

# Development and harmonisation of GMO detection methods

## Overview of EU activities



Molecular Biology and Genomics Unit of IHCP  
(EC Joint Research Centre, Ispra)

***Development and harmonisation of GMO detection methods***

***Overview of EU activities***

**European Commission**

**Joint Research Centre (JRC)**

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## Executive Summary

This report gives a general overview of activities regarding the development, validation and harmonisation of GMO detection methods within the European Union and, in particular, the role of the Joint Research Centre (JRC) of the European Commission (EC).

The EU legislative framework on genetically modified organisms (GMOs) requires the validation of an analytical method for the detection and identification of a GMO ahead of its authorisation in the EU. Furthermore, the enforcement of the EU legislation on GMO labelling necessitates reliable GMO detection methods for control laboratories in all Member States.

At the Joint Research Centre (JRC) of the European Commission (EC), scientific and technical support in relation to sampling, detection and control of GMOs is provided by:

- The Institute for Health and Consumer Protection (IHCP), where the European Union Reference Laboratory for GM Food and Feed (EURL-GMFF) is responsible for the provision of appropriately validated GMO testing methods
- The Institute for Reference Materials and Measurements (IRMM), which is the world leader in the production of Certified Reference Materials (CRM) for GMO analysis

Since 2003 the JRC is mandated as the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF) and is the driving force in the development and harmonisation of GMO detection methods within the EU. The EURL-GMFF validates testing methods according to a catalogue of “minimum performance requirements”, which defines the dynamic range and the limits of quantification and detection as well as reproducibility, standard deviation and trueness of detection methods.

The EURL-GMFF is also charged by the EC with the coordination of emergency measures in occasional cases of imports of unauthorised GMOs into the EU. In such cases, appropriate detection procedures and control samples for the relevant GMOs are developed and provided by the JRC. This is a clear example of the JRC capacity of response to an unforeseen emergency policy situation and of its capacity of support to the successful halting of illegal imports into the EU.

The EURL-GMFF is supported by the European Network of GMO Laboratories (ENGL). This network is formed by approximately one hundred national enforcement laboratories and provides a unique forum for experts throughout Europe. The coupling of the JRC with the ENGL serves a two-fold purpose: coordination between the bodies promotes the international harmonisation of analytical approaches and facilitates the solution of technical and analytical problems that confront enforcement laboratories in addressing the presence of GMOs in food/feed and the environment.

New sampling strategies and statistical tools are also being developed by the JRC and ENGL to overcome analytical problems posed by the occasional heterogeneous distribution of GM material in commodities.

Subsequently to its validation by the EURL-GMFF, a detection method may be incorporated into the catalogue of international standards established by the European (CEN) or international (ISO) standardisation bodies. CEN applies a set of six fundamental standards for methods of analysis for the detection of genetically modified organisms and products thereof. The establishment of international standards for the validation process of GMO detection methods is enhanced by contributions from individual EU Member States to the Committee on Methods of Analysis and Sampling (CCMAS) of the Codex Alimentarius Commission, the international harmonisation network addressing food, food production and food safety.

The improvement and harmonisation of current control systems remain a primary aim of research activities of the JRC and the associated networks. Main goals include the cost-effectiveness, the enhancement of efficiency, the simplification of methods for validation and detection and the provision of tools for the detection of unknown GMOs in the supply chain. Innovations include a modular approach to GMO testing that allows the independent validation of individual analytical steps, such as PCR or DNA extraction, which then may be used in a variety of detection tests.

Time and effort may be saved through the use of a new method of multiplex screening. This is of particular interest due to the expected increase in approved and non-approved GMO traits and new GMO crop species. The 'differential PCR' and the 'matrix' approaches currently are under development and are intended for use in the detection of unknown GMO products on the market. The first approach quantitatively induces the ratios of different genetic elements in sample DNA. The second approach is used to test simultaneously for the presence of a large number of possible DNA fragments. Protein-based methods (such as multiplex "ELISA Reverse" system and a high-throughput system based on the fluorescent covalent microsphere immunoassay) have also been developed by the Molecular Biology and Genomics (MBG) Unit of the JRC for the detection and quantification of specific GMOs. Such systems may be applied in the simultaneous screening of a large number of samples.

As an economical and reliable alternative to plant-derived CRMs, plasmids are of particular importance and are currently under promotion by the IRMM, IHCP and the ENGL. The first set of plasmids that carry the DNA sequence targeted by validated methods for event-specific quantification was placed on the market by the IRMM.

Finally, capacity-building and the dissemination of expertise related to GMO analysis is a core responsibility of the MBG Unit of the JRC, which conducts training for the staff of control laboratories, both inside and outside the European Union. In collaboration with the World Health Organisation, such training has been established since 2000 for laboratory personnel from countries both inside and outside the EU (EU Accession Countries, Central and Eastern European Countries, other parts of the world).

The "1st Global Conference on GMO Analysis", an initiative of the EC Joint Research Centre and of the ENGL, presented a major step forward in the dissemination and harmonisation of GMO detection approaches on the international level. More than 500 participants from over 70 countries worldwide attended the Conference in Como (Italy) in June 2008 and discussed the most recent developments in GMO detection. For the first time, the GMO detection community had a global platform for scientific exchange and the chance to encourage collaboration between laboratories from all over the world. The "2nd Global Conference on GMO Analysis" will be held in June 2011.



**Joint Research Centre (JRC) in Ispra (Italy).** Home of the European Union Reference Laboratory for Genetically Modified Food and Feed and of the Molecular Biology and Genomics Unit.

**Note:**

**In addition to serving as a compendium of current European activities in the field of GMO control and detection, this report provides access to a multitude of relevant original documents which may be retrieved through use of embedded links.**

# Content

<b>1</b>	<b>Introduction</b> .....	<b>7</b>
<b>2</b>	<b>GMO labelling regime in the European Union</b> .....	<b>9</b>
<b>3</b>	<b>Detection methods for GMOs - development and use</b> .....	<b>11</b>
3.1	General overview.....	11
3.2	PCR or protein-based detection? .....	11
3.3	Protein-based detection methods.....	12
3.3.1	Laboratory based ELISA-methods .....	12
3.3.2	Lateral flow strip test.....	12
3.4	PCR-based methods .....	13
3.4.1	Qualitative methods (screening and detection) .....	13
3.4.2	Quantitative methods.....	13
3.5	Biochips / Micro-arrays .....	14
<b>4</b>	<b>Validation and harmonisation of GMO detection methods in the EU</b> .....	<b>16</b>
4.1	Responsible bodies and supporting networks.....	16
4.1.1	The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF) .....	17
4.1.2	The European Network of GMO Laboratories (ENGL).....	20
4.1.3	Institute for Reference Materials and Measurements (IRMM) - Provision of Certified Reference Material .....	21
4.1.4	The European Committee for Standardization (CEN).....	22
4.1.5	Contributing to an internationally harmonised validation process: European activities at Codex Alimentarius.....	22
4.2	AMPE Software: A tool for a standardised validation of GMO detection methods .....	24
<b>5</b>	<b>Dissemination and training activities</b> .....	<b>25</b>
5.1	General dissemination activities .....	25
5.2	Harmonising GMO detection internationally: Global Conference on GMO Analysis .....	28
<b>6</b>	<b>Sampling strategies</b> .....	<b>29</b>
6.1	KeLDA (Kernel Lot Distribution Assessment).....	29
6.2	Supporting software tools .....	30
6.3	International Seed Testing Association (ISTA).....	31

<b>7</b>	<b>EU experience with GMO detection techniques</b> .....	<b>32</b>
7.1	Handling ‘emergency issues’ .....	32
7.1.1	Bt10 maize .....	33
7.1.2	LL RICE 601 .....	34
7.1.3	Bt63 Rice .....	35
7.1.4	FP967 Flax .....	36
7.2	Inspections on GMO controls .....	37
7.3	Pending issues on GMO detection and its harmonisation.....	39
<b>8</b>	<b>The next generation of detection methods</b> .....	<b>42</b>
8.1	GMO testing: the modular approach .....	42
8.2	Quantification of very low number of DNA targets using new real-time PCR approaches .....	43
8.3	GMO screening: the application of DNA chips and new real time PCR approaches .....	44
8.4	The detection of unknown GMOs in the supply chain .....	45
8.5	Novel approach for direct target quantification .....	46
8.6	Development of cloned DNA control samples and CRMs .....	46
8.7	ELISA Reverse method and device (ELISA-R m&d).....	47
8.8	High-throughput immunoassay.....	48
8.9	Non-PCR based approaches.....	48
<b>Annex I - Internet Guide to European bodies and research projects related to detection of GMOs in the supply chain (selection)</b> .....		<b>49</b>
 <b>Annex II – Publications and Posters</b> .....		<b>50</b>
 <b>Annex III – Register of validated GMO detection methods</b> .....		<b>57</b>
 <b>Annex IV - FVO missions regarding national GMO controls on food, feed and seeds</b> .....		<b>60</b>
 <b>Annex V – List of Certified Reference Materials</b> .....		<b>61</b>

# 1 Introduction

This report gives a general overview of activities regarding the development, validation and harmonisation of GMO detection methods within the European Union (EU) and, in particular, the role of the Joint Research Centre (JRC) of the European Commission (EC). It describes the established structures and research activities that have already led to a well-attuned control system for implementation of the EU legislation regarding placing on the market of authorised GMOs and GMO labelling throughout the EU. These activities also may serve as a model for other countries and regions seeking to achieve harmonisation of methods on an international level.

Complementing the major role of the JRC and the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF), individual European Member States have been active on the topic of detection and have established discussion and networks of experts within the region and beyond. The driving force behind such activities was the adoption in 2003 of comprehensive EU legislation on GM food and feed labelling, aiming at ensuring the freedom of choice for consumers. Since the enactment of this new EU legislation on GM labelling, food and feed containing more than 0.9 % of GMOs must be clearly labelled as "genetically modified" (see [chapter 2](#) - "GMO labelling regime in the European Union").

As a natural prerequisite for the proper enforcement of GM labelling regulations in the EU, extensive activities are underway to develop and harmonise GMO detection methods. An overview of GMO detection methods is given in [chapter 3](#) ("Detection methods for GMOs").

Since 2003 and the adoption of regulation (EC) 1829/2003 on genetically modified food and feed, the JRC is mandated as the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF) and is the driving force in the development and harmonisation of GMO detection methods within the EU. The EURL-GMFF is supported by the European Network of GMO Laboratories (ENGL). The ENGL is comprised of around 100 participating laboratories in the EU, Norway and Switzerland and represents a high-ranking resource for the solution of the technical challenges of GMO detection (see [chapter 4](#) - "Validation and harmonisation of GMO detection methods in the EU").

A further prerequisite for the successful harmonisation of GMO detection approaches is the dissemination of proper analytical methods. The Molecular Biology and Genomics (MBG) Unit of the JRC transfers its knowledge to collaborating laboratories and, in this context, holds a series of practical training courses for the staff of food control laboratories within the European Union and even beyond its borders. In this regard, the JRC is the leading player in the dissemination and harmonisation of GMO detection methods worldwide (see [chapter 5](#) - "Dissemination and Training Activities").

JRC and national laboratories in the EU countries have gathered broad experience in the practical application of GMO detection systems for the enforcement of labelling requirements. The implementation of control measures on national level is inspected regularly by the Food and Veterinary Office (FVO) of the EC. It serves as an independent control agency to promote the common and harmonised implementation of European legislation. In most cases, FVO inspectors have concluded that Member States have installed appropriate structures and competent staff to undertake GMO controls. Nonetheless, some authorities have been advised to extend their controls to all EU-approved GMO as well as unapproved GMO that illegally may enter the European market. The FVO is also running inspection visits to countries and control authorities outside the EU. These activities indicate a major challenge to control systems within the EU: the effective exclusion from the EU market of imports of illegal unapproved and possibly unknown GMO products. In cases in which illegal GMO products are detected and prompt the need for emergency measures, the MBG Unit of the JRC supports the EU Commission. The established procedures in such cases (for instance import of commodities containing illegal traces of Bt10 maize or LL Rice601) have

demonstrated efficiency in reacting to urgent matters (see [chapter 7](#) - “EU experience on GMO detection techniques”).

Such incidents also illuminate the gaps within the currently available methodology of GMO detection. Research is in progress to make GMO detection more robust and more economic, as well as to address the constantly increasing number of GMO events developed worldwide. An important objective is therefore the improvement of methods for the detection of unapproved (and therefore illegal) GMOs on the European market. In particular, such activities include the development of new high-throughput detection systems and the exploration of more reliable sampling strategies. Activities of the ENGL and EURL-GMFF have been supported by the research and development programmes FP5 and FP6 from the European Commission, such as Co-Extra and GMO-Chip (see [3.5](#) – “Biochips / Micro-arrays”, [chapter 6](#) – “Sampling strategies”, and [chapter 8](#) – “Next generation of detection methods”).

The Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) of the JRC develops, produces and certifies reference materials for GMO measurements according to the requirements laid down in EU legislation (see [4.1.3](#)). As a consequence of the embedment of this demand in the legislation, Certified Reference Materials (CRM) for appropriate quality assurance of GMO quantification measurements and the implementation of ISO 17025 are available for all GMOs authorised in Europe. At IRMM, the GMO CRMs are produced under accreditation of ISO Guide 34. They are supplied with a range of technical notes that explain the correct application of a CRM. Additionally, the IRMM offers regular training courses on the estimation of measurement uncertainty. After joint preparation with an ENGL working group, the IRMM has published a guidance document on measurement uncertainty, addressing the issue particularly for real-time PCR applications (see [5.1](#) – “General Dissemination Activities”).

Although not directly involved in GMO detection and sampling, the work of the JRC Institute for Prospective Technological Studies (IPTS, Seville, Spain) is significant in the field of co-existence and economic impacts of GMOs. Its mission is to provide customer-driven support to the EU policy-making process by researching science-based responses to policy challenges that have both a socio-economic and a scientific or technological dimension. In this context, the IPTS has provided much-noticed GMO-related reports, which, for example, analyse the feasibility of co-existence of GM and non-GM crops in European agriculture ([report](#)), as well as the economic impact of dominant GM crops worldwide ([report](#)) and the global pipeline of new GM crops (incl. implications of asynchronous approval for international trade, [report](#)).

Finally, particular attention shall be focused on the “[1st Global Conference on GMO Analysis](#)” hosted by the JRC and ENGL on 24-27 June, 2008 in Como, Italy. This conference presented a major step in the international harmonisation of approaches to GMO detection and addressed existing challenges in the fields of sampling for GMO analysis, the appropriateness of analytical tools and the consistency and interpretation of test results. Further and updated information is available at the [official website](#) of the Global Conference.

## 2 GMO labelling regime in the European Union

An overview of the EU legislation on GMOs, including requirements on GMO labelling is available on the European Commission [DG SANCO website](#) and in a [report](#) from the MBG Unit of the JRC.

The EU recognises the right of the consumer to information and labelling as a decisive tool in making an informed choice. GMO labelling was introduced in the EU to give consumers the freedom of choice between GMOs and conventional products. Since 1997, the labelling of GMOs, either as such or in a food product, has been mandatory.

On 18 April 2004, new regulations for the labelling and traceability of genetically modified food and feed came into effect in the EU. These reinforce labelling requirements and, for the first time, also addressed feed ([Regulation \(EC\) 1829/2003](#), [Regulation \(EC\) 1830/2003](#)).

Regulation (EC) 1829/2003 on genetically modified food and feed defines which items must be labelled with regard to applications of genetic engineering:

- GMOs for food and feed use (example: genetically modified tinned sweetcorn)
- food and feed produced from, or containing, ingredients or additives produced from GMOs (example: oil from GM soy beans or sugar from GM sugar beet)
- food, ingredients and additives which contain genetically modified organisms (example: wheat beer with GM yeast)

Food and feed which is produced with the aid of genetically modified organisms, or obtained using a genetically modified processing aid, do not have to be labelled. Therefore, for example, labelling is not required neither for meat, eggs, milk, and dairy products obtained from animals fed with genetically modified feed, nor is it required for additives, flavours and vitamins produced with the help of GM micro organisms.

The labelling requirements also do not apply to food and feed containing GMOs in a proportion not higher than 0.9 per cent of the food ingredients when considered individually, provided that this presence is adventitious or technically unavoidable.

This threshold applies only to GM content that has been authorised in the EU and which therefore is considered safe. Imported GMOs that have not yet received authorisation in the EU, but nevertheless have been subjected to scientific safety evaluations of the European Food Safety Authority (EFSA), had been transitionally tolerated at a threshold of 0.5 percent until April 2007. Since then, food and feed containing GMOs not approved by the EU are not tolerated on the EU market.

Provisions of Regulation (EC) 1829/2003 on GM labelling are stricter than the previous legislation and extend mandatory labelling to all food and feed produced from GMOs, without making a distinction between those which contain DNA or protein resulting from genetic modification and those which do not.

According to Regulation (EC) 1830/2003 on GMO traceability specialised traceability infrastructure had to be developed for the new process-oriented regulatory system. Each stakeholder who produces or trades GM raw materials, food or feed is obligated to forward relevant information to subsequent stakeholders in the food supply chain.

- Documentation must be retained for five years.
- It must always be possible to trace the route of a GMO from the farm to the final product.
- Upon authorisation, every GMO is assigned an ID number that can be used to identify it at all times (see [Regulation \(EC\) 65/2004](#) on the assignment of unique identifiers).

Member States are responsible for monitoring the GMO content of products and compliance with GMO labelling requirements. Analytical tests can best be used for enforcement at early stages in the food/feed supply chain, in which food/feed products have been subject to limited processing and retain sufficient intact DNA to enable testing.

In the case that analytical tests on a product are unable to confirm that labelling regulations have been upheld, indirect means of enforcement are needed. In such cases, monitoring is conducted through the request of written documentation, such as certificates or results of GMO testing from earlier stages in production.

Submission and validation of GMO detection methods by the EURL-GMFF are an integral part of the EU regulatory approval process for GMOs since Regulation (EC) 1829/2003 on GM Food Feed provides that the application for authorisation should include, amongst others:

- methods for detection, sampling (including references to existing official or standardised sampling methods) and identification of the transformation event and, where applicable, for the detection and identification of the transformation event in the food and/or in foods produced from it.
- samples of the food and their control samples, and information as to the place where the reference material can be accessed.

### **Labelling requirements for transboundary movements of GMO (EU exports)**

The Cartagena Protocol on Biosafety to the Convention on Biological Diversity establishes the importance of organising the supervision and control of transboundary movements of GMOs. This contributes to the conservation and sustainable use of biological diversity and, by taking into account risks to human health, enables citizens to make free and informed choices in regard to GMOs.

Provisions on imports of GMOs inside the EU as foreseen by the Cartagena Protocol on Biosafety are addressed in Directive 2001/18/EC and Regulation (EC) 1829/2003.

Until 2003, EU Community legislation did not contain specific requirements for exports of GMOs to third countries, [Regulation \(EC\) 1946/2003](#) was therefore adopted and established a common legal framework for such exports. Among other measures, it is necessary to ensure the identification of GMOs being exported from the Community. According to the Regulation, exporters shall ensure that the following information is stated in a document accompanying the GMO and is transmitted to the importer receiving the GMO: (a) confirmation that it contains or consists of GMOs and (b) the unique identification code(s) assigned to these GMOs if such codes exist.

#### **Further reading:**

[The EU Legislation on GMOs - An overview](#). D. Plan and G. Van den Eede (2010)

## 3 Detection methods for GMOs - development and use

### 3.1 General overview

GMO detection methods are essential not only to detect GMOs in food and feed. They also serve to identify particular GMOs and to quantify their amount in the various ingredients of food and feed.

All GM plants possess at least one new gene that has been inserted into their genomes. In most cases, the new gene or genes lead to the production of new proteins. Therefore, two classical approaches are used today to detect GMO compounds in crops and derived products: detection of the new transgenic DNA or of the new protein or proteins it prompts.

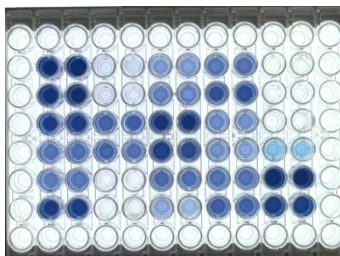
The first approach, the Polymerase Chain Reaction (PCR), is based on the detection of novel DNA sequences present in the genome of a crop. The method indicates the absence or presence of GMO-specific DNA in a given sample. The determination of a specific GMO in a sample allows if need be the segregation of its source and possibly the identification of unapproved GMOs on the market. Traceability thereby becomes possible throughout the supply chain of GM crops.

The second detection approach, called ELISA (Enzyme-Linked Immunosorbent Assay) uses antibodies that specifically bind the new protein compounds of GMOs.

A list of relevant publications on GMO detections methods is given in [Annex II](#).

### 3.2 PCR or protein-based detection?


Both methods can be used to quantify the amount of GMO compounds in a test sample. To date, DNA detection is the standard method used in the EU to determine the identity and amount of GMOs in a tested product. The reasons for its dominance include the comparatively high sensitivity of PCR-based detection methods and the inability of protein-based approaches to discriminate between varying GMOs that express the same or similar proteins. Additionally, industrial processing easily denatures proteins and impedes the use of ELISA methods for food products.




However, the ELISA-test can be a useful, economic and quick approach to the detection of GMOs, at least in raw products and on the field. A prerequisite of its use is that the GMOs in question produce new proteins in all stages of development and that these proteins also are present in harvested plants and their parts.

Qualitative detection methods can be used for the initial screening of food and feed products. Initially, the goal is to investigate whether GMO-specific compounds such as DNA elements and/or proteins are present in a particular product.

If the presence and identity of GMOs in a sample has been determined, a subsequent quantitative test must be executed in order to determine whether the GMO content in a food or feed sample complies with the EU labelling provisions.

 [Regulation \(EC\) No 1829/2003](#) on GM Food Feed provides that the application for authorisation should include, amongst others, "Methods for detection, sampling and identification of the transformation event". Today only PCR-based methods are applicable to fulfill this requirement for event-specific method. However, if novel protein-based methodologies satisfy legal requirements and are fit for use in GMO analysis, such methods will also become an integral element of official EU control measures. As cost- and time- efficient tools for the screening and traceability of GM events, they may have valuable practical applications. A selection of such activities conducted by the MBG Unit is described in [chapter 8](#) ("Next generation of detection methods").

**Further reading:**

 [The Analysis of Food Samples for the presence of Genetically Modified Organism. Course introduction \(training manual of MBG Unit, JRC\)](#)

### 3.3 Protein-based detection methods

The methods for GM plant detection that currently are commercially available have been developed mainly for insect-resistant Bt crops and for herbicide-tolerant GM plants.

#### 3.3.1 Laboratory based ELISA-methods

The most sensitive protein-based approach is the ELISA method, which is commonly a purely laboratory-based method. It can be used for detection and quantification and can be viewed as a useful tool for screening, for control purposes and for the implementation of traceability.

#### 3.3.2 Lateral flow strip test

The lateral flow strip test or dipstick kits are analyses which do not require a laboratory. The test can be carried out within 10-20 minutes and under field conditions. Typical samples for such a test are seeds or plant fragments. The test is semi-quantitative and therefore cannot be used for accurate quantification.

**Further reading and resources:**

 [Quantitative detection of Roundup Ready® Soybean by ELISA \(training manual "The Analysis of Food Samples for the Presence of Genetically Modified Organisms" of MBG Unit, JRC\)](#)


## 3.4 PCR-based methods

### 3.4.1 Qualitative methods (screening and detection)

Such methods are commonly used for GMO detection and identification. Detection typically is the first step in the analysis for GMO content. For screening purposes, PCR methods that can detect common genetic elements found in a range of GM plants are applied. Such elements include the 35S promoter and the *nos* terminator.

#### Further reading:

 [Characteristics of the qualitative PCR systems \(training manual “The Analysis of Food Samples for the Presence of Genetically Modified Organism” of MBG Unit, JRC\)](#)

 [Qualitative detection of MON810 Maize, Bt-176 Maize and Roundup Ready® Soybean by PCR \(training manual “The Analysis of Food Samples for the Presence of Genetically Modified Organisms” of MBG Unit, JRC\)](#)

Upon the detection of GMOs in a sample, their identity must be determined. For this purpose, PCR primers that are event-specific for the GMOs in question are used. Specialised PCR approaches are also available for specific applications including:

- Nested PCR: ‘nested’ sets of primers can be used to improve the sensitivity and specificity of a DNA amplification.
- Multiplex PCR: multiple pairs of primers are used simultaneously to detect a range of target sequences.

#### Further reading:

 [The Polymerase Chain Reaction \(training manual “The Analysis of Food Samples for the Presence of Genetically Modified Organisms” of MBG Unit, JRC\)](#)

Software tools recently have been developed to support the design of appropriate screening approaches. For example, GMOtrack is a utility that generates cost-effective testing strategies for traceability of genetically modified organisms (GMO). Given a table of GMOs (along with the probabilities of their presence, the genetic elements present in their genome and a linear cost function) GMOtrack computes the optimal set of screening assays for a two-phase testing strategy. GMOtrack is distributed free and can be [downloaded](#).

### 3.4.2 Quantitative methods

For the quantification of GMO content, the most widely used approach is based on real-time PCR. Real-time PCR-systems monitor the amplification of DNA by mean of a fluorescent signal that is correlated to the amount of PCR product generated by the PCR reaction. During the exponential phase of the PCR reaction, the fluorescent signal is directly correlated to the starting amount of DNA put in the PCR reaction and thus allows the quantification of the target DNA in a given sample.

#### Further reading:

-  [Quantitative PCR for the Detection of GMOs \(training manual “The Analysis of Food Samples for the Presence of Genetically Modified Organisms” of MBG Unit, JRC\)](#)
-  [Quantitative Detection of Roundup Ready® Soybean by Real time PCR \(training manual “The Analysis of Food Samples for the Presence of Genetically Modified Organisms” of MBG Unit, JRC\)](#)
-  [Real Time PCR based GMO quantification: limits and accuracy. C. Barbati, F. T. Weighardt, S. Kay, C. Paoletti, M. Querci, and G. Van den Eede \(2002\)](#)
-  [Review of GMO Detection and Quantification Techniques. L. Bonfini, H. Petra, S. Kay, and G. Van den Eede \(2002\)](#)
-  [Food Products Identity: the Need for a High Through-Put Approach in Food Analysis. A. Fantozzi, M. Marini, M. Ermolli, G. Van den Eede \(2003\)](#)
-  [Use of pJANUS™-02-001 as a calibrator plasmid for Roundup Ready soybean event GTS-40-3-2 detection: an interlaboratory trial assessment. A. Lievens, G. Bellocchi, D. De Bernardi, W. Moens, C. Savini & M. Mazzara & G. Van den Eede, M. Van den Bulcke \(2010\)](#)

### 3.5 Biochips / Micro-arrays

Micro-arrays based on DNA hybridisation are the most recent tools to be developed and validated in the EU for the detection of GMOs. Since most laboratories test their food and feed products by methods that do not allow a broad sample screening for GM crops, a major problem of the current GMO detection system has become increasingly visible. The number of GM crops worldwide constantly is rising and a corresponding increase of approved and unapproved GMOs in the food and feed chain must be expected. Consequently, there is an obvious need for screening tools that allow the simultaneous detection of different GMOs in a sample in one step. Considerable time and expense may be saved in GMO detection laboratories if an indication exists of which GMO is likely to be present in a sample.





In the course of the EU-funded Co-Extra project, a new method of multiplex screening – the DualChip GMO - has been developed.

A broad range of specific DNA molecules, corresponding to specific DNA elements of GMOs, are immobilized separately on glass slides. The immobilised DNA on the glass slide “captures” specific DNA elements of GMOs – if present in the sample – and bound DNA sequences of GMOs are made visible by a subsequent colorimetric reaction. The result is a pattern of visual spots on the glass slide.

The current version of the DualChip GMO detects six different DNA elements typical for a broad range of GM crops. GMOs in a given sample are identified by a software tool provided with the kit for the analysis of results. The current DualChip GMO micro-array can be used to discover most EU-approved GM crops and the spectrum of detectable GM crops continues to be expanded.

The suitability of the method recently was validated by a collaborative ring trial organised by the EC’s Joint Research Centre. The target DNA can be detected to a level of 0.1%.

**Further reading:**

-  [Microarray Method for the Screening of EU Approved GMOs by Identification of their Genetic Elements. Report of validation \(EURL-GMFF\)](#)
-  [Biochips: A powerful tool for multiple and fast analysis of genes and DNA sequences \(BATS\)](#)
-  [Project summary of GMOchips \(EU funded research project\)](#)
-  [EU funded research project DNA-Track](#)

## 4 Validation and harmonisation of GMO detection methods in the EU

### 4.1 Responsible bodies and supporting networks

Validation of a detection method is an integral part of the EU regulatory approval process since [Regulation \(EC\) No 1829/2003](#) on GM Food Feed provides that the application for authorisation should include, amongst others "methods for detection, sampling and identification of the transformation event". A GM food/feed cannot be authorised in the EU before a relevant detection method has been validated. The method validation process is conducted by the European Commission's Joint Research Centre (JRC) in its capacity of European Union Reference Laboratory for GM Food and Feed, assisted by the European Network of GMO Laboratories (ENGL).

Further to the GMO authorisation, the enforcement of the EU legislation on GMO labelling requires GMO detection methods that are sound, precise and robust. It is, therefore, an essential requirement to use validated methods for GMO detection and quantification. Only in this manner can it be assured that independent control laboratories achieve comparable analysis results and are able to fulfil regulatory tasks. [Regulation \(EC\) 882/2004](#) establishes that analytical methods used for food and feed control purposes must be validated by control laboratories before their use.

Consequently, a centralised validation procedure has been established to validate and harmonise GMO detections methods within European member states.

For this work, four institutions and networks mainly are responsible:

- The [European Union Reference Laboratory for Genetically Modified Food and Feed](#) (EURL-GMFF) in Ispra, Italy
- the [European Network of GMO Laboratories](#) (ENGL)
- the [Institute for Reference Materials and Measurements](#) (IRMM) in Geel, Belgium and
- the [European Committee for Standardization](#) (CEN).

#### 4.1.1 The European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF)



The Joint Research Centre (JRC) has been appointed as the [European Union Reference Laboratory for Genetically Modified Food and Feed \(EURL-GMFF\)](#) (formerly named Community Reference Laboratory; CRL-GMFF) in 2003.

Under [Regulation \(EC\) 1829/2003](#), the EURL-GMFF has the mandate to validate analytical methods for the detection of GMOs in food and feed. The operations of the EURL-GMFF are performed in line with:

- [Regulation \(EC\) 641/2004](#) (establishes the implementation of Regulation (EC) 1829/2003) and
- [Regulation \(EC\) 1981/2006](#) (outlines the implementation of Article 32 (European Union Reference Laboratory) of Regulation (EC) 1829/2003).

Within the current framework, detection methods are tested by EURL-GMFF for their 'fitness of purpose' and subsequently are validated by collaborative trials ([Overview of EURL-GMFF operations](#)).

Finally, the methods are published on the EURL-GMFF [website](#), facilitating their use by private detection laboratories and official control laboratories. Moreover, the methods are proposed for standardisation in CEN/ISO (see [4.1.4](#)).

Regulation (EC) 1829/2003 establishes that biotechnology companies must develop specific detection methods for GMOs (see box below). For validation, applicants must provide these to the EURL-GMFF as part of the complete application dossier.

##### **Improved conditions for the validation process by Regulation (EC) 1829/2003**

Due to the new GM Food and Feed Regulation (EC) 1829/2003 for the validation procedure within the EU, general and major progress has been achieved in this field. In the early years (1997-2002) of GMO authorisation, the applicant biotechnology company was not required to provide a method for the detection, identification and quantification of the GMO in question. Regulation (EC) 1829/2003 now obliges biotechnology companies to develop their own methods of detection and to provide these as part of the complete application dossier to the EURL-GMFF for validation.

In order to be accepted, the method submitted by the applicant must satisfy specific performance criteria. Failure to meet these criteria leads to rejection of the method and, consequently, to a delay in the authorisation of the GMO.

The criteria are recorded in a document provided by EURL-GMFF called ["Definition of minimum performance requirements for analytical methods of GMO testing"](#). This document was compiled by the European Network of GMO Laboratories (ENGL, see [4.1.2](#)).

It describes:

- the “Method Acceptance Criteria” for specific GMO detection approaches, which are to be fulfilled by applicants introducing new GMOs to the authorisation process in the EU (see general principle conditions in Annex I of [Regulation \(EC\) 641/2004](#)) and “Method Performance Requirements”, which must be met successively in a collaborative inter-laboratory study. A new CRL [document](#) on minimum performance requirements has been published end 2008.

### *Details on “Method Acceptance Criteria”*

Besides criteria such as applicability, practicability and specificity of the method submitted by the applicant and criteria regarding the purity of DNA extracts and the DNA fragmentation state, the following conditions also must be met (and are based on the EU’s labelling threshold of 0.9 % for the adventitious or technically unavoidable presence of GMO):

- The range of the standard test curve should allow reliable testing of GMO concentrations from 0.09 % to 4.5 % (m/m) (the required “dynamic range”)
- the accuracy of a given test should be within +/- 25 % of the reference value over the whole dynamic range
- the limit of quantification must be below 0.09 % (m/m)
- the limit of detection must be below 0.045 % (m/m)
- the results of a given test system should not deviate more than +/- 30 % and should be independent of the variety of instruments and operators as well as of the brand and concentration of reagents and the temperature of the reaction

The final assessment of sufficiency and suitability of the performance of a given GMO detection method is conducted in two independent steps:



- in-house evaluation of method performance characteristics (by EURL-GMFF)
- evaluation of method performance characteristics, executed through the analysis of inter-laboratory collaborative trial results (concerning dynamic range, precision, relative reproducibility standard deviation and trueness). ‘Trueness’ is defined as the closeness between the value obtained from collaborative inter-laboratory tests and the accepted reference value. It should be within +/- 25 % of the reference value over the whole dynamic range.


### Notes:


(1) Presently, the “minimum performance requirements” for GMO detection methods only refer to DNA-based analytical methodologies. [Commission Recommendation of 4 October 2004](#) establishes that results of quantitative analysis should be expressed as GM-DNA copy numbers in relation to target taxon specific DNA copy numbers calculated in terms of haploid genomes. Consequently, only PCR (DNA)-based methods are applicable. However, if novel methodologies fulfil legal requirements and are applicable to GMO analysis, the present document will be amended accordingly.

(2) EURL-GMFF provides additional documents for applicants to be used in the frame of the authorisation process of GMOs in the EU, including the contribution of suitable GMO detection methods:

**Guidance documents and further reading:**

-  [Guideline for the submission of DNA sequences to the EURL-GMFF](#)
-  [Explanatory notes to applicants \(Regulation EC 1981/2006\)](#)
-  [Explanatory Notes to applicants \(Regulation EC 1829/2003 and Regulation EC 641/2004\)](#)
-  [Note to the applicants on the type and nature of control samples according to Regulation \(EC\) 1829/2003](#)
-  [GMO Testing Methods: Analytical Approaches, Method Validation and Sampling Strategy \(JRC's Biotechnology and GMOs Unit\)](#)

The documents mentioned above are regularly under revision taken into account the latest technologies and experiences gained with the requirements set in the documents (see  [EURL-GMFF website](#)).

In 2004 the EURL-GMFF, appointed under Regulation (EC) 1829/2003, has also been nominated European Union Reference Laboratory under  [Regulation \(EC\) 882/2004](#) on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

Under Regulation (EC) 882/2004, the CRL aims at assisting the National Reference Laboratories (NRLs) in their area of competence, coordination of the activities of official laboratories responsible for the analysis of samples, in a context of health and consumer protection.

The CRL is granted a mandate of scientific support to the NRLs with three main objectives:

- solving scientific issues related to harmonisation and communication of scientific data among laboratories,
- monitoring the quality levels of the analytical laboratories for GMO detection,
- levelling the capacities through training, workshops and any common scientific normative tool available.

**Further reading:**

-  [Official controls performed to ensure the verification of compliance with feed and food law \(DG SANCO\)](#)
-  [Duties and tasks of the EURL-GMFF](#)

*In case of disputes*

Under  [Regulation \(EC\) 1981/2006](#), the EURL-GMFF also has the mandate to provide scientific and technical advice in the case of disputes between EU Member states concerning the results of GMO analysis. In such cases, the EURL-GMFF may reanalyse submitted samples and integrate appropriate procedures into the overall Quality System concerning GMO detection throughout Europe.

### Validated methods by EURL-GMFF

A list of validated GMO detection and DNA extraction methods (full method reports) within the frame of the Regulation EC 1829/2003 is given in [Annex III](#). The table list indicates “fit for use” methods for more than 50 GMOs. The validation reports and full method reports are available in pdf-format (see also: [status of dossiers at EURL-GMFF website](#)).

In addition to these methods validated by the EURL-GMFF under [Regulation \(EC\) 1829/2003](#), the JRC is going to publish end of 2010 a JRC Reference Report "Compendium of Reference Methods for GMO Analysis" which aims at providing a technical state of the art of the detection methods applied in GMO analysis that have been validated according to international standards. In short, 79 methods will be included in the upcoming Compendium (2010 edition) and will be DNA-based detection methods which have been validated through a collaborative trial according to ISO 5275 and/or the IUPAC protocol.

In this context, the EURL-GMFF is responsible for the provision of validated detection methods and control samples for GM products.

#### Further reading:

[Community register of genetically modified food and feed \(European Commission / DG Health and Consumer Protection\)](#)

## 4.1.2 The European Network of GMO Laboratories (ENGL)



In 2002, the European Network of GMO Laboratories for GM food and feed (ENGL) was established as a consortium of national enforcement laboratories. The network supports EURL-GMFF in evaluating new methods and is coordinated by [JRC's Molecular Biology and Genomics Unit](#) (Institute for Health and Consumer Protection).

A reason for the establishment of the network was dissatisfaction with the enforcement of labelling requirements in the EU. Prior to the ENGL, no systematic coordination existed between enforcement laboratories. In developing reliable GMO detection methods, these laboratories consequently were hampered by the lack of sufficient reference material and sequence information.

In regard to the sampling, detection, identification and quantification of GMOs, the ENGL represents a unique platform for experts from throughout Europe. The twin goals of such networking are at European level the harmonisation and standardisation of analytical approaches and the solution of the many technical and analytical problems faced by enforcement laboratories in addressing GMOs in food and the environment. Since 2004, ENGL provides assistance to the EURL-GMFF, particularly with respect to the validation of analytical methods for the event-specific quantification of GMOs.

The ENGL supports the EURL-GMFF in the following activities:

- development of methods for qualitative and quantitative analysis
- dissemination of proven detection technologies through training and capacity building
- harmonisation of control and exchange of information
- validation of screening and quantification methods for GMO detection

- development of sampling strategies for different GM commodities such as seeds, grains, raw material or processed food products
- development of supporting tools for reliable GMO detection, such as GMO sequence databases with GMO-specific molecular data and bioinformatics
- initiation of research on new detection methods
- provision of best practice information to worldwide stakeholders through international liaisons.

Today, the network is comprised of members approx. 100 laboratories (representing all 27 EU Member States as well as Norway and Switzerland). In addition, laboratories from other countries (e.g. China, Turkey) participate as observers in the network.

#### 4.1.3 Institute for Reference Materials and Measurements (IRMM) - Provision of Certified Reference Material



[The Institute for Reference Materials and Measurements \(IRMM\)](#) supports GMO testing laboratories with the production of certified reference materials and by delivering advice on the correct use of GMO CRMs. The delivery of GMO CRMs for further method validations provides additional direct support to the ENGL. Following Regulation (EC) 1829/2003, reference material (CRM) must be available by the requestor for authorisation for every GMO in the European food and feed chains. Building on its experience in the development of reference materials, IRMM offers the [service to GMO companies](#) of developing and certifying reference materials (CRM) for specific GMO events and products. Several of the world-leading Biotech companies have used the services of IRMM to fulfil the requirements of this Regulation.

Certified reference material (CRMs) are needed for reliable calibration and quality control of quantification methods. GMO certified reference materials for various GM events in maize, soybeans, cotton, potato and sugar beet have been produced by IRMM. Currently, 17 sets of CRMs are distributed worldwide by the IRMM to GMO testing laboratories (see [Annex V](#)). Research into the production procedure has resulted in major improvements that include a dry-mixing technique to avoid DNA degradation in ground GMO and non-GMO powders produced from seeds. Improvement of those techniques has led to long-term stability of matrix CRMs.

In 2004, the IRMM became accredited to ISO Guide 34, establishing the specific requirements for reference materials producers and to ISO 17025, with a flexible scope for the quantification of GMOs. All GMO CRMs are produced according to ISO Guide 34 and ISO 17025.

IRMM is official signatory of the Mutual Recognition Arrangement (CIPM MRA) for national measurement standards and for calibration and measurement certificates issued by National Metrology Institutes. It supports efforts to harmonise the reliable and comparable quantification of GMO at international level by its organisation of, and participation in, pilot studies for the Bioanalysis working group of the Consultative Committee for Amount of Substance ([CCQM-BAWG](#)).


##### Further reading and resources:

[Certified Reference Materials – Catalogue \(IRMM\)](#)


[Use of Certified Reference Material for the quantification of GMO in DNA copy number ratio \(IRMM\)](#)

[Reliable GMO analysis](#). S. Trapmann and H. Emons (2004)

#### 4.1.4 The European Committee for Standardization (CEN)

Once validated by EURL-GMFF in cooperation with ENGL, a GMO detection method may be accepted as an international standard by the  [European \(CEN\)](#) or international (ISO) standardisation body.

Standardisation of reliable detection methods is an important tool for fair trade under the umbrella of the World Trade Organization (WTO). The European and international standardisation organisations (CEN and ISO) have established common standards for GMO detection, including a general document on performance criteria and laboratory organisation requirements. These standards currently serve as models in several other detection areas, beginning with the [modular approach](#) and including the general requirements of PCR-based detection methods. In this way they save time and contribute to the global harmonisation of molecular biology based detection methods.

In order further to contribute to the harmonisation of sampling, the MBG Unit continues to provide technical advice to CEN towards the definition of new CEN sampling protocols, (CEN/TS 21568 : 2005), which is in line with the sampling protocol for grains proposed within the frame of  [EC Recommendation 787/2004](#).

CEN generally has approved a set of six general standards on methods of analysis for the detection of genetically modified organisms and derived products (CEN/TC 275 - Food analysis - Horizontal methods). The standards comprise methods of sampling, DNA extraction, and methods of protein and DNA analysis. All standards mentioned in the table below also have been accepted by the International Organization for Standardization (ISO), are now ISO Standards and have been adopted worldwide:


CEN/ISO methods of analysis for the detection of genetically modified organisms and derived products	
General requirements and definitions	EN ISO 24276:2006
Sampling strategies	CEN/TS 15568:2006
DNA extraction	EN ISO 21571:2005
DNA analysis (qualitative)	EN ISO 21569:2005
DNA analysis (quantitative)	EN ISO 21570:2005
Protein analysis	EN ISO 21572:2004

(Publications of the standard methods are commercially available)

##### Further reading:

 [CEN/TC 275 WG11 on Genetically Modified Foodstuffs \(IRMM\)](#)

#### 4.1.5 Contributing to an internationally harmonised validation process: European activities at Codex Alimentarius

Delegates of European Member States largely have contributed to the development of international standards for the validation process of GMO detection methods.  [Codex Alimentarius](#), the joint FAO/WHO Food Standards Programme, is an international harmonising network.

Discussions on GMO detection methods are being held in the Codex Alimentarius Committee on Methods of Analysis and Sampling (CCMAS), who generally meets once a year in March in Hungary. Papers from CCMAS aim to outline general considerations in regard to analytical methods for the detection and identification of foods derived from biotechnology (see [CX/MAS 02/9](#)).

This group also considers whether methods for the detection or identification of food ingredients from biotechnology fit existing criteria for analytical methodologies. The suitability of proposed methods to become Codex standards also is assessed.

The proposal currently under discussion provides comprehensive information required for the validation of quantitative and qualitative methods. Such information includes characteristics that could be used to consider existing validated methods as well as to assist laboratories in the determination of measurement uncertainty. Participants from the EU stated that international standards for GMO detection are needed to ensure traceability. Tracing requires adequate methods of analysis and, in light of several problems of methodology in the identification of foods derived from biotechnology, the EU participants further stressed the importance of such standards.

However, the inclusion of the CCMAS document in the stepwise procedure according to Codex rules currently is still under consideration. The Codex Commission approved in 2008 new work for the CCMAS on 'Guidelines on Criteria for Methods for the Detection and Identification of Foods Derived from Biotechnology' (see also: latest [meeting report](#) of CCMAS, 2010)

In the meantime important documents on foods derived from biotechnology were adopted by the Codex Alimentarius Commission in July 2008, e.g. one new annex to the 'Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants', namely 'Food Safety Assessment in Situations of Low-level Presence of Recombinant-DNA Plant Material in Food' (box below).

According to the [annex](#), the product applicant should provide a validated protocol for an event-specific or trait-specific detection method suitable for low level situations and appropriate reference materials (nonviable, or in certain circumstances, viable).

Additionally, Codex Members should make available to a publicly accessible central database to be maintained by FAO information on recombinant-DNA plants authorized in accordance with the Codex Plant Guideline. This information should include where detection method protocols and appropriate reference material (non-viable, or in certain circumstances, viable) suitable for low-level situation may be obtained for a specific GMO.

**Further reading:**

CCMAS meeting reports

[Report of the twenty-sixth session of the Codex Committee on methods of analysis and sampling - Budapest, Hungary, 2005](#)

[Report of the twenty-seventh session of the Codex Committee on methods of analysis and sampling - Budapest, Hungary, 2006](#)

[Report of the twenty-eighth session of the Codex Committee on methods of analysis and sampling - Budapest, Hungary, 2007](#)

[Report of the twenty-ninth session of the Codex Committee on methods of analysis and sampling - Budapest, Hungary, 2008](#)

 [Report of the thirtieth session of the Codex Committee on methods of analysis and sampling - Budapest, Hungary, 2009](#)


 [Report of the thirtieth-first session of the Codex Committee on methods of analysis and sampling \(Budapest, Hungary, 2010\)](#)

Low level presence of recombinant DNA plant material in food

 [Draft Annex on 'Food Safety Assessment in Situations of Low-level Presence of Recombinant-DNA Plant Material in Food' \(Codex ad hoc Intergovernmental Task Force on Foods derived from Biotechnology, 2007\)](#)

 [Adoption of draft Annex on 'Food Safety Assessment in Situations of Low-level Presence of Recombinant-DNA Plant Material in Food' \(Codex Alimentarius Commission, 2008\)](#)

## 4.2 AMPE Software: A tool for a standardised validation of GMO detection methods

 [AMPE \(“Analytical Method Performance Evaluation”\)](#) developed by the MBG Unit is a software tool designed to evaluate the performance of analytical methods under standardised conditions (method validation). The performance of a given detection method is evaluated by performance criteria that include the trueness, precision, and linearity of responses.



In the context of control purposes, method validation is requested by EU legislation for the acceptance of a specific method. It is important that method performance be evaluated across Member States in harmonised and standardised manner to provide comparable and reproducible results. Based on the principles of [ISO 5725 \(1994\)](#), AMPE supports standard validation procedures. Alternative procedures are also provided. The software enables the comparison of different detection methods and the evaluation of their adequacy with respect to user-specific analytical needs.

The software was developed using MS Visual Basic. It runs over Microsoft Windows operating systems and is available free of cost.

### Further reading:

 [Analytical Method Performance Evaluation \(AMPE\) - a software tool for analytical method validation. M. Trevisiol P, Confalonieri R, Bellocchi G, Grazioli E, Van den Eede G, Paoletti C. \(2007\)](#)

## 5 Dissemination and Training activities

### 5.1 General dissemination activities



Over years, the [Molecular Biology and Genomics \(MBG\) Unit of the JRC](#) has developed a profound knowledge on the different aspects related to GMO detection and quantification. The Unit also has designed, adapted or validated advanced methods for their detection and quantification.

A central task of the MBG Unit is the harmonisation and dissemination of proper analytical approaches for GMO detection. Knowledge on these techniques is transferred to collaborating laboratories through publications, collaborative projects, individual training or specific courses. Technical details are provided to trainees as oral presentations or brief written outlines.

Since 2000, the MBG Unit (JRC) and the World Health Organisation ([WHO Food Safety Programme in Europe](#)) have collaborated in the organisation of training courses for food control laboratory staff within



the European Union but also beyond the borders of European member states to promote issues related to food safety ([series of training courses](#)). The aim is to provide analytical biotechnology skills and to promote the use of validated and harmonised methods for the detection, identification and quantification of GMOs in food and feed.

The courses specifically address laboratory personnel who possess a good level of analytical knowledge but have little or no expertise in GMO detection.

As a response to the increasing collaboration with countries beyond the European borders, these training activities have been enlarged to address other continents like Asia for instance and various training courses have taken place under DG SANCO ([Better Training for Safer Food Programme](#))

Besides such regular training courses, the MBG Unit offers individual training according to specific needs. Training in this important area frequently has been requested, due to the increasing need to comply with current European legislative framework.

The training activities are supported by a written training manual, which describes a selection of basic techniques currently used in EU enforcement laboratories and which reflects the most updated and harmonised approaches. The subject matter covers a wide variety of techniques for the detection, identification, characterisation and quantification of GMOs, and includes important theoretical background information.

The following are the topics covered by the training courses:

- DNA extraction from raw and processed materials
- Screening of foodstuffs for the presence of GMOs by simple Polymerase Chain Reaction and by nested Polymerase Chain Reaction
- Quantification of GMOs in ingredients by Real-time Polymerase Chain Reaction

- Quantification of GMOs in ingredients by the Enzyme-Linked ImmunoSorbent Assay

**Further reading and resources:**

☞ [Training Manual](#) "The Analysis of Food Samples for the Presence of Genetically Modified Organisms" (last update: 2010)

📄 [Foreword](#)

📄 [Overview, general introduction on Genetically Modified Organisms \(GMOs\), EU legislation](#)

📄 [Manual presentation, working methods and course introduction](#)

📄 [Samples used during the course](#)

📄 [Extraction and purification of DNA](#)

📄 [Agarose Gel Electrophoresis](#)

📄 [The Polymerase Chain Reaction \(PCR\)](#)

📄 [Characteristics of Roundup Ready® Soybean, MON810 Maize and Bt-176 Maize](#)

📄 [Characteristics of the qualitative PCR systems described in the Manual](#)

📄 [Qualitative detection of MON810 Maize, Bt-176 Maize and Roundup Ready® Soybean by PCR](#)

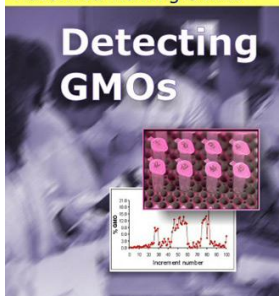
📄 [Quantitative PCR for the detection of GMOs](#)

📄 [Quantitative detection of Roundup Ready® Soybean by Real-time PCR](#)

📄 [Quantitative detection of Roundup Ready® Soybean by ELISA](#)



The JRC  
Advanced Training Series



**Interactive training course on DVD**

Additionally, the MBG Unit offers an interactive course on DVD ("Detecting GMOs", The JRC Advanced Training Series, KJ-53-03-491-EN-Z) that integrates the information provided in training courses and includes an overview of EU legislation, experimental set-up, sample preparation, agarose gel-electrophoresis, qualitative PCR, quantitative real-time PCR and a protein-based approach for GMO detection.

**Further reading:**

☞ [Information on DVD course](#)

📄 [Fact sheet](#)

## Workshops on Harmonisation of GMO Detection and Analysis (MBG Unit)

A series of [International Workshops on Harmonisation of GMO Detection and Analysis](#)\* aim at illustrating the EU legal framework on GMOs and the current situation in relation to analytical methods, as well as at sharing with representatives from the different countries the experiences derived from the implementation of the European Network of GMO Laboratories (ENGL). Moreover, these events aim at stimulating the participation of expert representatives of the national reference laboratories, for improving the overall scientific competence on official testing and, ultimately, for facilitating the adoption and application of testing methods validated in the EU. These events also involve representatives from Ministries and Regulatory Authorities in charge of the enforcement of GMO analysis.

\* in collaboration with the Directorate General for Health and Consumers (DG SANCO – [Better Training for Safer Food Programme](#)) and the Directorate General for External Relations (DG RELEX – TAIEX Programme).

## Creating and Supporting Regional Networks of Excellence

The JRC also plans to expand its supporting networks on an international level. In 2010 the project “Towards Global Harmonisation of GMO Analysis by Creating and Supporting Regional Networks of Excellence” has been launched. This [global capacity-building programme](#) aims at establishing ‘regional’ and ‘national’ reference laboratories and at providing required training support to strengthen reference laboratories’ position as centre of excellence in the regions, to give practical help to countries in improving their overall scientific competence on official testing services and, ultimately, to improve the adoption and application of testing methods validated in the EU. The improvement of third countries’ competence and capacity shall guarantee effective controls and facilitate international trade and, consequently, it assures the safety of food/feed and protects the consumers.

## Training courses on the use of reference materials and the estimation of measurement uncertainty (IRMM)

The Institute for Reference Materials and Measurements (IRMM, JRC) has long-term experience related to quality assurance of analytical methods and the estimation of measurement uncertainty. On a regular basis, the IRMM offers training courses on the use of reference materials and the estimation of measurement uncertainty. A measurement uncertainty guidance document outlining the specific aspects of measurement uncertainty estimation for real-time PCR has been elaborated in cooperation with a working group of ENGL ([Guidance on the estimation of measurement uncertainty for GMO testing laboratories](#)). As user support, several technical application notes describe issues relevant for GMO testing laboratories.

The following topics relevant for GMO analysis are covered by application notes:

- [Application Note 1](#): Comparison of a measurement result with the certified value
- [Application Note 4](#): Use of Certified Reference Materials for the quantification of GMO in food and feed
- [Application Note 5](#): Use of Certified Reference Materials for the quantification of GMO in DNA copy number ratio

## 5.2 Harmonising GMO detection internationally: Global Conference on GMO Analysis

The first "[Global Conference on GMO Analysis](#)", an initiative of the EC Joint Research Centre and of the European ENGL, was organised in June 2008 in Como, Italy. It presented a major step in the dissemination and the harmonisation of GMO detection approaches on an international level.



The growing worldwide production of GM crops and derived food and feed has led to increased challenges for producers and traders throughout the various supply chains. In order to secure identity preservation of GM and non-GM commodities according to specific market demands, further scientific and technical progress must be made to enable the successful functioning of global marketing. The conference addressed a broad range of topics related to a functional and internationally harmonised GMO control and analysis system. The conference brought together international experts to promote scientific dialogue across interdependent areas such as the following:

- Existing challenges of sampling for GMO analysis
- Analytical tools and applied procedures along the commodity production chains
- Consistency of test results, result interpretation and reporting
- Harmonisation standards for the detection of genetically modified traits

More than 500 participants from over 70 countries worldwide attended the Conference. For the first time, the GMO detection community had a global platform for scientific exchange and the chance to encourage collaboration between laboratories from all over the world.

At the end of the Conference the JRC organised a Workshop on EU Enlargement, Integration and International collaboration, which was attended by participants from the new Member States, the Candidate and Potential Candidate Countries, the European Neighbourhood Policy Partner Countries as well as further experts from other countries.

The second "[Global Conference on GMO Analysis](#)", has been announced for June 2011. So far the JRC is also planning a 1-day workshop ("crash course") on the day before the 2nd Global Conference in order to start wrapping-up the state-of-the-art knowledge regarding GMO testing. This will help participants to get a good overview about the topics which will be discussed in-depth during the Conference. Please keep an eye on the conference websites (see below) to stay informed on the planning.

### Conference details:

[1st Global Conference on GMO Analysis, Como, Italy \( 24-27 June 2008\)](#)

[Proceedings of 1<sup>st</sup> Global Conference on GMO Analysis](#)

[Programme and presentations \(Download page\)](#)

[Conference newsletter](#)

## 6 Sampling strategies

### The need for proper sampling procedures for reliable analysis

GMO detection aims to gain information on the composition of a large body of target material. Since only a small portion of sample material is subject to the analytical procedure, reliable results are guaranteed solely by appropriate sampling strategies.



A fundamental problem is presented by differing distribution of potential GMO components in the tested material. In seed lots, for example, a homogenous distribution may be assumed and available standard sampling strategies therefore are applicable. However, in cases involving bulk commodities or grain lots, a heterogeneous distribution of GM material must be expected. Appropriate new sampling guidelines must be devised.

Among all currently used sampling guidelines for GMO testing, only one ([📄 Recommendation \(EC\) 787/2004](#)) was specifically developed for GMO surveys. It is free of assumptions regarding distribution and therefore is applicable even in cases of heterogeneity.

Nonetheless, in respect to GMO legislative requirements in the EU, the extent of knowledge on distribution patterns in kernel lots is a pre-requisite to the development and recommendation of suitable sampling plans. The MBG Unit/IHCP and ENGL have launched several projects that support the understanding of distribution patterns in kernel lots. Their final goal is the availability of reliable statistical tools that accurately can predict sampling errors. In this manner, such tools support the design of appropriate and harmonised sample strategies for different commodities in all EU member states.

#### Further reading:

[📄 Statistics and Sampling \(MBG Unit / JRC\)](#)

### 6.1 KeLDA (Kernel Lot Distribution Assessment)



[📄 KeLDA](#) was an ENGL collaborative research project coordinated by the MBG Unit (JRC). KeLDA represents the first case study to assess the real distribution of GM materials in soybean grain lots. Its results indicated that GM material distribution in soybean lots is heterogeneous, which highlights the need to develop sampling protocols based on statistical models that contain no assumptions on the GMOs that potentially are present.

#### Further reading:

[📄 Kernel Lot Distribution Assessment \(KeLDA\): a study on the distribution of GMO in large soybean shipments. Claudia Paoletti et al.](#)

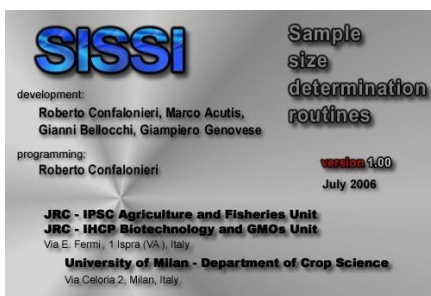
[📄 European Union Perspective – Sampling for Testing of Genetically Modified Impurities. C. Paoletti, M. Donatelli, A. Heissenberger, E. Grazioli, S. Larcher, and G. Van den Eede \(2005\)](#)


[📄 Sampling strategies for GMO detection and/or quantification. S. Kay and C. Paoletti \(2002\)](#)

 [Kernel Lot Distribution Assessment \(KeLDA\) a comparative study of protein and DNA-based detection methods for GMO testing \(Joint Research Centre, Institute for Health and Consumer Protection\)](#)

## 6.2 Supporting software tools

### OPACSA and SISSI: Calculation of optimal sample size and sampling strategy



Dedicated software tools to support sampling and sub-sampling plans aimed at GM detection through the food and feed chain were developed by the EU project  [Co-Extra](#): SISSI (Shortcut in Sample Size Identification) a novel approach to estimate the optimal sample size in experimental data collection and OPACSA (OPTimal ACceptance Sampling by Attributes) a new statistical optimisation software including a cost function to find the cheapest and most reliable mode of analysis by sub-sampling. The OPACSA software helps to find the cheapest and most reliable mode of analysis by

sub-sampling, and this is the first programme that takes economic factors into account and enables the use of inexpensive qualitative methods.

Within the project also a framework for the analysis of control plans, defined as a test procedure combined with a sample acceptance limit, has been developed in order to enable stakeholders to make objective choices about the effort that should be put into sampling and testing in order to make objective choices of sampling and testing strategies.

#### Further reading and resources:

 [The new software OPACSA: maximum control - minimal cost](#)

 [OPACSA software \(zip-file\)](#)

 [SISSI](#)

 [GMO sampling strategies in the food and feed chain \(Co-Extra\)](#)

 [Rationalization of GMO testing by appropriate sub sampling and control plans \(Co-Extra\)](#)

 [Existing sampling plans and needs for the development of novel sampling approaches for Genetically Modified Organisms \(GMO\) evaluation \(Co-Extra\)](#)

## 6.3 International Seed Testing Association (ISTA)

ISTA represents seed companies around the world and already has established seed testing schemes that also will be applicable to the GMO analysis. It is founded on a Performance Based Approach, under which laboratories are free to choose the methods they use. Minimum requirements for the performance of laboratories carrying out such tests are detailed in the [ISTA International Rules for Seed Testing](#).

### Further reading:

[Information Platform for GM Seed \(ISTA\)](#)

## 7 EU experience with GMO detection techniques

Due to the EU legislative requirements including in particular a 0.9% threshold for GM labelling, a tight control system for GMO detection and traceability has been well-established in the EU for several years. It benefits from the extensive research activities and validation work of the EURL-GMFF and the MBG Unit of JRC, from activities of the ENGL and from EU-funded research projects. Using validated methods and adapted sampling strategies for different kinds of commodities the established control system is able to enforce current legal tasks.

The control system is challenged by the occasional import of unauthorised GMOs. Therefore, the Commission has mandated the EURL-GMFF to coordinate emergency measures to exclude illegal imports from the EU market. This is to be executed through the rapid validation of appropriate detection procedures and the provision of control samples for unauthorised GMOs (see [7.1](#)).

In order to secure and to improve the practice of GMO control, EU inspections of responsible national authorities and enforcement laboratories regularly are conducted (see [7.2](#)).


Nevertheless, the incidence of emergency cases, ongoing discussions within expert networks and the results of national inspections reveal remaining bottlenecks and gaps in the practice of GMO control (see [7.3](#)). For this reason, research activities continue to be aimed at the improvement and harmonisation of current control systems (see [chapter 8](#)).

### 7.1 Handling ‘emergency issues’

#### Exclusion from the regional market of imports of illegal and possibly unknown GMO products.

In regard to genetically modified organisms, there have been several cases in which emergency measures\* were undertaken by European authorities to prevent the potential import of unauthorised GMO to the European market. They followed the appearance of unapproved GM strains known as maize Bt10, rice LL601, rice Bt63 and linseed FP 967. Such incidents, and the reactions of European authorities, may serve as case studies for the effectiveness of the emergency system.

##### \* Legal Basis for emergency measures

 [Regulation \(EC\) No 178/2002](#) establishes general principles and requirements of food law, as well as procedures in matters of food safety. As such, it provides the basis for the activities of reference laboratories and all other institutions that take part in the enforcement of food law.

**According to recital 10, “it is necessary to adopt measures aimed at guaranteeing that unsafe food is not placed on the market and ensuring that systems exist to identify and respond to food safety problems in order to ensure the proper functioning of the internal market and to protect human health. Similar issues relating to feed safety should be addressed.”**

To that purpose, **article 7** establishes the **Precautionary Principle**, according to which “in specific circumstances, where .... the possibility of harmful effects on health is identified but scientific uncertainty persists, provisional risk management measures ... may be adopted”.

The entire Chapter IV of the regulation (Articles 50 to 57) provides the basis for setting up an improved and broadened **Rapid Alert System**, including measures for Crisis Management and Emergencies. For the notification of direct or indirect

health risks deriving from food or feed, the Rapid Alert System for Food and Feed Safety (RASSF) was established as a network involving the Member States, the Commission and the EFSA. It is managed by the Commission.

**Article 53** establishes that “where it is evident that food or feed originating from the Community or imported from a third country is likely to constitute a serious risk to human health, animal health or the environment,” the Commission shall immediately adopt certain **emergency measures** such as the suspension of food or feed imports or laying down special conditions for import of the food or feed in question.

**The Commission may adopt such emergency measures provisionally after consulting the Member States concerned and informing the other Member States. At most within 10 working days, the measures taken shall be confirmed, amended, revoked or extended and the reasons for the decision of the Commission decision shall be made public without delay.**

☞ [EURL-GMFF](#) supports EU policy with assistance in cases of emergency measures, e.g. rapid validation of detection procedures and provision of control samples for unauthorised GMOs on the EU market.

### 7.1.1 Bt10 maize



On 22 March 2005, the European Commission was informed by the US mission to the European Union that, in the United States from 2001 to 2004, the Syngenta company inadvertently had marketed the genetically modified maize known as Bt10. In Europe and the USA, approval for Bt10 maize existed neither for planting nor for food and feed. However, maize seeds containing Bt10 may have been planted on approximately 37,000 hectares and approximately 1,000 tons of feed products containing Bt10 traces may have entered the European feed chain. On April 18, the Commission adopted the [Decision 2005/317/EC](#) to implement emergency measures, requiring all import shipments from the USA with corn gluten feed or brewers' grain to be certified as free of Bt10 maize.

#### Detection method

On April 22, the JRC published the validation report for an event specific detection method for Bt10 maize. Originally proposed by Syngenta for the testing of imports, the method then was validated by Genescan and, subsequently to in-house laboratory testing, was certified by [EURL-GMFF \(JRC\)](#) to become the official EU method for the detection of Bt10. The validation study assessed crucial performance characteristics of the method, including molecular specificity, limit of detection and repeatability of measurements. In October 2006, the [Standing Committee on the Food Chain and Animal Health](#) was informed that in previous months Syngenta and the European Union Reference Laboratory had performed further analysis on the molecular structure of Bt10. The status of the knowledge at that time led to the conclusion that the validated, event-specific method was appropriate for the implementation of the emergency measures regarding Bt10.

#### Review of implementation of measures

According to a [note](#) provided to the members of the Standing Committee on the Food Chain and Animal Health on 27<sup>th</sup> October 2005, approximately 1400 analytical tests were conducted between April and the end of September 2005 on corn gluten feed in the USA. Approximately the same number of tests was reported from EU Member States. The presence of Bt10 maize was indicated by none of these tests and this information was considered to prove the effectiveness of the emergency measures.

☞ [On 16<sup>th</sup> January 2007](#), the Commission and Member States voted in favour of lifting the emergency inspection measures for corn gluten feed and brewers' grain. The respective Commission Decision became effective on March 7, 2007.

#### Further reading and resources:

EURL-GMFF webpages and documents concerning Bt10 maize

🔗 [EURL-GMFF: Bt10 updates \(validated detection methods\)](#)

📄 [Scientific Report on a PCR assay for detection of maize transgenic event BT10 - Version 1 \(22/04/2005\)](#)

📄 [Scientific Report on a PCR assay for detection of maize transgenic event BT10 - Version 2 \(13/07/2005\)](#)

📄 [Scientific Report on a Detection Method for Event Bt10 using a qualitative PCR assay - Protocol for verification of positive results by restriction analysis \(23/06/2005\)](#)

📄 [Scientific Report on the in-house Validation of a detection method for event BT10 maize using a qualitative PCR assay - Version 1 \(22/04/2005\)](#)

📄 [Scientific Report on the in-house Validation of a detection method for event BT10 maize using a qualitative PCR assay - Version 2 \(13/07/2005\)](#)

📄 [Summary report on scientific data obtained at the JRC-GMO-CRL and an analysis of the data on Bt-10, obtained by Syngenta \(1/12/2006\)](#)

🔗 [Summary records of the Standing Committee on the Food Chain and Animal Health \(SCFAH\)](#)

The closed cases of Bt10 and LL RICE 601 provide clear examples of the contribution of the outstanding scientific capacity of the JRC in response to an unforeseen emergency policy situation. More recent examples of such emergency issues are the detection of unapproved 📄 [maize 32](#) from the USA in 2008, Bt63 rice originating from China (between 2006-2008), and FP967 flaxseed from Canada (2009) in the European food chain (see below).

### 7.1.2 LL RICE 601



On 18 August 2006, the US Department of Agriculture informed the European Commission that traces of the unauthorised, genetically modified rice strain LL RICE 601 developed by the predecessor company of Bayer CropScience (BCS) had been found in commercial rice samples in the US. They had entered marketing and export channels for long grain rice. The Commission immediately adopted an emergency decision on August 23 to ensure that no unauthorised rice entered the European market (📄 [Commission Decision 2006/578/EC](#)). To that purpose, shipments with long grain rice would be permitted for import into the EU only if they had been certified by an accredited laboratory to be free of LL601. In addition, to verify the absence of LL RICE 601 in rice products already on the market, appropriate control measures such as random sampling and analysis were to be undertaken at national levels. These provisional emergency measures were confirmed by the 📄 [Standing Committee for Food Chain and Animal Health \(SCFAH\)](#) two days later and resulted in the 📄 [Commission Decision 2006/601/EC](#), which replaced the first Decision. A list is available of thirteen types of rice products within the scope of the measures.

## Detection methods

At the time of enactment of the provisional emergency measures, Bayer CropScience made available two methods for detection of genetically modified rice LL RICE 601. These methods previously had been validated by the US Grain Inspection Administration (GIPSA) in collaboration with the European Union Reference Laboratory. On August 31, only 8 days after being provided with the two PCR-based detection methods, the JRC announced that it had validated the methods. The validation reports were published on the [JRC website](#) on the first day of September. Control samples were distributed to the members of the European Network of GMO Laboratories.

Sampling was directed to be undertaken in accordance with the relevant [Recommendation 2004/787/EC](#). This document establishes, among other specifications, sampling sizes in relation to the size of lots to be tested. The size of laboratory samples was set at 2.5 kg.

## Review of implementation of measures

On 16 January 2007, the Standing Committee on the Food Chain and Animal Health was informed that, since the disclosure of unauthorised LL RICE 601 in US imports, Member States had taken more than 1500 official samples upon import as well as from products that already were on the market. Initially, a significant number of positive results was reported. Affected lots were withdrawn from the market. However, since the enactment of Decision 2006/754/EC, which imposes mandatory counter-testing of every imported lot of long-grain rice, imports and the resulting incidence of positive test results virtually have ceased.

### Further reading and resources:

EURL-GMFF webpages and documents concerning LL601 Rice:

[EURL-GMFF: Detection methods for LLRICE601](#)

[Report on the verification of an event-specific detection method for identification of rice GM-event LLRICE601 using a real-time PCR assay \(CRL\)](#)

[Addendum to the Report on the Verification of an event-specific Detection Method for Identification of Rice GM-event LLRICE601 Using a Real-time PCR Assay \(CRL\)](#)

Rapid Alert System for Food and Feed (RASFF):

[RASFF Annual Report 2006; Health and Consumer Protection Directorate-General of the European Commission](#)

[RASFF Annual Report 2007; Health and Consumer Protection Directorate-General of the European Commission](#)

[RASFF Annual Report 2008; Health and Consumer Protection Directorate-General of the European Commission](#)


[RASFF Annual Report 2009; Health and Consumer Protection Directorate-General of the European Commission](#)

[Summary records of the Standing Committee on the Food Chain and Animal Health \(SCFCAH\)](#)


### 7.1.3 Bt63 Rice

In September 2006 rice products originating from China and contaminated with the unauthorised genetically modified rice “Bt 63” were identified in the United Kingdom, France and Germany and notified to the Rapid Alert System for Food and Feed (RASFF). A wide range of rice products are addressed, including rice noodles. Bt63 is not authorised for sale on the Chinese and EU market.

The Commission has been working closely with the Chinese authorities to ensure that products exported from China complied with European Community requirements. The measures taken by the Chinese authorities appeared to be effective initially but further contaminated products were identified in Germany and some other Member States in 2007. China was also unable to provide control samples and a protocol of a detection method that qualitatively and quantitatively were appropriate for use by the Joint Research Centre (JRC) of the Commission for validation of control methods used by Chinese authorities.

Consequently, the Commission has brought forward measures to control their import so that effective and consistent action is taken across Member States. The  [emergency measures](#), adopted on 3 April 2008, demand obligatory tests by an official or accredited laboratory using a specific testing method. Member States are responsible for the execution of necessary controls and the measure is valid for six months and will be monitored by the Commission.

## Detection method

In October 2006 the Joint Research Centre of the European Commission, namely the European Union Reference Laboratory for GM Food and Feed (EURL-GMFF), received samples possibly containing Bt rice as tested by GeneScan with a “Bt 63” construct-specific method. The EURL-GMFF, as a result of the analyses conducted, suggested to the European Network of GMO Laboratories (ENGL) and to the EU enforcement laboratories the use of the detection method developed by  [Mäde et al.](#)

In order to support official control activities within the EU, the EURL-GMFF provided, in October 2006 and in December 2006, control samples to enforcement laboratories; the EURL-GMFF continued to assist the laboratories in their needs for control samples and detection issues.

On 28 February 2008 the EURL-GMFF received a pooled sample of “Bt 63” DNA extracted from single plants previously tested for “Bt 63” presence. This DNA sample was used to assess the specificity and the sensitivity of the construct-specific detection method using Real-Time PCR developed by Mäde et al. On 9 April 2008, the JRC published the validation report for this method for Bt63 rice. The verification report confirms that the construct-specific method developed by Mäde et al specifically detects “Bt 63” rice. The limit of detection (LOD) of the method as experimentally established is at least 5 copies of haploid rice “Bt 63” genome.

### Further reading and resources:

EURL-GMFF webpages and documents concerning Bt63 rice

 [EURL-GMFF: Bt63 rice updates \(validated detection method\)](#)

 [Report on the verification of the performance of a method for the detection of "Bt63" rice using Real-time PCR](#)

 [Emergency measures regarding 'Bt 63' rice \(Commission Decision, 3 April 2008\)](#)

 [Detection of genetically modified rice: a construct-specific real-time PCR method based on DNA sequences from transgenic Bt rice. D. Mäde et al. \(2008\)](#)

 [Summary records of the Standing Committee on the Food Chain and Animal Health \(SCFCAH\)](#)

## 7.1.4 FP967 Flax

Beginning in July 2009, several European Union Member States detected the presence of unapproved genetically modified flax called CDC Triffid event FP967 in shipments of Canadian flaxseed. Due to its

zero tolerance for unapproved genetically modified material, the European Union halted Canadian flaxseed shipments. A first notification was sent through the [Rapid Alert System for Food and Feed \(RASFF\)](#) in September 2009. Event FP967 is approved for food, feed and cultivation only in the US and Canada

The Canadian Grain Commission and the Flax Council of Canada, along with other Canadian government departments and agencies, developed a protocol for sampling and testing Canadian flaxseed shipments to the European Union. The objective of the protocol is to help the Canadian flaxseed industry meet the European Union's import requirements. The protocol was submitted to European officials the week of October 19, 2009. The European Commission recommended to individual European Union Member States that the protocol be accepted. At present, the imposition of emergency measures by individual Member States in the European Union has been avoided.

### Detection method

On 21st August 2009, the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF) received from the German authorities a real-time PCR method for construct-specific detection of flax event FP967, developed by Genetic ID. On 11th September 2009, the EURL-GMFF received from the German authorities the FP967 positive control as DNA extracted from seeds. The EURL-GMFF carried out experiments on the control sample received in order to verify the specificity and sensitivity of the construct-specific method received for the qualitative detection of event FP967.

#### Further reading and resources:

EURL-GMFF webpages and documents concerning FP967 Flax

[EURL-GMFF: Bt63 rice updates \(validated detection method\)](#)

[Report on the verification of the performance of a method for the detection of FP967 Flax using Real-time PCR](#)

[Summary records of the Standing Committee on the Food Chain and Animal Health \(SCFCAH\)](#)

## 7.2 Inspections on GMO controls

The European Commission is responsible for ensuring that Community legislation on GMOs and derived food and feed is implemented and enforced properly (see [Official Controls, DG Health and Consumers, European Commission](#)). As a Commission service, the [Food and Veterinary Office \(FVO\)](#) plays an important role in fulfilling this task. The Office works to assure effective control systems on national levels and to evaluate the compliance with EU standards for food and feed that contain, consist of or are produced from genetically modified organisms.

In regard to GMO, the FVO evaluates, among others, the following:

- the supervision performed by the competent authority (CA) to ensure that market placement of genetically modified (GM) food and feed complies with [Regulation \(EC\) 1829/2003](#) of the European Parliament and the Council.
- the application of [Regulation \(EC\) 1830/2003](#) of the European Parliament and the Council, which concerns the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms;

For this purpose, mission teams of the FVO published about 50 mission reports on inspection visits addressing GMO controls between en 2001 and 2009(for more information see [Appendix IV](#)). Addressing the control of food, feed and seed material, the objective of the missions was to obtain insight into national practices of surveillance, sampling and GMO detection, as well as to identify problems or the need for improvement in current practices.

On the missions to EU Member States and other countries, the FVO team met with the Competent Authorities of the respective country as well as with local authorities and their staff responsible for the implementation for GMO surveillance. The mission team received documents on past surveillance activities and their results, accompanied inspectors during inspections of local import or production facilities and visited GMO analysis laboratories.

Among others, the following aspects were addressed during the missions:

- national legislation on GMO and the adequate implementation of EU legislation
- the structure and organisation of responsible authorities
- the training of inspectors
- the nature and effectiveness of communication among central/federal, regional and local authorities
- inspection plans and adherence thereto
- the number of inspections
- sampling procedures – sampling frequency, sample sizes – and adherence to sampling provisions established in [Commission Recommendation 2004/787/EC](#)
- the nature of controls, e.g., control of adherence to labelling and traceability obligations (by random or systematic sampling and testing and/or document checks), and checking for unapproved GMO by sampling and testing
- the nature of companies and facilities involved, e.g., port facilities, food and feed production factories, oil mills, plant breeders and seed production facilities
- the qualification of staff members, including inspectors and laboratory staff
- the accreditation of laboratories (under [ISO 17025](#))
- the use of validated detection methods, as well as activities regarding the development and evaluation of new methods, e.g., for the screening of unapproved GMOs
- the position of laboratories according to existing standards (ISO, [CEN](#)) and the membership of laboratories in the [European Network of GMO laboratories \(ENGL\)](#)
- technical equipment of laboratories
- the results of official controls, e.g., the number of GMO positive testing results and detected infringements of EU rules
- the reporting of results

Subsequently to the FVO missions, summary reports are written that, if necessary, include recommendations to the respective national authorities on the improvement of their control system and its alignment with EU requirements. Country authorities are invited to return commentary, and the mission reports as well as the comments thereto are published on the [website of the EU Commission](#) (see [Appendix IV](#)).

## General outcomes

In most cases, FVO inspectors concluded that Member States have installed appropriate structures and competent staff to undertake GMO controls. However, differences exist. For example, inspectors in some Member States do not follow standardized sampling procedures. In some countries, no central sampling and control plans exist, nor has a National Reference Laboratory been designated. Some authorities were advised to extend their controls to address all EU-approved GMO as well as unapproved GMO that illegally may enter the European market. In the opinion of the inspectors, the point of entry for food and feed imports from third countries deserve more attention in several Member States. In some cases, the post-processing of GMO detection below the labelling threshold of 0.9 per cent was found to be insufficient, since it is not considered if such traces are adventitious or technically unavoidable. Furthermore, the prosecution of infringements was not found to be sufficient in all countries, as deficiencies were noted in the quantification of GMO in food and feed samples. In singular cases, the amount of inspections and analyses of samples was insufficient and was seen to be due to limited financial and/or human resources.

## 7.3 Pending issues on GMO detection and its harmonisation

On the international level, problems in GMO control are mainly caused by the lack of harmonisation of GMO detection and by the lack of synchronicity between different countries and regions in regard to GMO approval processes. A selection of current problems of GMO detection and control is outlined below:

### 1. Detection of unapproved/unknown GMOs:

According to [Regulation \(EC\) 1829/2003](#), authorised GMOs must possess a corresponding and validated detection method. The detection of unauthorised or unknown GMOs is made difficult by the lack of molecular knowledge of their genetic contents. However, this is precisely this lack of knowledge and the illegality of the presence on the EU market of unapproved or unknown GMOs which make availability of detection tools for such unapproved GMOs needed.

### 2. Unit of measurement:

Three measurement scales/units of measurements can be used to express the quantity of GMOs in seed lots: %DNA, %mass and %seeds. Different interpretations of analytical results are to be expected due to different testing regimes in different countries. For example, the use of different units of measurements (e.g., mass percentage and DNA copy number) can cause inequality of test results.

Presently the regulatory situation in the EU can be summarised as follows:

- In EU legislation on GMOs, the unit of measurement to be used for GMO analysis is not specified.
- In EU legislation on food labelling, the unit of measurement to be used is mass.
- In EU legislation on seeds, the unit of measurement to be used is seeds.

The recommendation by the EC ([Recommendation EC/2004/787](#)) to support the use of DNA ratios to express GMO quantity was an important step towards coherence with the legal requirements. ENGL has

confirmed this approach in an [explanatory document](#) stating that DNA haploid genome copy number ratios are the only universally applicable unit to measure and express contamination levels. In the method validation process followed by the CRL, the Haploid Genome Equivalent (HGE) approach is followed i.e. DNA copy-numbers or %DNA is used as "unit of measurement".

However, this unit of measurement has not been fully implemented to date within the European Union and discussions are still on-going in the EU on the topic of unit of measurement and conversion factors between different units of measurement available (% of DNA, % of mass, % of seeds)

### **3. Reference materials**

Reference material is not available for all GMO events on the global market. The matrix Certified Reference Materials (CRMs) have demonstrated high stability and are available in appropriate concentration levels for the monitoring of the European legislation. However, the "classical" plant-derived CRMs may display such disadvantages as expense and limited availability for specific ranges of concentration. They also may contain traces of other GMOs and the applicability of these CRMs for highly processed food and feed samples must be evaluated case by case.

### **4. Botanical impurities**

Regulation (EC) 1829/2003 determines a threshold of 0.9% for adventitious presence of material derived from GMOs but there is an inconsistent legal status of products containing 'trace botanical impurities' derived from GMO. Discussions are on-going in the EU about the topic.

### **5. Detection of stacks**

An increasingly immanent situation is the occurrence of more than one transformation event in the same plant ('stacked' events). Unless a specific marker is introduced into the hybrid plant, the determination of whether a sample solely contains the hybrid itself or a mixture of two different GM plants is almost impossible when conducted on material other than seeds or grains. The currently available detection methods cannot distinguish "stacked" genetic material from a mixture of "individual" genetic material. At the moment the only available, but not practicable, approach in such cases is the analysis of single grains.

### **6. More user-friendly detection methods**

Generally, a considerable need exists for rapid and economic detection methods, which would not only benefit the EU control system but also particularly would enable developing countries to establish effective GMO control measures, like for instance GMO screening detection methods, allowing at one go the detection of a range of GMO events. The development of new, improved and innovative detection methods is a goal of the MBG Unit, the ENGL and several EC-funded research and development programmes on GMO traceability and detection. These programmes aim to deliver concepts and methods that are more efficient and more economical.


The next chapter provides a selection of related research topics that address the challenges of GMO detection mentioned above.


**Further reading:**

 [Evaluation of the EU legislative framework on GM food and feed \(DG Health and Consumers\)](#)

 [Evaluation of the EU legislative framework in the field of GM food and feed \(DG Health and Consumers\)](#)

 [Challenges to Achieve a Coherent GMO Legislation \(DG Environment\)](#)

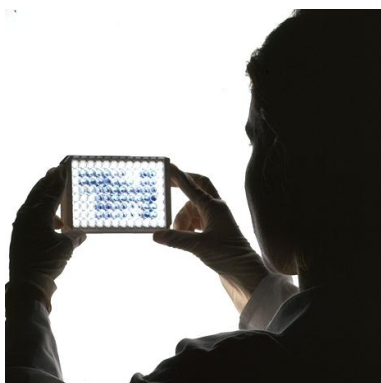
 [Explanatory Document on the use of “percentage of GM-DNA copy numbers in relation to target taxon specific DNA copy numbers calculated in terms of haploid genomes” as a general unit to express the percentage of GMOs \(ENGL, 2007\)](#)

 [Coherence Between Legal Requirements and Approaches for Detection of Genetically Modified Organisms \(GMOs\) and their Derived Products. A. Holst-Jensen, M. De Loose and G. Van den Eede \(2006\)](#)

 [Control of GMO Content in Seed and Feed - possibilities and limitations. Nordic Council of Ministers, Copenhagen 2004](#)

## 8 The next generation of detection methods

The MBG Unit (JRC) and several EU-funded projects address a range of topics that are aimed at improving the current system of GMO control and at filling the encountered gaps by contributing new approaches and techniques for GMO detection.



As part of its research and development programmes FP5 and FP6, European Commission has financed several research and development projects for the detection of genetically modified organisms (GMOs). These include:

- ☑ [QPCRGMFOOD](#): (2000-2003). Reliable, standardised, specific, quantitative detection of genetically modified foods. Coordinator: Arne Holst-Jensen
- ☑ [DNA-TRACK](#): (2001-2003). Traceability of DNA fragments throughout the food chain by DNA/PNA technologies. Application to Novel Foods. Coordinator: Nelson Marmioli.
- ☑ [GMOCHIPS](#): (2001-2004). Development of biochips to detect Genetically Modified Organisms (GMOs) in food. Coordinators: J. Remacle and Y. Bertheau.
- ☑ [Co-Extra](#): (2005-2009). GM and non-GM supply chains: their CO-EXistence and TRAcability. Coordinator: Y. Bertheau.
- ☑ [GMOseek project](#): (2009-2011). Development of screening methods for GMOs. This project also challenges the increasing number of unauthorized GM events needed to be detected and the need for better time- and cost-efficiency of analytical approaches. Coordinator: Dany Morisset

Most of the members of these EC GMO-traceability programmes, as well as the national competent authorities of all member states, collaborate as part of the European Network of GMO Laboratories (ENGL), which is under the chairmanship of ☑ [EC Joint Research Centre's Institute for Health and Consumer Protection \(IHCP\)](#).

A selection of research activities of the JRC and ENGL, as well as EU-funded research projects, is given below.

### 8.1 GMO testing: the modular approach

#### Time and cost efficiency and simplification of validation

A complete detection method correctly has been validated for only one particular GMO (and food/feed matrix) at a time. However, since several sub-tasks of GMO detection may be applied to a variety of GMOs, the idea of a modular approach to GMO testing is of interest. In such an approach, each procedure (for example, PCR or DNA extraction) would be validated independently and used

subsequently in a variety of detection tests. In addition to the cost-efficiency, the advantages of such an approach would include enhanced efficiency and the simplification of the task of validating tests for many different GMOs using different matrices. Presently, this modular approach is being applied by the JRC in collaborative studies only for the PCR part of GMO detection.

In addition, research was carried out in the context of the CO-EXTRA project to assess whether the analytical module of the GMO identification/quantification exercise is independent and at what conditions from the DNA extraction step. First results indicate that filtering criteria to remove inhibited DNA extracts can be necessary to eliminate interactions between quantification results in the real-time PCR tests from the extraction methods. If it proves possible to decouple each module of the analytical procedure, such an approach also may be considered for validation in other areas of detection that addresses such issues as food pathogens, mycotoxins and allergenic organisms.

#### Further reading:

[Co-Extra: Improving PCR based detection methods](#)

[Proceedings of Final Co-Extra Conference](#)

[The Modular Analytical Procedure and Validation Approach and the Units of Measurement for Genetically Modified Materials in Foods and Feeds. A. Holst-Jensen, K. G. Berdal \(2004\)](#)

[Critical points of DNA quantification by real-time PCR – effects of DNA extraction method and sample matrix on quantification of genetically modified organisms. K. Cankar, D. Štebih, T. Dreo, J. Žel and K. Gruden \(2006\)](#)

[Toward metrological Traceability for DNA Fragment Ratios in GM Quantification 1. Effect of DNA Extraction Methods on the Quantitative Determination of Bt176 Corn by Real-Time PCR. Corbisier P, Broothaerts W, Gioria S, Schimmel H, Burns M, Baoutina A, Emslie KR, Furui S, Kurosawa Y, Holden MJ, Kim HH, Lee YM, Kawaharasaki M, Sin D, Wang J. \(2007\).](#)

[Toward metrological traceability for DNA fragment ratios in GM quantification. 2. Systematic study of parameters influencing the quantitative determination of MON 810 corn by real-time PCR. Charels D, Broeders S, Corbisier P, Trapmann S, Schimmel H, Linsinger T, Emons H. \(2007\).](#)

[Qualitative and quantitative evaluation of the genomic DNA extracted from GMO and non-GMO foodstuffs with four different extraction methods. Peano, C.; Samson, M.C.; Palmieri, L.; Gulli, M.; Marmioli, N. \(2004\).](#)


## 8.2 Quantification of very low number of DNA targets using new real-time PCR approaches


There are new real-time PCR detection strategies under development using statistical approaches. For example, limitations in conventional realtime PCR applications to detect very low number of DNA targets have led to the development of digital PCR (dPCR). dPCR relies on single molecule detection since the PCR solution is distributed across a large number of partitions, and following amplification, linear, digital signals are used to estimate DNA copy number


Also“single molecule quantification” (SIMQUANT) has been developed for GMO quantification of samples with extremely low amounts of DNA. The approach is based on statistics and application of multiple qualitative parallel PCRs. According to the EU project Co-Extra this approach can yield a 100-fold improvement in the limit of quantification. Together with new protocols for DNA extraction from highly

processed samples, the efficient control of GMO presence is now possible in most of the processed soybean lecithins and oils.

**Further reading:**

 [A statistical approach for evaluation of PCR results to improve the practical limit of quantification \(LOQ\) of GMO analyses \(SIMQUANT\). K. G. Berdal, C. Bøydler, T. Tengs, A. H. Jensen. Eur Food Res Technol \(2008\) 227:1149–1157](#)

 [Single molecule detection in nanofluidic digital array enables accurate measurement of DNA copy number. S. Bhat, J. Herrmann, P. Armishaw, P. Corbisier, K. R. Emslie. Anal Bioanal Chem, 15. March 2009](#)

 [Absolute quantification of genetically modified MON810 maize \(Zea mays L.\) by digital polymerase chain reaction. P. Corbisier, S. Bhat, L. Partis, V. Rui Dan Xie, K. R. Emslie. Anal Bioanal Chem, 2009](#)

 [Summary of Co-Extra outcome](#)

 [Proceedings of Final Co-Extra Conference](#)


## 8.3 GMO screening: the application of DNA chips and new real time PCR approaches

### Improving laboratory economy and testing for a large numbers of target gene sequences in one step



A new method of multiplex screening (called Dual Chip®) has been developed by the Co-Extra project and the preceding GMO Chips project. This method will be commercialized by Eppendorf Array Technologies (Belgium). The aim of the project was to provide methods with which national control laboratories may infer the identity of a GMO that is likely to be found in a given sample. Necessitating a rapid screening method, such inferences allow great economy of time and effort. Multiple specific DNA capture probes, corresponding either to GMO elements, to species-specific targets or to control targets are mounted on glass slides. Through a colorimetric reaction of DNA hybridization and subsequent statistical analysis, various DNA elements present in a sample are detected and indicate the identity of the GMO. Target DNA can be detected to a level of 0.1% and the suitability of the method recently was validated by a collaborative ring trial organised by the EC's JRC. In addition to improving laboratory economy, the method is able to accommodate large numbers of target gene sequences. This trait is of particular interest, due to the expected increase in approved and non-approved GMO traits and new GMO crop species. The micro-array method is one of the applications of the "matrix approach" (see 8.5). The use of sub-sampling strategies also may allow the use of qualitative methods such as micro-arrays to determine if the GMO content of a sample exceeds a labelling threshold.












New real-time PCR approaches enable the multiple detection of different sequences specific of GM events. Examples are real-time PCR multiplex systems such as Pentaplex (five PCR in one tube) and CoSYP (Combinatory qPCR SYBR®Green screening). This is a cost-effective matrix-based approach for determining the presence of GM plant materials in products based on SYBR®Green technology.

A new  ["real-time PCR-based ready-to-use multi-target analytical system for GMO detection"](#) has been developed by the Joint Research Centre. This high-throughput analytical system, i.e., a unique screening tool for the unequivocal simultaneous identification of all currently EU-approved and all unapproved

genetically modified organisms (GMOs) known to the European Union Reference Laboratory for GM Food and Feed (EURL-GMFF), consists of 96-well prespotted plates containing lyophilized primers and probes for the individual detection of targets allowing the simultaneous identification of 39 GM events by the use of event-specific primers and probe combination. It represents the first analytical tool worldwide allowing the simultaneous detection of so many genetic modification events using event-specific targets. The use of the 96-well RTi-PCR platform was chosen to facilitate the immediate use of the proposed approach, which can be easily integrated in the laboratories' working routine, without the need for new instrumentation or new procedures.

Further research is currently carried out in the [GMOseek project](#). The GMOseek project is directed towards the challenges of detecting the increasing number of genetically modified organisms (GMOs) approved and unauthorized in the European Union (EU) that are potentially in the EU food supply. Moreover, GMOseek copes with the time- and cost-efficiency of analytical approaches in order to deliver inexpensive, fast, and broad-based screening tests.

#### Further reading and references:

-  [Validation Report: Microarray Method for the Screening of EU Approved GMOs by Identification of their Genetic Elements \(EURL-GMFF\)](#)
-  [Biochips: A powerful tool for multiple and fast analysis of genes and DNA sequences](#)
-  [Project summary of GMOchips \(EU-funded project\)](#)
-  [DNA-Track \(EU-funded project\)](#)
-  [GMOseek project \(funded by the German Federal Office of Consumer Protection and the Food Safety and the Food Standard Agency, UK\)](#)
-  [Real-Time PCR-Based Ready-to-Use Multi-Target Analytical System for GMO Detection. M. Querci, N. Foti, A. Bogni, L. Kluga, H. Broll, G. Van den Eede. Food Anal. Methods, 2009](#)
-  [A theoretical introduction to "Combinatory SYBR®Green qPCR Screening", a matrix-based approach for the detection of materials derived from genetically modified plants. M. Van den Bulcke, A. Lievens, E. Barbau-Piednoir, G. MbongoloMbella, N. Roosens, M.Sneyers, A. L. Casi. Anal Bioanal Chem, 2009](#)
-  [Development of an overall health strategy in the area of GMOs](#)
-  [New approaches in GMO detection](#)
-  [A novel quantitative high-throughput assay for multiplex GMOs quantification](#)
-  [Validation of the performance of a GMO multiplex screening assay based on microarray detection](#)

## 8.4 The detection of unknown GMOs in the supply chain

### Accelerating emergency measures against illegal GMOs on the market

Two concepts under development in the Co-Extra programme were the 'differential PCR' and the 'matrix' approaches. Both are aimed at the determination of the probable presence of unknown GMOs. Differential PCR quantitatively induces the ratios of different genetic elements in sample DNA which then are compared with expected ratios for known GMOs. The presence of an unknown GMO is indicated if the statistical result differs from zero. The matrix approach tests simultaneously for the presence of a large number of possible DNA fragments and compares the resulting combinations to a database of known

GMOs. A possible unknown GMO is indicated by the presence of unusual combinations of DNA targets (see above micro-array application of the “matrix approach”). This approach may be extended by the inclusion of a screening microarray that permits the detection of several thousand genetic elements. Such elements may include those that have not yet been used in an authorised or registered GMO. Application of this screening microarray also may facilitate further characterisation of the GMO in order to enable immediate preliminary risk assessment of the GMO.

**Further reading:**

[What is the future of GMO detection? A freely speaking scientist 's opinion \(EU-funded project Co-Extra\)](#)

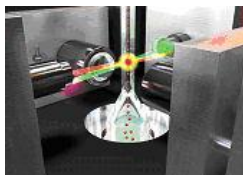
[Design of a DNA chip for detection of unknown genetically modified organisms \(GMOs\). H. Nesvold, A. Kristoffersen, A. Holst-Jensen and K. G. Berdal \(2005\)](#)

[Knowledge-technology-based discovery of unauthorized genetically modified organisms. T. Ruttink, D. Morisset, B. Van Droogenbroeck, N. Lavrač, G. L. M. Van Den Eede, J. Žel and M. De Loose \(2010\).](#)

[Molecular toolbox for the identification of unknown genetically modified organisms. T. Ruttink, R. Demeyer, E. Van Gulck, B. Van Droogenbroeck, M. Querci, I. Taverniers and M. De Loose \(2010\)](#)

## 8.5 Novel approach for direct target quantification

### Reducing the error rate of currently used PCR methods



The MBG Unit is seeking innovative DNA detection methods to reduce the error rate of currently used PCR methods. Today, quantification approaches imply an amplification of specific GMO DNA sequences by PCR. This leads to indirect GMO quantification and introduces uncertainty due to the intrinsic PCR error. The MBG Unit currently is evaluating the suitability of a novel direct DNA labelling system for detecting and quantifying DNA targets without the need of the amplification step. The Unit also is studying the reliability of the system for direct target measurements.

**Further reading:**

[Development and evaluation of methods for GMO analysis \(MBG Unit, JRC\)](#)

## 8.6 Development of cloned DNA control samples and CRMs

### Positive and negative control samples

Positive and negative control samples of GMOs for PCR-based detection methods are a legal prerequisite for the authorization process ([Regulation \(EC\) No 1829/2003](#)) and for compliance with the enforced threshold for labelling in the EU. The tasks of the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF) include the production and distribution of control samples to the national laboratories for its validated methods.

Currently, the MBG Unit and ENGL are advising the EC on positive and negative control samples. Plasmids have been demonstrated to represent a cheap and reliable alternative for use in qualitative applications. Cloned GMO-DNA as positive and negative control samples will be defined for the detection and quantification of GM products and for distribution to ENGL and to testing laboratories.

## Certified Reference Materials (CRMs)

In 2004, the European Commission recommended the expression of the content of genetically modified (GM) food and feed as the percentage of GM-DNA copy numbers in relation to target taxon-specific DNA copy numbers, calculated in terms of haploid genomes (Recommendation 2004/787/EC). For correct expression of the results and to comply with laboratory accreditation schemes (ISO 17025), calibrants are required for the determination of the ratio of copies of transgenic and taxon specific genes.

A first set of plasmids tested for quantitative applications and bearing the DNA sequence targeted by validated method for the event-specific quantification has been marketed by the IRMM ([ERM-AD413](#)). The material can be used primarily for calibration purposes, as well as for specificity testing.

### Further reading:

[Toward Metrological Traceability for DNA Fragment Ratios in GM Quantification 3. Suitability of DNA Calibrants Studied with a MON 810 Corn Model.](#)

[Development and evaluation of methods for GMO analysis \(MBG Unit, JRC\)](#)

[Development and application of a novel class of real-time PCR standards based on cloned sequences for GMO quantification \(JRC\)](#)

[Development and applications of real-time PCR standards for GMO quantification based on tandem-marker plasmids. E. Mattarucchi, F. Weighardt, C. Barbati, M. Querci, and G. Van den Eede \(2005\)](#)

[Event-specific plasmid standards and real-time PCR methods for transgenic Bt11, Bt176 and GA21 maize and transgenic GT73 canola. I. Taverniers, P. Windels, M. Vaitilingom, A. Milcamps, E. Van Bockstaele, G. Van den Eede, and M. de Loose \(2005\)](#)

[A Real-Time PCR Based Approach for the Quantification of the pat Gene in the T25 Zea mays Event. F. Weighardt, C. Barbati, C. Paoletti, M. Querci, S. Kay, M. De Beuckeleer, and G. Van den Eede \(2004\)](#)

[Use of Certified Reference Materials for the quantification of GMO in DNA copy number ratio](#)

[Use of pJANUS™-02-001 as a calibrator plasmid for Roundup Ready soybean event GTS-40-3-2 detection: an interlaboratory trial assessment. A. Lievens, G. Bellocchi, D. De Bernardi, W. Moens, C. Savini & M. Mazzara & G. Van den Eede, M. Van den Bulcke \(2010\)](#)

## 8.7 ELISA Reverse method and device (ELISA-R m&d)

### For a simultaneous screening of a large number of samples

In collaboration with Italian universities and institutions, the MBG Unit of IRC has developed innovative ELISA test systems for the detection and quantification of GMO containing the endotoxin Cry1Ab present in MON810 and Bt11 genetically modified (GM) maize lines and containing CP4EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) present in soy. Its application is proposed in cases in which a large number of samples must be screened simultaneously or when the simultaneous detection of different proteins is required. The last method mentioned currently is the only internationally accepted

protein-based detection method and is part of ISO standard 21572. For example, a protocol to quantify Cry1Ab protein in GM maize lines (MON810 and Bt11) with a limit of detection of 0.0056% (m/m) and a limit of quantification of 0.0168% (m/m) has been developed.

**Further reading:**

[Development and evaluation of methods for GMO analysis \(MBG Unit, JRC\)](#)

[Application of the ELISA Reverse Method and device to quantify CP4EPSPS protein in GM RUR Soya. M. Ermolli, A. Prospero, B. Balla, M. Querci, A. Mazzeo, and G. Van den Eede \(2006\)](#)

[ELISA Reverse m&d for Multiplex Detection and Quantification of Target Proteins in Food Analyses. A. Prospero \(2006\)](#)

[Implementation and optimization of the ELISA Reverse method and device. M. Ermolli, A. Mazzeo, S. Folloni, G. Petracca, M. Pettenati, D. Giordano, M. Querci, G. Van den Eede. European Commission • Joint Research Centre \(JRC\) • Institute for Health and Consumer Protection \(IHCP\) • Biotechnology & GMOs Unit](#)

## 8.8 High-throughput immunoassay

### The first application of a quantitative and protein-based high-throughput system

A covalent microsphere immunoassay using fluorescent beads coupled to a specific antibody was developed for the quantification of the endotoxin Cry1Ab present in the GM maize lines known as MON810 and Bt11. The limits of detection and quantification equal 0.018% and 0.054% (w/w) respectively. The present study describes the first application of quantitative high-throughput immunoassays in GMO analysis.

**Further reading:**

[First Application of a Micro-sphere Based Immunoassay to GMO Detection: Quantification of Cry1Ab Protein in GM Maize. A. Fantozzi, M. Ermolli, M. Marini, D. Scotti, B. Balla, M. Querci, S. Langrell, G. Van den Eede \(2007\)](#)

[Development and evaluation of methods for GMO analysis \(JRC\)](#)

## 8.9 Non-PCR based approaches

Several non-PCR based approaches were evaluated within Co-Extra to check for their performance. Among several alternatives tested, loop-mediated isothermal amplification (LAMP) combined with a Bioluminescent Assay in Real-Time (BART) detection system is promising. The sensitivity and quantification of this system is similar to that of PCR, but LAMP-BART is less sensitive to inhibitors and cheaper. A machine for on-site detection is available. An alternative for implementing on-site detection, such as cooperatives, was also successfully studied.

**Further reading:**

[Non-PCR based Alternative Analytical Methods \(CoExtra\)](#)

[Proceedings of Final Co-Extra Conference](#)

## Annex I - Internet Guide to European bodies and research projects related to detection of GMOs in the supply chain (selection)

### Higher-level EU institutions

- [Directorate General for 'Health and Consumers' \(DG SANCO\)](#)
- [European Commission - Joint Research Centre \(JRC\)](#)
- [European Food Safety Authority \(EFSA\)](#)

### Institution and networks for the development, validation and harmonisation of GMO detection methods

- [Molecular Biology and Genomics Unit \(JRC\)](#)
- [European Union Reference Laboratory \(JRC\)](#)
- [European Network of GMO Laboratories \(ENGL\)](#)
- [Institute for Reference Materials and Measurements \(IRMM\)](#)
- [European Committee for Standardization \(CEN\)](#)

### Training activities on GMO detection

- [Training courses of MBG Unit \(JRC\)](#)

### Databases / data collections

- [Guidance documents for the validation of GMO detection methods \(EURL-GMFF, JRC\)](#)
- [Validated GMO detection methods \(EURL-GMFF, JRC\)](#)
- [Information on notifications about deliberate field trials and placing on the market of genetically modified organisms \(MBG Unit, JRC\)](#)
- [Document collection MBG Unit \(JRC\)](#)

### Software tools for validation of GMO detection methods and sampling

- [AMPE: Analytical Method Performance Evaluation \(JRC\)](#)
- [Sampling Software: KesTE / CoDE \(JRC\)](#)

### Relevant EU research projects


- [CO-EXTRA: GM and non-GM supply chains: their CO-EXistence and TRAceability](#)
- [GMOseek project: Development of screening methods for GMOs](#)
- [DNA-TRACK: Traceability of DNA fragments throughout the food chain by DNA/PNA technologies. Application to Novel Foods](#)
- [Safe Foods: Promoting Food Safety through a New Integrated Risk Analysis Approach for Foods](#)


## Annex II – Publications and Posters


- General publications on GMO detection, quantification and validation
- PCR-based GMO detection methods, DNA standard material and reference material
- DNA-based Microarrays
- Protein-based GMO detection methods
- Harmonisation and validation of detection methods
- Sampling


### General publications on GMO detection, quantification and validation


 [The EU Legislation on GMOs - An overview](#). D. Plan and G. Van den Eede (2010)


 [The role of Community Reference Laboratories in the EU regulatory framework on GMOs](#)  
M. Mazzara, C. Charles-Delobel, C. Savini, E. Luque-Perez, G. Van den Eede  
European Commission, Joint Research Centre (JRC), Institute for Health and Consumer Protection (IHCP)


 [Analytical methods for Detection and Determination of Genetically Modified Organisms \(GMO's\) in Agricultural Crops and Plant-derived Food Products.](#)  
E. Anklam, F. Gadani, P. Heinze, H. Pijnenburg, and G. Van den Eede  
European Food Research and Technology (2002) 214:3-26


 [Review of GMO Detection and Quantification Techniques](#)  
L. Bonfini, H. Petra, S. Kay, and G. Van den Eede  
EUR 20384 EN (2002)


 [The limits of GMO detection.](#)  
S. Kay and G. Van den Eede  
Nature Biotechnology Vol 19 No 5 p 405 (2001)


 [Food Products Identity: the Need for a High Through-Put Approach in Food Analysis](#)  
A. Fantozzi, M. Marini, M. Ermolli, G. Van den Eede  
1st International Symposium on "Recent Advances in Food Analysis" - Prague 5-7 November 2003

 [Food products identity: the need for a high through-put approach for GMO screening](#)  
A. Fantozzi, M. Marini, M. Ermolli, and G. Van den Eede  
7th Food Authenticity and Safety International Symposium - Nantes, 15-17 October 2003

 [GMO Testing Methods: Analytical Approaches, Method Validation and Sampling Strategy](#)  
G. Bellocchi, M. Ermolli, C. Savini and G. van den Eede  
European Commission DR JRC, Institute for Health and Consumer Protection (IHCP)

 [New approaches in GMO detection](#)  
M. Querci, M. Van den Bulcke, J. Žel, G. Van den Eede, H. Broll  
Anal Bioanal Chem; 2009

 [Knowledge-technology-based discovery of unauthorized genetically modified organisms](#)  
T. Ruttink, D. Morisset, B. Van Droogenbroeck, N. Lavrač, G. L. M. Van Den Eede, J. Žel and M. De Loose (2010)

 [Molecular toolbox for the identification of unknown genetically modified organisms](#)  
T. Ruttink, R. Demeyer, E. Van Gulck, B. Van Droogenbroeck, M. Querci, I. Taverniers and M. De Loose (2010)

## PCR-based GMO detection methods, DNA standard material and reference material

### [Event-specific plasmid standards and real-time PCR methods for transgenic Bt11, Bt176 and GA21 maize and transgenic GT73 canola](#)

I. Taverniers, P. Windels, M. Vaitilingom, A. Milcamps, E. Van Bockstaele, G. Van den Eede, and M. de Loose

Journal of Agricultural and Food Chemistry (2005) 53: 3041–3052

### [A Real-Time PCR Based Approach for the Quantification of the pat Gene in the T25 Zea mays Event](#)

Journal of the AOAC International 87(6): 1342-1355 (2004)

F. Weighardt, C. Barbati, C. Paoletti, M. Querci, S. Kay, M. De Beuckeleer, and G. Van den Eede

### [Development and applications of a novel class real-time PCR standards based on cloned sequences for GMO quantification](#)

C. Barbati, F. Weighardt, E. Mattarucchi, M. Querci, and G. Van den Eede

6th Italian workshop in Real time PCR, Arrays Quantitative PCR and mutation detection - Florence, 18 November 2003

### [Real Time PCR based GMO quantification: limits and accuracy](#)

C. Barbati, F. T. Weighardt, S. Kay, C. Paoletti, M. Querci, and G. Van den Eede

Third CSL/JIFSAN Symposium on Food Safety and Nutrition” at the Central Science Laboratory, York, UK

### [Production of Reference Materials for the Detection of GMOs](#)

S. Trapmann, H. Schimmel, P. Brodmann, G. Van den Eede, K. Vorburger and J. Pauwels

Journal of the AOAC International, Vol. 85, No. 3 (2002) pp. 775-779

### [Development and applications of real-time PCR standards for GMO quantification based on tandem-marker plasmids](#)

E. Mattarucchi, F. Weighardt, C. Barbati, M. Querci, and G. Van den Eede

European Food Research and Technology 211: 511-519 (2005) ISSN: 1438-2385

### [A statistical approach for evaluation of PCR results to improve the practical limit of quantification \(LOQ\) of GMO analyses \(SIMQUANT\)](#)

K. G. Berdal, C. Bøydler, T. Tengs, A. H. Jensen

Eur Food Res Technol (2008) 227:1149–1157

### [Single molecule detection in nanofluidic digital array enables accurate measurement of DNA copy number](#)

S. Bhat, J. Herrmann, P. Armishaw, P. Corbisier, K. R. Emslie

Anal Bioanal Chem, 15. March 2009

### [Detection of genetically modified rice: a construct-specific real-time PCR method based on DNA sequences from transgenic Bt rice](#)

D. Mäde, C. Degner, L. Grohmann

Eur Food Res Technol (2006)

### [Detection of genetically modified rice: collaborative validation study of a construct-specific real-time PCR method for detection of transgenic Bt rice](#)

L. Grohmann, D. Mäde

Eur Food Res Technol (2008)

### [A theoretical introduction to “Combinatory SYBR@Green qPCR Screening”, a matrix-based approach for the detection of materials derived from genetically modified plants](#)

M. Van den Bulcke, A. Lievens, E. Barbau-Piednoir, G. Mbongolo Mbella, N. Roosens, M. Sneyers, A. L. Casi

Anal Bioanal Chem, 2009

### [Absolute quantification of genetically modified MON810 maize \(Zea mays L.\) by digital polymerase chain reaction](#)

P. Corbisier, S. Bhat, L. Partis, V. Rui Dan Xie, K. R. Emslie

Anal Bioanal Chem, 2009

 [Detection of genetically modified rice: collaborative validation study of a construct-specific real-time PCR method for detection of transgenic Bt rice](#)

L. Grohmann, D. Mäde  
Eur Food Res Technol (2008)

 [A novel quantitative high-throughput assay for multiplex GMOs quantification](#)

S. Folloni, M. Ermolli, M. Querci and G. Van den Eede  
European Commission • Joint Research Centre (JRC) • Institute for Health and Consumer Protection (IHCP) • Biotechnology & GMOs Unit

 [Real-Time PCR-Based Ready-to-Use Multi-Target Analytical System for GMO Detection](#)

M. Querci, N. Foti, A. Bogni, L. Kluga, H. Broll, G. Van den Eede  
Food Anal. Methods, 2009

 [Innovative Application of Fluorescent Microsphere Based Assay for Multiple GMO Detection](#)

A. Fantozzi, M. Ermolli, M. Marini, B. Balla, M. Querci, G. Van den Eede  
Food Anal. Methods (2008) 1:10–17

 [Event-specific Method for the Quantification of Cotton Line MON 1445 Using Real-time PCR](#)

M. Mazzara, A Bogni, G Van den Eede  
JRC Scientific and Technical Reports 2008

 [Event-specific Method for the Quantification of Cotton Line MON 531 Using Real-time PCR](#)

M.Mazzara, A Bogni, N. Foti, G Van den Eede  
JRC Scientific and Technical Reports 2008

 [Event specific Method for the Quantification of Cotton Line MON 15985 Using Real-time PCR](#)

C. Savini, M. Mazzara, B. Munaro, G Van den Eede  
JRC Scientific and Technical Reports 2008

 [Event specific Method for the Quantification of Maize Event Bt 11 Using Real-time PCR](#)

D. Delobel, S. Larcher, M. Mazzara, G Van den Eede  
JRC Scientific and Technical Reports 2008

 [Event specific Method for the Quantification of Cotton Line GHB614 Using Real-time PCR](#)

C. Savini, A. Bogni, M. Mazzara, G Van den Eede  
JRC Scientific and Technical Reports 2008

 [Event specific Method for the Quantification of Maize Line LY038 Using Real-time PCR](#)

D. Delobel, E. Grazioli, S. Larcher, M. Mazzara, G Van den Eede  
JRC Scientific and Technical Reports 2008

 [Event specific Method for the Quantification of Maize Line MON 88017 Using Real-time PCR](#)

D. Delobel, N. Foti, E. Grazioli, M. Mazzara, G Van den Eede  
JRC Scientific and Technical Reports 2008

 [Event specific Method for the Quantification of Maize Line MON 89034 Using Real-time PCR](#)


C. Savini, A. Bogni, E. Grazioli, B. Munaro, M. Mazzara, G Van den Eede  
JRC Scientific and Technical Reports 2008

 [Event specific Method for the Quantification of Maize Event 3272 Using Real-time PCR](#)

D. Delobel, N. Foti, M. Mazzara, G Van den Eede  
JRC Scientific and Technical Reports 2008

 [Event-specific Method for the Quantification of Soybean Line MON 89788](#)

C. Delobel, A. Bogni, G. Pinski, M. Mazzara, G. Van den Eede  
JRC Scientific and Technical Reports 2008

 [Report on the Verification of the Performance of 59122, 1507 and NK603 Event-specific Methods on the Hybrid Maize Line 59122x1507xNK603 Using Real-Time PCR](#)

C. Delobel, S. Price, M. Mazzara, C. Savini and G. Van den Eede  
European Commission, Joint Research Centre (JRC), Institute for Health and Consumer Protection (IHCP), Biotechnology & GMOs Unit

### DNA-based micro arrays


 [Microarray Method for the Screening of EU Approved GMOs by Identification of their Genetic Elements.](#)

Report of validation coordinated by the European Union Reference Laboratory for GM Food and Feed of the Joint Research Centre, Institute for Health and Consumer Protection, Biotechnology and GMOs Unit  
S. Hamels, S. Leimanis, M. Mazzara, G. Bellocchi, N. Foti, W. Moens, J. Remacle and G. Van den Eede


 [Development of testing and sampling approaches](#)

G. Bellocchi, W. Moens, M. Mazzara, G. van den Eede  
Joint Research Center – Institute for Health and Consumer Protection

### Protein-based GMO detection methods

 [First Application of a Micro-sphere Based Immunoassay to GMO Detection: Quantification of Cry1Ab Protein in GM Maize.](#)


A. Fantozzi, M. Ermolli, M. Marini, D. Scotti, B. Balla, M. Querci, S. Langrell, G. Van den Eede  
Journal of Agricultural and Food Chemistry, Vol. 55 (2007), 1071-1076

 [Development of an innovative immunoassay for CP4EPSPS and Cry1AB GM proteins detection and quantification.](#)

M. Ermolli, A. Prospero, B. Balla, M. Querci, A. Mazzeo, and G. Van den Eede  
Food Additives and Contaminants. Volume 23, Number 9/September 2006: 876-882

 [Food safety: screening tests used to detect and quantify GMO proteins.](#)

M. Ermolli, A. Fantozzi, M. Marini, D. Scotti, B. Balla, S. Hoffmann, M. Querci, C. Paoletti, and G. Van den Eede  
Accreditation and Quality Assurance - ACQUAL 11(1-2): 55–57. (2006)

 [Application of the ELISA Reverse Method and device to quantify CP4EPSPS protein in GM RUR Soya. The Safe Consortium.](#)

M. Ermolli, A. Prospero, B. Balla, M. Querci, A. Mazzeo, and G. Van den Eede  
The First Safe International Congress on Food Safety, Budapest Congress Centre, Budapest (Hungary), 11-14 June 2006

 [ELISA Reverse m&d for Multiplex Detection and Quantification of Target Proteins in Food Analyses.](#)

A. Prospero.  
University of Teramo, 11th Workshop on the Developments in the Italian PhD Research on Food Science and Technology, University of Teramo, Mosciano Sant'Angelo, 27-29 September 2006

 [An Innovative High-Throughput Assay for the Detection and the Quantification of GMOs Proteins and Sequences in Food Samples.](#)

M. Ermolli  
Facoltà di Agraria - Dipartimento di Scienze degli Alimenti  
The 10th Workshop on the Developments in the Italian PhD Research in Food Science and Technology, Foggia ( Italy), 7-9 September 2005

 [Protein Based Methods in GMO Detection and Quantification in Food.](#)

M. Ermolli, A. Prospero, M. Querci, and A. Mazzeo  
2nd International Symposium on recent advances in food analysis - Prague, 2-4 November 2005

 [Food Safety - Screening Tests Used to Detect and Quantify GMOs Proteins.](#)

M. Ermolli, A. Fantozzi, M. Marini, D. Scotti, S. Hoffmann, and G. Van den Eede  
Association of Analytical Communities (AOAC) Europe, Geel ( Belgium). The 3rd Association of Analytical Communities (AOAC) Europe Eurochem Conference, Brussels ( Belgium), 3-4 March 2005

 [An innovative high through put assay for the detection and the quantification of Mon 810 and Bt 11 in maize.](#)

A. Fantozzi, M. Marini, D. Scotti, M. Ermolli, and G. Van den Eede  
International Conference Rapid Methods Europe 2004 - Noordwijk, 25-26 March 2004

 [Implementation and optimization of the ELISA Reverse method and device](#)

M. Ermolli, A. Mazze, S. Folloni, G. Petracca, M. Pettenati, D. Giordano, M. Querci, G. Van den Eede  
European Commission • Joint Research Centre (JRC) • Institute for Health and Consumer Protection (IHCP) • Biotechnology & GMOs Unit


### Harmonisation and validation of detection methods

 [Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing](#)


M. Mazzara, C. Savini, D. Delobel, H. Broll, A. Damant, C. Paoletti, G. Van den Eede  
JRC Scientific and Technical Reports 2009

 [Method Validation for the Detection of GMOs in the Context of EU Regulation](#)

M. Mazzara, C. Charles Delobel, C. Savini, G. Van den Eede  
Scherago International, Inc, Plant & Animal Genome XV Conference, Town and Country Hotel, San Diego (USA), 13-16 January 2007

 [Coherence Between Legal Requirements and Approaches for Detection of Genetically Modified Organisms \(GMOs\) and their Derived Products.](#)

A. Holst-Jensen, M. De Loose and G. Van den Eede.  
Journal of Agricultural and Food Chemistry (2006), 54(8) pp 2799 – 2809. Amer Chemical Soc, Washington

 [The Role of the New European Union Reference Laboratory for Genetically Modified Food and Feed in the European Authorisation Procedure for Genetically Modified Food and Feedstuffs.](#)

S. Langrell and G. Van den Eede  
8th International Symposium on the Biosafety of Genetically Modified Organisms, INRA, Montpellier (FR), 26-30 September 2004

 [The European Network of GMO Laboratories as a Tool in Support of EU Legislation.](#)

S. Langrell  
Co-Operation with Universities and Research organisations of Baden-Württemberg, DG JRC - Karlsruhe (DE), 25 March 2004

 [Validation studies and proficiency testing of methods for the detection and quantification of Genetically Modified Organisms \(GMO's\).](#)

E. Anklam, P. Heinze, S. Kay, G. Van den Eede, and B. Pöpping  
Journal of the AOAC International, Vol. 85, No. 3, 2002 pp. 809-816 (2002)

 [Validation of the performance of a GMO multiplex screening assay based on microarray detection](#)

S. Leimanis, S. Hamels, F. Naze, G.M. Mbella, M. Sneyers, R. Hochegger, H. Broll, L. Roth, K. Dallmann, A. Micsinai, J.L. La Paz, M. Pla, C. Brünen-Nieweler, N. Papazova, I. Taverniers, N. Hess, B. Kirschneit, Y. Bertheau, C. Audeon, V. Laval, U. Busch, S. Pecoraro, K. Neumann, S. Rösel, J. van Dijk, E. Kok, G. Bellocchi, N. Foti, M. Mazzara, W. Moens, J. Remacle, G. Van Den Eede  
Eur Food Res Technol (2008) 227:1621–1632

 [Method Validation and Quality Management in the Flexible Scope of Accreditation: An Example of Laboratories Testing for Genetically Modified Organisms](#)

J. Žel, M. Mazzara, C. Savini, S. Cordeil, M. Camloh, D. Štebih, K. Cankar, K. Gruden, D. Morisset, G. Van den Eede. Food Anal. Methods (2008) 1:61–72

 [Report on GMO detection identification and quantification methods submitted to collaborative studies.](#)

L. Bonfini, S. Kay, H. Petra and G. Van den Eede  
EUR 20383 EN (2002)

 [Analytical Challenges: Bridging the gap from regulation to enforcement.](#)

G. Van den Eede, H. Schimmel, S. Kay and E. Anklam  
Journal of the AOAC International, Vol. 85, No. 3, 2002 pp. 757 - 761

 [Validation of a method based on polymerase chain reaction for the detection of genetically modified organisms in various processed foodstuffs.](#)


M. Lipp, A. Bluth, F. Eyquem, L. Kruse, H. Schimmel, G. Van den Eede and E. Anklam  
European Food Research and Technology (2001) 212 :497-504.

 [Validation of an analytical method for the detection of GMO-derived DNA in processed foodstuffs.](#) G.

Van den Eede, M. Lipp, F. Eyquem, and E. Anklam  
EUR 19676 EN (2000)

 [Validation of a double competitive polymerase chain reaction method for the quantification of GMO's in raw material.](#)

G. Van den Eede, M. Lipp, F. Eyquem, and E. Anklam  
EUR 19677 (2000)

 [Validation of an immunoassay for the detection and quantification of Roundup-Ready soy beans in food and food fractions.](#)

M. Lipp, E. Anklam, and J. Stave  
Journal of the AOAC International (2000) 83, 919-927

 [Results of an interlaboratory assessment of a screening method for the detection of genetically modified organisms in soy beans in maize.](#)

M. Lipp, E. Anklam, P. Brodmann, K. Pietsch, and J. Pauwels  
Food Control 10, 379 (1999)

 [Validity Assessment of the Detection Method of Maize Event Bt10 through Investigation of Its Molecular Structure](#)

A. Milcamps, S. Rabe, R. Cade, A. J. De Framond, P. Henriksson, V. Kramer, D. Lisboa, S. Pastor-Benito, M. G. Willits, D. Lawrence, and G. Van den Eede  
Journal of Agricultural and Food Chemistry, 2009, 57 (8)

 [IUPAC collaborative trial study of a method to detect genetically modified soy beans and maize in dried powder.](#)

M. Lipp et al.  
Journal of the AOAC International (1999) 82, 923-928

 [GMO analysis towards global harmonisation](#)

M. Querci, H. Broll, G. Van den Eede  
New Food, Issue 2, 2009, page 51-54

 [Statistical evaluation of Real-Time PCR performances using reference standard material and pENGL-03-012 plasmid](#)

M. Ermolli, G. Bellocchi, A. Prospero, M. Querci, W. Moens and G. Van den Eede  
European Commission • Joint Research Centre (JRC) • Institute for Health and Consumer Protection (IHCP) • Biotechnology & GMOs Unit

 [Expanding Horizons in the Validation of GMO Analytical Methods: Fuzzy-based Expert Systems](#)

G. Bellocchi, M. Acutis, C. Paoletti, R. Confalonieri, P. Trevisiol, E. Grazioli, C. Delobel, C. Savini, M. Mazzara, G. Van den Eede  
Food Anal. Methods (2008) 1:126–135

 [Use of pJANUS™-02-001 as a calibrator plasmid for Roundup Ready soybean event GTS-40-3-2 detection: an interlaboratory trial assessment](#)

A. Lievens, G. Bellocchi, D. De Bernardi, W. Moens, C. Savini & M. Mazzara & G. Van den Eede, M. Van den Bulcke (2010)

## Sampling

☞ [Kernel Lot Distribution Assessment – KeLDA - a study on the distribution of GMO in large soybean shipments.](#)

C. Paoletti, A. Heissenberger, M. Mazzara, S. Larcher, E. Grazioli, G. Van den Eede, P. Corbisier, N. Hess, G. Berben, P.S. Lübeck, M. De Loose, G. Moran, C. Henry, C. Brera, I. Folch, and J. Ovesna. European Food Research and Technology Journal

☞ [European Union Perspective – Sampling for Testing of Genetically Modified Impurities.](#)

C. Paoletti, M. Donatelli, A. Heissenberger, E. Grazioli, S. Larcher, and G. Van den Eede. Australasian Institute of Mining and Metallurgy, Australia. Second World Conference on Sampling and Blending Book. pp. 209-213 (2005)

📄 [Simulating kernel lot sampling: the effect of heterogeneity on the detection of GMO contamination.](#) C.

Paoletti, M. Donatelli, S. Kay, and G. Van den Eede  
Seed Science and Technology 31(3), 629-638 (2003)

📄 [Sampling strategies for GMO detection and/or quantification.](#)

S. Kay and C. Paoletti. EUR 20239 EN (2002)

📄 [Kernel Lot Distribution Assessment \(KeLDA\) a comparative study of protein and DNA-based detection methods for GMO testing](#)

M. Mazzara, C. Paoletti, P. Corbisier, E. Grazioli, S. Larcher, N. Hess, E. Jenssen, P. S. Lübeck, M. De Loose, G. Moran, C. Henry, R Onori, I. Folch, J. Ovesna, G. Van den Eede  
European Commission • Joint Research Centre (JRC) • Institute for Health and Consumer Protection (IHCP) • Biotechnology & GMOs Unit

## Other

📄 [A Qualitative Approach for the Assessment of the Genetic Stability of the MON 810 Trait in Commercial Seed Maize Varieties](#)

M. Aguilera, M. Querci, B. Balla, A. Prospero, M. Ermolli, G. Van den Eede  
Food Anal. Methods (2008) 1:252–258

📄 [Assessing Copy Number of MON 810 Integrations in Commercial Seed Maize Varieties by 5' Event-Specific Real-Time PCR Validated Method Coupled to 2  \$\Delta\Delta\text{CT}\$  Analysis](#)

M. Aguilera, M. Querci, S. Pastor, G. Bellocchi, A. Milcamps, G. Van den Eede  
Food Anal. Methods (2009) 2:73–79

📄 [DANA: a platform-independent component for data analysis and assessment](#)

G. Bellocchi, F. Foscarini, M. Canonico, G. Van den Eede  
1st Global Conference on GMO Analysis, Como (Italy), June 24-27, 2008

📄 [Web Components for Development of Computational Methods: Example with Fuzzy Logic Rules](#)

G. Bellocchi, F. Foscarini, M. Canonico, G. Van den Eede  
European Commission Joint Research Centre, Institute for Health and Consumer Protection, Biotechnology and GMOs Unit, 2008

## Annex III – Register of validated GMO detection methods

(as of September 2010; see also: [GMO community register](#))


### 1. Validated methods by EURL-GMFF (for GMO detection and DNA extraction)

Event	Unique identifier	Applicant	Validation Reports 	Validated Method 
1507 Maize	DAS-01507-1	Pioneer Hi-Bred, Dow Agrosciences, Mycogen Seeds	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
1507 x 59122 Maize	DAS-01507-1 x DAS-59122-7	Mycogen Seeds, c/o Dow AgroSciences LLC	<a href="#">Click here</a>	<a href="#">Method description (1507)</a> <a href="#">Method description (59122)</a>
1507 x NK603 Maize	DAS-01507-1 x MON-00603-6	Pioneer Hi-Bred, Mycogen Seeds	<a href="#">Click here</a>	<a href="#">Method description (NK603)</a> <a href="#">Method description (1507)</a>
3006-210-23/281-24-236 Cotton	DAS-24236-5 x DAS-21023-5	Dow AgroSciences	<a href="#">Click here</a>	<a href="#">Method description (281-24-236)</a> <a href="#">Method description (3006-210-23)</a> <a href="#">DNA extraction</a>
3272 Maize	SYN-E3272-5	Syngenta Crop Protection	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
59122 Maize	DAS-59122-7	Pioneer Hi-Bred; Mycogen Seeds c/o Dow AgroSciences	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
59122 x 1507 x NK603 Maize	DAS-5912-7xDAS-1507-1xMON-00603-6	Pioneer Hi-Bred	<a href="#">Click here</a>	<a href="#">Method description (TC1507)</a> <a href="#">Method description (NK603)</a> <a href="#">Method description (59122)</a>
59122 x NK603 Maize	DAS-59122-7xMON-00603-6	Pioneer Hi Bred	<a href="#">Click here</a>	<a href="#">Method description (59122)</a> <a href="#">Method description (NK603)</a>
A2704-12 Soybean	ACS-GM005-3	Bayer CropScience	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
A5547-127 Soybean	ACS-GM006-4	Bayer CropScience	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
Bt10 Maize	-	-	<a href="#">Click here</a>	<a href="#">Method description</a>
Bt11 Field Maize	SYN-BT011-1	Syngenta Crop Protection	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
Bt11 Maize	SYN-BT011-1	Syngenta	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
Bt11 Sweet maize	SYN-BT011-1	Syngenta Seeds	<a href="#">Click here</a>	<a href="#">Method description</a>
BT11 x GA21 Maize	SYN-BT011-1xMON-00021-9	Syngenta	<a href="#">Click here</a>	<a href="#">Method description (Bt11)</a> <a href="#">Method description (GA21)</a>
Bt11 x MIR604 maize	SYN-BT011-1xSYN-IR604-5	Syngenta	<a href="#">Click here</a>	<a href="#">Method description (Bt11)</a> <a href="#">Method description (MIR604)</a>
Bt11 x MIR604 x GA21	SYN-BT011-1xSYN-IR604-5xMON-00021-9	Syngenta	<a href="#">Click here</a>	<a href="#">Method description (Bt11)</a> <a href="#">Method description (MIR604)</a> <a href="#">Method description (GA21)</a>
Carnation (Dianthus caryophyllus L.) line 123.2.38	FLO-4Ø644-4	Florigene Ltd.	<a href="#">Click here</a>	<a href="#">Method description</a>

DP-305423-1 Soybean	DP-305423-1	Pioneer Hi-Bred	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
DP-356043-5 Soybean	DP-356043-5	Pioneer Hi-Bred	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
EH92-527-1 Potato	BPS-25271-9	BASF Plant Science Holding GmbH	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
GA21 Maize	MON-00021-9	Syngenta Crop Protection	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
GHB614 Cotton	BCS-GH-002-5	Bayer CropScience	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
GT73 Rapeseed	MON-00073-7	Monsanto	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
LLCOTTON25	ACS-GH001-3	Bayer CropScience	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
LLRICE601			<a href="#">Click here</a>	<a href="#">LLRice601 update</a>
LLRICE62 Rice	ACS-OS002-5	Bayer CropScience	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
LY038 Maize	REN-00038-3	Rehessen LLC	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
MIR 604 Maize	SYN-IR604-5	Syngenta	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
MIR604 x GA21 maize	SYN-IR604-5x MON-00021-9	Syngenta	<a href="#">Click here</a>	<a href="#">Method description (MIR604) Method description (GA21)</a>
MON 04032-6 Soybean	MON-04032-6	Monsanto	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
MON 1445 Cotton	MON-01445-2	Monsanto	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
MON 15985 Cotton	MON-15985-7	Monsanto	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
MON 15985 x MON 1445 Cotton	MON-15985-7 x MON-01445-2	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (MON 15985) Method description (MON 1445)</a>
MON 531 Cotton	MON-01445-2	Monsanto	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
MON 531 x MON 1445 Cotton	MON-00531-6 x MON-01445-2	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (MON 531) Method description (MON 1445)</a>
MON 810 maize	MON-00810-6	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (MON 810)</a>
MON 810 Maize	MON-00810-6	Monsanto	<a href="#">Click here</a>	<a href="#">Method description</a>
MON 863 x MON 810	MON-00863 x MON00810-6	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (MON 810) Method description (MON 863)</a>
MON 863 x MON 810 x NK603 Maize	MON-00863-5 x MON-00810-6 x MON-00603-6	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (MON 810) Method description (NK603) Method description (MON 863)</a>
MON 863 x NK603 Maize	MON-00863-5 x MON-00603-6	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (NK603) Method description (MON 863)</a>
MON 88913 Cotton	MON-88913-8	Monsanto	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>
MON 89034 Maize	MON-89034-3	Monsanto	<a href="#">Click here</a>	<a href="#">Method description DNA Extraction</a>

MON 89034 x MON 88017 Maize	MON-89034-3 x MON-88017-3	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (MON 89034)</a> <a href="#">Method descriptio (MON 88017)</a> <a href="#">DNA Extraction</a>
MON 89034 x NK603 Maize	MON-89034-3 x MON-00603-6	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (MON 89034)</a> <a href="#">Method description (NK603)</a>
MON 89788 Soybean	MON-89788-1	Monsanto	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
Mon863 Maize	MON-00863-5	Monsanto	<a href="#">Click here</a>	<a href="#">Method description</a>
MON88017 Maize	MON-88017-3	Monsanto	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
MON88017 x MON810 Maize	MON-88017-3xMON00810-6	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (MON 88017)</a> <a href="#">Method description (MON 810)</a>
MON89034 x 1507 x MON88017 x 59122 maize	MON-89034-3 x DAS-Ø1507-1 x MON-88017-3 x DAS-59122-7	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (MON89034)</a> <a href="#">Method description (1507)</a> <a href="#">Method description (MON88017)</a> <a href="#">Method description (59122)</a>
MON89034 x 1507 x NK603 maize	MON-89034-3 x DAS-Ø1507-1 x MONØØ603-6	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (MON89034)</a> <a href="#">Method description (1507)</a> <a href="#">Method description (NK603)</a>
Ms8 Rapeseed	ACS-BN005-8	Bayer CropScience	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
Ms8xRf3 Rapeseed	ACS-BN005-8 x ACS-BN003-6	Bayer CropScience	<a href="#">Click here</a>	<a href="#">Method description (Ms8)</a> <a href="#">Method description (Rf3)</a>
NK603 Maize	MON-00603-6	Monsanto	<a href="#">Click here</a>	<a href="#">Method description</a>
NK603 x MON 810 Maize	MON-00603-6 x MON-00810-6	Monsanto	<a href="#">Click here</a>	<a href="#">Method description (MON 810)</a> <a href="#">Method description (NK603)</a>
PT73 <i>E. coli</i> (TM) dried killed bacterial biomass	Not applicable	Ajinomoto Eurolysine SAS	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
Rf3 Rapeseed	ACS-BN003-6	Bayer CropScience	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
RUR H7 Sugar beet	KM-000H71-4	KWS SAAT AG. Monsanto	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>
T25 Maize	ACS-ZM003-2	Bayer CropScience	<a href="#">Click here</a>	<a href="#">Method description</a>
T45 Rapeseed	ACS-BN008-2	Bayer CropScience	<a href="#">Click here</a>	<a href="#">Method description</a> <a href="#">DNA Extraction</a>



## Annex IV - FVO missions regarding national GMO controls on food, feed and seeds



Period	Country	Inspection number	FVO reports and comments from national authorities (  )
04/2009	Brazil	8301/2009	<a href="#">FVO report</a> , <a href="#">Comments Brazil 1</a> , <a href="#">Comments Brazil 2</a>
03/2009	Romania	8138/2009	<a href="#">FVO report</a> , <a href="#">Comments Romania 1</a> , <a href="#">Comments Romania 2</a>
11-12/2008	China	7834/2008	<a href="#">FVO report</a> , <a href="#">Comments China 1</a> , <a href="#">Comments China 2</a>
06/2008	United States	7857/2008	<a href="#">FVO report</a> , <a href="#">Comments United States</a>
11/2007	Greece	7199/2007	<a href="#">FVO report</a> , <a href="#">Comments Greece 1</a> , <a href="#">Comments Greece 2</a>
04/2007	Romania	7186/2007	<a href="#">FVO report</a> , <a href="#">Comments Romania</a>
03/2007	Brazil	7180/2007	<a href="#">FVO report</a> , <a href="#">Comments Brazil</a>
12/2006	Argentina	8118/2006	<a href="#">FVO report</a> , <a href="#">Comments Argentina</a>
06/2006	United Kingdom	8116/2006	<a href="#">FVO report</a> , <a href="#">Comments United Kingdom</a>
05/2006	Hungary	8109/2006	<a href="#">FVO report</a> , <a href="#">Comments Hungary</a>
05/2006	Czech Republic	8110/2006	<a href="#">FVO report</a> , <a href="#">Comments Czech Republic</a>
05/2006	France	8086/2006	<a href="#">FVO report</a> , <a href="#">Comments France</a>
03/2006	Poland	8106/2006	<a href="#">FVO report</a> , <a href="#">Comments Poland</a>
03/2006	Germany	8105/2006	<a href="#">FVO report</a> , <a href="#">Comments Germany</a>
03/2006	Slovenia	8104/2006	<a href="#">FVO report</a> , <a href="#">Comments Slovenia</a>
02/2006	Belgium	8102/2006	<a href="#">FVO report</a> , <a href="#">Comments Belgium</a>
02/2006	Slovak Republic	8100/2006	<a href="#">FVO report</a> , <a href="#">Comments Slovak Republic</a>
10/2005	Portugal	7669/2005	<a href="#">FVO report</a> , <a href="#">Comments Portugal</a>
09/2005	The Netherlands	7666/2005	<a href="#">FVO report</a> , <a href="#">Comments of the Netherlands</a>
06/2005	Italy	7653/2005	<a href="#">FVO report</a> , <a href="#">Comments Italy</a>
03/2005	Spain	7632/2005	<a href="#">FVO report</a> , <a href="#">Comments Spain</a>
10/2003	United Kingdom	9249/2003	<a href="#">FVO report</a> , <a href="#">Comments United Kingdom</a>
06/2003	Austria	9141/2003	<a href="#">FVO report</a> , <a href="#">Comments Austria</a>
03/2003	Spain	9103/2003	<a href="#">FVO report</a> , <a href="#">Comments Spain</a>
04/2002	Sweden	8605/2002	<a href="#">FVO report</a> , <a href="#">Comments Sweden</a>
10/2001	Germany	3233/2001	<a href="#">FVO report</a> , <a href="#">Comments Germany</a>

Source: [http://ec.europa.eu/food/fvo/ir\\_search\\_en.cfm](http://ec.europa.eu/food/fvo/ir_search_en.cfm)

## Annex V – List of Certified Reference Materials

**Certified Reference Materials produced by the JRC-IRMM for quality assurance of GMO quantification** (as of January 2010)

Event	Unique identifier	Applicant	Certification reports 	Concentration levels 
1507 maize	DAS-Ø15Ø7-1	Pioneer Hi-Bred International Inc.	<a href="#">ERM-BF418</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a>
1507 x 59122 maize	DAS-Ø15Ø7-1xDAS-59122-7	Pioneer Hi-Bred International Inc.	<a href="#">ERM-BF418</a> <a href="#">ERM-BF424</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> <a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a>
1507 x NK603 maize	DAS-Ø15Ø7-1xMON-ØØ6Ø3-6	Pioneer Hi-Bred International Inc.	<a href="#">ERM-BF418</a> <a href="#">ERM-BF415</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> <a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a>
281-24-236 x 3006-210-23 cotton	DAS-24236-5 x DAS-21Ø23-5	Dow AgroSciences LLC	<a href="#">ERM-BF422</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a>
3272 maize	SYN-E3272-5	Syngenta Crop Protection AG	<a href="#">ERM-BF420</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a>
305423 Soybean	DP-3Ø5423-1	Pioneer Hi-Bred International Inc.	<a href="#">ERM-BF426</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a>
356043 Soybean	DP-356Ø43-5	Pioneer Hi-Bred International Inc.	<a href="#">ERM-BF425</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a>
59122 maize	DAS-59122-7	Pioneer Hi-Bred International Inc.	<a href="#">ERM-BF424</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a>
59122 x 1507 x NK603 maize	DAS-59122-7xDAS-Ø15Ø7-1xMON-ØØ6Ø3-6	Pioneer Hi-Bred International Inc.	<a href="#">ERM-BF424</a> <a href="#">ERM-BF418</a> <a href="#">ERM-BF415</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> <a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> <a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a>
59122 x NK603 maize	DAS-59122-7xMON-ØØ6Ø3-6	Pioneer Hi-Bred International Inc.	<a href="#">ERM-BF424</a> <a href="#">ERM-BF415</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> <a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a>
98140 maize	DP-Ø9814Ø-6	Pioneer Hi-Bred International Inc.	<a href="#">ERM-BF427</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a>
Bt11 maize	SYN-BTØ11-1	Syngenta Crop Protection AG	<a href="#">ERM-BF412</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a>
Bt176 maize	SYN-EV176-9	Syngenta Seeds S.A.S	<a href="#">ERM-BF411</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a>
EH92-527-1 potato	BPS-25271-9	BASF Plant Science GmbH	<a href="#">ERM-BF421</a>	<a href="#">Blank</a> , <a href="#">1</a>
GA21 maize	MON-ØØØ21-9	Monsanto	<a href="#">ERM-BF414</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a>
GA21 x MON 810 maize	MON-ØØØ21-9 x MON-ØØ81Ø-6	Monsanto	<a href="#">ERM-BF414</a> <a href="#">ERM-BF413</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a> <a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a>
GHB119 cotton	BCS-GHØØ5-8	Bayer BioScience N.V.	<a href="#">ERM-BF428</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a>
GTS 40-3-2 soybean	MON-Ø4Ø32-6	Monsanto	<a href="#">ERM-BF410</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a>
H7-1 RR Sugar Beet	KM-ØØØH71-4	KWS	<a href="#">ERM-BF419</a>	<a href="#">Blank</a> , <a href="#">1</a>

Event	Unique identifier	Applicant	Certification reports 	Concentration levels 
<u>LY038</u> x MON 810	REN-ØØØ38-3 x MON-ØØ81Ø-6	Monsanto	<a href="#">ERM-BF413</a> <a href="#">ERM-AD413</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a> <a href="#">plasmid</a>
MIR604 maize	SYN-IR6Ø4-5	Syngenta Crop Protection AG	<a href="#">ERM-BF423</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a>
MON 810 maize	MON-ØØ81Ø-6	Monsanto	<a href="#">ERM-BF413</a> <a href="#">ERM-AD413</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a> <a href="#">plasmid</a>
MON 863 maize	MON-ØØ863-5	Monsanto	<a href="#">ERM-BF416</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a>
MON 863 x MON 810 maize	MON-ØØ863-5 x MON-ØØ81Ø-6	Monsanto	<a href="#">ERM-BF417</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a>
MON 863 x MON 810 x NK603 maize	MON-ØØ863-5 x MON-ØØ81Ø-6 x MON-ØØ6Ø3-6	Monsanto	<a href="#">ERM-BF416</a> <a href="#">ERM-BF413</a> <a href="#">ERM-AD413</a> <a href="#">ERM-BF415</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> <a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a> <a href="#">plasmid</a> <a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a>
MON 863 x NK603	MON-ØØ6Ø3-6 x MON-ØØ81Ø-6	Monsanto	<a href="#">ERM-BF416</a> <a href="#">ERM-BF415</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> <a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a>
MON 88017 x MON 810 maize	MON-88Ø17-3 x MON-ØØ81Ø-6	Monsanto	<a href="#">ERM-BF413</a> <a href="#">ERM-AD413</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a> <a href="#">plasmid</a>
NK603 maize	MON-ØØ6Ø3-6	Monsanto	<a href="#">ERM-BF415</a>	<a href="#">Blank</a> , <a href="#">1</a> , <a href="#">2</a> , <a href="#">3</a> , <a href="#">4</a> , <a href="#">5</a>