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# The EU Legislation for GMOs; role and function of the Joint Research Centre and of the European Union Reference Laboratory for GM Food & Feed


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**1st International Workshop on Harmonisation of GMO Detection and Analysis in MENA Region**
**Jordan, 4-5 June 2012**

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# CONTENT

- ***EU GMO legislation (in short)***
- **European Commission and GMO detection (EURL and ENGL)**

**The Joint Research Centre (JRC) is a Directorate-General of the European Commission** under the responsibility of the European Commissioner for Science and Research.

The JRC role is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies.

# EUROPEAN COMMISSION AND GMOs (in short)

- **DG Health and Consumer Protection (SANCO)**
  - EU legislation on GMOs, including GMO approvals (for Cultivation and/or for Food Feed Processing) and GMO controls;
  - International Agreements, Standardisation etc.

- **DG Joint Research Centre (JRC)**
  - Scientific support to implementation of EU legislation on GMOs;
  - GMO detection methods;
  - Socio-Economic Studies
  - Certified Reference Materials
  - Capacity-building for GMO analysis

## EU Legislation on GMOs – some key texts …

- **Regulation (EC) No 1829/2003 on genetically modified food and feed**
- **Regulation (EC) No 882/2004 on official controls** performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
- **Regulation (EU) No 619/2011** on official controls of feed about presence of GM material for which an authorisation is pending **(so-called Low Level Presence (LLP) Regulation)**

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# Regulation (EC) No 1829/2003 on genetically modified food and feed

- **Mandatory approval of GM Food / Feed** before placing on the EU market: a GM food/feed can be placed on the EU market only once it is covered by an authorisation granted according to Regulation (EC) No 1829/2003 (the EU authorisation process is based on an independent EU risk assessment carried out by the European Food Safety Authority - EFSA);

- **A validated quantitative event-specific method and certified reference materials are required;**

- **Certified Reference materials need to be generally available**

- **Mandatory labelling of GM Food / Feed** once approved for placing on the market (incl. labelling threshold of 0.9% to exempt from GM labelling the adventitious or technically unavoidable presence of GM material in food or feed);

- **Zero tolerance for non-authorised GMOs.**

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# Regulation (EC) No 1829/2003 on genetically modified food and feed

- **The EU Reference Laboratory for GM Food and Feed is responsible for validation** of the GMO detection methods and **is assisted by** National Reference Laboratories, as members of the Consortium referred to as **the "European Network of GMO laboratories" (ENGL)**

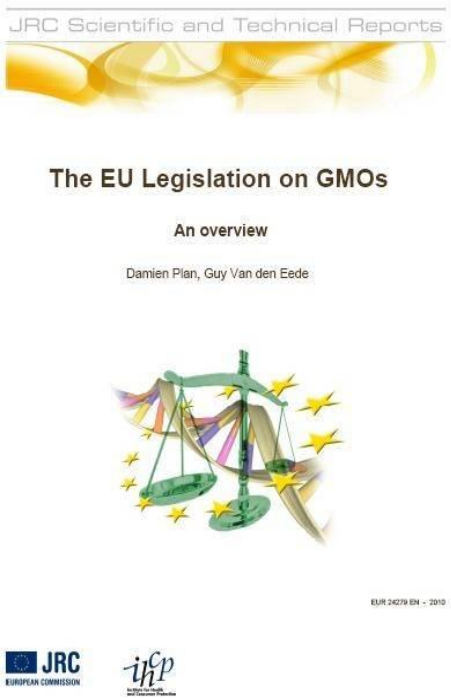
- **The EU-RL GMFF is the Commission Joint Research Centre (JRC)**

# Information on EU GMO approvals available on EU GM Food Feed register

*http://ec.europa.eu/food/dyna/gm_register/index_en.cfm*

**As of January 2012, 42 GMOs approved for food/feed use in the EU**

(incl. 2 for cultivation): 26 maize, 8 cotton, 3 soya, 3 oilseed rape, 1 starch potato, 1 sugarbeet





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# CONTENT

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EURL
European Union Reference Laboratory
for GM Food & Feed

## The European Union Reference Laboratory for GMOs: two legal mandates defined in two EU regulations

1) European Union Reference Laboratory under **Regulation (EC) No 1829/2003** on GM food and feed

2) European Union Reference Laboratory under **Regulation (EC) No 882/2004** on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules

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# 1st mandate of the EU-RL under Reg. (EC) No 1829/2003

**Core activity:** validation of GMO detection methods as part of the EU GMO approval process under Reg. (EC) 1829/2003 : > 60 methods have been validated by the EU-RL GMFF since April 2004

Provision of **control samples**

Provision of **guidance documents**

Role in **dispute settlements**

# 2nd mandate of the EU-RL under Reg. (EC) No 882/2004

Providing National Reference Laboratories (NRLs) in the EU with **reference analytical methods**

**Role in emergency situations (unauthorised GMOs on EU market)**

Coordinating application of the methods by organising **comparative testing** and by ensuring an appropriate follow-up

**Conducting training** courses for the benefit of staff from NRLs in the EU and of laboratories responsible for analysing feed and food **in third countries.**

Note: **EU-RL and NRLs need to be accredited according to ISO 17025**

# ENGL and EU-RL – two European partners in GMO detection

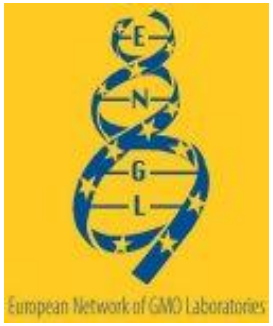
**EU-RL - the European Union Reference Laboratory**

**1 central lab hosted by the European Commission JRC**

**ENGL – the European Network of GMO Laboratories**

**97 labs hosted by 27 EU Member States (+ 4 non-EU)**

EURL
European Union Reference Laboratory
for GM Food & Feed




All EU-RL and ENGL activities are based on EU GMO legislation

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# The European Network of GMO Laboratories (ENGL)

**Operational since December 2002 under the JRC chairmanship**

**Members are appointed by Competent Authorities: 97 laboratories** from 27 EU Member States (+ Norway, Switzerland, Turkey, Croatia) + observers from non-EU countries

**Working Procedures laid down in a ENGL Consortium Agreement** signed by all ENGL members

**Two Plenary Meetings (and two Steering Committee meetings) a year + Different WGs established by the ENGL Steering Committee** on topics like Unauthorised GMOs, Method Verification….

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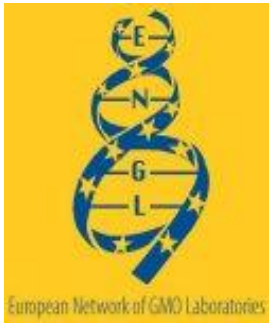
# The European Network of GMO Laboratories (ENGL) today :

## A network of 96 labs chaired by the European Commission JRC

## All ENGL members sign a Consortium Agreement incl.

- Objectives (art. 1)
- Membership (art. 2)
- Work Programme (art. 3)
- Responsibilities of Parties (art.4)
- Plenary Meetings and Working Groups (art. 5)
- Steering Committee (art. 6)
- Secretariat (art.7)
- Reports (art.8)
- Confidentiality (art. 9)
- Liability (art. 10) …



## ENGL Objectives (art. 1):

- **Support the EU Reference Laboratory** defined in Regulations (EC) No 1829/2003 and No 882/2004

- **Improve at European level harmonization** and standardisation of methods for the identification and quantification of GMOs

- **Act as a network of scientific excellence** for the detection of GMOs and related scientific issues

- **Provide information to worldwide stakeholders** through international relations and active communication policy

## ENGL Membership (art. 2)

- **ENGL members are designated by National Competent Authorities operating under legislation related to GMOs** in the EU Member States and/or EEA + EFTA and/or EU Candidate Countries

- New membership applications must be made in writing to the ENGL President who shall refer them to the ENGL Steering Committee

- The list of ENGL members is made publicly available and is regularly updated

- **ENGL observer status is also available for countries non-eligible to full membership**

## ENGL bodies

- **Plenary:** all ENGL members (+ observers) - 2 meetings a year to discuss scientific issues and review progress of the Work Program

- **Steering Committee:** composed of one ENGL member per Member State - 2 meetings a year to manage the strategic operations of the ENGL, approve the annual working plan, install the appropriate working groups and monitor execution

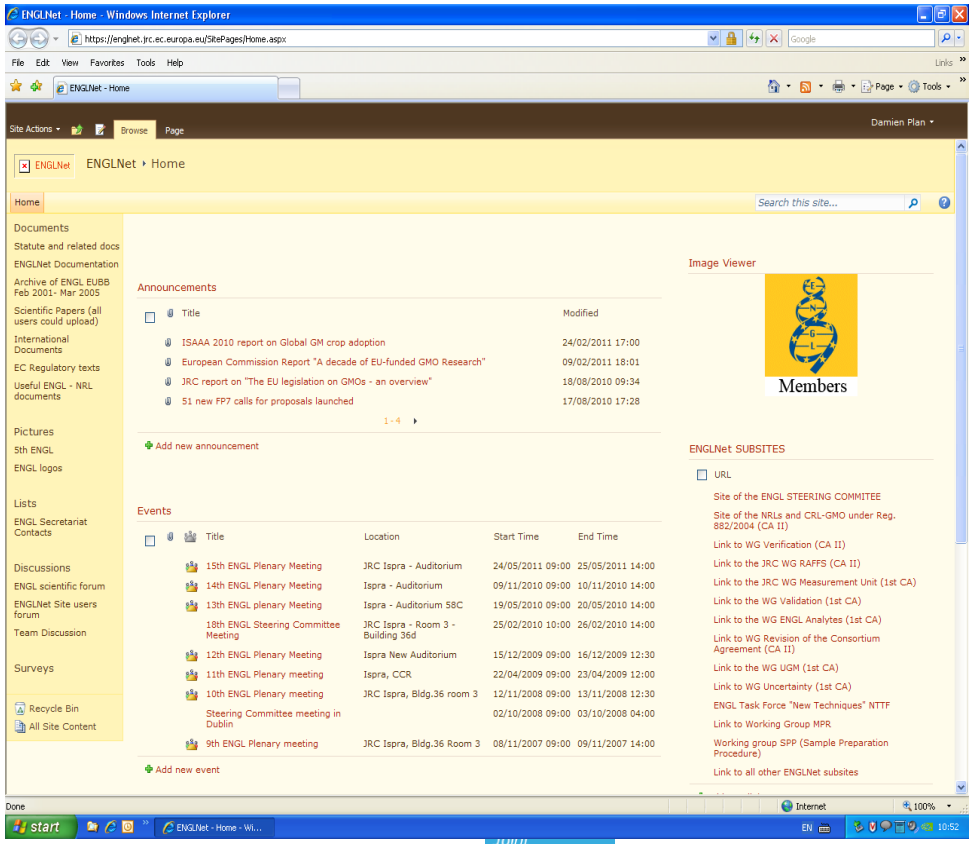
- **Chairmanship and Secretariat :** European Commission JRC

- **Working Groups:** mandate approved by the ENGL Steering Committee (usually 1-2 year work programme and +/- 10 ENGL members)

In 2011, 4 ENGL WGs : Unapproved GMOs (UGM-WG), Method Verification (MV-WG), Method Performance Requirements (MPR-WG), Sample Preparation Procedure (SPP-WG)

# ENGL Internal Communication : ENGLNet

- Based on a Share Point system with access restricted to ENGL members
- Structure based on ENGL structure (eg subsites for meetings, Steering Committee, WGs)



# More than 60 GMO detection methods validated by the EU-RL/ENGL publicly available at *http://gmo-crl.jrc.ec.europa.eu/*

Legal Notice Privacy statement English (EN)

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JOINT RESEARCH CENTRE

European Union Reference Laboratory for GM Food and Feed

European Commission > JRC > IHCP > EU-RL GMFF

Home | Legal basis | Guidance documents | Status of dossiers | Methods database | Capacity building | ENGL | Contacts

## Status of dossiers

**EU-RL GMFF validation process**

The following table lists the EU-RL GMFF validation process carried out within the frame of the Regulation (EC) No 1829/2003, providing details on the current status of the validation process.

The following links provide information about additional validation studies conducted by the EU-RL GMFF in support to notifications submitted according to Directive 2001/18/EC, about GMO authorised in the EU, notifications submitted according to Directive 2001/18/EC and opinions issued by the European Food Safety Authority (EFSA).

Detection methods validated in support to notifications submitted under Directive 2001/18/EC

European Commission information on GM authorizations, legislation and alike

Information about the notifications submitted in the context of Directive 2001/18/EC

Opinions of the EFSA Scientific Panel on Genetically Modified Organisms

Last updated 29/03/2012 Watch this page for changes

| Event | Crop | Unique identifier | Applicant | Status/Progress | Reports | Validated Method |
|---|---|---|---|---|---|---|
| Bt10 | maize | Not applicable | Not applicable | Validation completed | Validation report Published on: 13/07/2005 | Validated method Published on: 13/07/2005 |
| Bt11 | sweet maize | SYN-BT011-1 | Syngenta Crop Protection AG | Validation completed | Validation report Published on: 05/08/2004 | Validated method Published on: 05/08/2004 |
| NK603 | maize | MON-00603-6 | Monsanto Company | Validation completed | Validation report Published on: 10/01/2005<br>Validation report Published on: 30/01/2008 | Validated method Published on: 10/01/2005 |
| GA21 | maize | MON-00021-9 | Monsanto Company | Validation completed | Validation report Published on: 17/01/2005 | Validated method Published on: 17/01/2005 |
| Mon863 | maize | MON-00863-5 | Monsanto Company | Validation completed | Validation report Published on: | Validated method Published on: 16/02/2005 |

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# Various guidance documents developed by the EURL/ENGL publicly available at *http://gmo-crl.jrc.ec.europa.eu/*

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JOINT RESEARCH CENTRE

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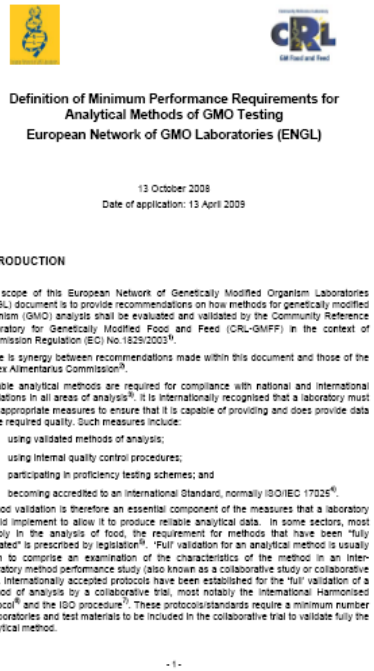
## Guidance documents

| Title | Date inserted / modified | Download |
|---|---|---|
| Overview on the detection, interpretation and reporting on the presence of unauthorised genetically modified materials | 23/03/2012 | |
| Technical guidance document from the European Union Reference Laboratory for Genetically Modified Food and Feed on the implementation of Commission Regulation (EU) NO 619/2011 | 01/09/2011 | |
| Verification of analytical methods for GMO testing when implementing interlaboratory validated methods | 22/07/2011 | |
| Explanatory notes to applicants (Reg. EC No. 1981/2006) | 13/04/2010 | |
| Definition of minimum performance requirements for analytical methods of GMO testing | 13/10/2008 | |
| Explanatory notes to applicants (Reg. EC No. 641/2004) | 13/10/2008 | |



**Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing**

**European Network of GMO Laboratories (ENGL)**

13 October 2008

Date of application: 13 April 2009

**INTRODUCTION**

The scope of this European Network of Genetically Modified Organism Laboratories (ENGL) document is to provide recommendations on how methods for genetically modified organism (GMO) analysis shall be evaluated and validated by the Community Reference Laboratory for Genetically Modified Food and Feed (CRL-GMFF) in the context of Commission Regulation (EC) No.1829/2003[1].

There is synergy between recommendations made within this document and those of the Codex Alimentarius Commission[2].

Reliable analytical methods are required for compliance with national and international regulations in all areas of analysis[3]. It is internationally recognised that a laboratory must take appropriate measures to ensure that it is capable of providing and does provide data of the required quality. Such measures include:

- using validated methods of analysis;
- using internal quality control procedures;
- participating in proficiency testing schemes; and
- becoming accredited to an international Standard, normally ISO/IEC 17025[4].

Method validation is therefore an essential component of the measures that a laboratory should implement to allow it to produce reliable analytical data. In some sectors, most notably in the analysis of food, the requirement for methods that have been "fully validated" is prescribed by legislation[5]. 'Full' validation for an analytical method is usually taken to comprise an examination of the characteristics of the method in an inter-laboratory method performance study (also known as a collaborative study or collaborative trial). Internationally accepted protocols have been established for the 'full' validation of a method of analysis by a collaborative trial, most notably the International Harmonised Protocol[6] and the ISO procedure[7]. These protocols/standards require a minimum number of laboratories and test materials to be included in the collaborative trial to validate fully the analytical method.



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## Concept of “Reference Methods"

- Not strictly defined in EU GMO legislation

- ENGL & EU-RL GMFF criteria:

1: DNA-based detection methods (Recommendation EC/2004/787) - Polymerase Chain Reaction (PCR) based methods

2: Validated through collaborative trial according to the principles of and in compliance with ISO 5725 standard and/or the IUPAC guidelines

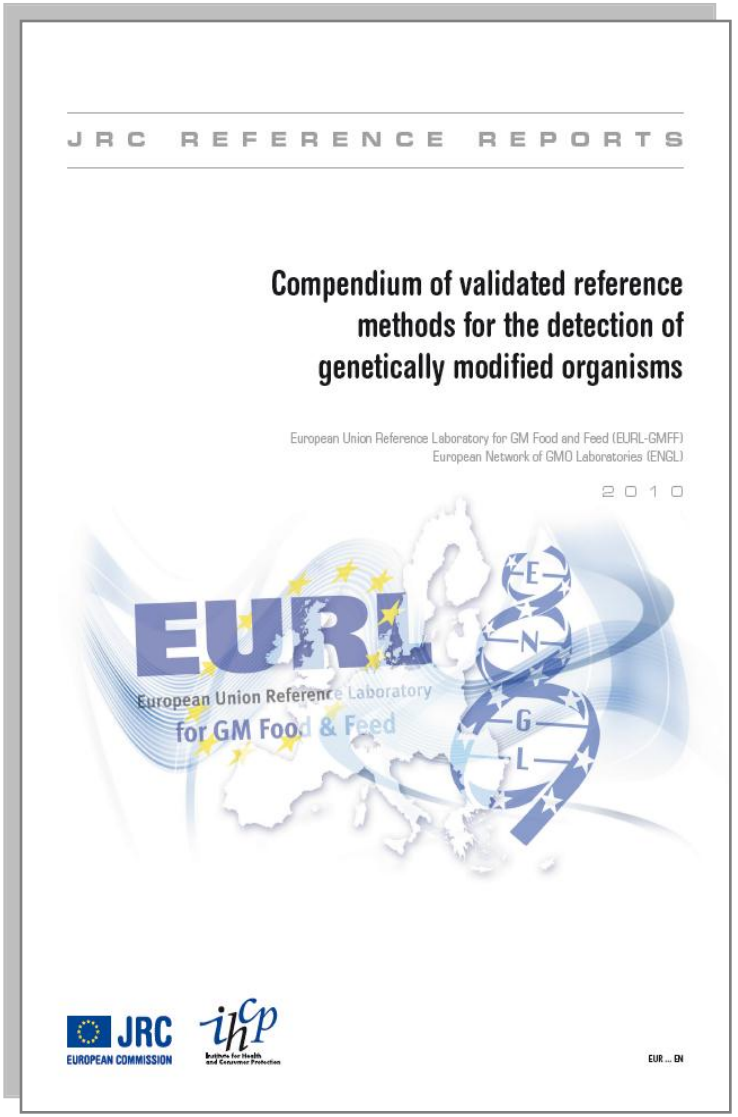
**EURL-ENGL Report published in November 2010 (updated in April 2011):**

## Compendium of validated reference methods for the detection of Genetically Modified Organisms

**Searchable method database GMOMethods** (based on compendium) **on-line since May 2011** *at http://gmo-crl.jrc.ec.europa.eu/*

iPad Application freely available

Both Compendium and Database linked to BCH

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# GMO detection PCR Methods:

***Chapter 1: Quantitative GMO detection PCR methods (48)***

*Maize quantitative PCR methods (20)*
*Soybean quantitative PCR methods (9)*
*Cotton quantitative PCR methods(8)*
*Oilseed rape quantitative PCR methods (4)*
*Potato quantitative PCR methods (1)*
*Rice quantitative PCR methods (1)*
*Sugar beet quantitative PCR methods (1)*
*Element- and Taxon-specific quantitative PCR methods (4)*

***Chapter 2: Qualitative GMO detection PCR methods (31)***

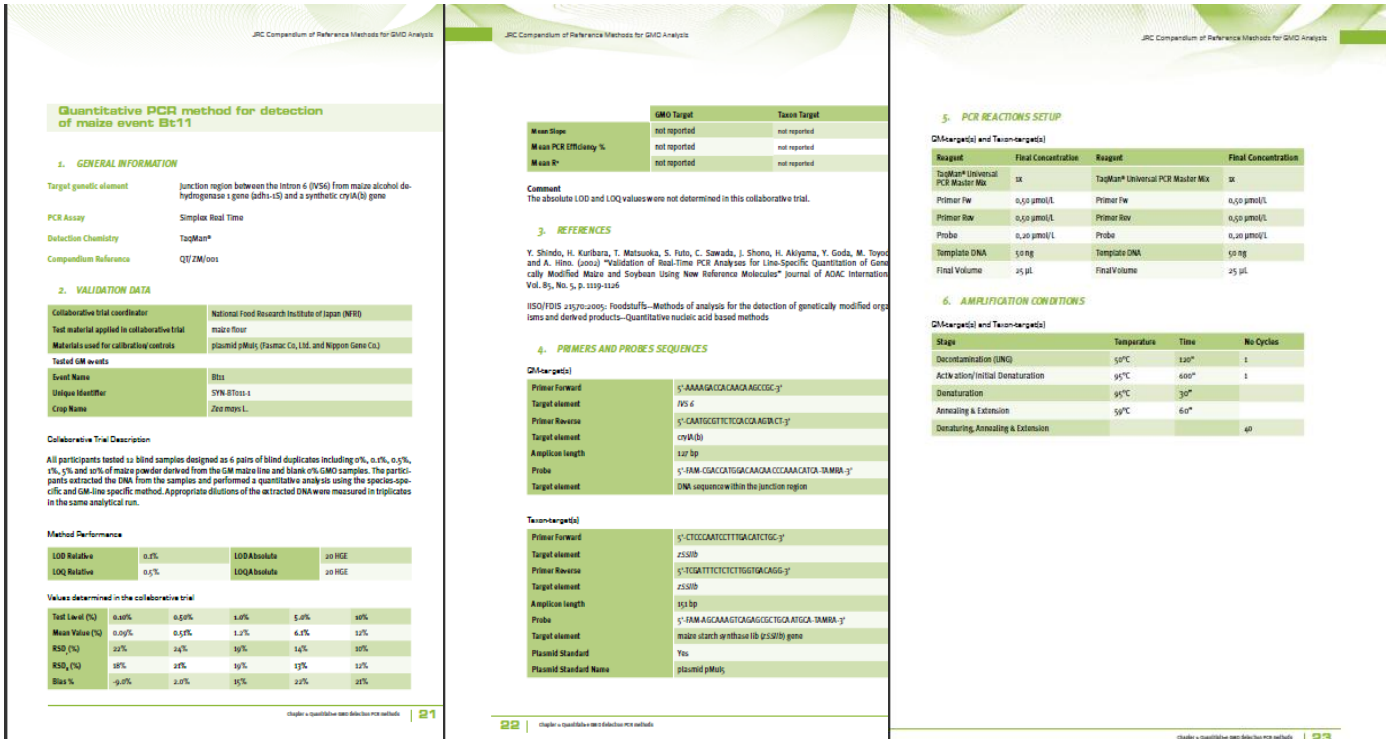
*Element-specific qualitative PCR methods (15)*
*Construct-specific qualitative PCR methods (8)*
*Event-specific qualitative PCR methods (2)*
*Taxon-specific qualitative PCR methods (6)*

# Quantitative GMO detection PCR Methods:

- Type: Event-specific (EU-RL GMFF), Element- & Taxon-specific methods

- Method Performance Parameters:

Trueness & Bias
Reproducibility
Repeatability
PCR efficiency,
LOD, LOQ



JRC Compendium of Reference Methods for GMO Analysis

Quantitative PCR method for detection of maize event Bt11

1. GENERAL INFORMATION

| | |
|---|---|
| Target genetic element | Junction region between the intron 6 (IVS6) from maize alcohol dehydrogenase 1 gene (adh1-1S) and a synthetic cryIA(b) gene |
| PCR Assay | Simplex Real Time |
| Detection Chemistry | TaqMan® |
| Compendium Reference | QT/ZM/001 |

2. VALIDATION DATA

| | |
|---|---|
| Collaborative trial coordinator | National Food Research Institute of Japan (NFRI) |
| Test material applied in collaborative trial | maize flour |
| Materials used for calibration/controls | plasmid pMul5 (Fasmac Co, Ltd. and Nippon Gene Co.) |

Tested GM events

| | |
|---|---|
| Event Name | Bt11 |
| Unique Identifier | SYN-BT011-1 |
| Crop Name | Zea mays L. |

Collaborative Trial Description

All participants tested 12 blind samples designed as 6 pairs of blind duplicates including 0%, 0.1%, 0.5%, 1%, 5% and 10% of maize powder derived from the GM maize line and blank 0% GMO samples. The participants extracted the DNA from the samples and performed a quantitative analysis using the species-specific and GM-line specific method. Appropriate dilutions of the extracted DNA were measured in triplicates in the same analytical run.

Method Performance

| | | | |
|---|---|---|---|
| LOD Relative | 0.1% | LOD Absolute | 20 HGE |
| LOQ Relative | 0.5% | LOQ Absolute | 20 HGE |

Values determined in the collaborative trial

| Test Level (%) | 0.10% | 0.50% | 1.0% | 5.0% | 10% |
|---|---|---|---|---|---|
| Mean Value (%) | 0.09% | 0.51% | 1.2% | 6.1% | 12% |
| RSD$_r$ (%) | 22% | 24% | 19% | 14% | 10% |
| RSD$_R$ (%) | 38% | 28% | 19% | 13% | 12% |
| Bias % | -9.0% | 2.0% | 15% | 22% | 21% |

21

| | GMO Target | Taxon Target |
|---|---|---|
| Mean Slope | not reported | not reported |
| Mean PCR Efficiency % | not reported | not reported |
| Mean R² | not reported | not reported |

Comment
The absolute LOD and LOQ values were not determined in this collaborative trial.

3. REFERENCES

Y. Shindo, H. Kuribara, T. Matsuoka, S. Futo, C. Sawada, J. Shono, H. Akiyama, Y. Goda, M. Toyoc and A. Hino. (2002) "Validation of Real-Time PCR Analyses for Line-Specific Quantitation of Gene cally Modified Maize and Soybean Using New Reference Molecules" Journal of AOAC Internation Vol. 85, No. 5, p. 1119-1126

ISO/FDIS 21570:2005: Foodstuffs--Methods of analysis for the detection of genetically modified orga isms and derived products--Quantitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

QM-target(s)

| | |
|---|---|
| Primer Forward | 5'-AAAAGACCACAACAAGCCGC-3' |
| Target element | IVS 6 |
| Primer Reverse | 5'-CAATGCGTTCTCCACCAAGTACT-3' |
| Target element | cryIA(b) |
| Amplicon length | 127 bp |
| Probe | 5'-FAM-CGACCATGGACAACAACCCAAACATCA-TAMRA-3' |
| Target element | DNA sequence within the junction region |

Taxon-target(s)

| | |
|---|---|
| Primer Forward | 5'-CTCCCAATCCTTTGACATCTGC-3' |
| Target element | zSSIIb |
| Primer Reverse | 5'-TCGATTTCTCTCTTGGTGACAGG-3' |
| Target element | zSSIIb |
| Amplicon length | 153 bp |
| Probe | 5'-FAM-AGCAAAGTCAGAGCGCTGCATGCA-TAMRA-3' |
| Target element | maize starch synthase IIb (zSSIIb) gene |
| Plasmid Standard | Yes |
| Plasmid Standard Name | plasmid pMul5 |

22

5. PCR REACTIONS SETUP

QM-target(s) and Taxon-target(s)

| Reagent | Final Concentration | Reagent | Final Concentration |
|---|---|---|---|
| TaqMan® Universal PCR Master Mix | 1x | TaqMan® Universal PCR Master Mix | 1x |
| Primer Fw | 0,50 µmol/L | Primer Fw | 0,50 µmol/L |
| Primer Rev | 0,50 µmol/L | Primer Rev | 0,50 µmol/L |
| Probe | 0,20 µmol/L | Probe | 0,20 µmol/L |
| Template DNA | 50 ng | Template DNA | 50 ng |
| Final Volume | 25 µL | Final Volume | 25 µL |

6. AMPLIFICATION CONDITIONS

QM-target(s) and Taxon-target(s)

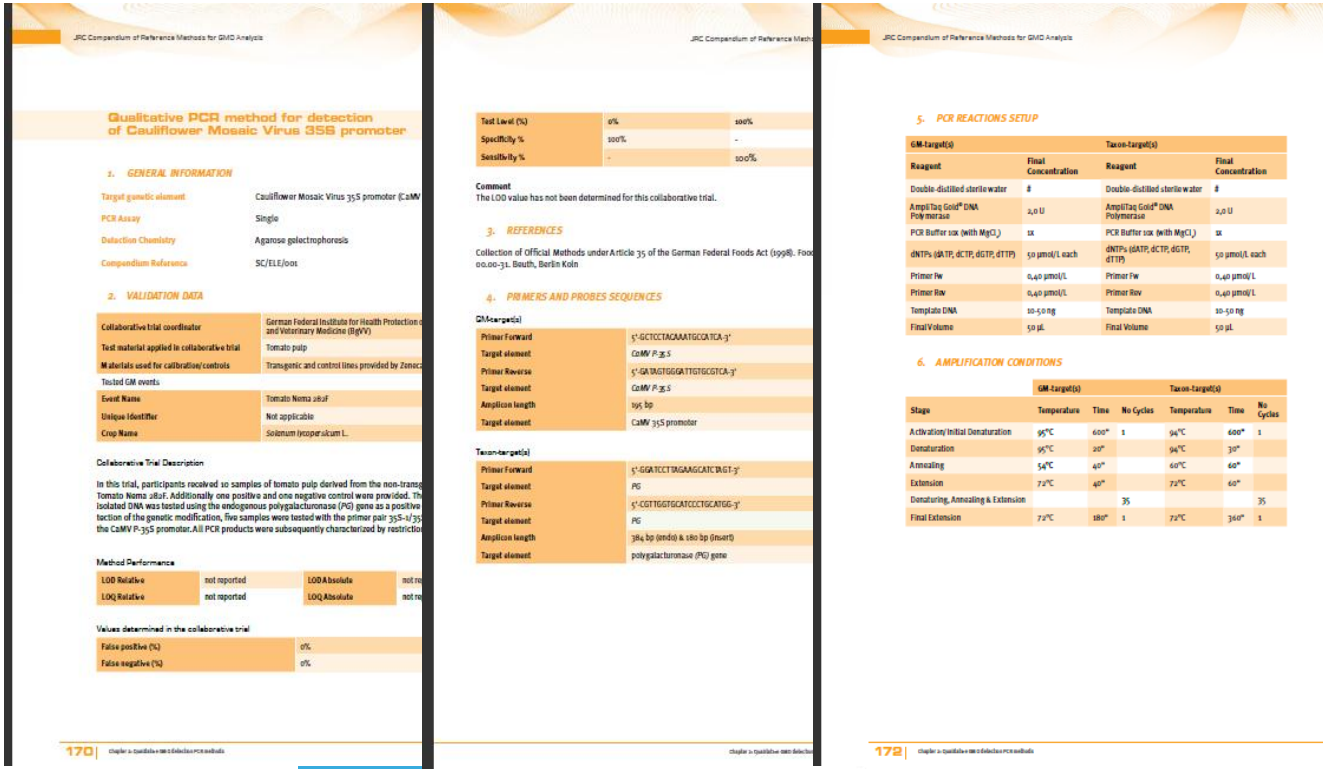
| Stage | Temperature | Time | No Cycles |
|---|---|---|---|
| Decontamination (UNG) | 50°C | 120" | 1 |
| Activation/Initial Denaturation | 95°C | 600" | 1 |
| Denaturation | 95°C | 30" | |
| Annealing & Extension | 59°C | 60" | |
| Denaturing, Annealing & Extension | | | 40 |

# Qualitative GMO detection PCR Methods (screening)

- Type: Element-, Construct-, Event-, & Taxon-specific methods

- Method Performance Parameters:

False positive/
negative results
LOD



JRC Compendium of Reference Methods for GMO Analysis

Qualitative PCR method for detection of Cauliflower Mosaic Virus 35S promoter

1. GENERAL INFORMATION

| | |
|---|---|
| Target genetic element | Cauliflower Mosaic Virus 35S promoter (CaMV |
| PCR Assay | Single |
| Detection Chemistry | Agarose gelectrophoresis |
| Compendium Reference | SC/ELE/001 |

2. VALIDATION DATA

| | |
|---|---|
| Collaborative trial coordinator | German Federal Institute for Health Protection and Veterinary Medicine (BgVV) |
| Test material applied in collaborative trial | Tomato pulp |
| Materials used for calibration/controls | Transgenic and control lines provided by Zeneca |
| Tested GM events | |
| Event Name | Tomato Nema 28oF |
| Unique Identifier | Not applicable |
| Crop Name | Solanum lycopersicum L. |

Collaborative Trial Description

In this trial, participants received 10 samples of tomato pulp derived from the non-transg Tomato Nema 28oF. Additionally one positive and one negative control were provided. Th isolated DNA was tested using the endogenous polygalacturonase (PG) gene as a positive tection of the genetic modification, five samples were tested with the primer pair 35S-1/35 the CaMV P-35S promoter. All PCR products were subsequently characterized by restriction

Method Performance

| | | | |
|---|---|---|---|
| LOD Relative | not reported | LOD Absolute | not re |
| LOQ Relative | not reported | LOQ Absolute | not re |

Values determined in the collaborative trial

| | |
|---|---|
| False positive (%) | 0% |
| False negative (%) | 0% |

170 | Chapter 2: Qualitative GMO detection PCR methods

| | | |
|---|---|---|
| Test Level (%) | 0% | 100% |
| Specificity % | 100% | - |
| Sensitivity % | - | 100% |

Comment
The LOD value has not been determined for this collaborative trial.

3. REFERENCES

Collection of Official Methods under Article 35 of the German Federal Foods Act (1998). Foo 00.00-31. Beuth, Berlin Koln

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

| | |
|---|---|
| Primer Forward | 5'-GCTCCTACAAATGCCATCA-3' |
| Target element | CaMV P-35S |
| Primer Reverse | 5'-GATAGTGGGATTGTGCGTCA-3' |
| Target element | CaMV P-35S |
| Amplicon length | 195 bp |
| Target element | CaMV 35S promoter |

Taxon-target(s)

| | |
|---|---|
| Primer Forward | 5'-GGATCCTAGAAGCATCTAGT-3' |
| Target element | PG |
| Primer Reverse | 5'-CGTTGGTGCATCCCTGCATGG-3' |
| Target element | PG |
| Amplicon length | 384 bp (endo) & 180 bp (insert) |
| Target element | polygalacturonase (PG) gene |

JRC Compendium of Reference Methods for GMO Analysis

5. PCR REACTIONS SETUP

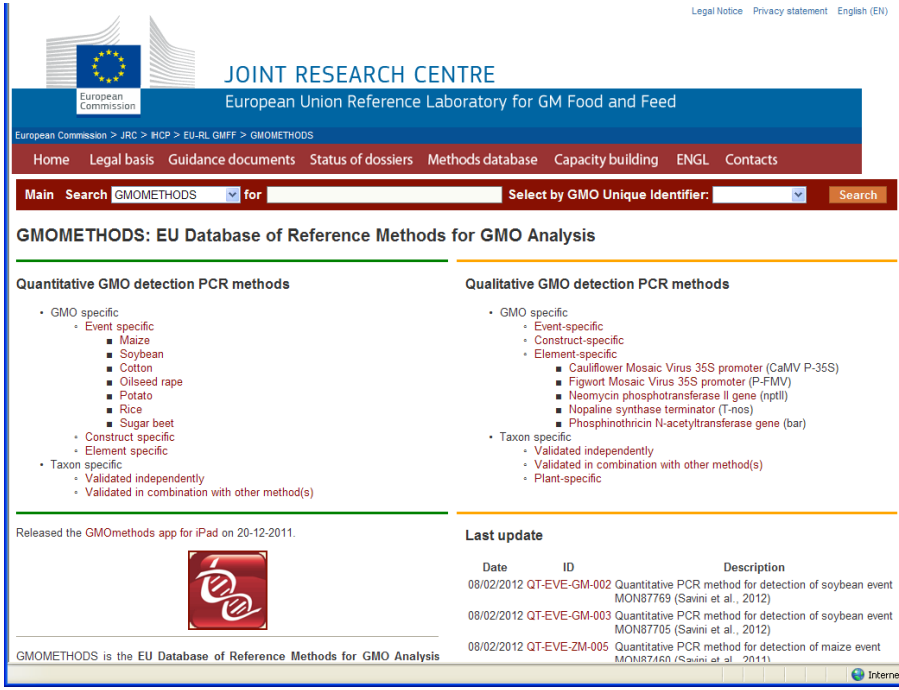
| GM-target(s) | | Taxon-target(s) | |
|---|---|---|---|
| Reagent | Final Concentration | Reagent | Final Concentration |
| Double-distilled sterile water | # | Double-distilled sterile water | # |
| AmpliTaq Gold® DNA Polymerase | 2,0 U | AmpliTaq Gold® DNA Polymerase | 2,0 U |
| PCR Buffer 10x (with $MgCl_2$) | 1x | PCR Buffer 10x (with $MgCl_2$) | 1x |
| dNTPs (dATP, dCTP, dGTP, dTTP) | 50 µmol/L each | dNTPs (dATP, dCTP, dGTP, dTTP) | 50 µmol/L each |
| Primer Fw | 0,40 µmol/L | Primer Fw | 0,40 µmol/L |
| Primer Rev | 0,40 µmol/L | Primer Rev | 0,40 µmol/L |
| Template DNA | 10-50 ng | Template DNA | 10-50 ng |
| Final Volume | 50 µL | Final Volume | 50 µL |

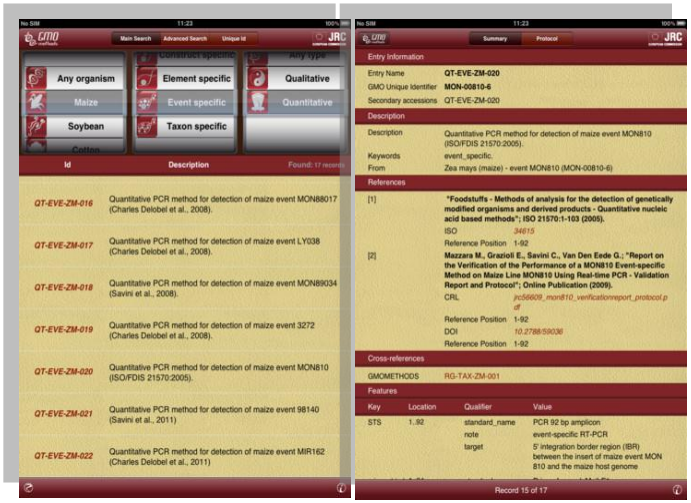
6. AMPLIFICATION CONDITIONS

| | GM-target(s) | | | Taxon-target(s) | | |
|---|---|---|---|---|---|---|
| Stage | Temperature | Time | No Cycles | Temperature | Time | No Cycles |
| Activation/Initial Denaturation | 95°C | 600" | 1 | 94°C | 600" | 1 |
| Denaturation | 95°C | 20" | | 94°C | 30" | |
| Annealing | 54°C | 40" | | 60°C | 60" | |
| Extension | 72°C | 40" | | 72°C | 60" | |
| Denaturing, Annealing & Extension | | | 35 | | | 35 |
| Final Extension | 72°C | 180" | 1 | 72°C | 360" | 1 |

172 | Chapter 2: Qualitative GMO detection PCR methods

**GMOMETHODS:** EU Database of Reference Methods for GMO Analysis
*http://gmo-crl.jrc.ec.europa.eu/gmomethods/*




**GMOmethods app for iPad**
released on 20-12-2011
*http://itunes.apple.com/us/app/gmomethods/id481988894?mt=8*

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# Thank you for your attention