New approaches in GMO detection: Detection of Unapproved GMOs

Cristina Aleixo

National Institut of Biological Resources

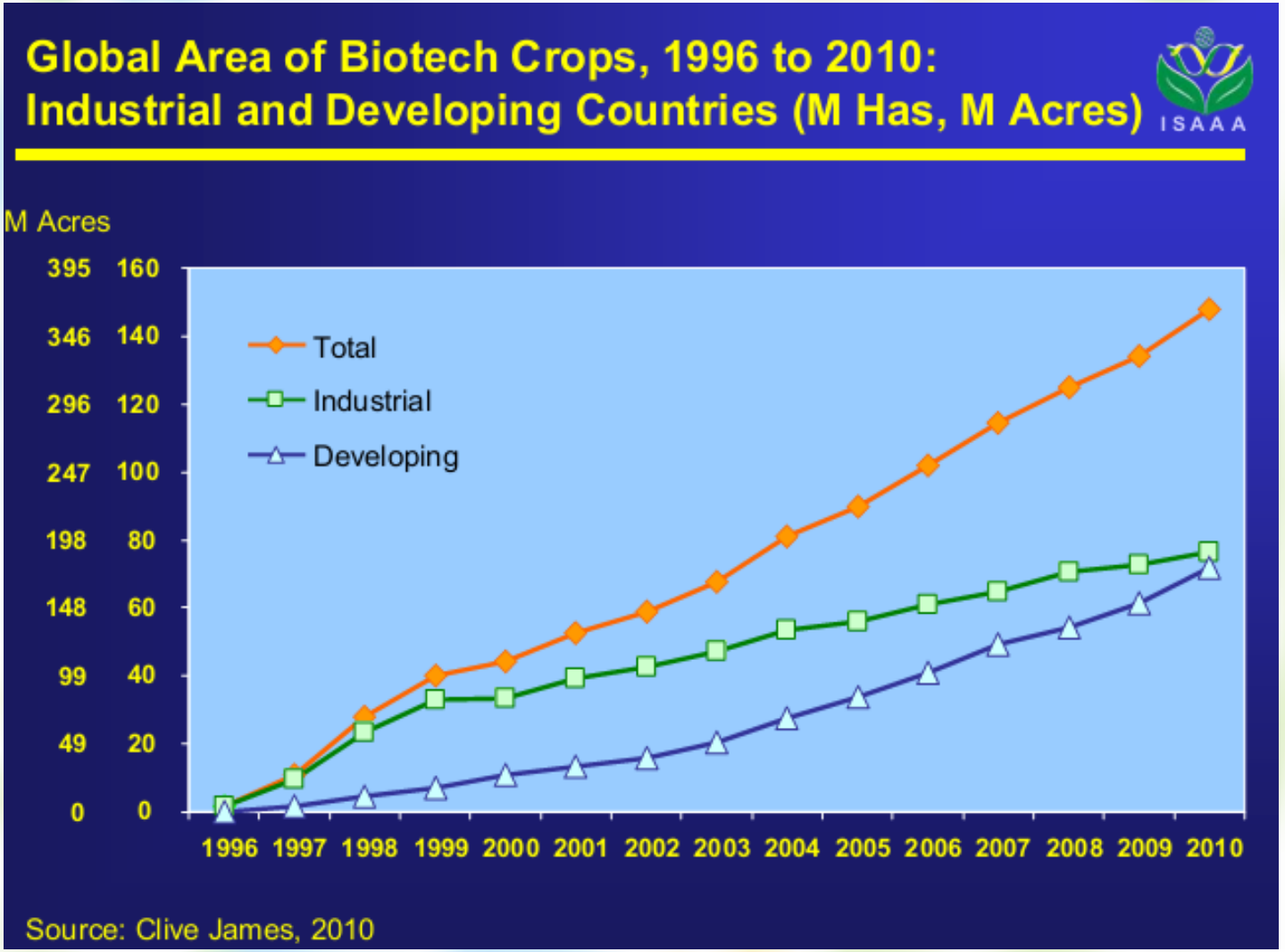
Research Unit of Food Technology

Lisboa- Portugal

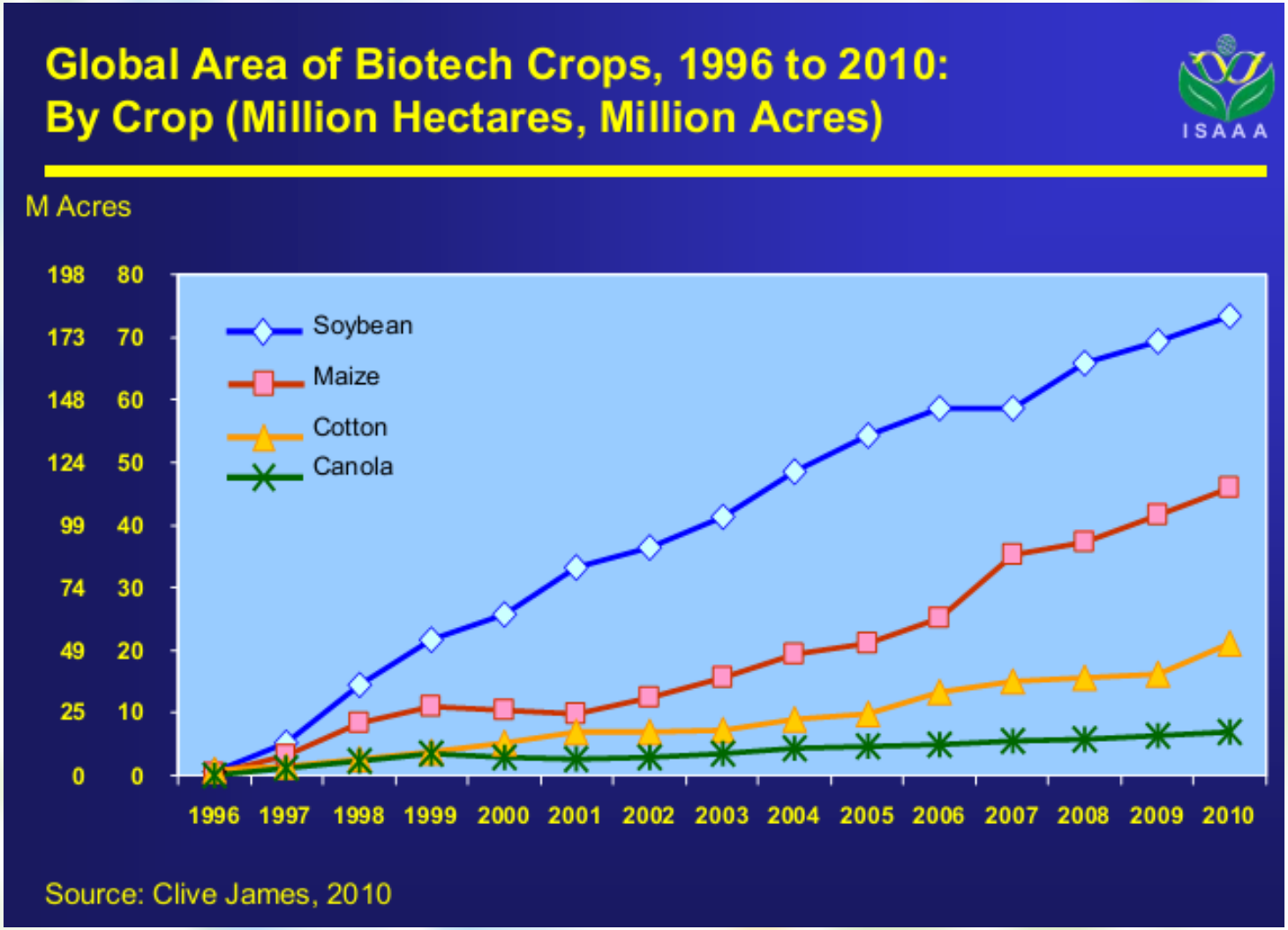
2nd International Workshop on Harmonisation of GMO Detection and Analysis

7-8 February 2012, Nelspruit - South Africa

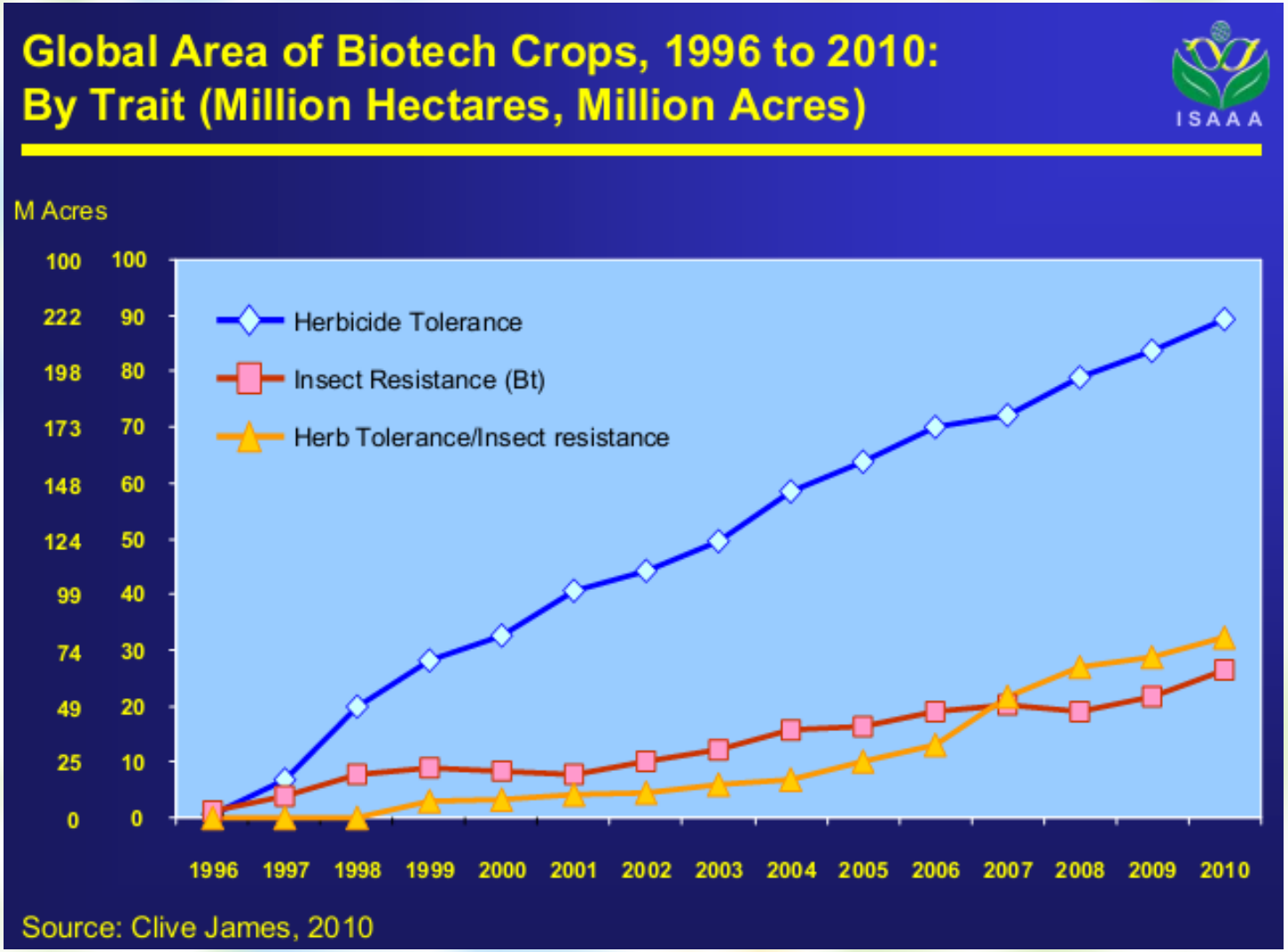
Overview


Global Area of Biotech Crops, 1996 to 2010:
Industrial and Developing Countries (M Has, M Acres)
ISAAA
M Acres
395 160
346 140
296 120
247 100
198 80
148 60
99 40
49 20
0 0
Total
Industrial
Developing
1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010
Source: Clive James, 2010

Overview


Global Area of Biotech Crops, 1996 to 2010:
By Crop (Million Hectares, Million Acres)
ISAAA
M Acres
198 80
173 70
148 60
124 50
99 40
74 30
49 20
25 10
0 0
Soybean
Maize
Cotton
Canola
1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010
Source: Clive James, 2010

Overview



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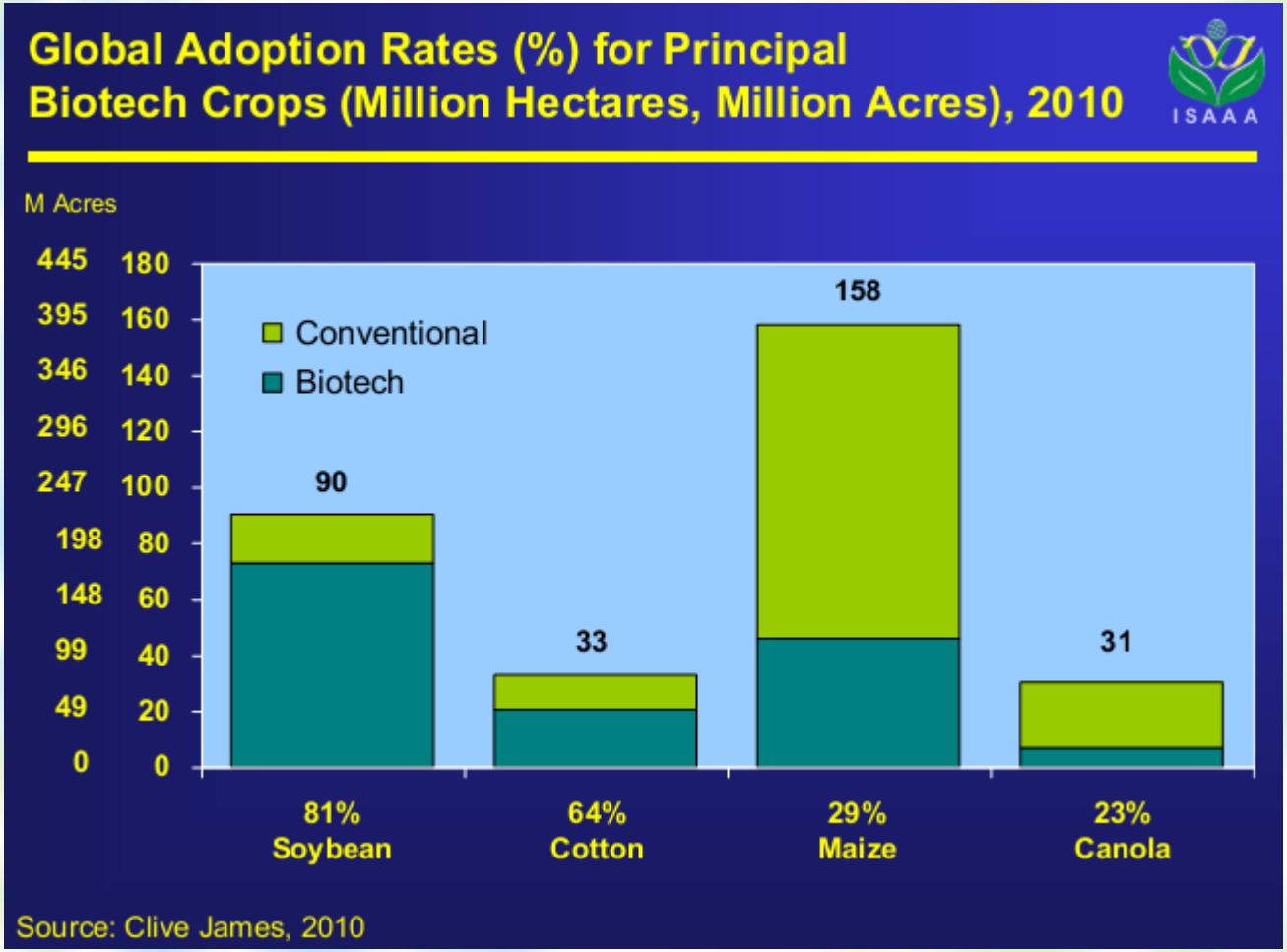
Overview



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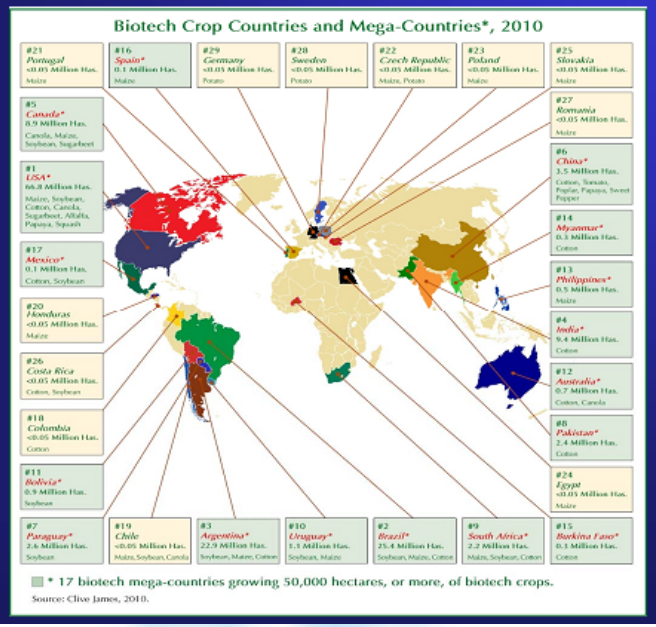
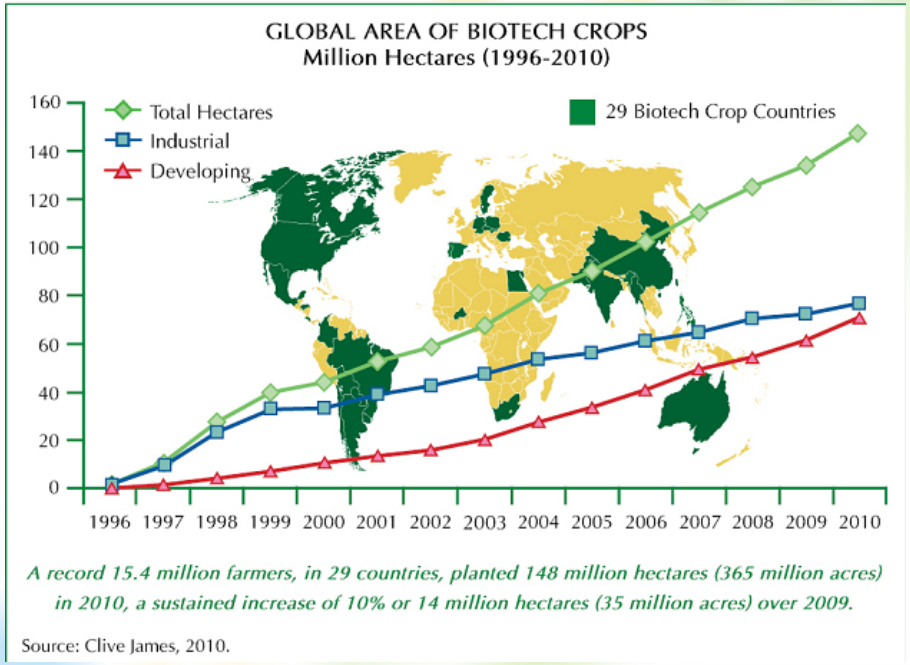
Overview

Biotech Crop Countries and Mega-Countries, 2010





European food safety and quality concerns

According to Eurobarometer:

- European public opinion globally against GMO
- Lack of public confidence in food safety and government administration, and even scientists to some extent

Public opinion may change: at present consumers' attitudes are divided into:

- +/-30% for GM food
- +/-30% against GM food
- +/-30% wait and see what are the benefits for the consumer

Labeling is a strong European demand by the EU population (similar requests in third countries)

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Traceability and Labeling: a growing European request

To ensure the free choice to the consumer facing new products (GMO, irradiated food…)

Quality and authentication of the products

Necessary for compliance with EC Directives and Regulations on labeling of GM food

Necessary for traceability of GM plants under the new EC Directives and Regulation on approvals of GM crops and imports

European background

- Mandatory labeling above a threshold of fortuitous presence of
  - 0.9% for approved GMOs
  - New threshold for unapproved GMOs?
- Obligation (01/18 et 1829/03) made to notifiers to provide sampling plans, control samples and quantitative identification method
- Forecasted increase of GMO pressure in EU
  - Growing number and acreages of GMOs for food and feed
  - Non-food and non-feed-GMO not having to enter the food supply chains

European background

- **Cartagena Protocol on Biosafety to the Convention on Biological Diversity** establishes the importance of organizing the supervision and control of transboundary movements of GMOs.

- Regulation (EC) 1946/2003 - legal framework for exports to third countries
  - to ensure the identification of GMOs exported from the Community.
  - exporters shall ensure in the document ation accompanying 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

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European background

- **Exclusion from the regional market of imports of illegal and possibly unknown GMO products**

- **Several cases in which emergency measures were undertaken by European authorities to prevent the potential import of unauthorized GMO to the European market.**
  - **maize Bt10,**
  - **rice LL601,**
  - **rice Bt63**
  - **linseed FP 967**

European background

Legal Basis for emergency measures

Regulation (EC) No 178/2002 - provides the basis for the activities of reference laboratories and all other institutions that take part in the enforcement of food law.

"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."

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European background

Legal Basis for emergency measures

Regulation (EC) No 178/2002 - cont.

article 7 establishes the Precautionary Principle,

"in specific circumstances, where the possibility of harmful effects on health is identified but scientific uncertainty persists, provisional risk management measures … may be adopted".

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.

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What are UGM?

Unauthorised GMO

All GMO not authorised for commercial release within relevant jurisdiction

May divide into several subgroups

Useful to provide terminology for stakeholders
Legal status in a global perspective
Information about availability of knowledge (decision support)

Detectability

availability of a detection method and reference material

Safety issue – Risk assessment

To what extent has a UGM been risk assessed?
Is the information available, accessible and reliable?
Are some UGM safer than other?

Legal issue

By definition, presence of UGM is illegal
Are some UGM “more illegal” than other?

Sources of UGM

"Asynchronous" authorisation and failure to segregate

Usually risk assessed where authorised. Relevance to other jurisdictions?
Several cases e.g. of US authorised (deregulated) GMOs found in EU
cf. Rapid Alert System for Food and Feed
(ec.europa.eu/food/food/rapidalert/)

New GMO developing and releasing states – assessments/controls
Illegal use in countries where not authorised of GMOs
authorised somewhere else

Escapes from field trials and laboratories, etc.

Usually not risk assessed and information limited or unavailable
e.g. LL601 and Bt63 rice, Bt10 and E32 maize, pig vaccine
Transparency vs. confidentiality (IP, public awareness, trust, etc.)
Pollen, bird/rodent, human error, etc.

Sources of UGM

- **Presence of a UGM in the food chain**
  - escapes from field trials
  - inefficient segregation on commercial chain
  - accidental escapes from laboratories or green-houses
  - intended releases - very rare and unlikely incidents
- **Socio-economic impact of UGM**
  - presence of UGM is by definition illegal
  - Lots may be rejected upon arrival to importing harbours
  - consumer trust and preference
  - international trade

European Backgorund

Lack of synchronicity between different countries and regions in regard to GMO approval processes

Need for detecting unknown GMO

A need for
rapid and cost-effective methods not impacting end product's prices

Filling EU regulations gaps

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European Backgorund

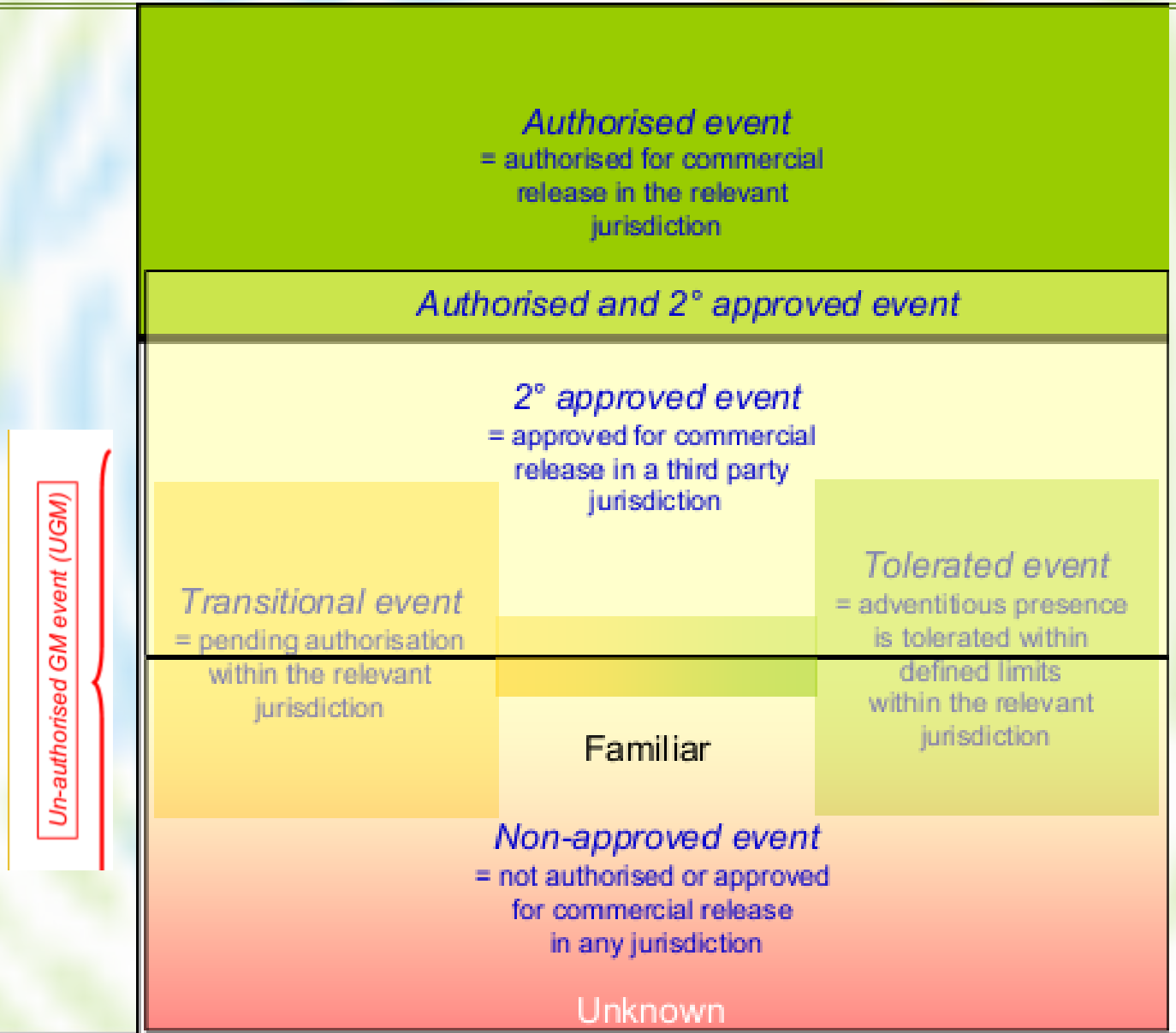
Need for detecting unknown GMO

Difficulties on detection of unauthorized or unknown GMOs

- lack of molecular knowledge of their genetic contents - the data on inserted constructs and nucleotide sequences are usually proprietary

- reference materials are not available,

Defining the problem of UGM



Approaches for detecting GM material

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 harmonization of GMO detection methods within the EU

As the number of GMOs on the global market has increased the ability to perform GMO testing has been challenged

Improvement and harmonization of current control systems main goals

- cost-effectiveness,
- enhancement of efficiency,
- simplification of methods for validation and detection
- provision of tools for the detection of unknown GMOs in the supply chain

Approaches for detecting GM material – Some traceability related FP5 research programs

The control system benefits from

- research activities and validation work of the EURL-GMFF,
- activities of the ENGL and
- EU-funded research projects

DNAtrack: N. Marmiroli

QPCRGMOFOOD: 2000-2003 A. Holst-Jensen

GMOCHIPS: 2001-2004 J. Remacle. Y. Bertheau

ENTRANSFOOD Cluster: H. Kuyper

- **provided first insights on GMO detection**
- **Evidenced issues on GMO detection**
- **Influenced the European regulation**

Approaches for detecting GM material – Some traceability related FP5 research programs

Several programs on food safety and quality, detection methods…

Results:

Provided first insights on GMO detection

Evidenced issues on GMO detection

Influenced the European regulation: 1829/03, 1830/03

Approaches for detecting GM material - EC FP6 programs on Co-existence and/or Traceability

SIGMEA (FP6, STREP)Sustainable Introduction of GMOs into European Agriculture: 2004-2007

J. Sweet & A. Messéan INRA

Co-Extra (FP6, IP): 2005-2009 Co-existence and traceability in the GM and non-GM supply chains

Y. Bertheau, INRA

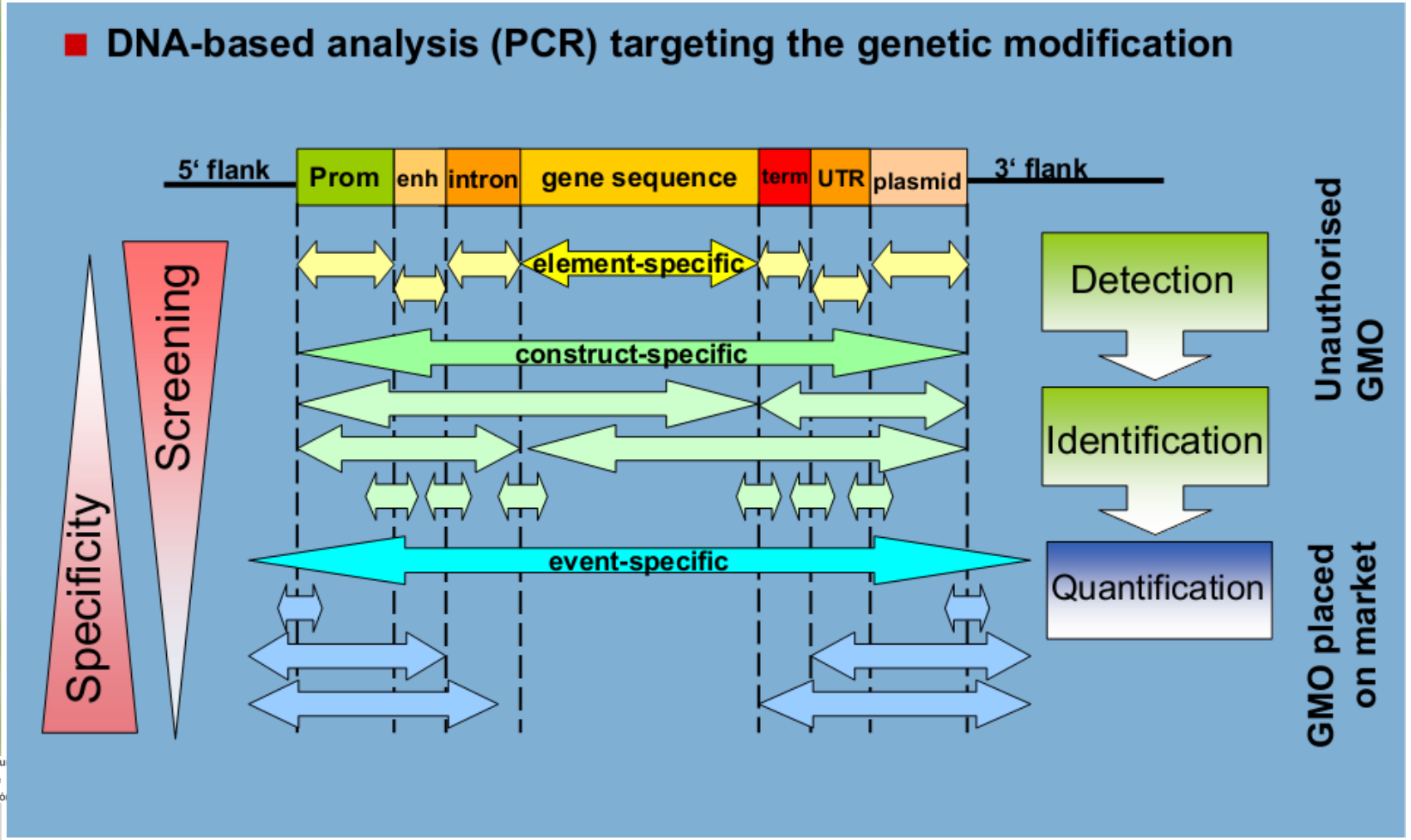
PETER (FP6 Specific Support Action) Promoting EC traceability research

M. Debord, CCI Gers

Transcontainer (FP6, STREP) (program on tools for biological containment)

R. De Maagd, Wageningen Univ.

Approaches for detecting GM material



Approaches for detecting unknown GMOs

Two concepts from Co-Extra project

“Differential PCR”

“Matrix Approach”

“Differential PCR”

induces the ratios of different genetic elements in sample DNA which are compared with expected ratios for known GMOs.

- presence of an unknown GMO - statistical result differs from zero

“Matrix Approach”

- tests simultaneously for the presence of a large number of DNA fragments.
- compares the resulting combinations to a database of known GMOs

Approaches for detecting unknown GMOs

- Qualitative differential analysis
  - **Qualitative differential PCR**
  - **Mutation/substitution screening**
  - **The “matrix” approach (micro-arrays, SNPlex)**
  - **Fingerprinting approach (Anchored PCR)**
  - **High density microarray approaches**
  - **Transcript sequencing and subtraction analysis**
- Quantitative differential analysis
  - **Quantitative differential PCR**

Approaches for detecting unknown GMOs

Qualitative differential analysis

Qualitative differential PCR

- Sequence detected e.g. P35S
- None authorized GMO with such sequence detected
- No donor organism for this sequence detected, eg. CaMV

Suspicion of presence of unknown GMO !

Approaches for detecting unknown GMOs

Qualitative differential analysis

Mutation/substitution screening

Observations of small nucleotide changes may indicate divergent origin

Comparison of DNA strands between reference and possible UGM

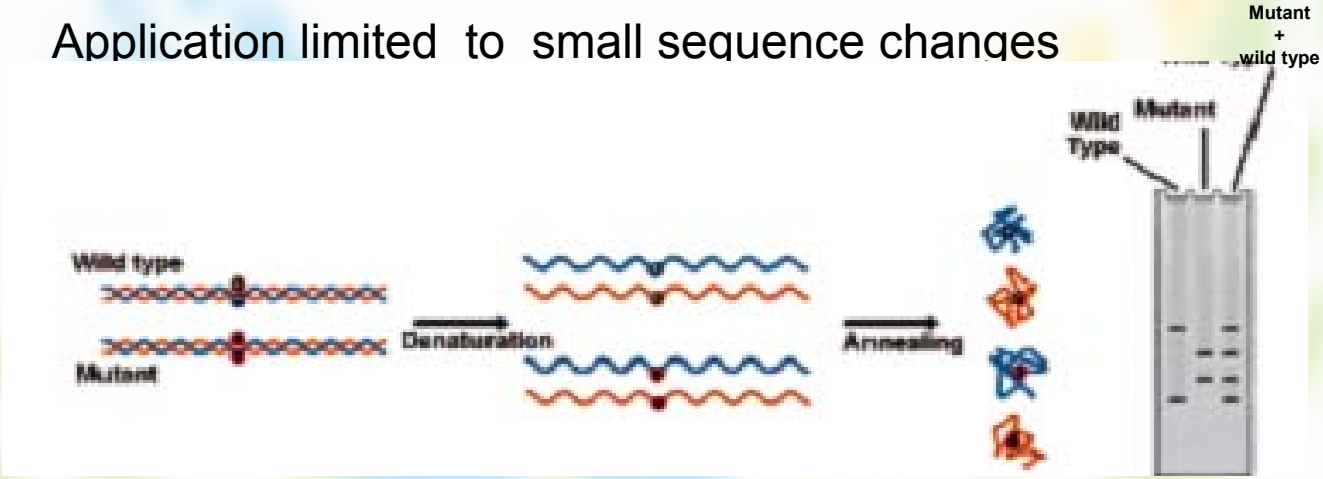
Divergent origins often associated with substitutions / sequence changes

Substitutions / sequence changes alter migration in electrophoresis

Applicable to commonly used elements, eg. P35S, 3'-nos, EPSPS

Can tabulate data from reference materials for comparison

Application limited to small sequence changes



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Approaches for detecting unknown GMOs

Qualitative differential analysis

The" Matrix approach"

First proposed for GMOchips project in FP5.
Explored in multiple variants

Screening for potentially introduced elements

- Promoters, terminators, trait genes,vector & potentially fusion elements
- Simplex, oligoplex or multiplex detection method
- Allelic diversity may result in false negatives

Apriori, a relation table between GMOs and the elements screened is established

the "matrix"

shows the expected response by individual GMOs to specific tests

Approaches for detecting unknown GMOs

Qualitative differential analysis

The" Matrix approach"

	GM O-1	GM O-2	GM O-3
P35S	x	x	x
Tnos		x	x
T35S	x	x	
cryI(A)b	x	x	x
bar		x	
nptII			

A posteriori comparing the results of the screening with the "matrix"

The result is a list of GMOs that may be present in the product

Perfect matches vs partial matches vs incompatible results, relative to "matrix"

Detection of elements not found in any authorised GMO → presence of UGM

Provided non-GM source can be excluded

The principle is already implemented in many laboratories

Issue: numerous different sequences with similar names

Need of reliable publicly available information on sequences

Approaches for detecting unknown GMOs

Qualitative differential analysis

The" Matrix approach"

The principle is already implemented in many laboratories

Screening results predicting the presence of UGM should be verified by use of construct-specific or event-specific methods, as by donor specific control methods

Negative test result will not rule out the presence of a UGM

Matrix approach has been developed already in various formats for GMO screening in a wide range of products

"Matrix-based approach"

- the most efficient and cost effective strategy to detect accidental occurrence of UGM,
- is equally useful for the general detection of authorized GMOs
- does not require a new GMO detection paradigm.

Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

Current applications

- Qualitative PCR: up to 9plex
- Screening for GMO based on the combination of generic and construct-specific markers
- CoSyps
- "Pre-spotted" plates for event-specific screening
- Micro-arrays: DualChip® first inter-laboratories validated chip
- SNPlex™: up to 48 targets amplified in a time
- Whole genome amplification and micro-arrays detection

Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

Anal Bioanal Chem
DOI 10.1007/s00216-009-3173-2

ORIGINAL PAPER

A practical approach to screen for authorised and unauthorised genetically modified plants

Hans-Ulrich Waiblinger · Lutz Grohmann · Joachim Mankertz · Dirk Engelbert · Klaus Pietsch

Analytical and Bioanalytical Chemistry
Volume 396, Number 6 (2010) pp. 2065-2072
(issue on "GMO Analysis")

Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

Screening for 35S promoter and nos terminator alone is not sufficient

X1 = authorized
X2 = pending
- = not authorized

S = data from sequence information (e.g. plasmid map)
R = analytical verification with reference material

Name of Event	Plant	Authori-zation EU	P35S		T-nos		CTP2-CP4EPSPS		bar		35S-pat		CRM
			S	R	S	R	S	R	S	R	S	R	
305423	soybean	x[2]	-	wp	-	wp	-	-	-	-	-	-	x
356043	soybean	x[2]	(+)	wp	-	wp	-	-	-	-	-	-	x
A2704-12, A2704-21, A5547-35 (LibertyLink)	soybean	x[1]	+	+	-	-	-	-	-	-	+	+	x
A5547-127 (LibertyLink)	soybean	-	+	+	-	-	-	-	-	wp	+	+	x
G94-1, G94-19, G-168 (Optimum)	soybean	-	+		+		-		-		-		
GTS 40-3-2 (Roundup Ready)	soybean	x[1]	+	+	+	+	-	-	-	-	-	-	x
GU262 (LibertyLink)	soybean	-	+		-		-		-		+		
MON 89788	soybean	x[1]	-		-		+	+	-		-		
W62, W98 (Liberty Link)	soybean	-	+		+		-		+		-		

Comment:
here: information from application (FDA, 2007): parts of P35S included

wp = unexpected signal, weak positive (Ct = 35 and higher)

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Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

Data sources: CERA GM-Crop Database

BATS Report

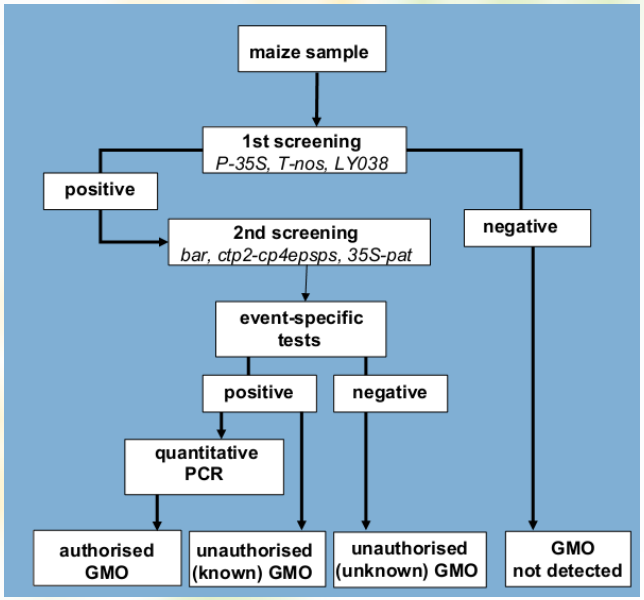
EU-Register of GM Food & Feed

GMDD

NCBI

All commercially available reference materials have been used

Screening is either done step-by-step or simultaneously

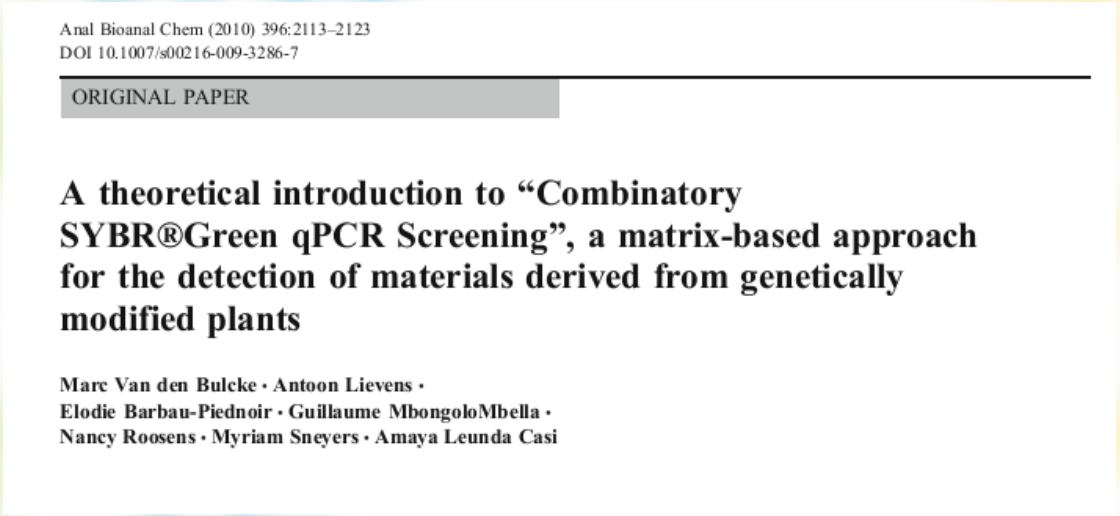


https://www.gdch.de/netzwerk-strukturen/fachstrukturen/lebensmittelchemische-gesellschaft/arbeitsgruppen/biochemische-und-molekularbiologische-analytik.html

Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

COSYPS



Anal Bioanal Chem (2010) 396:2113–2123
DOI 10.1007/s00216-009-3286-7

ORIGINAL PAPER

A theoretical introduction to "Combinatory SYBR®Green qPCR Screening", a matrix-based approach for the detection of materials derived from genetically modified plants

Marc Van den Bulcke · Antoon Lievens · Elodie Barbau-Piednoir · Guillaume MbongoloMbella · Nancy Roosens · Myriam Sneyers · Amaya Leunda Casi

enable the multiple detection of different sequences specific of GM events.

cost-effective matrix-based approach based on SYBR®Green technology

applies a limited set of real-time PCR methods

Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

COSYPS

Target four different types of DNA elements:

1) a **generic plant-DNA** denominator (plastid rbcL isolated from cotton, rape seed and maize),

2) **species-specific** elements (soy, maize, oilseed rape, cotton, sugarbeet and rice),

3) **generic recombinant DNA** elements (P-35S from CaMV, T-nos from *Agrobacterium*), and

4) recombinant **trait-specific** elements (cp4-epsps, cryIAb, pat and bar)

Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

COSYPS

- RbcL: Ribulose-1, 5-bis-phosphate carboxylase — Plant-specific marker
- ADH alt: Alcohol deshydrogenase 1: Maize marker
- SLTM: Lectin: Soybean marker
- Cru: Cruciferine : Oilseed Rape marker — Taxon-specific markers
- p35S : 35S promotor (CaMV) marker
- tNOS : Nopaline synthase terminator (*A. tumefaciens*) marker — Generic recombinant markers
- CP4-EPSPS : Glyphosate (herbicide) tolerance gene marker
- CryIAb : Maize European corn borer resistance gene marker
- PAT/pat : Glufosinate (herbicide) tolerance gene marker
- PAT/bar : Other glufosinate tolerance gene marker — Trait-specific markers
- CRT 2: Cauliflower Mosaic Virus (CaMV) Reverse Transcriptase marker — Control of CaMV presence

	RbcL	ADH	Lectine	Cru	p35S-short	T-NOS	CP4-EPSPS	CryIAb	Pat/Pat	Pat/Bar	CRT 2	
Plate-setup	1	2	3	4	5	6	7	8	9	10	11	12
A	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	
B	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	
C	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	
D	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	
E	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	
F	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	
G	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	
H	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN	

Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

COSYPS

- amplicons cloned Into a pUC18 vector
- all plasmid vectors can be used in a plasmid-mix set-up as reference material.

- a mathematical model has been developed that allows for identification of possibly present GM events in a sample applying a prime-number based GMO identification algorithm

- model is developed in a Microsoft Excel format

- fully operational within an ISO 17025 evaluated system since September 2006.
- July 2010 - successfully applied in
  - GEMMA "Food Ingredient" proficiency tests or ISTA "Seed" proficiency tests
  - in more than 350 different Food/Feed samples by the Belgian GMO enforcement framework under control of the Belgian Federal Agency for Food Safety.

Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

"Pre-spotted" plates for event-specific screening

A Ready-To-Use Multi-Target Analytical System for GM Soy and Maize Detection for Enforcement Laboratories

Linda Kluga, Marc Van den Bulcke, Silvia Folloni, Jean-Michel Gineste, Thomas Weber, Nicoletta Foti, Marco Mazzara, Guy Van den Eede and Maddalena Querci
European Commission – Joint Research Centre, Institute for Health and Consumer Protection (IHCP), Molecular Biology and Genomics Unit, Via Fermi 2749, 21027 Ispra (Va) - Italy

Methodological approach:
real-time PCR (probe based)

Format:
96-well plate format

Analytical target(s):
event-specific targets of EU
Approved and unapproved GM events

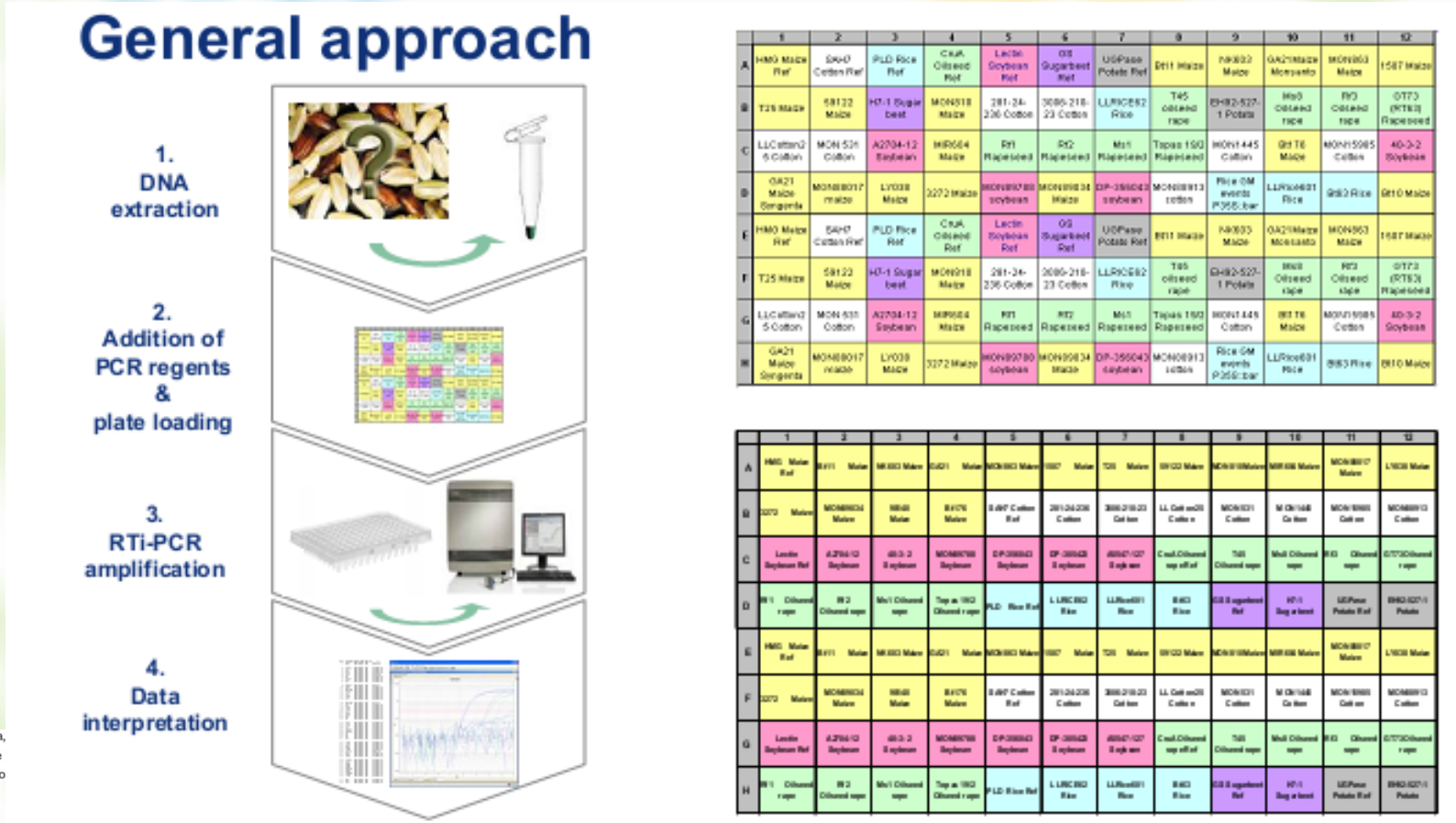
Product format:
ready-to-use pre-spotted plates containing, in lyophilized format, primers and probes for all methods

Maize	Oilseed rape
Bt11	T45
NK603	Ms8
GA21	Rf3
MON863	GT73
1507	Rf1
T25	Rf2
59122	Ms1
MON810	Topas 19/2
MIR604	Rice
Bt176	LLRICE62
MON88017	LLRice601
LY038	Bt63 Rice
3272	Sugar beet
MON89034	H7-1 Sugar beet
Bt10	Cotton
Soybean	MON1445
A2704-12	MON88913
40-3-2	LLCotton25
MON89788	MON 531
DP-356043	281-24-236X3006-210-23
Potato	MON15985
EH92-527-1	

Approaches for detecting unknown GMOs

Qualitative differential analysis - “Matrix Approach”

"Pre-spotted" plates for event-specific screening



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Approaches for detecting unknown GMOs

Qualitative differential analysis - “Matrix Approach”

"Pre-spotted" plates for event-specific screening

the first analytical tool worldwide allowing

simultaneous detection of so many genetic modification events using event-specific targets.

use of the 96-well RTi-PCR platform

easily integrated in the laboratories' working routine,

without the need for new instrumentation or new procedures

The plates **contain all necessary reagents** to screen the EU-authorized GMOs and a number of unauthorized GMOs

highly efficient,

time-saving,

low cost

methods validated

Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

"Pre-spotted" plates for event-specific screening

Great potential for increasing harmonisation in GMO testing:

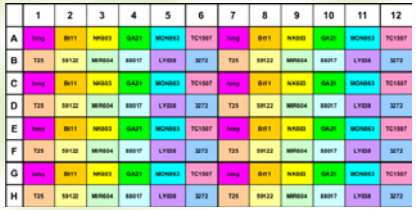
–Tool to test many events/targets at once (need for constant updating)

–Unique tool/provider for all control laboratories;

–Harmonised set of targets / methods;

–Flexibility to be adapted according to needs;

–Same tool - if used by different laboratories →comparable results.



Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

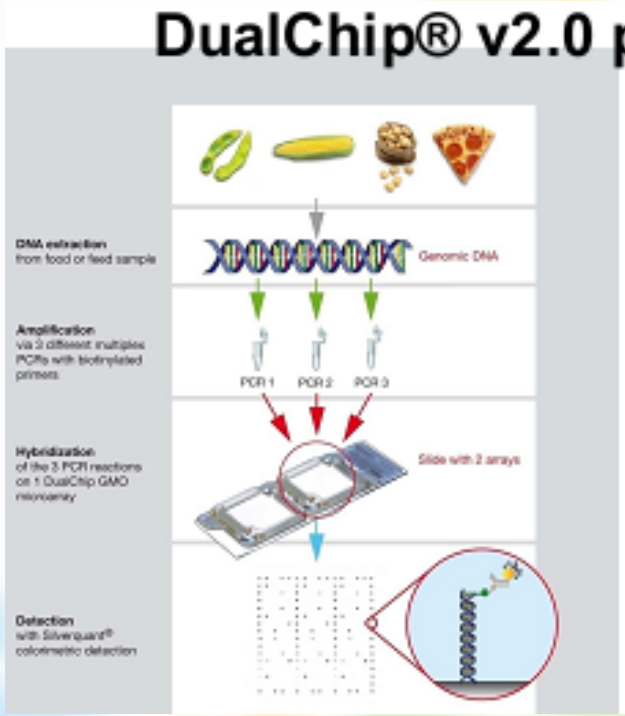
- Micro-arrays: DualChip® first inter-laboratories validated chip

Multiple specific DNA capture probes to species-specific targets or to control targets are immobilized separately on glass slides.

The immobilised DNA on the glass slide "captures" specific DNA elements of GMOs – if present in the sample

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



32 genetic elements to be detected

3 multiplex PCR

Collaboration started through GMOchips with Namur's Univ. and AAT (spin-off) then EAT

eppendorf

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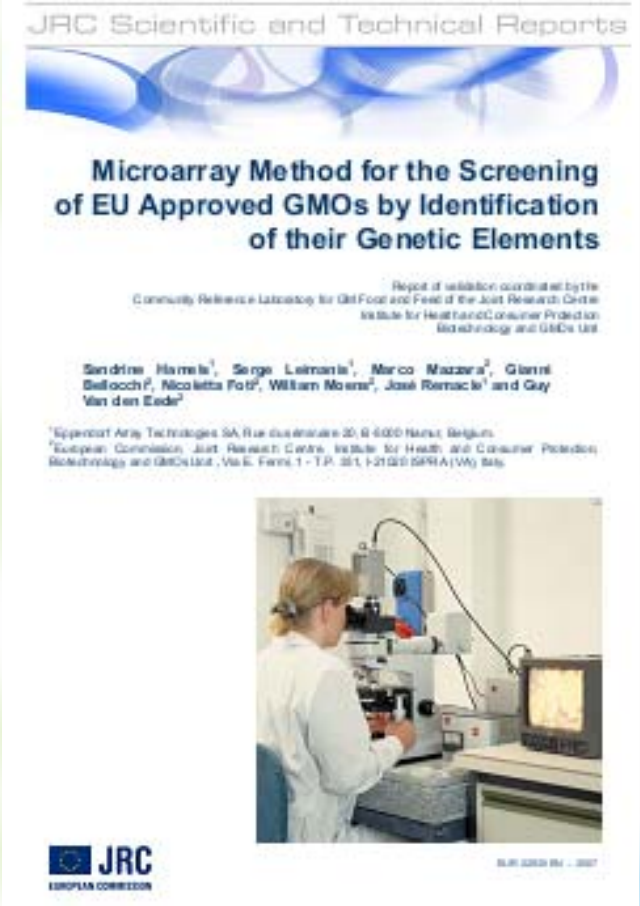
Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"


JRC Scientific and Technical Reports

Microarray Method for the Screening of EU Approved GMOs by Identification of their Genetic Elements

Report of validation coordinated by the Community Reference Laboratory for GM Food and Feed of the Joint Research Centre Institute for Health and Consumer Protection Biotechnology and GMOs Unit

Sandrine Hamels[1], Serge Leimanis[1], Marco Mazzara[2], Gianni Bellocchi[2], Nicoletta Foti[2], William Moens[2], José Remacle[1] and Guy Van den Eede[2]

[1]Eppendorf Array Technologies SA Rue du Séminaire 20, B-6000 Namur, Belgium
[2]European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Biotechnology and GMOs Unit, Via E. Fermi, 1 - T.P. 201, I-21020 ISPRA (VA), Italy

JRC
EUROPEAN COMMISSION

Eur Food Res Technol (2008) 227:1621–1632
DOI 10.1007/s00217-008-0886-y

ORIGINAL PAPER

Validation of the performance of a GMO multiplex screening assay based on microarray detection

Serge Leimanis · Sandrine Hamels · Florence Nazé · Guillaume Mbongolo Mbella · Myriam Sneyers · Rupert Hochegger · Hermann Broll · Lillian Roth · Klára Dallmann · Adrienn Micsinai · José Luis La Paz · Maria Pla · Claudia Brünen-Nieweler · Nina Papazova · Isabel Taverniers · Norbert Hess · Britta Kirschneit · Yves Bertheau · Colette Audeon · Valérie Laval · Ulrich Busch · Sven Pecoraro · Katrin Neumann · Sibylle Rösel · Jeroen van Dijk · Esther Kok · Gianni Bellocchi · Nicoletta Foti · Marco Mazzara · William Moens · José Remacle · Guy Van Den Eede

Received: 21 December 2007 / Revised: 29 April 2008 / Accepted: 4 May 2008 / Published online: 27 May 2008

Drawbacks to this technology

i) a lower flexibility with respect to inclusion of novel targets on an ad-hoc basis,

ii) the need to purchase (relatively) expensive commercial reagents and novel equipment in addition to the PCR apparatus

iii) the increased risk of carry over contamination resulting from the dependence on post-PCR pipetting of amplified DNA

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Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

SNPlex

two major drawbacks with the use of multiplex PCR methods:

- appearance of amplification artifacts,

or

- nonspecific amplification products

SNPlex technology

a high-throughput genotyping method.

48 signature sequences are detected that correspond to

- sequences of GMO construction
- sequences of plant reference genes, and
- sequences of donor organisms such as

Agrobacterium tumefaciens , *Bacillus thuringiensis*, and cauliflower mosaic virus.

Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

11596 J. Agric. Food Chem. 2008, 56, 11596–11606

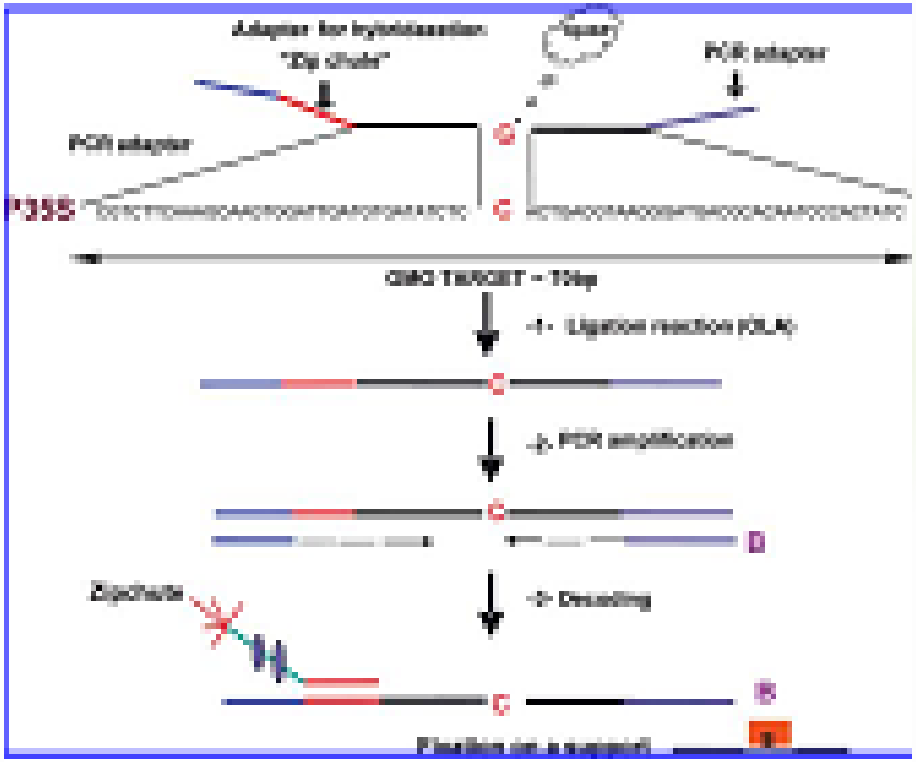
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

A High-Throughput Multiplex Method Adapted for GMO Detection

Maher Chaouachi,[†,§] Gaëlle Chupeau,[†] Aurélie Berard,[†] Heather McKhann,[†] Marcel Romaniuk,[§] Sandra Giancola,[†] Valérie Laval,[§] Yves Bertheau,[§,#] and Dominique Brunel*[,†]

CEA/Institut de Génomique/Centre National de Génotypage/INRA UR EPGV, 2 rue Gaston Crémieux, CP 5724, 91057 Evry cedex, France, and INRA, Laboratoire de Méthodologies de la Détection des OGM, Unité PMDV, Route de Saint Cyr, RD10, 78026 Versailles cedex, France

SNPlex



Ligation mediated PCR approach

binding sites for universal primers are coupled to the terminal-ends of two primers for a single strand of the GM template.

two primers are ligated to a single stranded novel template with universal primer sites only in the presence of the appropriate template.

After the ligation reaction PCR with universal primers is performed.

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Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

SNPlex

Detection and identification of the amplification products is done by solid phase capture (biotin-streptavidin) and hybridisation with labelled probes

simultaneous detection of up to 48 targets

can be applied to 384 well microtiter plate formats, with one sample per well.

The assay sensitivity lower than the EU threshold for labelling (0.9%),

the level of multiplexing superior.

The assay requires particular equipment and software.

Detectable targets

SNPlex

Table 2. Description of the Multiplexed Targets Grouped into the Two Studied Panels[a]

	TARGETS				
PANEL 1 (47 markers)	Taxa	Screening	Construct specific	Event specific	Donor organisms
PANEL 2 (48 markers)	TARGETS				
	Taxa	Screening	Construct specific	Event specific	Donor organisms

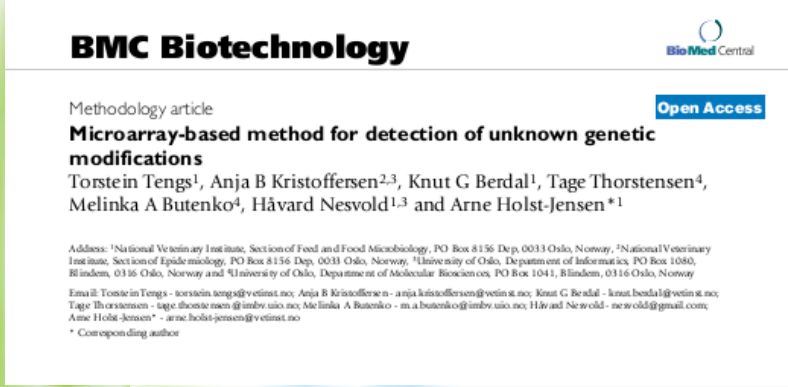
[a] CP4EPSPS1 (soybean event RR sequence), CP4EPSPS2 (sugar beet event GTSB77 sequence); Cry1Ab1, 2 and 3 correspond to Bt11, Bt176, and MON810 sequences. SS, signature sequence. For the targets P35S and P35S/pat, the signature sequences were different between the first and second panels.

Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

High density microarrays – detecting vectors sequences

Direct hybridisation of genomic DNA to "profiling" microarrays



BMC Biotechnology

BioMed Central

Methodology article

Open Access

Microarray-based method for detection of unknown genetic modifications

Torstein Tengs[1], Anja B Kristoffersen[2,3], Knut G Berdal[1], Tage Thorstensen[4], Melinka A Butenko[4], Håvard Nesvold[1,3] and Arne Holst-Jensen*[1]

Address: [1]National Veterinary Institute, Section of Feed and Food Microbiology, PO Box 8156 Dep, 0033 Oslo, Norway, [2]National Veterinary Institute, Section of Epidemiology, PO Box 8156 Dep, 0033 Oslo, Norway, [3]University of Oslo, Department of Informatics, PO Box 1080, Blindern, 0316 Oslo, Norway and [4]University of Oslo, Department of Molecular Biosciences, PO Box 1041, Blindern, 0316 Oslo, Norway

Email: Torstein Tengs - torstein.tengs@vetinst.no; Anja B Kristoffersen - anja.kristoffersen@vetinst.no; Knut G Berdal - knut.berdal@vetinst.no; Tage Thorstensen - tage.thorstensen@imbv.uio.no; Melinka A Butenko - m.a.butenko@imbv.uio.no; Håvard Nesvold - nesvold@gmail.com; Arne Holst-Jensen* - arne.holst-jensen@vetinst.no

* Corresponding author

This approach developed a strategy for detection and characterization of unknown genetic modifications

The approach relies on direct hybridization of total genomic DNA to high density microarrays designed to have probes tiled throughout a set of reference sequences

PCR-independent,

applies direct hybridization of total genomic DNA

takes advantage of the high degree of recycling and sequence similarity between elements

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Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

Anchor PCR fingerprinting approach

Each GMO produces a specific anchor PCR fingerprint. Fragments can be sequenced

Anchor PCR – Semitargeted PCR, captures fragment adjacent to anchor

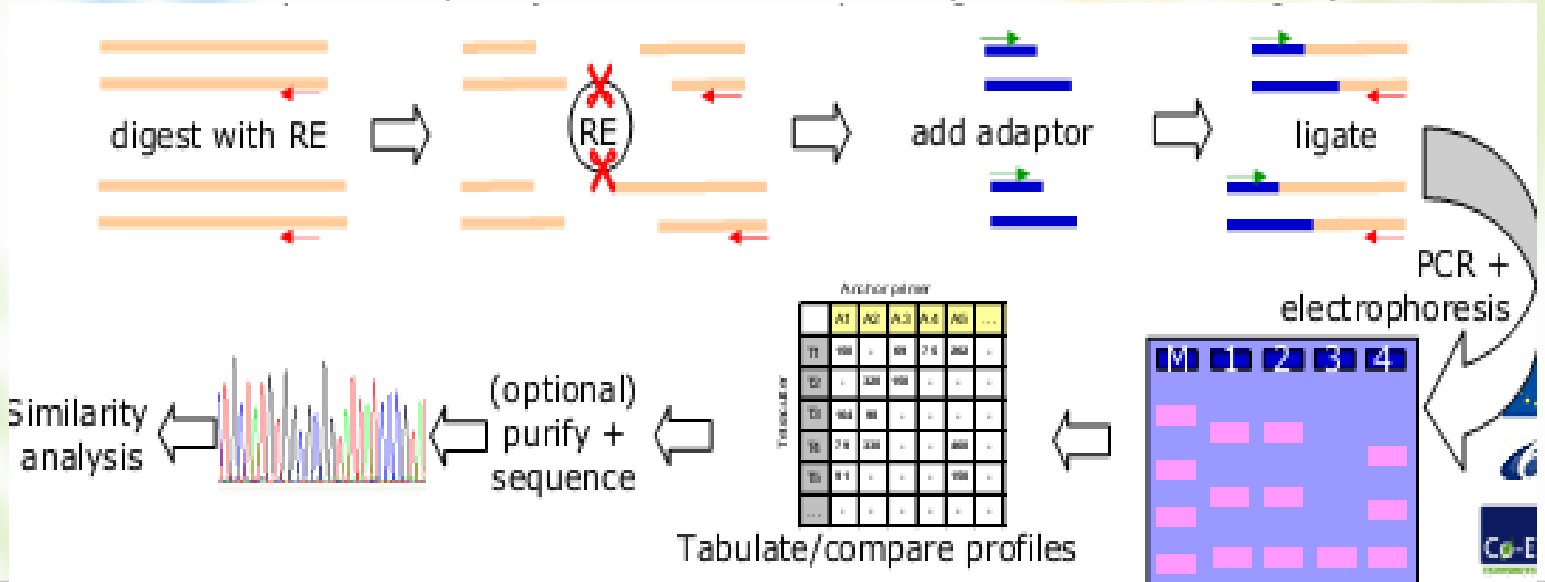
DNA fragmented with restriction enzyme (RE), adaptor ligated to fragment

PCR with anchor primer ← and adaptor primer →

Result = fingerprint profile specific for GMO + RE + adaptor + anchor

Fingerprint profiles can be tabulated (size per fragment per profile)

Suspected UGM subject to anchor PCR profiling. Profile matched against known



Approaches for detecting unknown GMOs

Qualitative differential analysis - "Matrix Approach"

Transcriptome sequencing

High throughput sequencing: GM subtracted from non-GM transcriptome

Isolate mRNA from suspected UGM, convert to cDNA with reverse transcriptase

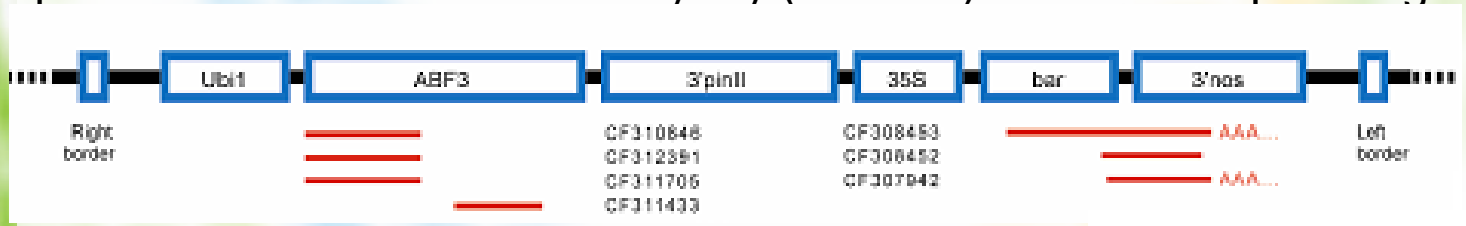
Option 1: Subtract against cDNA library from non-GM

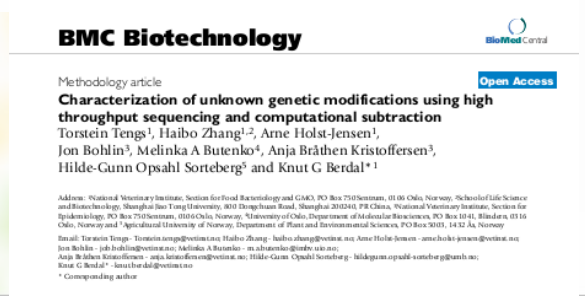
Perform high-throughput DNA sequencing on the suspect cDNA library

Option 2: Subtract against DNA sequence database with non-GM sequences

Apply bioinformatics to identify potential GMO-derived sequence motifs

Exploit identified motifs to verify by (anchor-)PCR and sequencing




BMC Biotechnology

Methodology article

Open Access

Characterization of unknown genetic modifications using high throughput sequencing and computational subtraction

Torstein Tengs[1], Haibo Zhang[1,2], Arne Holst-Jensen[1], Jon Bohlin[3], Melinka A Butenko[4], Anja Bråthen Kristoffersen[3], Hilde-Gunn Opsahl Sorteberg[5] and Knut G Berdal*[1]

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Approaches for detecting unknown GMOs

Quantitative differential analysis

Quantify at least two targets – hypothetically equal quantities

Significant difference in quantity means that hypothesis is falsified

Example: screening element and multiple GMO events

For screening element S: [S] = QS

For all authorised GMOs (A, B, …) containing S: [A + B + …] = QAuth = QA + QB +

Taking into consideration all measurement uncertainty factors

Hypothesis: μ = QS - QAuth = 0

Detecting unknown GMOs: the differential quantitative PCR

Analytical Biochemistry 376 (2008) 189–199

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

ELSEVIER

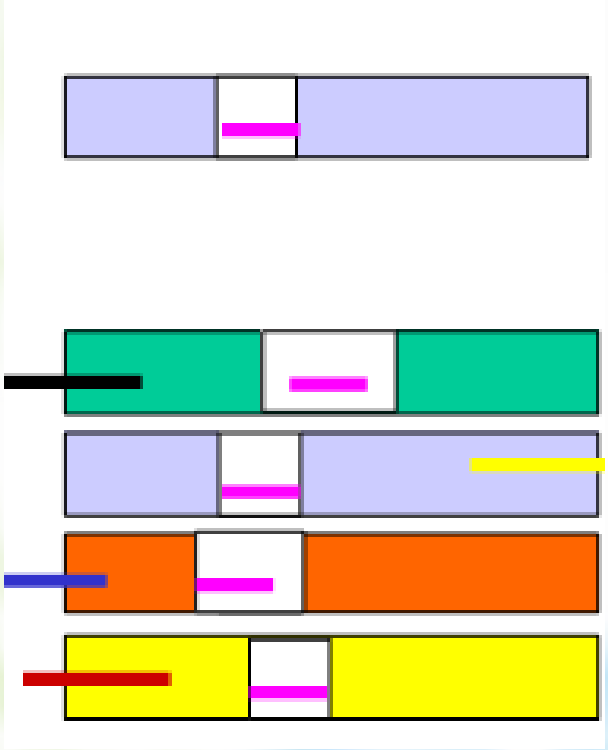
Detection of nonauthorized genetically modified organisms using differential quantitative polymerase chain reaction: application to 35S in maize

Katarina Cankar[a,1,2], Valérie Chauvensy-Ancel[b,1], Marie-Noelle Fortabat[b,1], Kristina Gruden[a], André Kobilinsky[c], Jana Žel[a], Yves Bertheau[d,*]

Approaches for detecting unknown GMOs

Quantitative differential analysis

Quantitative differential approach (dQ PCR): principle



Detect and quantify a consensus element common to a group of GMOs

set of primers specific of the consensus element, eg. p35S

Detect and quantify all the approved GMOs presenting this consensus element

sets of event-specific primers for each of these GMO (e.g. edge-fragments

Approaches for detecting unknown GMOs

Quantitative differential analysis

Method could be easily transposed to other common GMO sequences,

Usable for determining the number of copies of any sequence

Use of existing equipment and reagents without the need for additional personnel training

Approaches for detecting unknown GMOs

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

Taking decisions in uncertain environment

- Matrix data can detect several GMO in a time: interpretation of data
- Matrix approach can detect unknown GMO

Need of Decision Suport System for

- Harmonization of data interpretation
- Reporting
- Decision making
- In combination with doc traceability

The global dimension

UGMs affect

- domestic supplies,
- international trade,
- reduce the trust in industry and authorities,
- pose risks to human and animal health and the environment.

the effective way to inspect unauthorized GMOs is a big challenge for detection laboratories.

Various analytical methods have been developed and collected in various databases .

current testing methods also need improvements

- cost,
- in-field application
- specificity and
- ability to quantify the commercial GMOs.

The global dimension

For the incidents of unauthorized events reported so far, no evidence of significant harm to human health has been provided.

these incidents challenge the present regulations in many countries that require authorisation

a number of detection approaches have been developed and
additional approaches are under development.

The “ideal” - event specific methods for unauthorized events.

alternative is application of combinations of screening methods and comparing the results with tabulated data

The global dimension

Limitations of this approach

the presence of unknown events is only inferred
the evidence for unknown events is indirect

it does **not provide conclusive evidence** of the presence of unauthorized GMO it yields only indirect evidence for unknown events;

the screening method by itself does not identify the causative event per se.

an unknown event may remain unnoticed if the presence of known events does explain the detection of screening elements.

products with LLP of one or more known events may “mask” the presence of novel UGMs.

The global dimension – Future trends

Actual trend

initial screening applying the "matrix approach", followed by "ad hoc" results verification using more specific PCR and/or DNA sequencing methods

Remaining gaps – Steps for harmonization

ability to conclude on the absence or presence of UGM
GMO reference framework - "GMO Reference Matrix"

availability of validated screening methods and appropriate reference materials

development of decision support systems that are open to a large community through web applications

The global dimension

Future trend

development of faster and cheaper analytical methods

Methods allowing high-throughput, miniaturization, automation and quantification.

Owing to the differences in labeling regulations among different countries,

standardization,

exchange of information,

international cooperation on GMO analytical methods will be also extremely important:

facilitate monitoring GMOs,

reduce possible disputes for global trade.

Final remarks

Unauthorised GMOs represent a significant and growing challenge to stakeholders

Co-existence between GM and non-GM supply chains is difficult

.

The negative impact can only be reduced if the problems are given increased attention by the involved stakeholders.

Resources for control and enforcement may need to be increased

research and development on suitable and reliable detection methods.

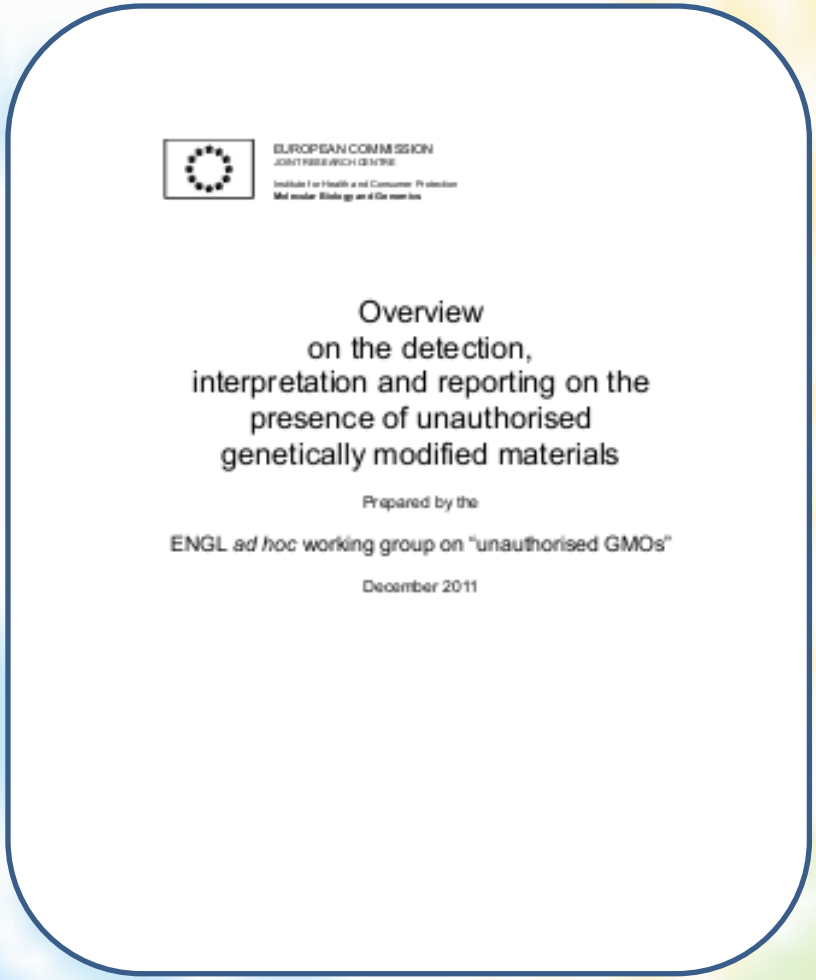
International collaboration

facilitate information and material exchange

harmonise analytical approaches and traceability

Transparency facilitates monitoring and identification and may reduce risks!

Final remarks



chaired by Arne Holst-Jensen, National Veterinary Institute (NVI), Oslo, Norway

MEMBER OF

European Network of GMO Laboratories

Thanks for your attention

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