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Review article

Research progress in techniques for detecting genetically modified organisms

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Abstract

The techniques used to detect genetically modified organisms (GMO), including qualitative polymerase chain reaction (PCR), quantitative PCR, enzyme-linked immunosorbent assay (ELISA) and many others, are systematically described and discussed. The application progress of GMO in species-specific detection, endogenous genes, standard substances and restraining factors influencing detection are reviewed. The ongoing problems and development prospects of detection techniques of GMO are also pointed out.

Keywords: GMO; detection; PCR; progress

Introduction

With the foundation of a labelling system for genetically modified organisms (GMO) and the improvement of public concern about GMO in many countries, it is necessary to bring forward the stringent requirements for sensitivity and accuracy of GMO detection techniques. Therefore, all kinds of GMO detection techniques have become a research hotspot. Over the past decade, the technology of agricultural GMO in China has been developed rapidly: insect pest-resistant transgenic cotton, delayed-ripening tomato, antivirus peppers, insect pest-resistant transgenic poplar and antivirus papaya have been certified and are produced for marketing in China. Research on transgenic detection technology has registered tremendous advances outside China.

The purpose of this review is to provide scientists with the up-to-date information on trends in GMO detection techniques. The development, promotion and supervision of these techniques are also widely presented and discussed in this paper.

Qualitative PCR detection techniques

Methods of polymerase chain reaction (PCR)-based GMO qualitative detection, in accordance with their specific differences, can be divided into four groups: screening PCR, gene-specific PCR, construct-specific PCR and event-specific PCR.

The screening PCR method is used to detect the generic components for genetically modified products, including the promoters, the terminators and marker genes. The most common method was to detect the promoter of the *Cauliflower mosaic virus* (CaMV35S), the *Agrobacterium tumefaciens* nopaline synthase (T-NOS) terminator of *Agrobacterium* rouge base synthase or NPT-II reporter gene (neomycin phosphotransferase-II). However, identifying only screening genes, such as the 35S promoter,

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NOS terminator or NPT-II reporter gene, does not include all the requirements for detecting GMO. This is because both 35S promoter and virus NOS terminator respectively exist naturally in *Cauliflower mosaic virus* and in the plant virus Ti plasmid. The antibiotic labelling gene is also widespread in nature. Therefore, the use of screening PCR only can lead to false-positive results and, consequently, compromise the detection of genetically modified plants.

The gene-specific PCR method detects the target gene. The target gene may be derived from nature, but is often slightly modified, such as sequence shortening and codon changes (Hemmer, 1997). In addition, the number of obtained genes is far higher than numbers of promoters and terminators. Therefore, the gene-specific method for the target gene appears to be more rigorous than screening PCR.

The primers for construct-specific PCR are designed in the gene cassettle of a complete transformation vector expressing the target gene and the method is more specific and determines the construct type accurately. For example, the construct-specific gene of GM corn Bt176 is *DPK-Cry1A* (b), GM corn Bt176 is *1 VS-CryA* (b), GM Roundup Ready has *35S-CTP* and *CP4-CTP* structural genes, and so on. By this method, the positive signals appear only in material from the GMO (XD Xu *et al.*, 2005).

However, different GM crops often contain the same foreign DNA. For example, GM plants Mon809 corn and Mon810 corn have the same plasmid insert, pV-ZMBK07. The construct-specific PCR method will not succeed in distinguishing these.

Event-specific PCR can identify different GMOs with the same foreign DNA through the amplification of the flanking sequence between the inserted receptor gene and the plant genome sequence (Anklam *et al.*, 2002). When a foreign gene can lead to several different inserted situations, the best method of detecting the specific GMO is to amplify its flanking sequence, i.e. the connecting region between the plant receptor genome and inserted DNA fragment. Thereby, the flanking sequence is favourable for the specificity of GMO detection.

For qualitative detection of genetically modified products, high specific nested PCR and semi-nested PCR are often used. Nested PCR, developed on the basis of the general PCR, has been used mainly in molecular biology and medical research. The principle of nested PCR involves the design of two pairs of primers to detect target genes by a double PCR reaction. The primer pair of the second PCR is designed using the amplified fragment from the first PCR. Usually the first PCR adopts the primer pair amplifying the larger fragments. The first amplified product is used as a template for the second PCR in 20 to 30 cycles. The principle of semi-nested PCR is basically the same as nested PCR, except that the semi-nested utilizes only three primers. One primer is used in both the first and the second PCRs. These two methods can reduce the appearance of false positives, but also can enable a fall in the detection threshold by several orders of magnitude. Therefore, both nested and semi-nested PCRs can be used to detect genetically modified products efficiently. Huang and Luo (2003) detected GM soybeans and highly processed products by running these two techniques, showing that nested PCR and semi-nested PCR with high sensitivity can still detect GMO with seriously degraded DNA.

In addition, multiplex PCR has widely been used to identify GMO. The basic principle of multiplex PCR is the same as that of the general PCR, except that, here, more than one pair of primers are required in the same reaction system. The primers can specifically combine their corresponding DNA template and more than one DNA fragment will be amplified in one tube. Faster and more economic multiplex PCR has similar specificity and sensitivity as simplex PCR and shows greater flexibility in the design of primers and PCR reaction conditions. Multiple PCR could provide internal controls and appreciation of the quantity and quality of amplified templates (Xu *et al.*, 2006a).

Huang and Pan (2004) simultaneously detected GM corn MON810 and NK603 by multiplex PCR with a detection limit lower than 0.5% (50 ng template in 25 µl system). Feng *et al.* (2006) built multiplex PCR with nine kinds of primer pairs to detect relatively stable transgenic components from GM soybeans, corn and canola, and applied it to 185 samples (soybean, corn, canola and their processed products) in 1–2 days. Zhao *et al.* (2004) also employed multiplex PCR to detect Roundup Ready soybean and Bt176 GM corn. All the above research indicates that multiplex PCR is a fast, reliable, sensitive, accurate, specific, simple, low-cost technology for detecting GMO.

Quantitative PCR

Quantitative PCR is a method often used to detect and label GMO. Traditional quantitative PCR methods, such as the endo-reference method, competitive PCR and PCR-ELISA method, are endpoint detection methods with poor reproducibility.

The real-time PCR technique was first reported by Higuchi *et al.* (1993) and the world's first fully automated real-time PCR instrument was produced by ABI company (United States) in 1996. This technology requires the addition of fluorescence into the PCR reaction system. The accumulation of fluorescent signal is monitored in the whole process and finally the generated standard curve is used for quantitative analysis of the unknown template. This method involves a dual fluorescent

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labelling probe to detect the accumulation of PCR product and is a very precise quantitative method. Compared to conventional PCR, this PCR method represented a leap from qualitative to quantitative detection. Moreover, it is very specific, highly automated and effective for resolving PCR contamination.

Real-time quantitative PCR mainly uses a fluorescent labelling probe and a corresponding fluorescence signal detection device. The principle is based on fluorescence resonance energy transfer phenomenon (FRET): when the excitation spectrum of a fluorescent molecule (the donor molecule) overlaps with that of another fluorescent molecule (the receptor molecule), self-fluorescence intensity of the donor molecule decays. As real-time PCR can achieve the quantitative detection of nucleic acid, it has been widely used for quantitative GMO detection (Holck *et al.*, 2002; Hernández *et al.*, 2003; Xu *et al.*, 2007a).

The fluorescent probe of real-time quantitative PCR mainly includes TaqMan fluorescent probe, hybrid probe and molecular beacon. The TaqMan fluorescent probe is the most widely used. The reaction system includes a primer pair and a probe. The probe only combines at specific sites with the templates between the primer pair. The reporter (R), such as FAM and VIC, is tagged at the 5′ tag of the probe and fluorescence quenching group (Quencher, Q), such as TAMRA, is tagged at the 3′ tag of the probe. When the probe is integrated, the fluorescence signal cannot be detected. When PCR takes action, *Taq* enzyme can cut off the probe and a fluorescent signal appears because the reporter energy cannot be absorbed. Therefore, both the fluorescent signal and the target fragment have an exponential increase with the increase of PCR cycle number. The signal strength is proportional to the copy number of the DNA template.

In the real-time quantitative PCR technique, a very important concept is the cycle threshold (Ct). Ct stands for the cycle number as the fluorescence signal reaches the set threshold of fluorescence (10 times standard deviation of fluorescence signal from 3 to 18 cycles). The baseline means that the fluorescence signal is still below the sensitivity of the detection equipment. Fluorescence signal beyond the fluorescence threshold is to be regarded as the real-time signal of sample Ct. Research suggests that Ct values of each sample stay in a negative linear relationship with the original copy number of the sample and the higher the original copy number, the smaller Ct value will be.

At present, real-time quantitative PCR has been used as an external standard quantitative method; that is, the standard curve is generated with different concentrations of a known gene template at certain times. Once the Ct value of an unknown sample is obtained, its original copies can be calculated from the standard curve.

Enzyme-linked immunosorbent assay detection technique

Enzyme-linked immunosorbent assay (ELISA) is a primary method of protein-based detection. The method relies on a specific combination between both an antigen and antibody.

ELISA is most commonly used in immunology. The technique has many advantages including high specificity, high sensitivity, easy preservation, easy observation, quantitative detection, simple equipment and more. At present, this method has been widely used to analyse the exogenous target proteins of genetically modified crops at gene expression level. These proteins include the Bt insecticide protein Cry1Ab (MON810) (Stave, 2002), EPSPS protein (Rogan *et al.*, 1999; Lipp *et al.*, 2000), npt II protein (McKenzie *et al.*, 2000), herbicide-resistant plant protein (WT Xu *et al.*, 2005).

In recent years, scientists have established test strips, such as the lateral flow and rapid detection kit, on the basis of ELISA. The test strip only directly contacts the extracted protein antigens during detection, which makes the process simple and automatic, highly sensitive, easily adapted for fast detection and diagnosis in the field and suitable for semi-quantitative analysis of GMO (Stave, 1999).

However, ELISA presents some limitations for detecting genetically modified products:

(**1**) Foreign genes cannot express the specific recombinant protein or express the protein at a low level. In general, the expression accounts for only 0–2% of the soluble protein. The maximum expression due to a strong promoter has also been found to be below 2% (Hemmer, 1997); however, the expression level usually amounted to less than the limit, and was thus undetected.

(**2**) Specific proteins are sometimes only synthesized in specific parts of the crops or at a certain physiological stage. Therefore, the expression level in different parts is inconsistent. For example, the expression quantity of EPSPS protein in Roundup Ready soybeans leaves and seeds was 459 and 288 μg/g, respectively.

(**3**) As only the protein antigen with intact tertiary or quaternary structure can be identified by a specific antibody, ELISA is not applied to detect the highly processed or antigen-variable genetically modified products.

(**4**) Numerous antibodies must be prepared because of various inserted target genes.

(**5**) ELISA cannot distinguish two different kinds of transgenic plants with the same exogenous protein.

(**6**) The accuracy of ELISA is affected by impurities such as the saponins, phenol complex, fatty acids and endogenous phospholipase in mixed samples.

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(7) ELISA has a low detection limit. Commercial ELISA detection kits can only account for 0.3–5% GM soybeans of the total sample.

Therefore, this protein detection method for GMO cannot be used as the preferred method.

Cultivar-specific detection of genetically modified products

For genetically modified products with a single trait, cultivar-specific detection is an event-specific transgenic detection technology. For stacked transgenic products with complex characters, the cultivar-specific detection technology is not always the same as event-specific transgenic detection technology, depending on the way transgenic strains with complex traits are obtained. According to the definition of the Organization for Economic Co-operation and Development (OECD), a stacked GMO could be a re-transformation of an existing transgenic line or hybridization between two or more GM transgenic lines. For the hybridization transgenic lines, event-specific transgenic detection does not work, and up to now, there are still no good methods for cultivar-specific detection; for re-transformation, the transformation event-specific testing methods can be used.

Event-specific transgenic detection has been used successfully in investigations of many transgenic crops, such as GTS 4032 soybean (Berdal and Holst-Jensen, 2001; Taverniers *et al.*, 2001; Terry and Harris, 2001), MON1445 and MON531 cotton (Yang *et al.*, 2005), MON810 (Holck *et al.*, 2002; Hernández *et al.*, 2003), Bt11 (Berdal and Holst-Jensen, 2001), MON863 (Yang *et al.*, 2005), GA21 (Hernández *et al.*, 2004), NK603 (Nielsen *et al.*, 2004) and T25 corn (Collonnier *et al.*, 2005) and GT73 rapeseed (Yang *et al.*, 2007), etc. In addition, more and more flanking sequences of transgenic crops have also been tested. The key to event-specific transgenic detection is to obtain flanking sequences of transgenic crops. At present, several flanking sequence analysis methods, based on PCR technology, have been developed: inverse PCR (I-PCR), thermal asymmetric interlaced PCR (TAIL-PCR), ligation-mediated PCR (LM-PCR), etc.

I-PCR is a genome walking method based on conventional PCR, developed by Ochman *et al.* (1998). This method amplifies fragments of unknown sequence outside the two primers by the use of reverse complement primers, while conventional PCR amplifies the DNA fragments of known sequence between the two primers. During the experiment, the restriction endonuclease, which has no cut-off point within the known sequence, is chosen to digest DNA fragments. Then, the target sequence with cohesive end is self-looped and ligated, and a couple of reverse primers are used for PCR reaction. The amplified products contain the unknown sequence outside the two primers, which can be analysed. Zimmermann *et al.* (2000) obtained the flanking sequences of Bt11 corn by the use of I-PCR.

TAIL-PCR is a method based on the thermal asymmetric PCR developed by Liu *et al.* (1999). TAIL-PCR refers to a reaction where the flanking sequence of known sequence is amplified by the guidance of a series of nested sequence-specific primers and a short arbitrary primer; it is a semi-specific PCR reaction. As annealing temperatures of the two primers are different, the amplification of specific and non-specific products can be controlled by varying the annealing temperature in the process of the reaction. In TAIL-PCR, the nested sequence-specific primers are longer than the arbitrary primers and the annealing temperature higher. The long primers can anneal with a specific sequence at both high and low temperatures; however, the short arbitrary primers can only anneal with unknown sequence (flanking of known sequences) at the low annealing temperature. Target genes are effectively amplified by alternating high and low annealing temperatures.

The TAIL-PCR method can reduce the background of specific products of the non-flanking region and, at the same time, it can produce more than two nested fragments. Compared with other methods, TAIL-PCR method is simple, specific, efficient, rapid and sensitive. Hernández *et al.* (2003) and Yang *et al.* (2005) obtained the flanking sequences of MON810 and MON863 corn, respectively, by TAIL-PCR.

LM-PCR was created by Pfeifer *et al.* (1989). This technology uses restriction enzymes to digest a specific site of DNA, connects the synthetic joints, and uses two co-rotating primers and adapter primers to amplify the unknown sequence by double PCR. Holck *et al.* (2002) obtained the flanking sequence of genetically modified corn MON810 by the method. In essence, LM-PCR only needs to specifically anneal one primer, and the other is the common adapter through the ligation reaction. The two primers can amplify any DNA fragments of a flanking gene. The method is efficient, sensitive and specific. But LM-PCR has some limitations: (1) LM-PCR only directly detects a gap or rupture of DNA and thus needs enzymic or chemical treatment; (2) the 5′ end of DNA template molecules must be phosphorylated and have connectivity; (3) the primer can take part in the even connection only as the primer extends to the end of templates, so the target fragment cannot be detected in LM-PCR if the extended molecule is stopped earlier.

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Species-specific detection of genetically modified products

Species-specific detection of endogenous genes

In order to prevent false-negative results during GMO detection, the endogenous (reference) genes of many crops have been widely accepted and applied to transgenic detection. So far, endogenous genes that have been studied and developed include: soybean housekeeping gene *Lectin* (Meyer, 1999) and β-*actin* gene (Delano *et al*., 2003), corn *invertase* (Holst-Jensen *et al*., 2003) and *zein* genes (Zimmermann *et al*., 1998; Vollenhofer *et al*., 1999), rape gene *BnACCg8* (Hernández *et al*., 2001), rice gene *sps* (Ding *et al*., 2004) and cotton housekeeping gene *sad 1* (Xu *et al*., 2006b).

Endogenous genes are indicators for both the species characters and the PCR reactions. In other words, there must be some problems with the PCR reaction system if the endogenous genes are not detected: for instance, wrong reagents added to the reaction system, or PCR inhibitors present in the PCR reaction system. However, the endogenous genes are mainly used for the quantitative detection of genetically modified crops. Some factors, such as copy number of the endogenous gene, sensitivity and specificity of probes and primers, may affect the properties of an endogenous gene.

Nowadays, studies on endogenous genes in many species are being carried out. The resulting information, including identification of various species, shows that the ongoing research is still limited to qualitative research, but not to quantitative research. Although previous investigations studied their own housekeeping genes for each species, few studies were conducted on gene copy numbers, which directly affect the sensitivity of detection and quantitative accuracy. As research on GM food has increased, basically every GM species has been found to have its own (at least one) suitable housekeeping gene and the copy number for these genes has been identified.

Preparation technology of reference material

It is necessary to test samples of known concentration as reference samples when adopting external quantitative methods. Certified reference materials (CRMs) approved by authorities (e.g. the European Institute for Reference Materials and Measurements, IRMM) should be used as much as possible in the detection of genetically modified samples. Available CRM transgenic crops are very limited (e.g. Roundup Ready soybean, corn Bt176 with insect resistance). Nevertheless, research on this issue is increasing year by year. CRMs are prepared from raw materials. Meanwhile, some factors, including matrix effects, loss of intact DNA during the process, etc., lead to great limitations and problems of adopting CRMs as the quantitative reference, including:

(**1**) All the quantification PCR reactions must be performed on the basis of genomes or similar equivalence (the relative proportion of DNA) in the detection of the genetically modified ingredient, and this conflicts with the CRM, which is treated by weight as its unit. The weight of target gene per unit in genome and non-genome varies very much, due to differences in copy number.

(**2**) CRM preparation is time consuming and labour intensive, thus costly.

(**3**) It is essential to grind CRM materials in order to diminish the volume bias and ensure the homogeneity, but this process will cause DNA degradation.

(**4**) A method should be available with all the detection levels of materials from 0 to 100%, but CRMs can only be used at lower concentrations.

(**5**) CRMs are pure particles of a single component. To depend only on the confirmatory test of CRMs may lead to a wrong limit of detection (LOD) and especially a wrong limit of quantification (LOQ). For instance, the limitation of absolute quantification changes following the variation of total amount of template DNA. In recent studies on the real-time quantification of corn Bt11, the detection limit changed when soybean DNA was added. A similar conclusion has been drawn by Berdal and Holst-Jensen (2001).

In order to overcome the above disadvantages, standard molecules, such as PCR amplification products and linear plasmids, are gradually replacing CRMs as reference materials of quantitative PCR (Stave, 1999; Pardigol *et al*., 2003). The reference materials of PCR products are convenient and simple, but inaccurate and unstable. On the other hand, linear plasmids as references are not only easy to prepare, but also stable and accurate. Currently, the methods of constructing standard plasmids mainly include conventional DNA recombination performance (including operations such as cleavage and ligation by enzymes) and the recombination technique of PCR products. Huang and Pan (2004) have successfully applied the PCR recombination method to the quantitative detection of corn MON810 and corn NK603. Kuribara *et al*. (2002) have also constructed standard molecules as reference material for the quantitative detection of transgenic corn and soybean.

Techniques of elimination of PCR inhibitors

During the detection of genetically modified food, inhibitors acting on the PCR reaction have been encountered,

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for example in rice (Xu *et al.*, 2007b). Most PCR inhibitors (such as polysaccharide, urea, humic acid and haem) have similar solubility to the DNA. These substances (detergent, protease, chloroform-isoamyl alcohol) cannot be easily and completely removed by classical DNA extraction methods, and thus contaminate the final extraction. Many methods have been developed to remove these contaminants, including dialysis for the removal of most reagents and CsCl gradient density centrifugation for the purification of DNA (Maniatis *et al.*, 1982). Both methods are simple, but cause great loss of the original DNA template. Other methods, such as ion-exchange chromatography, glass bead transformation, immunomagnetic separation, molecular sieve separation, anion binding resin and rotational cylindrical centrifugation (Wilson, 1997), aim at a specific contaminant; they are effective but also expensive. Moreover, the further purification performances these methods involve increase the risk of cross-contamination, consequently leading to false-positive results (Vaneechoutte and Van Eldere, 1997).

A decrease of detection sensitivity can be attributed to the presence of PCR inhibitors, often found in blood and many other similar complex biological media. PCR reaction inhibitors suppress the activity of PCR polymerase, cause degradation of DNA templates or interfere with them or with cell devision (Lantz *et al.*, 2000). It was revealed that some inhibitors actually operate on the DNA polymerase, because the inhibition phenomenon can be alleviated by using enzymes less insensitive to inhibitors (Shames *et al.*, 1995; Al-soud and Radstrom, 2001).

As PCR inhibition appears to be the main obstacle in detection techniques and processes, many scientists are trying to find appropriate solutions to the problem (Abbaszadegan *et al.*, 1993; Lantz *et al.*, 1997; Cavallini *et al.*, 2000), or to improve the upper limit of PCR detection (Zhang *et al.*, 2000).

Other techniques

With the availability of novel techniques and instruments, GMO detection techniques are being increasingly developed, among which the application of biochips is a technique at the frontier of research. Thousands of samples can be treated simultaneously and analysed by this method, resulting in a high efficiency and low cost (XD Xu *et al.*, 2005). Feriotto *et al.* (2002, 2003) have successfully accomplished real-time quantitative assays for Roundup Ready transgenic soybean and Bt176 corn. Recently, the European firm GeneScan has commercialized a GMO chip kit, which allows the qualitative detection of several transgenic ingredients in certain transgenic crops.

If the chemical composition of the genetically modified product is greatly different from that of the non-transgenic one, it is recommended to apply chromatography to analyse the chemical components and identify transgenic products. In addition, for some special transgenic products, for instance plant oil, which cannot be tested by traditional detection of exogenous genes or proteins, the chromatographic technique can be used to analyse the composition of fatty acids and triglycerides in samples. Byrdwell and Neff (1996) analysed the components of triglycerides in three kinds of oilseed rapes by combining high pressure liquid chromatography and mass spectrometry techniques. The results showed that the content and antioxidant capacity of triglycerides in transgenic rapeseed oil were both significantly higher than in other varieties. Because of its qualitative property, this method lacks accuracy in testing products from mixed transgenic and non-transgenic samples.

Furthermore, infrared spectrum analysis can be used to screen the transgenic crops, due to the fact that some of their fibre structures change during the transgenic process. Hurburgh *et al.* (2000) successfully distinguished transgenic from non-transgenic soybean with a correct detection rate of up to 84% using a near infrared spectroscopic method. Rui *et al.* (2005) have also successfully identified transgenic and non-transgenic corn using the same method, with a correct detection rate of 100%.

The immunoassay technique is always attracting scientists' interest due to its high specificity and sensitivity. More attention is focused on how to detect transgenic components quantitatively and, at the same time, reduce the analysis cost attached to this method. PCR-ELISA can immuno-detect PCR products. Primers are labelled by digoxin or biotin and amplified products are, thus, attached to the specific probe on a fixed plate, with the addition of digoxin or biotin–horseradish peroxidase labelled antibody, and eventually a colour change due to the reaction of enzyme and substrate. Conventional PCR-ELISA is just a qualitative experiment; however, the objective of quantitative detection can be realized if a standard curve is established with internal standard material.

Prospect

The micro-, high-throughput and automatic techniques, like biochips and biosensors, can meet the increasing demand for transgenic detection. Meanwhile, the research focus is on how to improve the accuracy of these techniques and apply them to quantitative detection. The future goals of transgenic detection techniques are high accuracy, high throughput, automaticity and low cost (Gu *et al.*, 2006).

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In order to improve the PCR technique, which has been most widely used, scientists are aiming to enhance the limit of detection (LOD), limit of quantification (LOQ) and detection of PCR amplification products by, for example, using biosensors to replace the electrophoresis technique, which is time-consuming and easily contaminated.

It will be a research challenge to determine a specific detection method for stacked transgenic crops with multiple traits. Standard molecules and substances will remain a hot and difficult topic in the field of GMO detection. This significant factor will determine whether quantitative detection can be extended, or not, in developing countries.

With the continuously increasing number of transgenic species, studying the species endogenous genes will be of great value. In addition, studies on universal internal genes will be also highly relevant, as they could help in controlling DNA preparation quality and PCR amplification efficiency. For example, 5s DNA has been developed as a specific internal gene (Doveris, 2007). There is still much potential for the intensive study of this gene, its copies and the possibility of using it in quantitative detection.

In view of the great breakthrough of gene deletion techniques (Luo *et al.*, 2007), we can count on the realization of producing non-transgenic plant tissue from transgenic crops in the near future, and the detection of novel transgenic species using gene deletion techniques will become a new challenging topic.

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