

State-of-play – available **methods** for the detection, identification, and quantification of GM-material in food and feed

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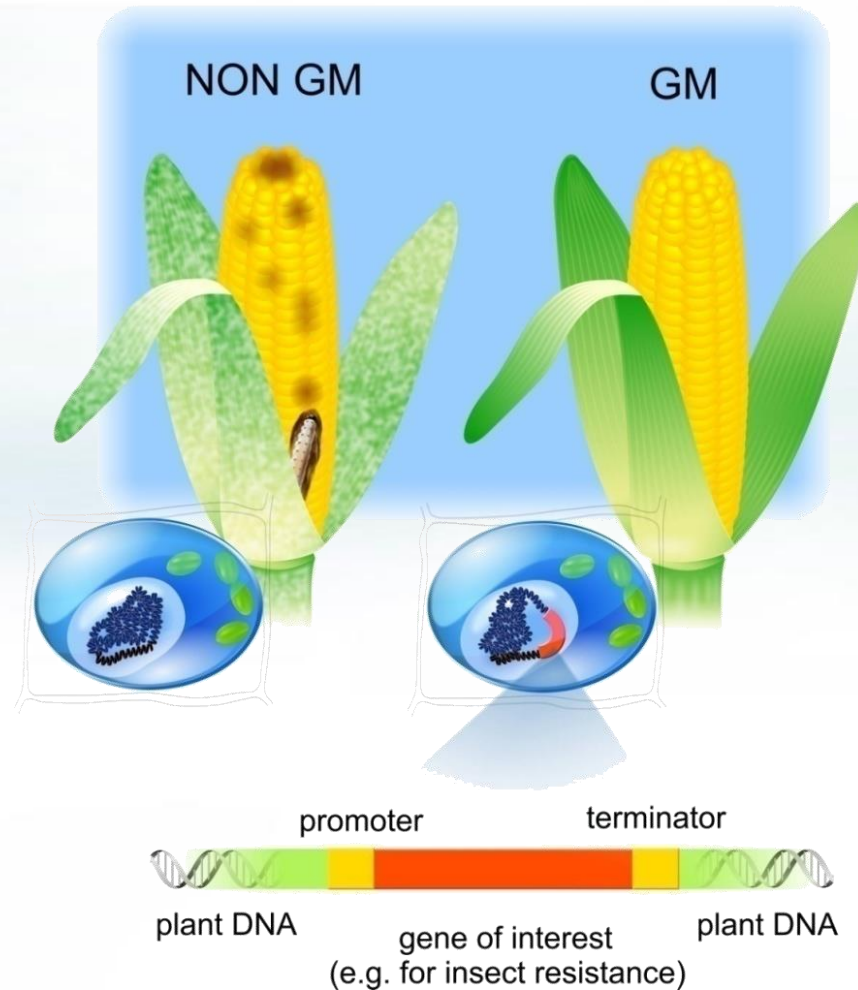


Galanthus nivalis L.

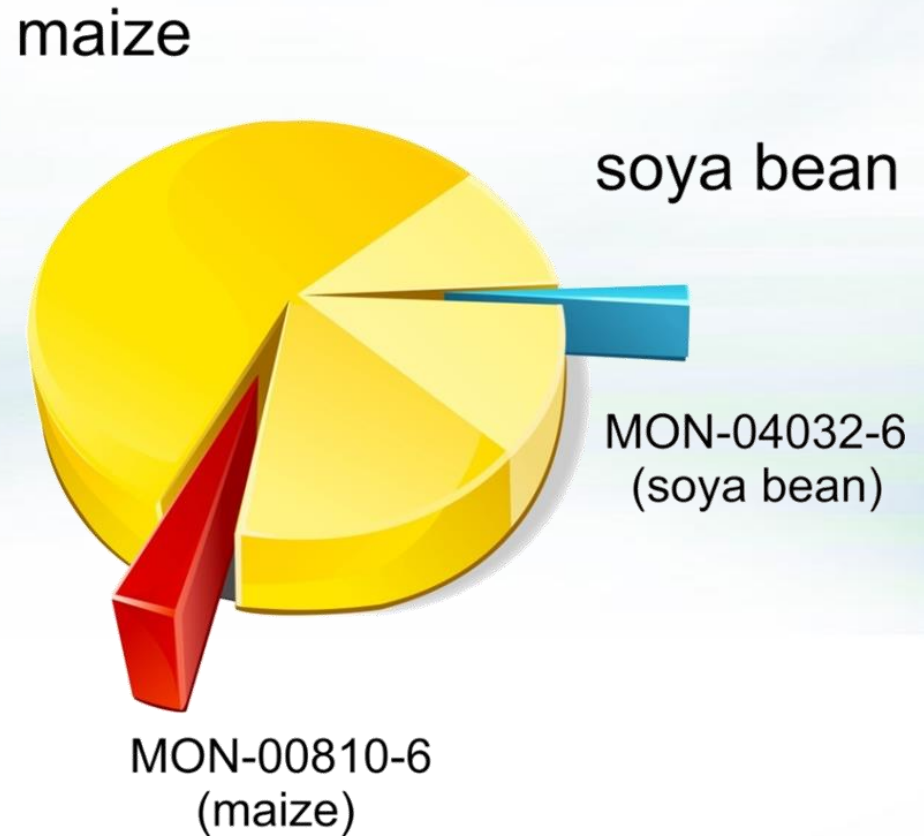
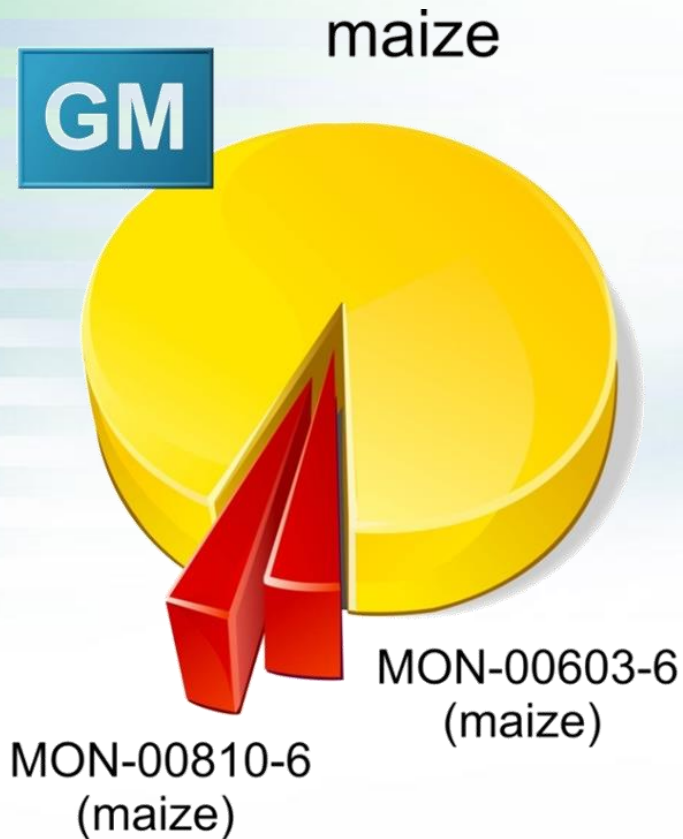


More than 200 varieties

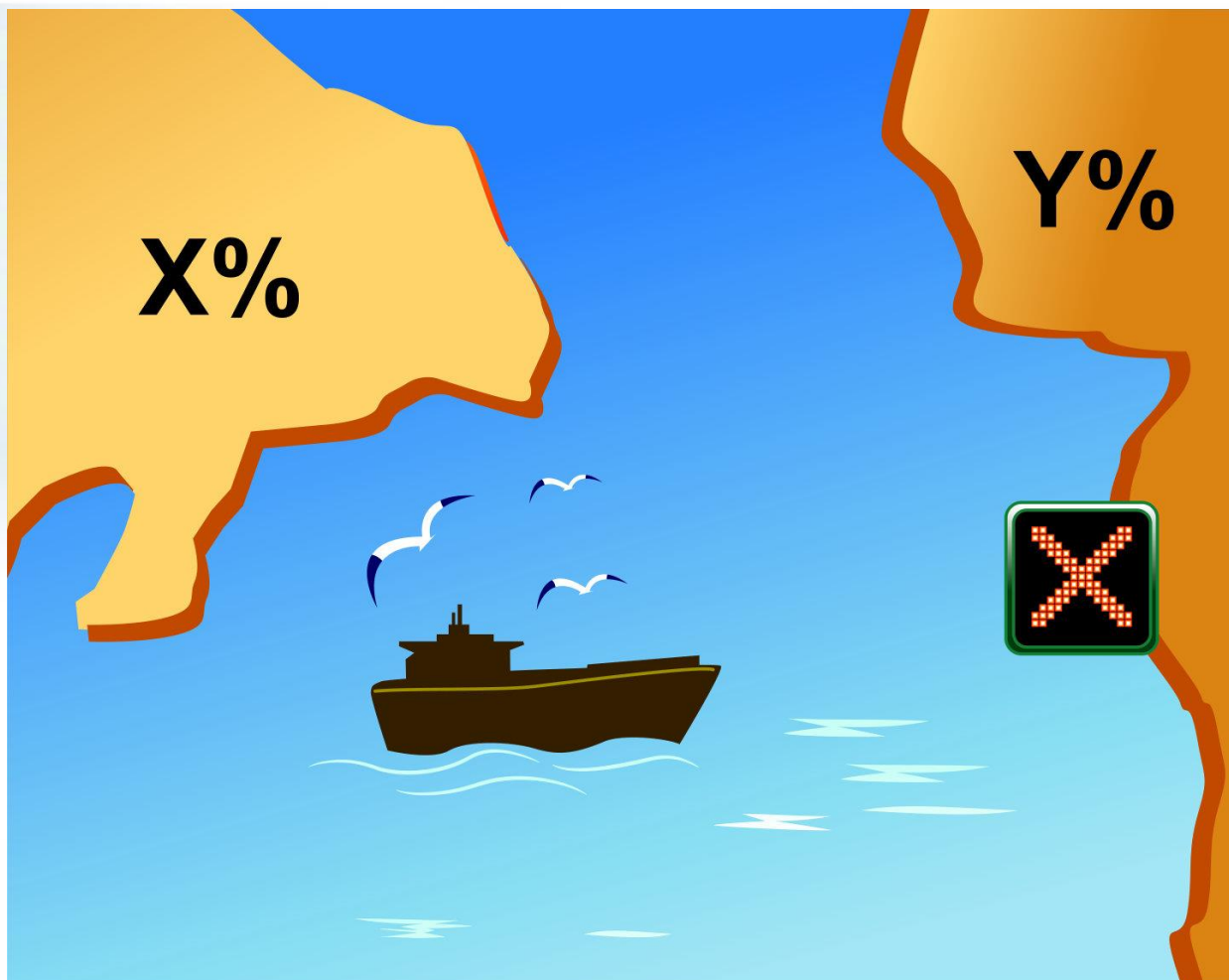
GMOs



Different labeling requirements



Harmonization of GMO detection



Harmonization of GMO detection



Needs for methods

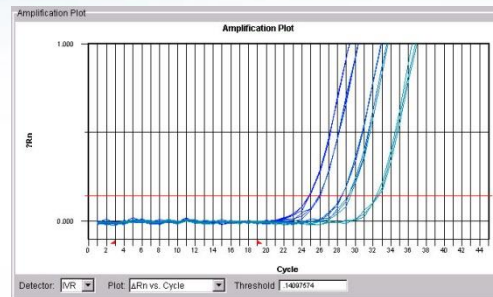
- Qualitative (presence/absence)
- Quantitative (treshold)

Methods

- Immunological



- Molecular



Standards

- ISO/CEN



- EN ISO/IEC 24276:2006 – General requirements and definitions (International Organization for Standardization 2006)
- EN ISO/IEC 21571:2005 – Nucleic acid extraction (International Organization for Standardization 2005c)
- EN ISO/IEC 21569:2005 – Qualitative nucleic acid-based methods (International Organization for Standardization 2005a)
- EN ISO/IEC 21570:2005 – Quantitative nucleic acid-based methods (International Organization for Standardization 2005b)

Standards

- Codex



- Codex Committee On Methods Of Analysis And Sampling (2010) Guidelines On Performance Criteria And Validation Of Methods For Detection, Identification And Quantification Of Specific DNA Sequences And Specific Proteins In Foods. CAC/GL 74–2010. Rome: Codex alimentarius commission – WHO.

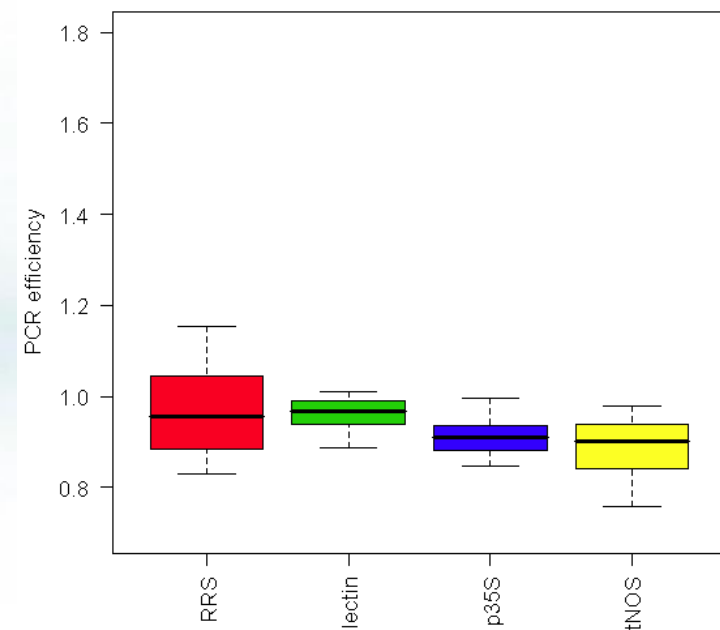
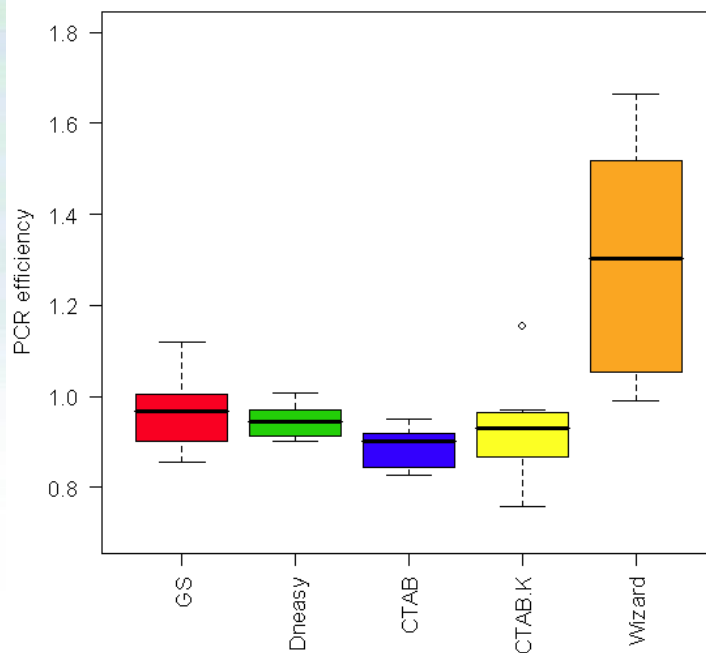
Modular approach

Modules validated separately

Moduls:

- Method for isolation
- Method for PCR

Methods for isolation

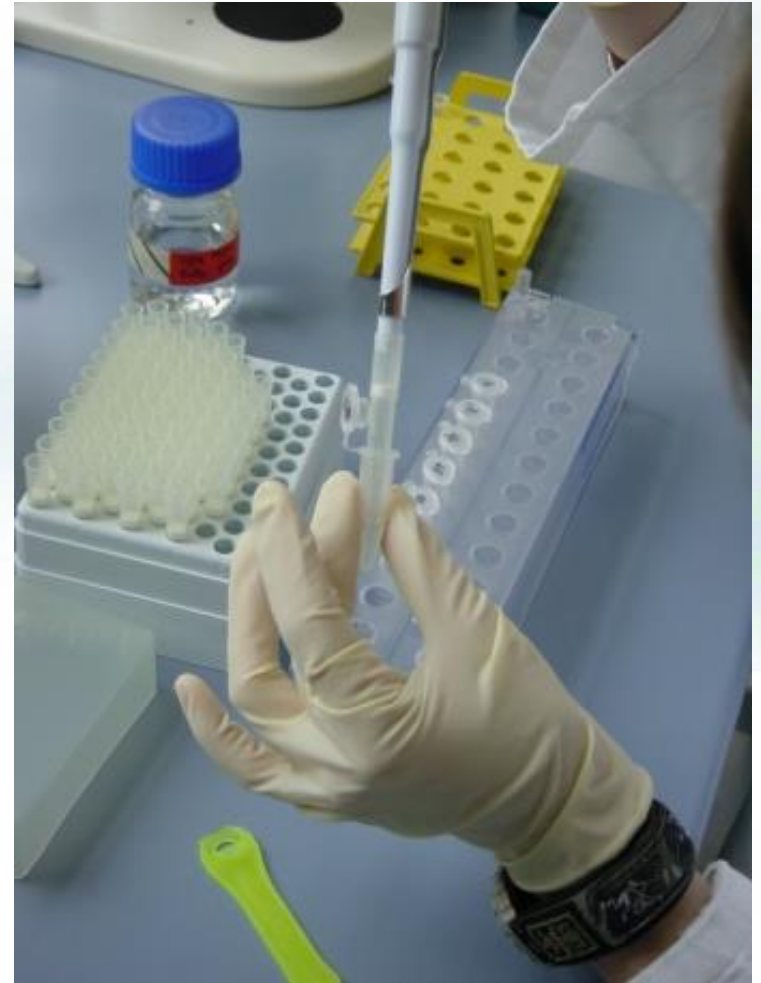


Influence of the DNA extraction method on PCR efficiency. (A) Variability of PCR efficiency for different DNA isolation

methods. Outlier for the CTAB procedure with proteinase K and RNase A treatment is shown as circle above the boxplot. (GS = GENESpin, CTAB.K = CTAB procedure with proteinase K and RNase A treatment). (B) The distribution of PCR efficiencies of 4 tested amplicons on different DNA extracts is presented in boxplots (efficiency data for DNA isolated with Wizard method was excluded because of high variability of results). Cankar et al, *BMC Biotechnology* 2006, 6:37, <http://www.biomedcentral.com/1472-6750/6/37>

Methods for isolation

- Type of sample
- Composition (inhibitors present)
- Processing degree
- Low amounts



PCR - Reliability of the method



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.1829/2003¹⁾.

New version in preparation

By ENGL WG on methods performance requirements

- also qualitative aspects will be considered

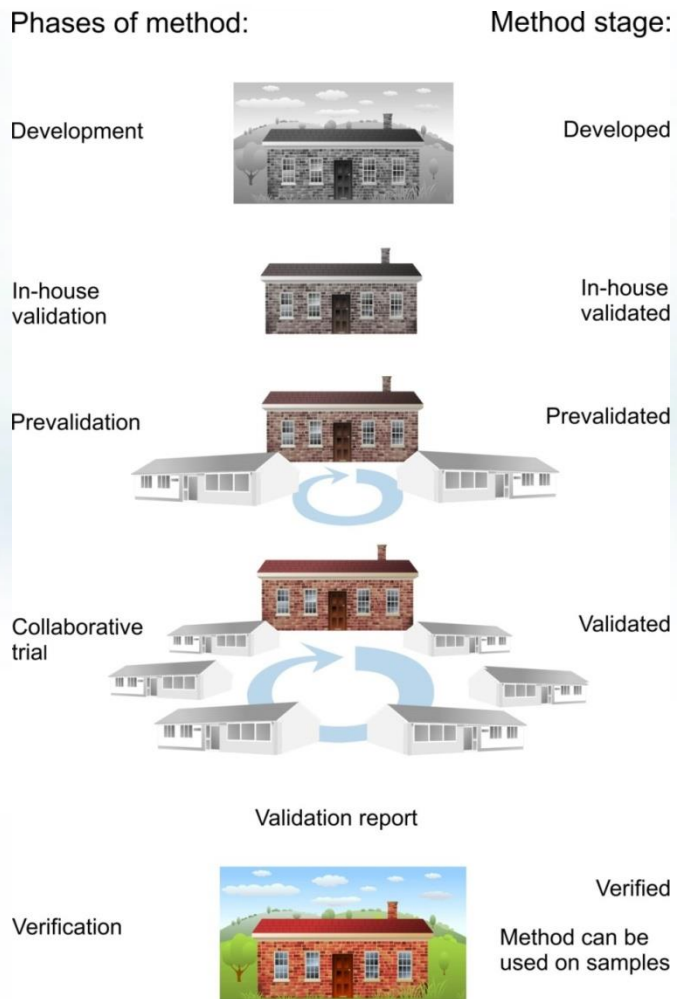
Validation (def)

- Validation is the confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled (ISO 17025 section 5.4.5.1).

Performance criteria

- Applicability
- Practicability
- Specificity
- Dynamic range
- Trueness
- Amplification efficiency
- R²
- Precision
- False - False +
- LOQ
- LOD
- Robustness

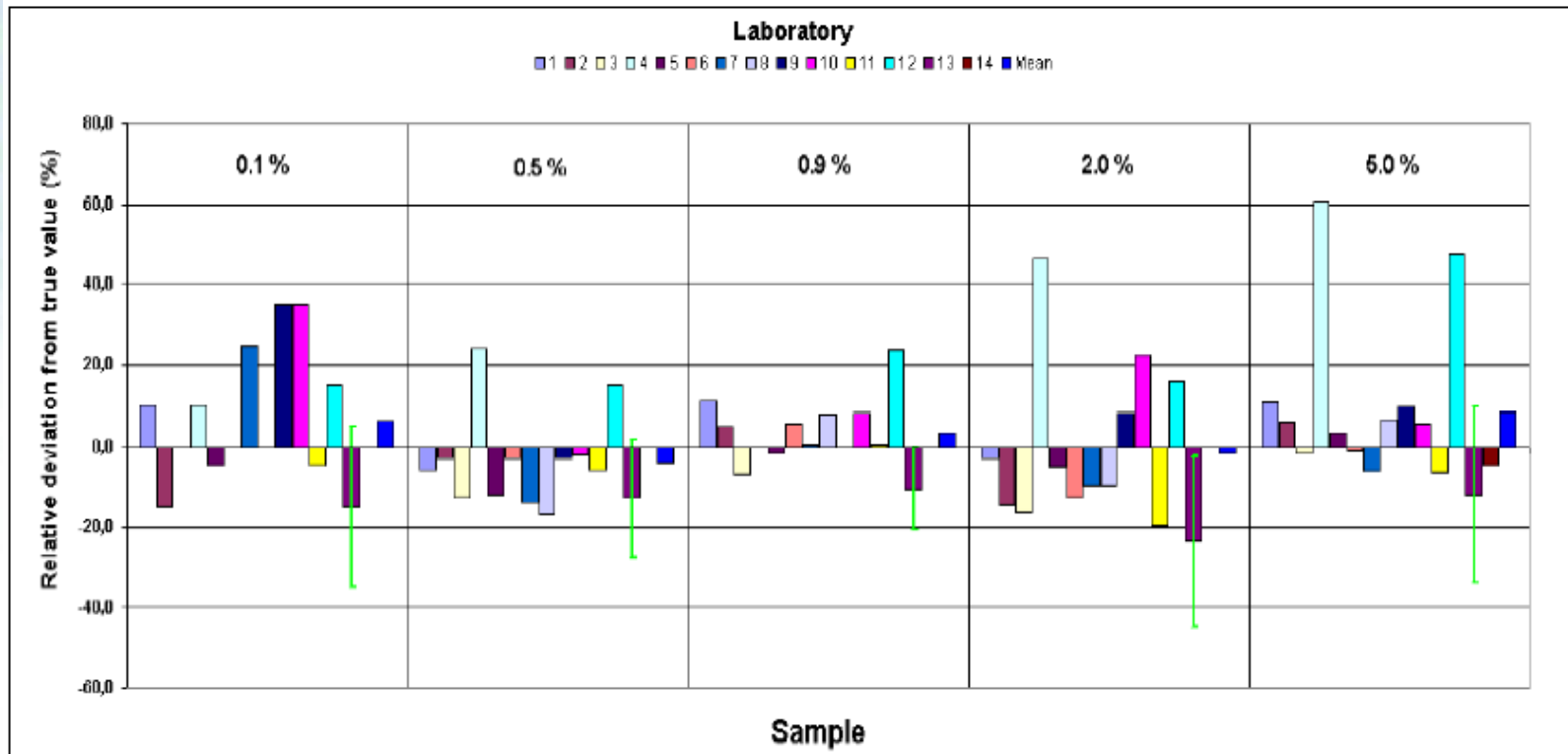
Ring trial



In EU

- Applicant propose the method and availability of reference material.
- The EURL-GMFF - the European Commission's Joint Research Centre – is responsible for testing and validating the method.
- Assisted by National Reference Laboratories (NRLs), members of a consortium of laboratories referred to as the 'European Network of GMO laboratories' (ENGL).

Example of collaborative study



Verification of the method

Verification of analytical methods for GMO testing when implementing interlaboratory validated methods

Guidance document from the European Network of GMO laboratories (ENGL)

Prepared by the ENGL working group on “Method Verification”



Verification (def)

- Verification is the confirmation, through the provision of objective evidence, that specified requirements have been fulfilled [ISO 9000:2000 section 3.8.4]. Verification that a laboratory can adequately operate a standard method requires that the laboratory provides objective evidence that the performance parameters specified in the test method have been met for the sample matrices to which the method is being applied.

Verification

- “The method should work in your lab (staff, machines, chemicals...) as it did in ring trial”

Issues

- Using same master mix for different methods (usefull, but not same method – specificity can be changed)
- Methods for species specific genes (should be stable, 1 copy.....not yet for all species)
- ...

Reference method

A reference method is the one designated method recommended for use in cases of dispute and for calibration purposes (Codex Alimentarius Commission 2010) .

Following this definition, the quantitative event-specific methods validated by EU-RL GMFF are gaining the status of reference methods for GMO detection.

GMOMETHODS

EU Database of Reference Methods

Developed by the Joint Research Centre as European Union Reference Laboratory for GM Food and Feed (EU-RL GMFF), in collaboration with the European Network of GMO Laboratories (ENGL).

It aims at providing a list of reference methods for GMO analysis that have been:

- validated in a collaborative trial, according to the principles and requirements of ISO 5725 and/or IUPAC protocol or
- verified by the EU-RL GMFF in the context of compliance with a EU legislative act.

GMOMETHODS

EU Database of Reference Methods

The application is referred to by the Biosafety Clearing House, a global mechanism set up by the Cartagena Protocol on Biosafety to facilitate the exchange of information on Living Modified Organisms.

Biosafety Clearing-House



Convention on
Biological Diversity



GMOMETHODS

EU Database of Reference Methods

Web | gmo-cr1.jrc.ec.europa.eu/gmomethods/ | Legal Notice Privacy statement English (EN)

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European Union Reference Laboratory for GM Food and Feed

European Commission > JRC > IHCP > EU-RL GMFF

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GMOMETHODS:
EU Database of Reference Methods for GMO Analysis

Home

Search: for Select by GMO Unique Identifier:

Quantitative GMO detection PCR methods

- GMO specific
 - Event specific
 - Maize
 - Soybean
 - Cotton
 - Oilseed rape
 - Polato
 - Rice
 - Sugar beet
 - Construct specific
 - Element specific
- Taxon specific
 - Validated independently
 - Validated in combination with other method(s)

Qualitative GMO detection PCR methods

- GMO specific
 - Event-specific
 - Construct-specific
 - Element-specific
 - Cauliflower Mosaic Virus 35S promoter (CaMV P-35S)
 - Figwort Mosaic Virus 35S promoter (F-FMV)
 - Neomycin phosphotransferase II gene (nptII)
 - Nopaline synthase terminator (T-nos)
 - Phosphinothricin N-acetyltransferase gene (bar)
- Taxon specific
 - Validated independently
 - Validated in combination with other method(s)
 - Plant-specific

Released the GMOMethods app for iPad on 20-12-2011.

GMOMETHODS is the EU Database of Reference Methods for GMO Analysis based on the "Compendium of Reference Methods for GMO Analysis", assembled by the Molecular Biology and Genomics Unit (MBG) of the Institute for Health and Consumer Protection (IHCP), nominated European Union Reference Laboratory for Genetically Modified Food and Feed (EU-RL GMFF), in collaboration with the European Network of GMO Laboratories (ENGL).

Last update

Date	ID	Description
25/01/2013	BCS-GH004-7	Quantitative PCR method for detection of cotton event T304-40 (Nardini et al., 2012).
24/01/2013	QT-EVE-ZM-004	Quantitative PCR method for detection of maize event DAS-40278-9 (Savini et al., 2012).
26/11/2012	QL-CON-00-010	Qualitative PCR method for detection of flax event FP967 (Grohmann L. et al., 2011).
26/11/2012	QT-EVE-GH-008	Quantitative PCR method for detection of cotton event GHB119 (Nardini et al., 2012).

Publication

"GMOMETHODS: The European Union Database of Reference Methods for GMO Analysis"
Authors: Bonfni, Laura; van den Bulcke, Marc H.; Mazzara, Marco; Ben, Enrico; Patak, Alexandre
Source: Journal of AOAC International, Volume 95, Number 6, November-December 2012, pp. 1713-1719(7).

Internet version

Printed version

Ipad application

GMOMETHODS

EU Database of Reference Methods

Quantitative GMO detection PCR methods

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Example

JOINT RESEARCH CENTRE
European Union Reference Laboratory for GM Food and Feed

CP > EU-RL GMFF

GMOMETHODS:
EU Database of Reference Methods for GMO Analysis

Home

Search for Search Select by GMO Unique Identifier:

View Data provided by <http://gmo-rl.jrc.ec.europa.eu/gmomethods/>

Entry information

Entry name **QT-EVE-GM-001;**
GMO Unique Identifier **MST-FG072-2**

Description

Description Quantitative PCR method for detection of soybean event FG72 (Savini et al., 2012)
Keywords event_specific.
From Glycine max (soybean) - event FG72 (MST-FG072-2)

References

1 Savini C., "Event-specific Method for the Quantification of Soybean FG72 Using Real-time PCR - Validation Report and Protocol" Online Publication (2012)
EURL_GMFF EURL-VL-04-10 VR.pdf
EURL_GMFF EURL-VL-04-10 VP.pdf
Reference Position 1-70

2 "PCR reactions set up and amplification conditions" Online Publication (2012)
PCR QT-EVE-GM-001.pdf
Reference Position 1-70

Cross-references

GMOMETHODS QT-TAX-GM-020;

Features

Key	Location	Qualifier	Value
STS	1..70	standard_name	PCR 70 bp amplicon
		note	event-specific RT-PCR
		target	3'Integration border region (IBR) between the insert of soybean event FG72 and the soybean host genome
primer_b	1..20	standard_name	Primer forward: MAE071
		note	AGATTTGATCGGGCTGCAGG
		target	insert
primer_b	25..43	standard_name	RT-PCR probe: TM325
		note	FAM-AATGTGGTTCATCCGTCCTT-MGBNFQ
primer_b	complement (49..70)	standard_name	Primer reverse: SHA097
		note	GCACGTATTGATGACCCGATTA
		target	3'-host genome

Sequence information

Length: 70 BP, A Count: 15, C Count: 12, T Count: 25, G Count: 18

```

agatttgatc gggctgagg nnnnaatgty gttcatccgt cttmmnnta atcgcytcat 60
caatactgac                                     70
    
```

References

Primers, probes

Sequences

http://gmdd.shgmo.org/



GMO Detection method Database (GMDD)

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Introduction

GMO Detection Method Database (GMDD) is one part of Shanghai GMO platform, our platform mainly engages in the detection of GMOs, and it provides the following services.

1. GMO Detection Method Database

GMDD is a database of GMO detection methods, which provide detailed information of nucleic acid-based methods & protein-based methods, including primer sequences, amplicon length, endogenous reference gene primers, validation information, PCR programs and references etc. Besides, the database also contains information of GMO insertion sequences, certified reference materials.

By registration, users can submit their own methods and GMO inserted sequences to GMDD. Other browsers could obtain the newly updated information after the web administrator's confirmation. We hope this database would be a platform for researchers exchanging their ideas on GMO detection methods, and could save their time developing or validating GMO detection methods.



2. GMO Detection Services

In China, our platform also provide services for GMO content detection, new method validation, detection technique training. For detailed information, please visit <http://www.shgmo.org>.



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If you used data from GMDD, please cite the paper of GMDD: a database of GMO detection methods.

http://gmdd.shgmo.org/



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Navigation

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Username: *

Password: *

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About Us

This GMO detection method database (GMDD) is being set up by **GMO Detection Laboratory in Shanghai Jiao Tong University (GMODL-SJTU)** in collaboration with **RIKILT, Shanghai Academy of Agricultural Sciences, Shanghai Food and Drug Administration, Shanghai Entry-Exit Inspection and Quarantine Bureau, and Shanghai University Bioinformation Center**. This platform is funded by **Science and Technology Commission of Shanghai Municipality, Chinese Ministry of Science and Technology**.

The aim of our database is to provide detailed information of GMO detection methods, and also information of gene insert. These information will be very useful in the development and standardization of GMO detection methods.

GMO Detection Laboratory, Shanghai Jiao Tong University



The research fields of our lab focus on some research projects listed as follows:

- 1) Development of novel detection methods of GMOs products, including qualitative and quantitative PCR and ELISA, etc;
- 2) Identification and validation of endogenous reference genes;
- 3) Development of novel reference molecules.

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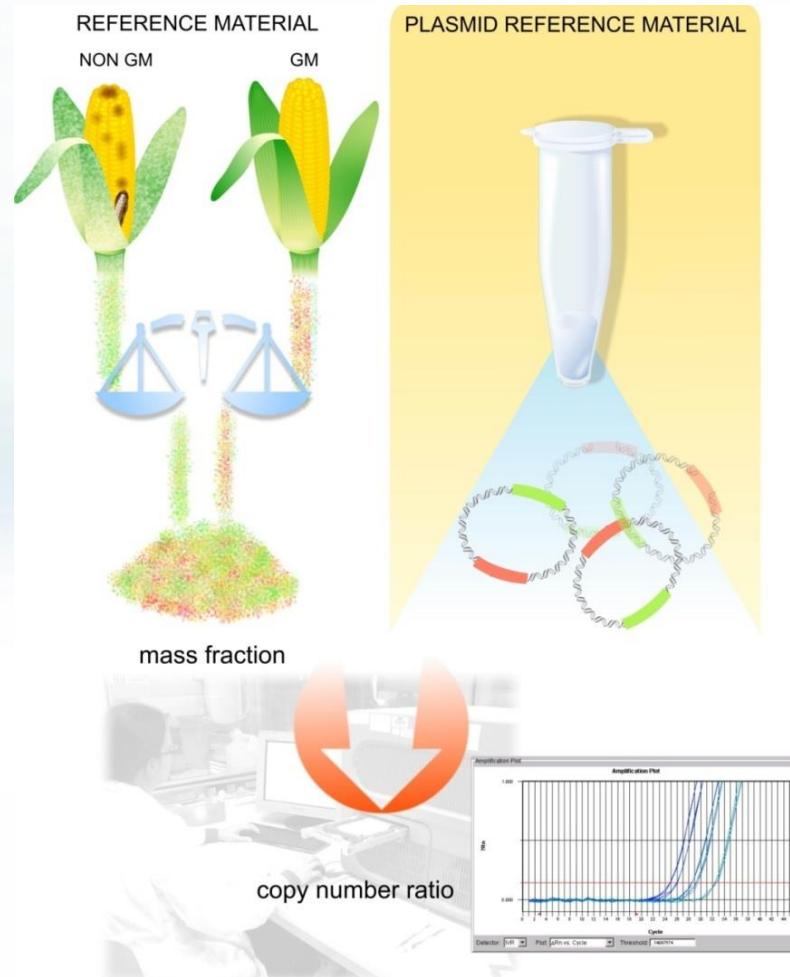
If you used data from GMDD, please cite the paper of **GMDD: a database of GMO detection methods**.

EUGENIUS – in preparation

EUGINIUS (European GMO initiative for a unified database system)

Initiative of the Federal Office of Consumer Protection (BVL, Germany) and of the Institute of Food Safety (RIKILT, Netherlands).

Reference materials needed



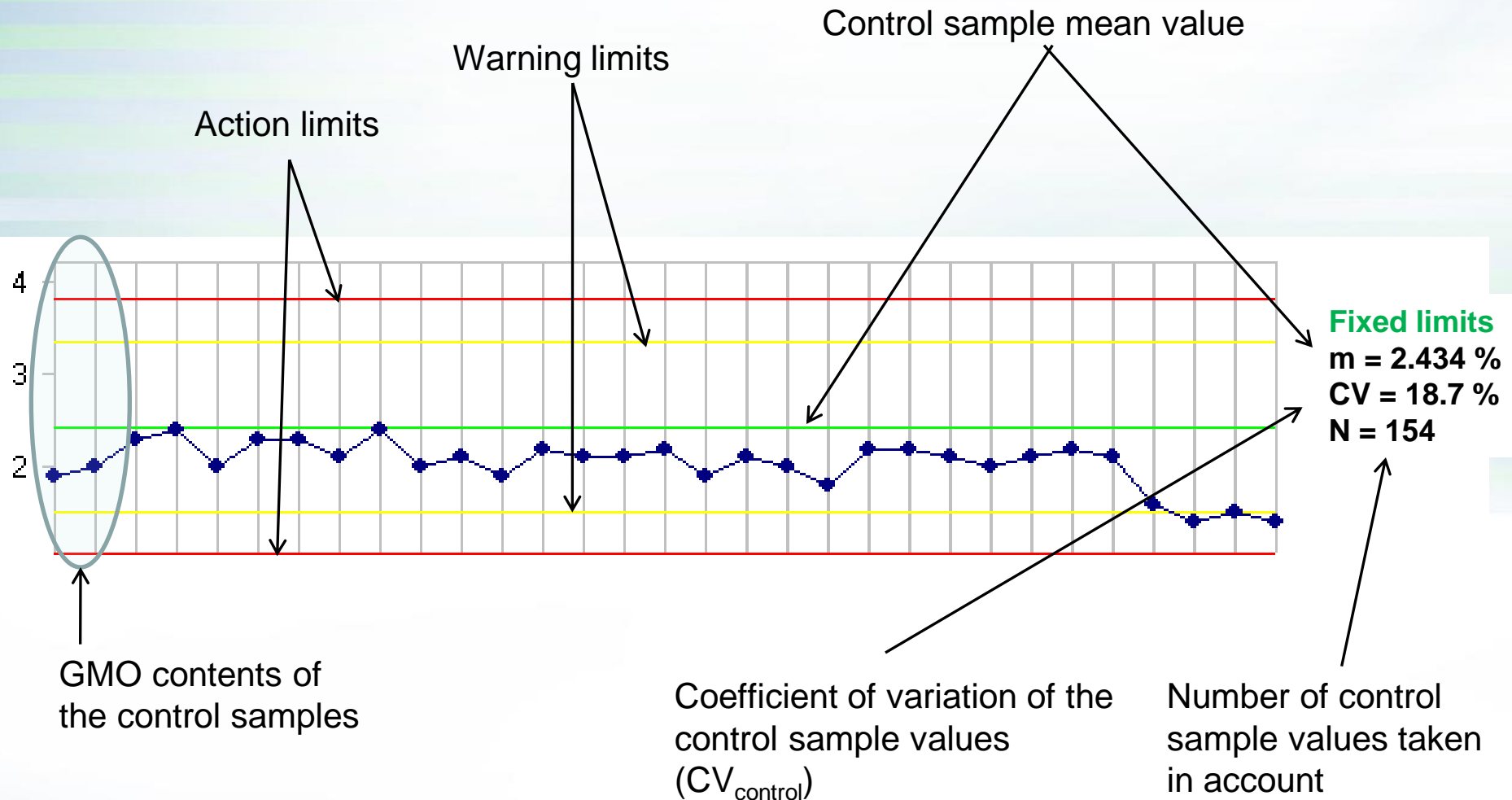
Using methods on samples

- Take into account practical LOD and practical LOQ

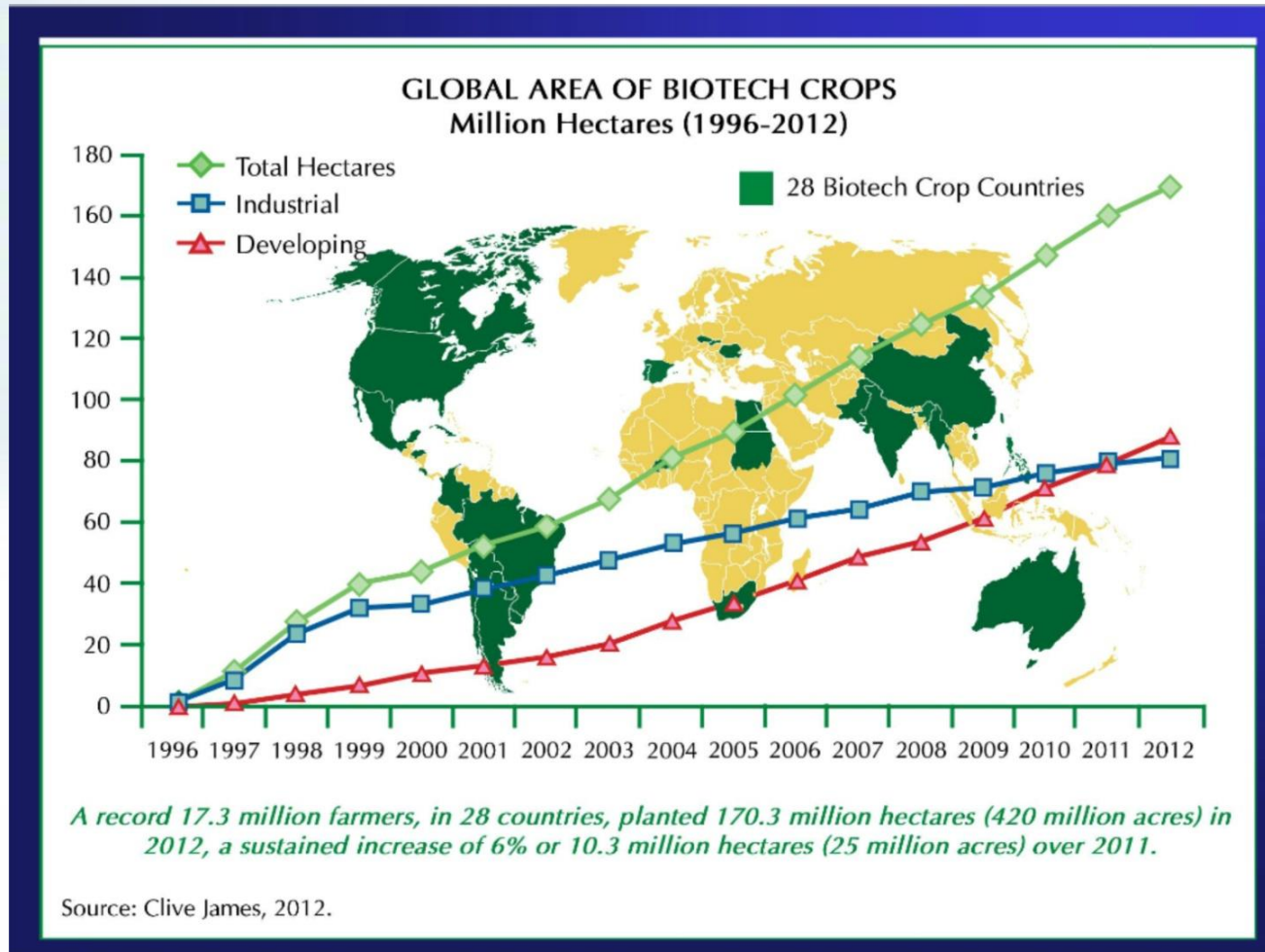
Assurance of quality of testing

- Different controls to be used during testing
- Proficiency tests

Control charts



Presence of GMOs



Advances

Real time PCR



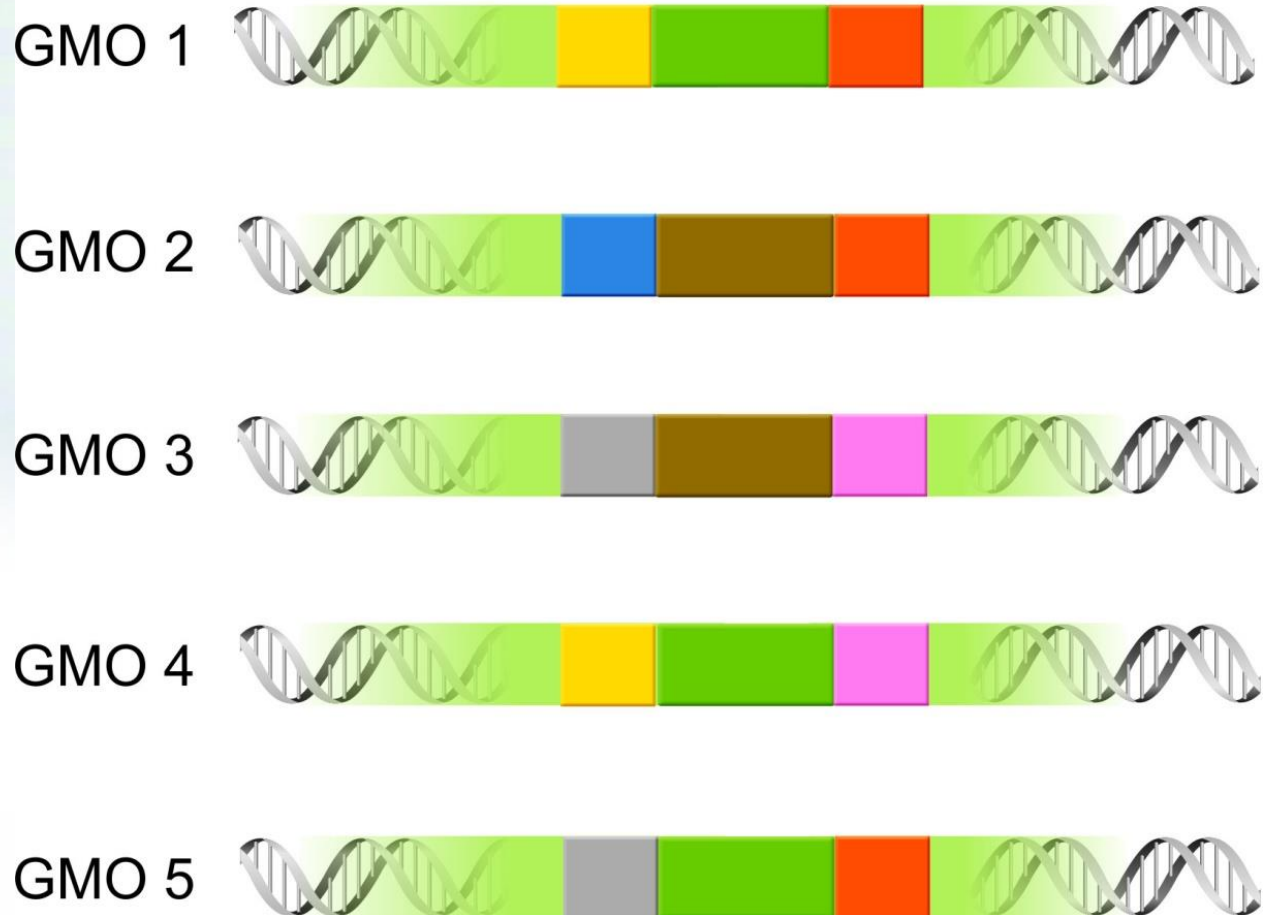
Multiplexing



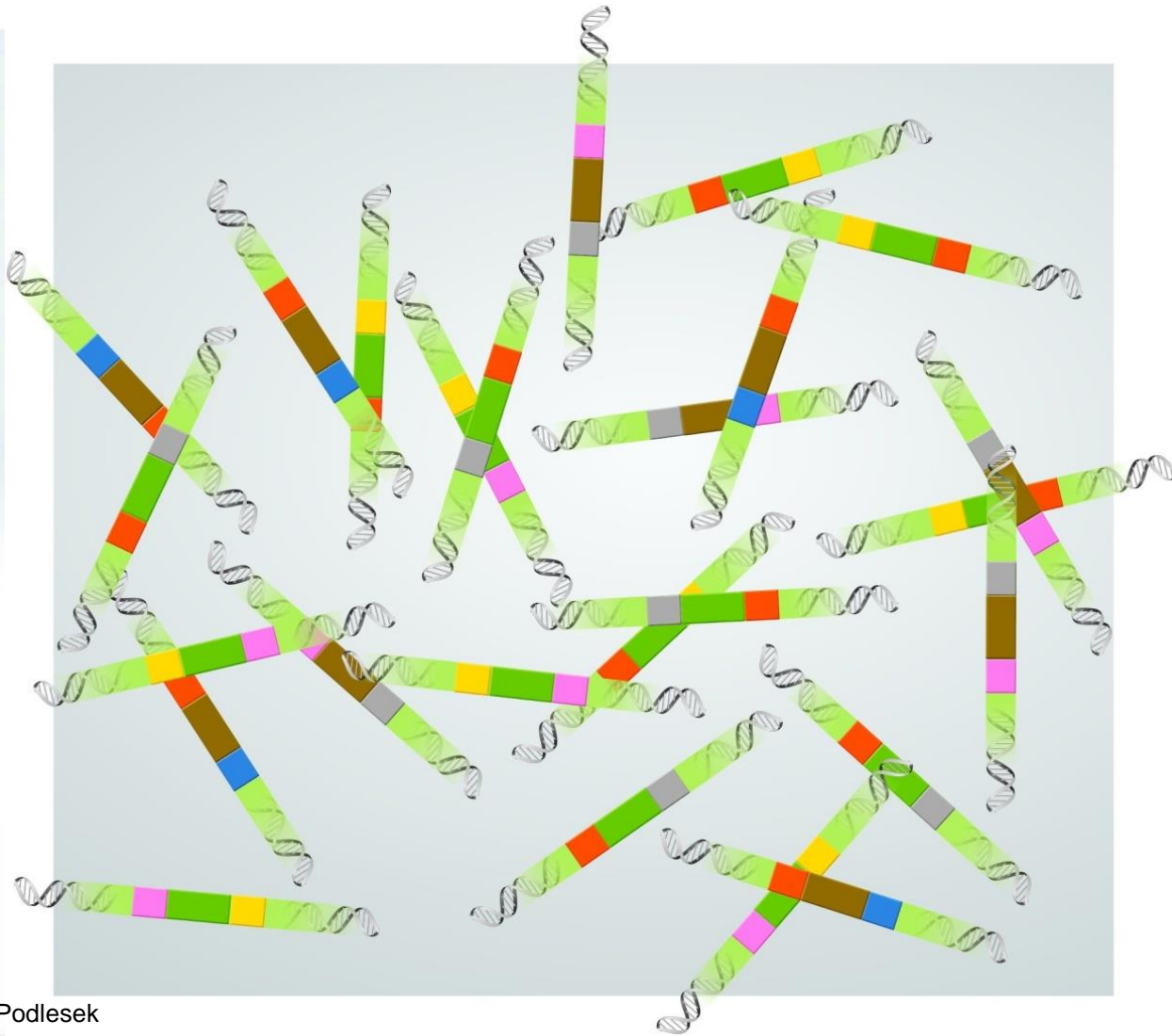
<http://www.platr-pipetting.com/>

Easier pipeting

Matrix approach



Matrix approach



Smart selection of screening elements (Cosyps, GMOseek)

	Yellow	Orange	Brown	Grey	Blue	Pink
GMO 1	+	+				
GMO 2		+	+		+	
GMO 3			+	+		+
GMO 4	+					+
GMO 5		+		+		

More screening elements tested in first phase

Example: approved GM maize in EU

	2 elements e.g. 35S in NOS	5 elements
No. of event specific GMOs to be tested after screening	11	5

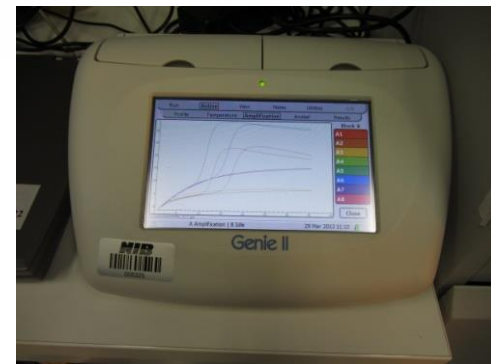
New technologies

Isothermal methods (e.g. LAMP - Loop-Mediated Isothermal PCR) - quicker

Digital PCR – absolute quantification

NGS – data on sequences

Combinations with bioinformatic tools



Reliable result



Questions

