Tracking genes from seed to supermarket: techniques and trends

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Analytical techniques to track plant genes in the environment and the food chain are essential for environmental risk assessment, government regulation and production and trade of genetically modified (GM) crops. Here, I review laboratory techniques to track plant genes during pre-commercialization research on gene flow and post-commercialization detection, identification and quantification of GM crops from seed to supermarket. At present, DNA- and protein-based assays support both activities but the demand for fast, inexpensive, sensitive methods is increasing. Part of the demand has been generated by stringent food labeling and traceability regulations for GM crops. The increase in GM crops, changes in GM crop design, evolution of government regulations and adoption of risk-assessment frameworks will continue to drive development of analytical techniques.

The use of genetically modified (GM) crops in large-scale farm production in the past decade has created a demand for laboratory techniques that can track plant genes and transgenes in the environment and through the food chain. GM crops were grown on 58.7 million hectares in 2002, 99% of which was grown in the USA, Argentina, Canada and China (http://www.isaaa.org/). In most cases, this first generation of GM crops has been modified by the insertion of one or a few novel genes to produce valuable agronomic input traits such as herbicide tolerance or insect resistance. In the development, regulatory review and commercialization of a GM crop, there are crucial research projects and analytical methods that provide information to business, government, and other organizations (Figure 1). Pre-commercialization research activities are conducted before regulatory approval, including biosafety research and confined field tests (see Glossary) [1,2]. Post-commercialization activities occur after a government approves the GM crop for field production and/or use as an ingredient in human food or animal feed. Here, I review the laboratory techniques that are used to track plant genes during two important activities: (i) precommercialization research on gene flow to support ecological risk assessments; and (ii) post-commercialization detection, identification and quantification of GM crops from seed to supermarket.

One event that pushed the issue of gene flow to greater prominence was the detection of transgenes in maize landraces in Mexico [3]. Although crops and wild plants have exchanged genes throughout the history of agriculture, GM crops have raised concerns that gene flow will lead to negative environmental impacts in agricultural systems and/or natural areas [4,5]. The discovery of StarLink corn in human food and the subsequent recall of hundreds of food products highlighted the difficulty of separating and tracking GM crops through the food chain (http://pewagbiotech.org/resources/issuebriefs/starlink/ and http://pewagbiotech.org/research/postmarket/). The European Union (EU) is currently enacting more stringent policies requiring traceability for all GM crops and mandatory labels on all food and feed products if the proportion of GM ingredient exceeds a threshold level of 0.9% [6] (http://europa.eu.int/). Because GM crops and the regulations pertaining to them are proliferating around the world, the current system of global agricultural trade demands laboratory techniques that support regulations, risk assessment frameworks and contracts between trading partners.

Analytical techniques for tracking genes and transgenes (Tables 1,2) must be chosen based on the research question and a combination of other factors [7–9]. The accuracy, precision, reproducibility, sensitivity and specificity of the method must be understood in relation to the research question. Practical considerations include the cost and time per sample, the chemicals and equipment required, sample handling and processing, adaptability to field conditions, and technical expertise. For post-commercialization traceability and food labeling activities,
methods must be practical for testing points at the farm, during transport and in food processing. Regardless of the technique, appropriate experimental controls, \textit{REFERENCE MATERIALS}, and information about parental crop lines and transgenes must be available [10] (http://pewagbiotech.org/research/postmarket/).

**Techniques for understanding gene flow**

Although crops and wild plants have exchanged genes throughout the history of agriculture, the development of GM crops has released a flood of research and debate about gene flow from many different crop species and the possibility of negative environmental impacts in different regions of the world [4,5,11–15]. In response to these concerns, regulatory agencies in all countries require an environmental risk assessment on a case-by-case basis before approving confined field trials or unconfined release of GM crops [6]. These environmental risk assessments generally require information about the probability of pollen movement from GM crops to fields of the same crop, closely related crops, wild ancestors in centers of biodiversity, intermediate weed species and wild relatives in natural areas [4,5,11–13,15]. Gene flow through seeds and vegetative propagules must also be addressed.

In general, gene flow research can be divided into two types of studies: (i) descriptive studies of past introgression events between conventional (non-GM) crops and their wild relatives; and (ii) experimental studies demonstrating the likelihood of hybridization and introgression. Descriptive studies using conventional crops are important when public policy or a high level of risk prohibit any experimental field trials. The three most widely used laboratory methods are DNA-based molecular techniques to characterize genetic markers, isozyme analysis of protein profiles and marker genes that produce a selectable phenotype (Table 1) [8,9,16,17]. An example of

Figure 1. Overview of activities requiring laboratory techniques to track plant genes and transgenes in the environment and food chain. Pre-commercialization research activities are conducted by industry, academia and government to support the development and risk assessment of GM crops. Post-commercialization activities focus on risk management, agricultural trade, food processing and regulatory compliance.

### Table 1. Comparison of the principal laboratory techniques used to characterize gene flow

<table>
<thead>
<tr>
<th>Laboratory technique</th>
<th>Analyte</th>
<th>Useful for conventional or GM crop</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isozyme (allozyme) analysis</td>
<td>Enzyme profile</td>
<td>Conventional</td>
<td>9,18</td>
</tr>
<tr>
<td>ELISA</td>
<td>Novel protein</td>
<td>GM</td>
<td>27</td>
</tr>
<tr>
<td>Selectable marker genes</td>
<td>Resistance phenotype</td>
<td>GM</td>
<td>50–52</td>
</tr>
<tr>
<td>Antibiotic resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbicide resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visible marker genes</td>
<td>Fluorescence or colored stain</td>
<td>GM</td>
<td>21–23</td>
</tr>
<tr>
<td>GUS, GFP, Luc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular marker assays</td>
<td>DNA sequences as genetic markers</td>
<td>Conventional</td>
<td>8,16</td>
</tr>
<tr>
<td>AFLP, RAPD, RFLP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microsatellite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphological character</td>
<td>Morphological trait or plant phenotype</td>
<td>Conventional</td>
<td>24</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Nuclear DNA content</td>
<td>Conventional</td>
<td>25,26</td>
</tr>
<tr>
<td>Bioassay</td>
<td>Resistance phenotype</td>
<td>GM</td>
<td>27,28</td>
</tr>
</tbody>
</table>

Abbreviations: AFLP, amplified restriction fragment polymorphism; ELISA, enzyme-linked immunosorbent assay; GFP, green fluorescent protein; GUS, \(\beta\)-glucoronidase; Luc, luciferase; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism.

http://plants.trends.com
how different methods can be integrated to create a detailed picture of gene flow is presented for cultivated beets (*Beta vulgaris ssp. vulgaris*) (Box 1).

DNA-based molecular techniques to identify genetic markers and describe genetic relationships have become a powerful tool for crop breeding, plant systemsatics, population genetics and descriptive studies on gene flow [8,9,16]. Many DNA sequence markers and assays have been developed for nuclear DNA or chloroplast and mitochondrial DNA that can trace maternal lines. Molecular markers are advantageous because they are abundant in the plant genome, are not affected by environment, can be based on noncoding sequences that are selectively neutral and can provide a high level of resolution between closely related plants. Disadvantages of molecular markers include the requirements for expensive laboratory equipment, costly reagents and technical expertise. The most useful molecular techniques to describe genetic relationships include amplified fragment length polymorphisms (AFLP), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and microsatellite markers [8,16]. AFLP and RAPD have an advantage in that they do not require prior information about DNA sequences or a large investment in primer/probe development.

Isozymes are related enzymes that catalyse the same reaction but have different structural, chemical or immunological characteristics. Isozyme (allozyme) analysis uses the isozyme profile to distinguish between related plant taxa, an approach that has been documented for many crop species [9,16,18]. Although laboratory equipment and cost are modest, isozyme variation will not always be sufficient to discriminate between taxa and might not be selectively neutral. Plant samples must be handled carefully to protect enzyme activity and activity is affected by tissue type, developmental stage and environmental conditions.

In experimental research on gene flow, GM crops containing selectable marker genes can simplify the identification of hybrids and the screening of large numbers of plants. The most common selectable markers are antibiotic resistance and herbicide resistance, both of which are routinely used in the initial selection of transformed plant cells and plant propagation [19,20] (http://www.defra.gov.uk/environment/accre/bestpractice/guidance/index.htm). Other types of selectable markers include genes for resistance to cytotoxic agents, for auxotrophic markers to complement mutants deficient in a growth factor and for the use of mannose or xylose sugars [20]. Visible markers or reporter genes can be inserted to study gene flow, including green fluorescent protein (GFP), β-glucuronidase and luciferase. The family of GFP genes provides the advantage of real-time, non-invasive identification of GM plants and pollen in the laboratory or field [21,22]. For example, tobacco plants expressing GFP...

### Box 1. Beets – a case study for gene flow

Beets (genus *Beta*) provide a good example of how laboratory techniques in descriptive and experimental research projects can be integrated to characterize gene flow in an important crop species. The potential for gene flow between the six cultivated beets (*Beta vulgaris* subspecies) and wild *Beta* species has been recognized for many years [1,46,47] (http://www.oecd.org/document/51/0,2340,en_2649_34387_1889391_1_1_1,00.html). Since the development of GM sugar beet (*Beta vulgaris ssp. vulgaris*) in the early 1980s, there has been special concern about transgene movement into EU populations of wild sea beets (*Beta vulgaris ssp. maritima*) and the potential for negative ecological impacts. Sugar beets have been categorized as a high risk for gene flow because they out-cross and are wind pollinated and sexually compatible with wild species, and grow in contact zones with wild beets. Adding to the concern is the existence of conspecific ‘weed beet’, a hybrid form that can bolt and flower in one year and create a weed problem in sugar beet seed-production areas.

Early studies inferred gene flow based on the introgression of the annual lifecycle of weed beet into cultivated biennial beets and the transfer of morphological traits [47]. A study with cultivated beets and wild sea beets used isozyme analysis and RFLP to demonstrate gene flow from crops to wild beets near beet farms, although these wild populations increased in genetic diversity compared with distant wild beet populations [48]. A study of the genetic structure of one sea beet population in the UK using isozyme analysis suggested that long-distance seed dispersal could contribute to gene flow [49]. A similar result was obtained in France, where genetic analysis of weed beet, wild beet and an intermediate contact-zone population was performed using two types of molecular markers – a maternally inherited chloroplast PCR-RFLP marker and biparental nuclear microsatellite markers (J-F Arnaud et al.’). The maternal chloroplast markers showed that the intermediate contact zone contained both wild and weed beets, suggesting that weed beets could move to disturbed habitats via human dispersal. Using genetically modified sugar beets expressing three traits [herbicide resistance (*bar*), virus resistance (*BNYVV*) and antibiotic resistance (*nptII*)], hybridization was measured in field conditions and hybrid F1 plants were easily identified by the herbicide-resistance trait [50]. Understanding the potential for gene flow in beets provides some scientific basis for environmental risk assessments and decision making.


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**Table 2. Comparison of the principle laboratory techniques used to track transgenes from seed to supermarket**

<table>
<thead>
<tr>
<th>Laboratory techniques</th>
<th>Analyte</th>
<th>Type of measurement</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>Novel protein</td>
<td>Qualitative, semi-quantitative, quantitative</td>
<td>31</td>
</tr>
<tr>
<td>Lateral flow test strip</td>
<td>Novel protein</td>
<td>Qualitative</td>
<td>31</td>
</tr>
<tr>
<td>PCR-based methods</td>
<td>Novel DNA sequence</td>
<td>Qualitative, semi-quantitative, quantitative</td>
<td>34,36,37,45</td>
</tr>
<tr>
<td>RTQ-PCR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QC-PCR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiplex PCR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA microarray</td>
<td>Novel DNA sequence</td>
<td>Qualitative, semi-quantitative, quantitative</td>
<td>35,45</td>
</tr>
<tr>
<td>Spectroscopy and chromatography</td>
<td>Plant biochemical trait</td>
<td>Qualitative</td>
<td>7</td>
</tr>
</tbody>
</table>

Abbreviations: ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; QC-PCR, quantitative competitive PCR; RTQ-PCR, real-time quantitative PCR.
under the control of a promoter for anther and pollen expression demonstrated that a hand-held ultraviolet (UV) light can detect transgenic pollen carried by bees [22]. GFP expression could support direct monitoring of pollen movement over different large distances and research on containment strategies [21–23]. However, government approval would be required before unconfined release of the gene encoding GFP into the environment.

In a few cases, distinctive morphological traits with mendelian inheritance have been used to study gene flow. For example, a study with wild and cultivated carrots (Daucus) in Denmark used root color [24]. Hybridization between crops and weeds with different ploidy levels can sometimes be detected by flow cytometry, as demonstrated in studies of wheat [25] and Brassica [26]. If a GM trait confers insect or disease resistance, bioassays measuring insect or disease attack can identify transgenic plants and changes in plant fitness [27,28].

**Techniques for tracking GM crops from seed to supermarket**

Post-commercialization activities conducted by industry, government agencies and independent groups (Figure 1) require fast, accurate, sensitive, inexpensive methods to track transgenes from the planting of GM seed to the production of food products (Table 2). Business operators need analytical methods to support seed certification, IDENTITY PRESERVATION, traceability and food labeling [10, 29]. Government agencies need laboratory tests for programs related to stewardship, seed quality, food safety, food labeling, environmental monitoring and regulatory enforcement [1,2].

Post-commercialization tracking of GM crops requires three types of tests: (i) a rapid detection assay to determine whether a GM crop is present in a sample of raw ingredients or food products; (ii) an identification assay to determine which GM crop is present; and (iii) quantitative methods to measure the amount of GM material in the sample [7,10,30]. The first stage can be accomplished by qualitative methods (presence or absence of transgene), whereas the third stage uses semi-quantitative (above or below a threshold level) or quantitative (weight/weight % or genome/genome ratio) methods. Currently, the two most important approaches are immunological assays using antibodies that bind to the novel proteins and PCR-based methods using primers that recognize DNA sequences unique to the GM crop.

The two most common immunological assays are enzyme-linked immunosorbent assays (ELISA) and immunochromatographic assays (lateral flow strip tests) [7,31,32]. ELISA can produce qualitative, semi-quantitative and quantitative results in 1–4 h of laboratory time [33] (http://www.ers.usda.gov/publications/aib762/ and http://anrcatalog.ucdavis.edu/pdf/8077.pdf). The lateral flow strip tests produce qualitative results in 5–10 min in any location for less than US$10. However, sufficient protein concentrations must be present for antibody detection and protein levels can be affected by the plant’s environment, tissue-specific patterns of transgene expression, protein extraction efficiency, matrix effects and food processing techniques that degrade proteins [7,31].

The most powerful and versatile methods for tracking transgenes use PCR [7,30,34,35]. PCR has many advantages but it requires DNA sequence information to design primers to identify a crop (e.g. Lec1 lectin gene for soybean), to detect a DNA sequence common to many GM crops (e.g. cauliflower mosaic virus 35S promoter), to detect a specific transgene or to identify a specific transformation event using the unique transgene–genome border region. Some sequence information can be found in biosafety databases (http://www.agbios.com/), genome databases (e.g. GenBank), patent applications and government documents. Theoretical detection limits for PCR have been calculated for various grain crops [30]. Estimates for cost are US$150 to US$1050 and for time from 4 h to several days [33] (http://www.ers.usda.gov/publications/aib762/ and http://anrcatalog.ucdavis.edu/pdf/8077.pdf).

Although basic PCR and gel electrophoresis protocols can be used, more sophisticated techniques have been developed to provide higher sensitivity and transformation-event-specific identification. Real-time quantitative PCR (RTQ-PCR) systems rely on the quantitation of fluorescent reporter molecules (e.g. Taqman, Molecular Beacons) that increase in direct proportion to the amount of PCR product in the reaction. RTQ-PCR equipment automates analysis by quantifying the PCR products at time points during the reaction [7,34]. For example, a study of MON810 Bt maize identified the unique 3’ region of the cryIA(b) gene and the flanking maize genomic sequence, allowing event-specific quantification with RTQ-PCR primers [36]. Multiplex PCR can measure multiple DNA sequences in one sample using fluorescent dyes with different emission spectra attached to different probes. A study with herbicide-resistant soybeans co-amplified the transgene sequence and the Lec1 lectin gene, providing a ratio of transgenic DNA to soybean genomic DNA in one sample tube [37]. Quantitative competitive PCR (QC-PCR) works through the co-amplification of an unknown amount of a target gene and a known amount of internal control template in one sample tube with one set of primers [34].

In addition to PCR and protein-based methods, chromatography, mass spectrometry and near-infrared spectroscopy can be used in some situations, such as GM crops that have significant changes in chemical composition [7]. However, these methods can fail when alterations in GM crop biochemistry are within the range of natural variation found in conventional crops.

**Science and policy challenge laboratory techniques**

**Changing the design of GM crops**

As we move towards the agricultural production of more sophisticated GM crops, there must be new innovations in the techniques for tracking genes and transgenes. For example, genomics projects are providing new phenotypes, genes and promoters based on native or homologous DNA sequences [38]. Modest overexpression of native or homologous genes will make standard immunosassays ineffective and complicate PCR methods. Downregulation of native gene expression through antisense or RNA interference strategies will also complicate testing of crops and food products [32]. A GM tobacco containing an antisense
construct that decreases the production of a key enzyme in the nicotine biosynthesis pathway and creates a low-nicotine tobacco has already been approved for commercialization in the USA (United States Federal Register Notice, 2002, 67 FR 71929–71930; http://www.gpoaccess.gov/fr/index.html). Tissue-specific promoters will also affect analytical methods because protein expression could be eliminated from the harvested seed or fruit.

Research and policy groups in the EU and USA comprising representatives from academia, government, industry and non-governmental organizations are promoting the careful design of future GM crops to reduce exposure pathways, to decrease identified or perceived risks and to simplify risk assessment [19,20,39,40] (http://www.defra.gov.uk/environment/acre/bestprac/guidance/index.htm). One group has recommended guiding principles that include avoiding or minimizing unnecessary transgenes and DNA sequences (e.g. selectable markers), using tissue-specific gene expression, and minimizing or eliminating transgene dispersal in the environment (http://www.defra.gov.uk/environment/acre/bestprac/guidance/index.htm). At present, many commercial PCR-based detection tests rely on common sequences such the nptII antibiotic resistance gene or cauliflower mosaic virus 35S promoter sequence.

The need to trace and identify GM crops has led to the suggestion that a universally accepted, noncoding DNA sequence be incorporated adjacent to the transgene to provide a unique identification tag [41]. The identification tag sequence could contain information in an encrypted, artificial triplet-based code and would not produce a protein or change plant fitness. The unique non-coding identification tag would be read by PCR amplification and sequencing of the PCR product. If one identification tag system were widely adopted, stakeholders in the food chain could detect GM crops without precise knowledge of each transformation event in every crop species.

**Government policies on food labeling and traceability**

Should food products carry labels specifying that ingredients were derived from GM crops? This question has been part of the international debate about agricultural biotechnology. At present, the EU, Australia, Brazil, China, Japan and Switzerland mandate labels for some or all food products containing GM crop ingredients (http://www.isaaa.org/kc/issues/labling/countries.htm). By contrast, the USA and Canada have adopted voluntary labeling policies. In other areas of the world, regulations are only just being developed. Regulations are also imposed by international treaties (e.g. the Cartagena Protocol on Biosafety) and international organizations (e.g. United Nations Codex Alimentarius Commission). Food labeling and traceability regulations are largely determined by economic, political and social issues, leaving business operators and researchers to develop analytical methods that support compliance with the regulations within the existing system for crop production, international trade and food processing.

The debate on food labeling gained new attention in 2003, when the European Parliament approved legislation creating more stringent labeling and traceability procedures for human food and animal feed derived from GM crops (http://europa.eu.int/comm/food/fs/biotech/biotech08_en.pdf and http://europa.eu.int/comm/food/fs/biotech/biotech09_en.pdf). If there is final approval from the 15 member states, EU food processors and supermarkets will be required to label all food products containing approved GM crops above a 0.9% threshold level for each ingredient. The establishment of a threshold acknowledges that conventional crops, such as bulk shipments of maize or soybeans, are never 100% pure and a low level of commingling with GM crops is expected. At present, only two transformation events (a herbicide-tolerant soybean and an insect-resistant Bt maize) are authorized for human consumption in the EU. There is no acceptable threshold level for unauthorized GM crops, although GM crops that have received a favorable scientific assessment but are not authorized can be present below a 0.5% threshold. Labels will carry the words, 'This product contains genetically modified organisms' or 'Produced from genetically modified (name of organism)'. Information about EU policies and their implications can be obtained from various sources [42] (http://www.europa.eu.int/, http://bob.nap.edu/issues/17.4/p_laget.htm, http://bob.nap.edu/issues/17.4/p_moore.htm, http://europa.eu.int/comm/dgs/health_consumer/library/press/press298_en.pdf and http://pewagbiotech.org/resources/issuebriefs/europe.pdf).

Supporters of labeling maintain that it will provide consumers with information to make choices between GM and non-GM foods, increase consumer confidence in the food system and government agencies, facilitate withdrawal of products if unexpected problems become apparent, support monitoring of effects on humans and the environment, and help to verify claims on labels. Critics of mandatory labeling policies believe that labels are unnecessary because government agencies conduct adequate risk assessments before market entry, current GM crops are substantially equivalent to conventional crops and there is no scientific evidence that GM crops present different health risks than conventional crops. Food labels could be perceived as a warning that decreases consumer acceptance (http://www.isaaa.org/kc/issues/labeling/labeling.htm). Many business operators argue that stringent thresholds and traceability requirements are unmanageable for globally traded products, too expensive to implement and create new liability for exporters and food processors. Compliance will necessitate testing and record keeping by all farmers, not just those producing GM crops [33] (http://www.ers.usda.gov/publications/aib762/). Ultimately, companies will pass on the cost of compliance to consumers, although the effect on food prices is largely unknown [42]. In the end, stringent labeling and traceability requirements could ensure that GM crops are unlikely to enter EU supermarkets, creating a significant barrier to agricultural trade. In May 2003, the USA, Argentina and Canada initiated a case with the World Trade Organization (WTO; http://www.wto.org/) asking for a dispute settlement panel to determine whether the EU's moratorium on approval of new GM crops creates an unfair trade barrier. The WTO case
does not directly address EU policies on labeling and traceability.

In addition to food labels, the new EU legislation imposes traceability on GM crops, requiring business operators to provide documentation through each step of the agricultural production and food processing system, and to retain that information for five years. Traceability depends on record keeping with some testing at key points in the food chain. An understanding of grain production, handling, transport and marketing reveals the challenges associated with traceability and identity preservation [29] (http://anrcatalog.ucdavis.edu/pdf/8077.pdf).

Record keeping will be essential for some food ingredients, because the new law requires labeling regardless of the ability to differentiate between GM and conventional ingredients. For example, highly refined products (such as vegetable oils or sugars derived from GM crops) must be labeled even if they are physically and chemically identical to products made from conventional crops and do not contain any transgenic DNA or novel protein. However, products such as beer, cheese and wine that are made with the aid of enzymes derived from GM microorganisms will not be labeled, with the rationale that enzymes are ‘processing aids’ that cannot be detected in the final food product. Critics of the EU legislation believe that this provision was enacted to protect important European products from labels. In addition to labeling and traceability rules, the EU is creating a new regulatory agency to authorize GM crops and requires applicants to supply methods for detection, sampling and identification based on the transformation event.

Although the EU legislation will call for better analytical techniques, business operators will rely on contract specifications dictating the acceptable threshold for GM crops, detailed documentation between buyer and seller, quantification of GM content at the ingredient level, and some analysis of food products to meet regulatory requirements [43] (http://www.ers.usda.gov/publications/ailb762/). Recommendations from working groups have focused on four aspects of crop and food testing: (i) creating internationally standardized and validated methods that are inexpensive, fast and accurate; (ii) providing DNA and protein reference materials to support methods development; (iii) improving sampling methods for crop shipments and food products; and (iv) creating a more manageable unit of measurement for threshold values [10,30] (http://pewagbiotech.org/research/postmarket/ and http://www.cast-science.org/).

One major challenge is to create standardized and validated methods that will quantify GM crops in whole grains, flours, processed ingredients and complex food products [30,44]. For quantification of GM content, there are problems with gene copy number and food products made with more than one GM crop carrying the same transgene [30]. For example, a GM maize cultivar could contain more than one copy of a transgene because of the initial transformation process or because of hybridization using two different transformed parental lines. Immunoassay procedures will overestimate GM content because they cannot distinguish between two different transgenic events that produce the same protein. A PCR method with primers designed for a genetic element (e.g. selectable marker, promoter or trait gene) will also overestimate the GM crop content. If a product contains GM maize and GM soybeans with the same herbicide-resistance gene, many current methods will not be able to measure the proportional GM content for each ingredient.

Another challenge for EU business operators and government enforcement agencies will be screening grain shipments, ingredients and food products for the presence of all unauthorized GM crops. In the future, the number of GM crop species, traits, transformation events, transgenic constructs, food products and animal feeds is likely to increase [5,6] (http://www.isaaa.org/). At present, the USA and Canada are considering approval of herbicide-resistant wheat cultivars that could lead to the screening of all wheat shipments to the EU. Governments do not always control the production of GM crops, as in Brazil, where up to 15% of the soybean crop is believed to be herbicide-resistant cultivars in spite of an official ban on the production of GM soybeans (http://www.realities.com/mbd/krwashington/news/columnists/kevin_g_hall/6673931.htm). As previously discussed, methods to screen for the presence of unauthorized crops require prior knowledge about all of the novel proteins or DNA sequences that might be present. Food processing can remove all DNA or protein in a sample [44]. Screening presents a formidable challenge and there is probably no laboratory method that can prove a crop or food product is absolutely ‘GM free’.

To address these challenges, new laboratory techniques are being developed to achieve the rapid, low cost, accurate detection of an increasing range of transgenes [7,35]. DNA microarrays can detect, identify and quantify many different transgenes and crops in a complex sample using just one assay [35]. Recently, a hybrid method using multiplex PCR and microarray technology was able to test a variety of food and feed products purchased in the USA for seven different maize transformation events at levels as low as 0.1% GM [45]. EU-funded projects to improve laboratory techniques include the European Network of GMO Laboratories (http://engl.jrc.it/), Entransfood (http://www.entransfood.com/) and GMO Chips (http://www.gmochips.org/). Reference materials, sequence information and detection methods can be linked to the regulatory process, although they can be treated as confidential business information by government agencies. The EU Institute for Reference Materials and Methods (http://www.irmm.jrc.be/) provides some reference materials.

It is important to realize that large-scale production of GM crops is a recent phenomenon and government regulations are still evolving to address the social, biological and agricultural factors associated with biotechnology. However, it is clear that a range of laboratory methods will continue to be essential for providing science-based information to stakeholders involved in all aspects of the development and safe use of GM crops.

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