Fig. 14.4). Results should be expressed in compliance with current legislation.

Apart from increasing the likelihood of accurate quantification, the other main advantages of real-time PCR are in its speeding up of sample throughput (Kubista et al., 2006) and minimization of false results (Vaïtilingom et al., 1999).

Currently, real-time PCR is considered to be the most powerful tool for the detection and quantification of GMOs across a wide range of agricultural and food products (Bonfini, Heinze, Kay, & Van den Eede, 2001).

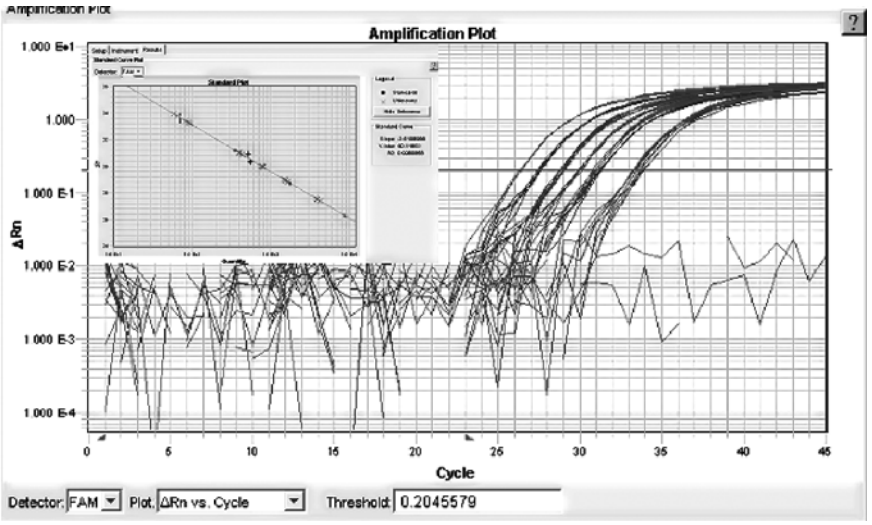

Fig. 14.4 An example of the result of quantitative analysis. Real-Time PCR results are shown in the form of curves that identify a Ct value (a PCR cycle, in which the amount of amplified DNA is higher than the background). Ct values enable the content of GM to be calculated from a calibration curve (b).

Other DNA-Based Methods

About 10 years ago, GM-based food/feed production was rare, but today the number of GM products approved or awaiting approval is growing rapidly. While a few years ago it was enough to use the PCR method for the detection of single GM events, such as 'Roundup Ready' soybean, nowadays we must employ tens of them, and in the near future it is likely that hundreds of such methods will be needed (Table 14.3). Already there is an increasing demand for more sophisticated methods capable of capturing different GMOs in a single reaction. One such option may be the use of DNA chips (arrays) that are based on the hybridization of analyzed DNA with specific probes on a glass support (Deisingh & Badrie, 2005; Nesvold, H., Kristoffersen, Holst-Jensen, & Berdal, 2005). Thousands of DNA sequences can be accommodated on one glass support giving rise to the possibility of being able to simultaneously identify multiple GMOs. The DNA chips (arrays) currently available have primarily been used for research purposes: to evaluate gene expression (e.g. Affymetix DNA chips); to identify the methylation status of a genome (e.g. TILLING arrays); and to differentiate among genotype [DArT (Diversity Array Technology) arrays recognizing SNPs (single nucleotide polymorphism), e.g. APEX arrays] (Khan et al., 1998; Roy & Sen, 2006; Schrijver, Külm, Gardner, Pergament, & Fiddler, 2007; Wenzl et al., 2004). Such analytical procedures require costly equipment, and even with it, analyte processing is not fully resolved in the case of GMO. However, Chen et al. (2006) described a method for the realization of full analyte processing using a cDNA array as a prototype for the detection of multiple GMOs. Their procedure requires further testing and validation. Although a cheaper version of the DNA array technique recently appeared, utilizing scaled-down equipment and multiplex PCR to process the analyte (Kok, Aarts, Van Hoef, & Kuiper, 2002; Wenijn, Siyang, Minnan, & Guangming, 2003), the very use of multiplex PCR constitutes the limitation of this approach.

Table 14.3 Basic websites addresses of international bodies dealing with GMOs

Institution	URL
European Food Safety Authority (EFSA)	http://www.efsa.europa.eu
Institute for Consumer Health Protection in Ispra, Italy	http://ihcp.jrc.ec.europa.eu
Europen Network of GMO Laboratories. (ENGL)	http://gmo-crl.jrc.it/statusofdoss.htm
World Trade Organization (WTO)	http://www.wto.org
Organization for Eonomic Co-operation and Development (OECD)	http://www.oecd.org
Food and Agriculture Organization of the United Nations (FAO)	http://www.fao.org
Codex Alimentarius (FAO/WHO Food Standards)	http://www.codexalimentarius.net
Agbios database	http://www.agbios.com/dbase.php
International Organization for Standardization	http://www.iso.org
United States Department of Agriculture	http://www.usda.gov
U.S.Food and Drug Administration	http://www.fda.gov/
U.S.Environmental Protection Agency	http://www.epa.gov/
Institute for Reference Materials and Measurements	http://www.irmm.jrc.be

Another approach, based on Ligation Probe Amplification (LPA), has been recently introduced. LPA allows simultaneous event-specific detection and the relative quantification of DNA from several GMOs in a single reaction. However, this approach requires careful primer setup and the optimization of reaction conditions just for the system to detect a mere 40–50 targets per reaction (Carrino, 1996; Mezzelani et al., 2002).

Novel approaches to DNA analysis based on micro and nanoparticles, as well as on mass spectrometry, may open new possibilities in DNA detection. The possible advantages of some of these approaches have been described by Sato, Hosokawa, and Maeda (2007); Einspanier (2006), amongst others.

Other Methods of GMO Detection

Where the composition of GMO, e.g. fatty acids or triglycerides, has been modified, conventional chemical methods based on chromatography can be used to detect differences in the chemical profile. Metabolites, where they are the product of transgenic protein activity, can be detected by physical methods, such as NMR (Nuclear Magnetic Resonance), HPLC (High Performance Liquid Chromatography) or Near Infrared Spectroscopy (NIRS) (Heller, 2006; Roussel, Hardy, Hurburgh, & Rippke, 2001; Von Holst et al., 2004).

Data Quality and Interpretation

Any procedure used in GMO analysis for control purposes should be based on validated methods, some of which have been published by ISO (International Organization for Standardization http://www.iso.org). All methods should be reproducible, repeatable, precise and accurate and should reflect the true value. These analytical parameters are critical when applied to any method used for the detection of GMOs in foodstuffs. The limits of detection (LOD) and quantification (LOQ) should be determined in accordance with the method being used and the matrix of the sample being analyzed (Berdal & Holst-Jensen, 2001). Full details of how to report and issue results are described in *ISO 24276: "Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - General requirements and definitions"*. To ensure data quality, all control laboratories should be accredited according to the ISO 17025:2005 (Žel, Cankar, Ravnikar, Camloh, & Gruden, 2006).

Despite the prescribed procedures, the Food Analysis Performance Scheme (FAPAS), which operates a proficiency testing scheme for GM food analyses in the UK, has reported the following findings: a tendency for laboratories to overestimate GM levels; significant differences between the results obtained using PCR and ELISA; data being skewed and not normally distributed until undergoing mathematical treatment (Powell & Owen, 2002).

These findings indicate the need to better understand the processes underlying GM detection methods, as well as the need for considerable investment in the research of more advanced techniques. Further development of methods used for the detection and quantification of GMOs in agricultural commodities is essential to monitor the implementation of regulatory compliance concerning the use of GM food/feed. Such research should focus on improving the analytical parameters of high-throughput methods so that they are fit for the purpose.

It is likely that new methods will not only be used to control GMOs and accommodate consumer requirements, in some countries, but also to protect breeders' rights and intellectual properties worldwide.

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Bonfini, L., Heinze, P., Kay, S., & Van den Eede, G. L. M. (2002). *Review of GMO Detection and Quantification Techniques*. EUR 20384/EN.

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