A Brief Review of Current Bioinformatics Decision Support System (DSS) Tools for Screening for GMOs in the EU using PCR-Based Approaches

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Summary

The development and commercial use of Genetically Modified Organisms (GMOs) is subject to strict legal regulation in many countries around the world[1]. Key to the enforcement of legislation is the availability of appropriate methods and analytical approaches to detect, identify and quantify GMOs. To date, GMO screening for enforcement purposes is predominately performed using PCR-based methods for the detection of specific DNA sequences[2]. A large number of methods have been developed, with many being subject to inter-laboratory validation and made available in the public domain (eg by the EU Reference Laboratory for GM Food and Feed). However, the availability of detection methods in isolation is not sufficient to cope with the increasing number and complexity of GMOs now available. In order to provide for an efficient and comprehensive detection strategy, an informed decision needs to be made on choosing the least number of assays to provide the greatest likelihood of successfully identifying a GMO event.

To address this problem, a number of bioinformatics-based decision support system (DSS) tools and resources have been developed. These often define the relationship between the DNA sequence to be targeted and the GMO identity in matrix or tabular form. Such approaches can be used to identify a GMO based on the minimum number of targets that need to be screened for. The following article briefly describes some of the current bioinformatics-based DSS tools and related resources available for PCR-based approaches for GMO screening, in support of EU labelling legislation enforcement[3,4]. This review will be of interest to analytical laboratories who wish to augment their GMO screening approaches for EU-authorised varieties and who seek guidance in identifying the most appropriate means to achieve this goal.

Introduction

Following the commercialisation of Genetically Modified Organisms (GMOs) in 1996 there has been a progressive increase in the share of the global food chain market that they occupy[5]. More than 30 countries now commercially grow GMO crops and many more have performed field trials. As a result of public and scientific concern for this novel technology[6], the development and commercial use of GMOs is subject to strict legal regulation in many of

the countries around the world, including all European Union (EU) member states[3,7]. Infringement of such legislation can lead to severe penalties including the worldwide recall of a GMO or GMO-containing product resulting in potential economic loss for both developer and suppliers.

The EU has established a strict regulatory framework to trace all GMOs and their derived products[3,8]. As part of this framework a mandatory labelling requirement of any GMO-derived food or feed has been introduced. However, due to adventitious contamination that can occur along the supply and production chains, a labelling threshold of 0.9% (w/w) of an EU-approved GMO ingredient has been established for all products[9]. In addition, a minimum level of 0.1% (w/w) has been established for feed containing EU non-approved GMOs. Based on these regulations[3,4], UK and EU official control laboratories are required to be able to detect the low level presence of GMO materials, evaluate their EU authorisation status and, where appropriate, quantify the GMO content to check for compliance with legal provisions.

GMO detection, identification, and quantitation follows a complex multistep procedure that currently utilises quantitative real-time PCR (qPCR), a well characterised technique that is regularly used in both UK and other EU official control laboratories[10]. In the first phase of conventional GMO analysis an initial screening step is performed which focuses on the detection of genetic elements common to many GMO constructs, in order to determine whether a sample may contain GMO material. In terms of analytical costs and time this initial step is of critical importance, particularly when the presence of a large number of GMOs needs to be investigated[11]. For these scenarios a screening strategy needs to be able to detect the presence of all EU authorised and un-approved GMOs present in a sample and reduce the number of samples that will require further evaluation. If a GMO is detected then the next step in the analytical process is to identity which GMO is present and what its EU authorisation status is. In order to achieve this GMO-specific methods are used which target the unique sequences generated during the insertion of the exogenous GMO DNA into the plant genome. Finally, in the case of EU-authorised GMOs, and those with pending authorisation, a third step is required in order to quantify their presence. In the case of non-authorised GMOs no further action is needed as their presence violates the zero tolerance legislation that applies to products on sale within the EU.

Multi-target approaches combined with decision support system (DSS) tools are now widely acknowledged as the most practical means to improve on time and cost-effectiveness of PCR-based GMO analysis[12-14]. A number of bioinformatics-based DSS tools and resources are now available for PCR based analysis of GMOs which can provide guidance on selecting the most appropriate screening and identification strategies. This review will briefly describe some of the bioinformatics-based DSS tools and resources for PCR-based approaches for GMO analysis in support of EU labelling which are currently freely available[15]. This review will be of interest to analytical laboratories who wish to augment their GMO screening approaches for EU-authorised varieties and who seek guidance in identifying the most appropriate means to achieve this goal.

Current Bioinformatics Decision Support System Tools for use with PCR-Based Screening Approaches for GMOs in the EU

In response to the growing need for official control laboratories to perform routine PCR-based screening for GMOs a new generation of analytical platforms have been developed to assist with the testing process[11,14,16-19]. These provide varying levels of guidance for establishing rapid cost-effective screening strategies, interpreting results and reporting analytical findings. These platforms use a combination of different screening strategies which are then followed by the integration of the results into data matrices and/or dedicated DSS tools[19,20].

Dependent on the DSS tool used and sample type to be analysed (rice, maize, soya etc) the DSS will either provide the analyst with an initial screening list of genetic elements which are common to those GMOs historically associated with a particular sample type (eg soya or maize-containing products), or provide a universal list of genetic elements which can be used in the screening of any sample. Based on the results obtained for this initial screening a list of putative GMOs present in a sample is generated by the DSS tool enabling the analyst to make an informed decision as to which GMOs to test for using event-specific methods in order to confirm the presence of specific GMOs.

In the following section, a number of the analytical platforms that have been developed which specifically incorporate a DSS capability for PCR-based analytical approaches will be described. The main characteristics of these have been summarised in Table One.

Table One – Key Features and Scope of the Principle Open-access, Bioinformatics-based DSS Tools Available for GMO Detection Using PCR-based Approaches

Platform	Allows species Identification[1]	Species-specific screening design[2]	Considers single and stacked events[3]	Considers masked Events[4]	Considers false Positives[5]	Indicates EU validation status of GMO[6]	Evaluates method Specificity[7]	Allows simultaneous running of methods[8]	Considers cost Effectiveness[9]	Considers missing Data[10]	Methods used are EU traceable[11]	Food product related GMO likelihood[12]	Allows integration of historical data[13]
Universal Screening Approach	-	+/-	-	-	-	+	-	+	-	-	+	-	-
CoSYPS	+	+/-	+	-	+	+	-	+	-	-	+	-	-
Euginius	+	+/-	NS	NS	-	+	+	NS	-	-	+	-	-
GMO Finder	+	+	+	+	+	-	-	NS	-	+	+	-	+
GMTrack	-	+	+	-	-	-	-	NS	+	-	NS	+	-
GMOseek	+	+	+	-	-	+	+	+/-	+	-	+	+	+
JRC GMO Matrix	+	+/-	+	+	-	+	+	+/-	-	-	+	-	-

(+) Possess attribute; (-) Lacks attribute; (+/-) Permits dual capability, (**NS**) Not Specified

(**1**) Provides taxon specific information; (**2**) "+" denotes species specific screening strategy (screens only for GMOs found in a specific taxon) and "-" denotes universal screening strategy (panel of common GMO screening markers), +/- indicates manual selection of species specific required; (**3**) Where single designates the presence of a single GMO event per genome, and stacked indicates the presence of more than one GMO per genome; (**4**) Where the presence of multiple GMOs mask the presence of one another; (**5**) Screens for CaMV or *Agrobacterium* contamination which can result in false positives; (**6**) Provides information regarding the EU validation status of detected GMOs; (**7**) Evaluates the specificity of the methods used; (**8**) Permits running of all PCR assays on one 96 well plate; (**9**) Considers cost of consumables and time; (**10**) Takes missing data into consideration; (**11**) Methods deposited in EURL-GMFF GMO detection method database; (**12**) Takes into account product related GMO likelihood; (**13**) Allows historical laboratory data to be incorporated into DSS tool to improve detection capability.

Universal Screening Approach (German Laboratory Network Screening Table)

Waiblinger *et al*,[21] have published details of a matrix-based approach that used a combination of singleplex assays for five target DNA sequences to screen for 81 GM plant events. Since the original publication, a further three DNA target sequences have been included to extend the screening capacity of the analysis platform to approximately 160 EU approved and unapproved GMO events. All of the quantitative real-time PCR (qPCR) methods used in this approach have been validated and are available on the EU Database of Reference Methods for GMO Analysis[22]. Following an initial round of screening qPCR the results can be interpreted using a Microsoft Excel screening table that describes the presence or absence of the eight target sequences in the listed GMO plant events. The data in the table has been verified either theoretically with the use of databases or experimentally using reference materials. The results can be used to filter the columns of the screening table in order to sort the list of candidate GMO into those that are positive (demonstrate amplification) and negative (no amplification observed) for each of the methods. This provides a list of possible GM events that may be present in the sample for which it will need to be screened in order to confirm a positive identification of the GMOs present.

CoSYPS (Combinatory SYBR Green qPCR Screening)

The Combinatory qPCR SYBR® Green screening platform (CoSYPS) is a "GMO method matrix" developed at the Wetenschappelijk Instituut Volksgezondheid, Institut Scientifique de Santé Publique (WIV-ISP), and combines a SYBR® Green qPCR method for detecting the presence of common GM genetic elements with a DSS which operates at the GMO screening level[19]. It uses a SYBR® Green qPCR-based approach in order to reduce both the cost of the analysis and enable the generation of DNA duplex melting temperature (T_m) data[23]. Currently, the platform uses 18 validated singleplex methods to detect:

(i) a universal plant target to facilitate the identification of plant material in a sample
(ii) taxon specific targets to assist in the identification of which plant taxa are present
(iii) GMO targets
(iv) cauliflower mosaic virus (CaMV) specific target to enable detection of CaMV contamination

The results are interpreted on the basis of the DNA duplex melting temperature (T_m) which is the temperature at which a DNA duplex denatures, the PCR quantification cycle (C_q), the limit of detection (LOD) and the limit of quantitation (LOQ). Of these, the T_m values are the primary criterion used and should ideally match the T_m value of a known target obtained by testing an appropriate reference material. A benefit of using a T_m analysis is that it can enable the post-PCR verification of amplification from expected DNA targets and also of closely related targets. The analytical results obtained with the CoSYPS matrix are then interpreted and evaluated in combination with a DSS which utilises a prime number-based algorithm

developed by Van den Bulcke *et al*[24]. Briefly, a unique prime number is assigned to each target/method so that the prime number represents amplification and "1" represents no amplification. Once a specific set of methods have been applied to a sample, the following calculations are used:

(i) the prime numbers generated from applying the methods to the sample are multiplied in order to generate a product termed Gödel's Prime Product (GPP)
(ii) the resulting GPP is then divided by the GPP of a list of GMOs that have been screened using the same methods

When the result of dividing the sample GPP with the GPP of a known GMO is zero then there is a high probability that the sample contains the specified GMO. In order to confirm the presence of a certain GMO or GMOs a further round of qPCR analysis is required using methods validated and published by the EURL-GMFF[25]. The Decision Support System tool, which incorporates a dedicated analytical algorithm, has been patented by the WIV-ISP-GMOlab[26]. Further details regarding the basis and development of the CoSYPS platform have been published in Van den Bulcke *et al*., 2010[19].

EUginius

The European GMO Initiative for a unified database system (EUginius) is an integrated analytical platform developed as part of an initiative to produce a primary reference resource for use by the European Union (EU)[27] and undertaken by the Federal Office of Consumer Protection and Food Safety (BVL) (Berlin, Germany) and RIKILT Wageningen University and Research (Wageningen, Netherland).

As an analytical tool the platform is designed to guide the user in determining the specificity and coverage of a method for a range of GMO targets and is comprised of a number of components, including:

(i) GMO Database
(ii) Detection Method Database
(iii) Method Verification Database
(iv) GMO data analysis tools

For completeness, a brief description of each of these separate resources is provided in the following sections.

(i) GMO Database

The GMO Database component contains a list of known GMOs and is structured according to identifiers, traits and genetic elements.

The lists can be searched and filtered in order to obtain information on the producer, tradename, EU authorisation status, traits and genetic elements present and can be searched

independently in order to compile lists of GMOs that can be filtered on the basis of any of the attributes listed above.

(ii) Detection Methods Database

The Detection Methods Database component can be used to search for appropriate methods for screening, identification and quantification.

Three sets of methods can be searched for, depending upon the level of filtering the end-user wishes to apply. These are (i) all methods (where no restrictions are applied to the list of available methods), (ii) screening methods (a list of methods used to detect specific GMO elements or constructs only) and (iii) ABC methods (list of methods restricted to a user-defined specific sub-set only)

In addition, the methods can be searched and filtered according to criteria including validation and standardisation status and detection method type and target.

The resultant table gives both the method and the GM events which contain the selected target.

(iii) Method Verification Database

The Method Verification Database component can be used to search the same three sets of methods referred to in the Detection Methods Database in order to get an indication of method specificity. The same filters can be applied in conjunction with optional additional filters in order to target GMO event and GMO authorisation status. The output table describes whether a GMO is detected by a method, indicated by "+" for amplification and "-" for no amplification. The results can also be ranked according to how the data has been verified either experimentally, through sequence alignments, or through use of other EUginius databases.

(iv) GMO Analysis Tool

The GMO Analysis Tool component allows the user to enter detection methods that provide a positive or negative result when applied to a sample. The resultant table then lists those GM events that are most likely to be present in the sample.

GMOFinder

GMOFinder is a Microsoft Access-based database application which was originally available only on request from the developer[17] due to a number of intellectual property issues. The database combines a tabular matrix of genetic elements commonly found in GMO constructs with a series of analysis algorithms that facilitate the interpretation of results obtained during a screening analysis. The platform has been designed to use 15 qPCR methods to screen for GMOs the results from which are interpreted using the database. The database describes the presence or absence of the 15 targets in the user-selected GMOs with the data ranked (0-9) according to reliability (data derived either *in-silico* and/or experimentally), where experimentally obtained data is generally ranked higher than data derived from theoretical

interpretation alone. Additional information is also used in the database, which may provide the platform with some advantages over other matrix-based bioinformatics approaches. This information includes:

(i) false positives due to contamination with CaMV and *Agrobacterium* are considered and highlighted
(ii) possible masking of events can also be identified by selecting an appropriate function
(iii) species selection can be made based on information about the samples or the results of species specific screening assays

However, running all of the suggested specified assays may be resource intensive and could involve using multiple 96 well PCR plates. In addition, some of the methods that the platform refers to are only available from the service provider of GMOFinder. Although access to the analysis platform was initially restricted due to the confidential nature of the information it contained the software is currently freely available.

GMOTrack

GMOTrack[11] is a freely available, command line driven utility which generates cost-effective testing strategies for use in the traceability of GMOs. When provided with:

(i) a table of GMOs to be screened for
(ii) the probabilities of their presence in the product being evaluated
(iii) the genetic elements present in the genomes of the target GMOs
(iv) a linear cost function

GMOTrack will compute the optimal set of screening assays that would be required in order to implement a two-phase testing strategy. The system was originally designed to be adaptable to the current GMO market when provided with updates to the GMO tables it uses. It was also designed to provide the analyst with an automatic interpretation of the experimental results obtained to assist the operator in performing an analysis. The approach was primarily aimed at reducing the cost and the time needed for each individual analysis thus simplifying GMO testing and increasing throughput.

GMOseek

GMOseek[18] is a freely-available software platform that identifies the most cost effective testing strategy for a sample-centred two phase analysis comprising of an initial screening phase and an event specific identification phase. The software guides the user through the selection of the screening assays, the selection of the event specific assays and the interpretation of results for both. The user selects one of two GMO databases, either all known GM events or EU-approved and GMO varieties subject to EU Regulation 619/2011 (low level presence of unauthorised GMOs in feed). Detailed information regarding the sample can also be entered (e.g. taxon) and the software then considers the assays that

provide the best coverage for all the possible GMOs in the sample. The software also takes into account anticipated costs from materials and labour. If the suggested screening approach is used the results can be entered into the software platform and used to identify which event-specific assays are appropriate. Based on the results of the event-specific assays the software will both interpret and check for any data inconsistencies. All of these functionalities can be used together or separately.

JRC GMO Matrix

More recently, the European Union Reference Laboratory for GM Food and Feed (EURL-GMFF) has developed a set of bioinformatics tools in order to provide support for official enforcement laboratories in the detection of existing and new GMOs. The EURL-GMFF also hosts the EU Database of Reference Methods for GMO Analysis (the GMOMethods database), the JRC GMO-Amplicons database as well as a website where specific information can be located regarding the current status of new GMOs. More detailed information regarding these resources have been described elsewhere[28,29] however some of the key features for each of the resources is summarised below.

(i) GMO Database

The GMO Database consists of the JRC GMO Matrix database and bioinformatics tool set, which was created using DNA sequence information from the Central Core DNA Sequence Information System (CCSIS) and primer and probe sequence data available from the GMOMethods database[28]. The platform enables *in-silico* modelling of both PCR amplification and probe binding to be performed using the bioinformatics tools referred to as "reverse-PCR" and "Nucleotide Matcher". Use of the "reverse-PCR" tool enables the location of the genomic binding site to be predicted as well as the amplicon size and specificity for user supplied primer pairs. The "Nucleotide Matcher" tool enables the similarities in two input sequences to be identified in order to test the annealing characteristics of the probe. The platform also permits an *in-silico* simulation of the detection of each GMO to be performed for each of the assigned detection methods and provides a value which represents the extent of matching between the methods primers and probe and the GMO sequence, indicative of no amplification detected, amplification detected with imperfect primer and probe binding or perfect annealing of both primers and probe.

(ii) GMO Event Finder

The GMO Event Finder interface allows identification of potential GMOs present in the sample based on a set of positive and negative detection method results from experimental testing. This tool allows the user to select the detection method(s) that have previously been reported to provide a positive or negative result for previously characterised samples when used in an analysis. Based on the results returned for a sample challenged with a panel of selected methods the Event Finder tool generates a list of potential GM events which fit the pattern of results obtained.

(iii) Event/Method Matrix

The Event/Method Matrix allows the user to select both the target (by taxon or specific GMO) and detection method (event, construct or element-specific) following which the approach shows the results of the *in-silico* simulations. This enables the user to identify the coverage of the selected detection method(s) for the selected taxon and provides an assessment of specificity of the selected detection method(s) for a selected GMO. In addition it can be used to compare specificity of a number of different methods for the same event, construct or element and can be used to select an appropriate range of element or construct-specific methods to screen for a specified GMO or GMO events.

The comprehensive nature of the JRC GMO Matrix approach, its accessibility and the support available for its use, means that the JRC GMO Matrix is a very useful bioinformatics resource.

A list of additional technical specifications associated with the principle open access bioinformatics-based DSS resources for PCR-based approaches described in this review are presented in Table Two and are provided in order to assist the reader in making an informed decision as to which platform would best fit the requirements of their analytical laboratory.

Table Two – Additional Specifications Associated with the Principal Open-access Bioinformatics DSS Resources Available for Use with GMO Detection using PCR-Based Approaches

Screening Platform	Method(s)[1]	Screening Design[2]	Availability[3]	GMOs Detected[4]
Universal Screening Approach	qPCR	Samples screened using a panel of 5 methods	Open access: http://www.bvl.bund.de/SharedDocs/Downloads/09_Untersuchungen/screening_tabelle_gvoNachweis.xls?__blob=publicationFile&v=2/	A total of 160 events are listed for the version of screening table available from 26/05/2015
CoSYPS	SYBR® Green	Samples screened using a panel of 18 methods	Platform has been patented and the DSS is available on request from WIV-ISP	At the time of the 2009 inter-laboratory trial 42 of the 48 EU-authorised GMOs could be detected by this platform
EUginius	qPCR and/or SYBR® Green	Samples screened using a panel of user defined methods	Open access: http://www.euginius.eu/euginius/pages/home.jsf/	A total of 337 events listed for release 1.6.2 (22/01/2016) 253 of which are unauthorised in EU
GMOFinder	qPCR	Samples screened using a panel of 15 methods	Restricted access: http://gmo-finder.soft112.com/	Lists a total of 324 GMO events from 29 plant species (as of 2012)
GMOTrack	qPCR	Samples screened using a panel of 7 methods	Open access: http://kt.ijs.si/software/GMOtrack/	All EU authorised GMOs for platform release date 2008
GMOseek	qPCR	Samples screened using a panel of user defined methods	Open access: http://www.gmoseek.com/gmoseek/	All EU authorised GMOs for platform release date 15/02/2010
JRC GMO Matrix	qPCR and/or SYBR® Green	Samples screened using a panel of user defined methods	Open access: http://gmo-crl.jrc.ec.europa.eu/jrcgmomatrix/	All GMOs currently authorised and pending authorisation in the EU

1 **Method:** Detail included to provide guidance on instrumentation compatibility (e.g., CoSYPS uses both C_q and T_m determination)
2 **Screening Design:** Example plate layout design to guide in analytical costing estimates
3 **Availability:** Indicates the level of public access assigned to the platform and URL where applicable
4 **GMOs Detected**: Indicates the number of GMO events that can be detected with use of the platform

Additional GMO Bioinformatics Resources

In addition to the bioinformatics-based DSS tools for PCR based analysis in support of EU labelling described in the previous section a number of additional resources (including GMO-related databases and websites) are currently freely available for use with GMO analysis[30], many of which are based on protein[31] or DNA sequence[32] approaches.

A detailed description of these is beyond the scope of the current review article, which is based on Decision Support Systems for screening for GMOs in the EU using PCR based approaches. However, the key features of those resources that are more relevant to GMO analysis are summarised in the following section.

JRC GMO-Amplicons Database

The JRC GMO-Amplicon database[29] is comprised of a comprehensive collection of PCR products (amplicons) which has been assembled by screening public nucleotide sequence databases by using *in-silico* determination of PCR amplification from reference methods for GMO analysis. The database currently supports more than 240,000 amplicons which can be searched via a publically-accessible web interface. The resource has been designed to support official control laboratories in the design and evaluation of GMO methods, by providing *in-silico* predictions of primer specificities and GM targets coverage. As a resource it offers a number of tools that can aid with the analysis of a range of complex issues, including the detection and identification of unauthorised GMOs. In addition, it can help with the annotation of poorly recorded GMO sequences and in identifying new GMO-related sequences from publicly accessible databases. The JRC GMO-Amplicons is a freely available, web-based platform that is accessible through the web-based portal at the following URL: http://gmo-crl.jrc.ec.europa.eu/jrcgmoamplicons/

GMO Detection Method Database (GMDD)

The GMO Detection Method Database (GMDD)[33] was developed and maintained by the GMO Detection Laboratory at Shanghai Jiao Tong University in China. The database is an open-access web-based resource which allows the user to screen for GMO methods on the basis of event name, gene name and protein information. In response to user queries, sequence information for exogenous inserts, if available, is provided as well as associated endogenous reference genes, the availability of standard reference materials and details of respective detection methods. In addition, registered users can submit information concerning any new GMO method that has been developed and validated. However, some of the methods available through this database have not been subject to full EU validation. The database is accessible at the following URL: http://gmdd.shgmo.org/

Biosafety Clearing-House

This database contains a registry of known GMOs, which includes:

(i) detailed descriptions for each GMO
(ii) a unique identifier, if available
(iii) detailed information on the transformation method used
(iv) details of the modifying genetic elements including the vector

Each entry in the GMO registry also contains links to relevant detection methods for many of the commercialised GMOs. The Biosafety Clearing House (BCH) also contains two other registries, the Organism Registry and the Genetic Element Registry. The Organism Registry includes information on the donor organisms and the recipient or parental organisms for the registered GMOs. The Genetic Element Registry contains details of the genes and other genetic elements that were modified in the GMOs. Due to the confidential nature of the information DNA sequence information is provided for a limited number of entries. The resource is available at the following URL: http://bch.cbd.int/database/organisms/

BioTrack Product Database

This database is maintained by the Organisation for Economic Co-operation and Development (OECD). It is comprised of a list of Unique Identifiers (UIs) for GMO plants that have specifically been approved for commercial application in at least one country. The UI codes consist of an alphanumeric sequence and are specific for a single transformation event. The resource has been solely compiled for the purposes of accessing and sharing information on any particular GMO. The database can be accessed at the following URL: http://www2.oecd.org/biotech/default.aspx/

CERA LM Crop Database

The LM crop database was established by The Centre for Environmental Risk Assessment (CERA) and was established by the International Life Science Institute Research Foundation (ILSI). The resource is comprised of a registry of plants produced using both recombinant DNA technologies (eg genetically engineered or transgenic plants) and more traditional methods, such as accelerated mutagenesis or plant breeding. The database provides information on the genetic elements present in the construct, details of the vector used as well as a description of each GMOs characteristics (traits, common use etc), respective risk assessments and regulatory decisions. The database can be accessed at the following URL: http://cera-gmc.org/GMCropDatabase/

GMO-Compass

The GMO-Compass[34] database provides an overview of the current status of all EU approved GMOs as well as those pending approval. No molecular data or validated methods for their detection are provided. The resource can be accessed at the following URL: http://www.gmo-compass.org/eng/home/

GMO Checker

GMO Checker is an analytical platform that was developed by Mano *et al*[35] to screen for unapproved GMOs in Japan using a PCR-based approach. The platform uses a universal detection approach comprising a panel of thirty real-time PCR assays for screening purposes. Results include the approval status of any GMOs detected. Where the presence of an unauthorised GMO is determined additional details of any recombinant DNA elements identified are also provided to assist in the identification of potential GM events. As GMO Checker is associated with screening for unapproved GMOs in line with Japanese legislation its functionality may have more limited use within the EU. The analytical spreadsheet is an open-access resource currently available at the following URL: http://cse.naro.affrc.go.jp/jmano/index.html

Concluding Remarks and Future Perspectives

The number of GMOs that are grown for commercial and research purposes continues to increase at a rapid rate. This creates an ever-increasing analytical challenge for those laboratories required to test for GMOs, as well as being a significant drain on time and resources. There is therefore a need for the continued development of new and novel detection strategies in order to help address this issue. As long as the continuing development of GMOs relies on the introduction of genetic material into a host, specifically in the form of DNA derived from regulatory and desirable trait elements, then the currently-used analytical strategies for identification based on qPCR can be applied. Furthermore, the use of matrix-based bioinformatics strategies in combination with high-throughput screening strategies and DSS platforms to help augment these PCR based approaches has been demonstrated to be an effective tool in the screening for the increasing number of GMOs currently available[36].

A number of freely-accessible bioinformatics DSS tools are now available to guide official control and screening laboratories in the detection and identification of the ever-increasing number of GMOs present in the market place. From a practical stand point a number of these DSS driven, matrix based platforms have gained acceptance with the enforcement community. These range in their complexity from the streamlined platform described by Waiblinger *et al*[21] to the comprehensive modular system of the JRC GMO Matrix platform[28].

These platforms, which rely heavily on the use of bioinformatics DSS tools to select appropriate detection methods and interpret results have been demonstrated to be effective in screening for those GMO currently available. The modular infrastructure on which they are all based enables a degree of flexibility to be adopted with respect to the implementation of any additional markers that might be required to identify new GMOs following their introduction. In addition, it may be possible to expand the capability of many of these platforms to include aspects of data mining and web search engines in order to perform automated searches to help identify new GMOs.

However, the introduction of new molecular biology-based plant breeding techniques and genetic modification (eg products arising from CRISPR genome editing[37,38]) will make the

detection of unauthorised GMO challenging. Without access to information detailing the DNA sequence for these classes of modification and/or their flanking sequences detection of these small modifications may be unlikely without the application of more complex technologies such as whole genome sequencing[39]. The establishment of additional bioinformatics resources, which include the provision for automated web trawling including of patent applications etc, would be one strategy that could be adopted in order to remain abreast of the introduction of these emerging products and to guide in the development of new methods and strategies to enable their detection.

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