

Towards higher throughput GMO testing: matrix-based screening approaches and multi-target detection systems



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The need for screening in traceability of GMOs

The number of new GMOs is increasing constantly

Employing only event-specific methods for GMO testing thus becomes impractical

Time and cost effective strategies include the development of **screening approaches** using element- or construct-specific detection methods

Screening strategies for GMO testing: main concerns

1. Planning.

How can I make sure the methods used in the screening strategy will not “miss” one of the GMO I want to screen for?

2. Drawing conclusions

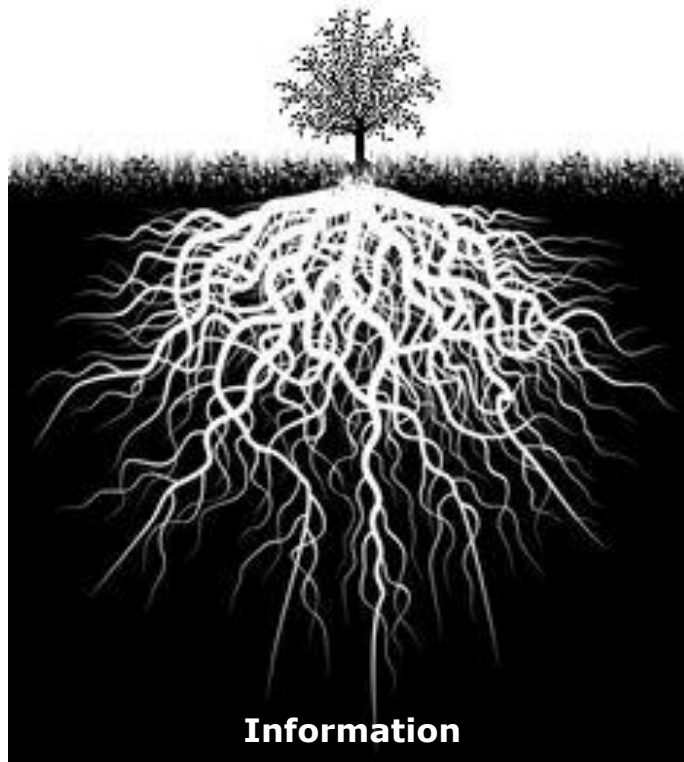
How can I gain the most information from the results of the screening results, i.e. narrowing down the pool of potential candidates.

A number of "**GMO target matrices**" have been developed to support the planning and evaluation of screening approaches



European
Commission

Detection



Information

Information on GMO detection by screening methods

Event annotation



- + Many public sources of information (eg the BCH LMO registry)
- Elements given the same name in various GM events can differ at the level of the DNA sequence

Information on GMO detection by screening methods

Event annotation

Event sequence



- + Allows direct prediction of detection by bioinformatics tools
- Event sequences difficult to obtain

Information on GMO detection by screening methods

Event annotation

Event sequence

Laboratory testing



- + Gives a "formal" answer for the detection of an event by a detection method (i.e. not a prediction)
- Very high confidence in the experiment is crucial (sample purity, quality of execution, ...). Significant work required.

Examples of available matrices

GMOfinder

Specific profile of 15 elements and constructs for >350 GMOs
Information taken from experimental data, literature, applications for
GMO authorisation and other sources

Additional information: Gerdes, Lars, Ulrich Busch, and Sven Pecoraro. "GMOfinder—a GMO screening database." *Food Analytical Methods* 5.6 (2012): 1368-1376.

GMOseek

Provides an overview of >270 genetic elements and their occurrence in
>320 GMOs

Additional information: Block, Annette, et al. "The GMOseek matrix: a decision support tool for optimizing the detection of genetically modified plants." *BMC bioinformatics* 14.1 (2013): 256.

The JRC GMO-Matrix application

Predicts (in silico) **which GMO detection method(s)** will detect **which GMO event(s)**

Links the information between

1. **The JRC GMOMETHODS** database
2. The internal **Central Core Sequence Information System (CCSIS)**, a database that hold sequences of GMO inserts.

GMOMETHODS: The European Union Database of Reference Methods for GMO Analysis

Legal notice Privacy statement English (EU)



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

European Commission > JRC > MCP > EU-RL GMFF

EU-RL GMFF Home

Legal basis

Tasks and duties

Guidance documents

Status of dossiers

Methods database

Capacity building

ENGL

Emergencies/
Unauthorised GMOs

Contacts

GMOMETHODS: EU Database of Reference Methods for GMO Analysis

Home

Search GMOMETHODS for Search Select by GMO Unique Identifier:

Quantitative GMO detection PCR methods

- GMO specific
 - Event specific
 - Maize
 - Soybean
 - Cotton
 - Oilseed rape
 - Potato
 - 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)
 - Flavour Mosaic Virus 35S promoter (P-FMV)
 - Neomycin phosphotransferase II gene (nptII)
 - Nopaline synthase terminator (T-nos)
 - Phosphinobitrin 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.



Last update

Date	ID	Description
28/06/2013	OT-ELE-00-004	Quantitative PCR method for detection of Cauliflower Mosaic Virus 35S promoter
25/01/2013	BCS-GH004-7	Quantitative PCR method for

Available at: <http://gmo-crl.jrc.ec.europa.eu/gmomethods/>

More information: Bonfini *et al.* Journal of AOAC international (2012)

Mining of the public DNA sequence databases for GMO sequences

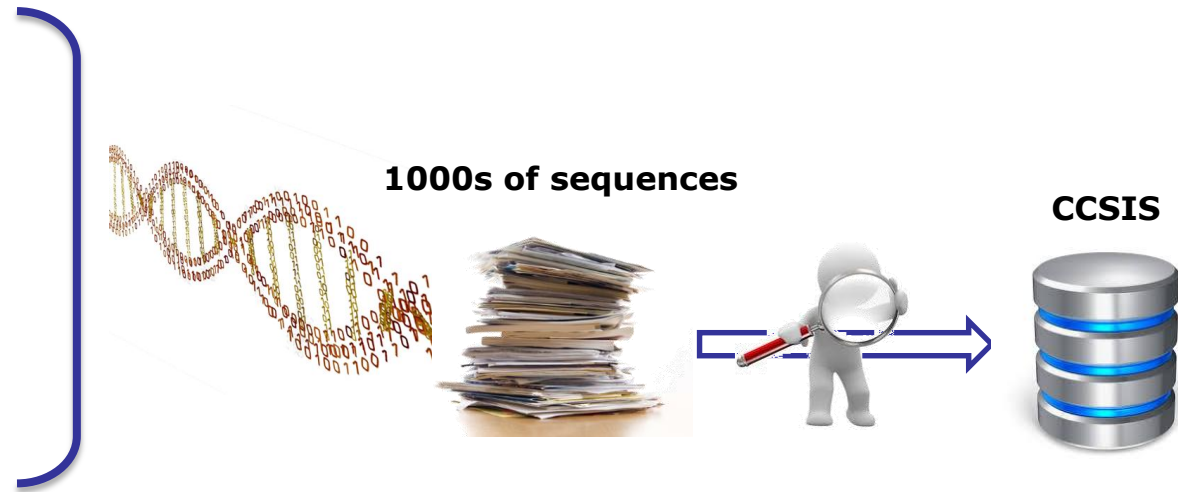


Scientific literature



Patents

1 000 000 000s of sequences



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JRC GMO-Matrix

1) Select GMO(s):

By taxon(s)

and/or

Specific GMO(s)

2) Select method(s):

Event-specific

and/or

Construct-specific

and/or

Element-specific

Please select at least one taxon

Please select at least one method

Try an example matrix: All cotton events vs 'pat' and 'bar' element-specific methods

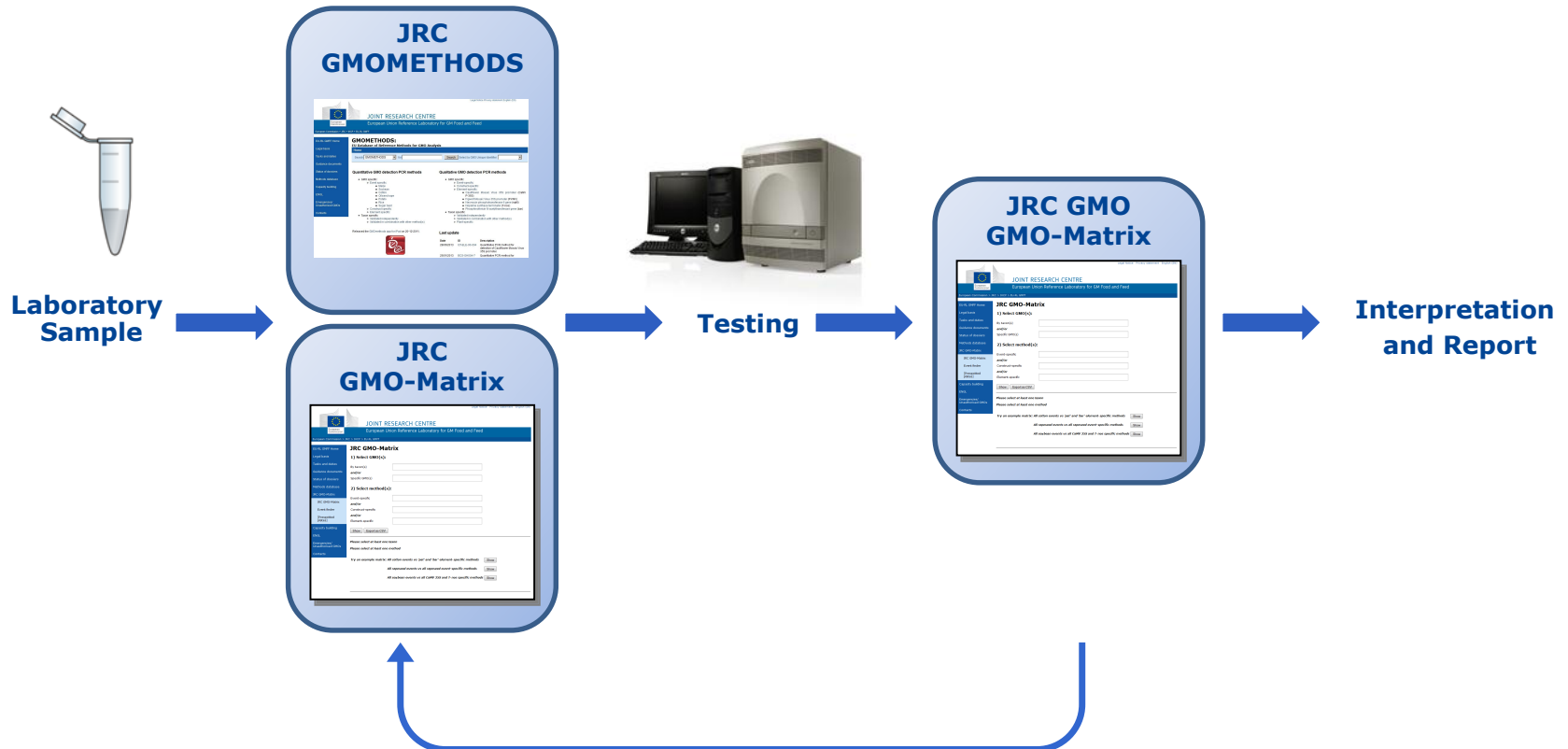
All rapeseed events vs all rapeseed event-specific methods

All soybean events vs all CaMV 35S and T-nos specific methods

Available at: <http://gmo-crl.jrc.ec.europa.eu/jrcgmomatrix/>

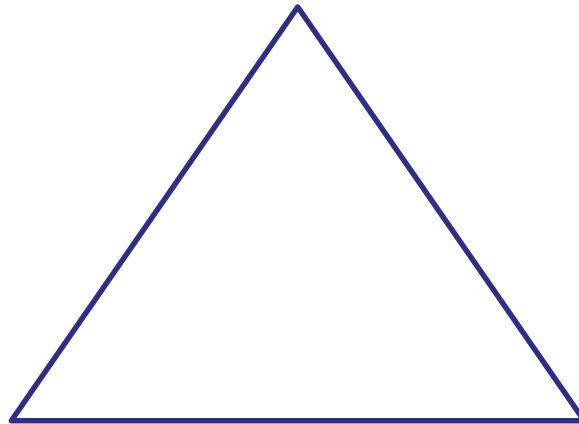
More information: Angers-Loustau et al. BMC bioinformatics (2014)

From planning to analysis



EURL-GMFF support to harmonisation of GMO screening

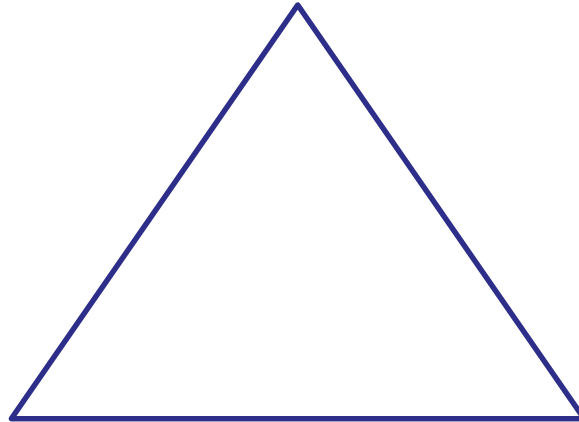
Reference methods
(Validation, GMOMETHODS database)



Decision-support tools
(JRC GMO-Matrix, JRC GMO-Amplicons)

EURL-GMFF support to harmonisation of GMO screening

Reference methods
(Validation, GMOMETHODS database)



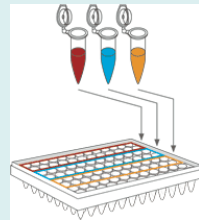
Decision-support tools
(JRC GMO-Matrix, JRC GMO-Amplicons)

Ready-to-use system
(Pre-spotted plates)

Pre-Spotted Plates (PSP) for GMO detection



PSP



qPCR plastic support whose wells are spotted with dried primers & probes (GMO detection assays)

Advantage

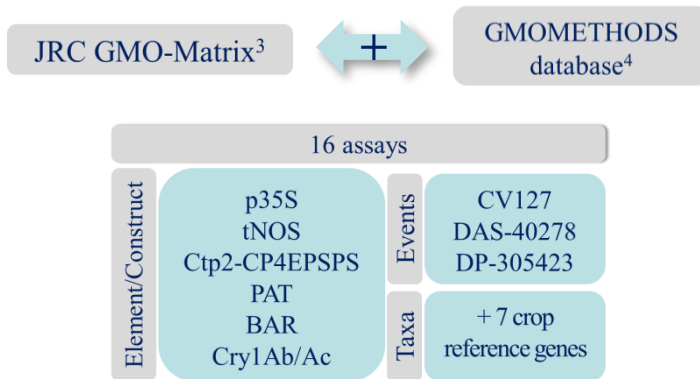


Perform up to 96 assays in a single experiment, with limited steps

Development of a GMO Screening System

I. Which assays?

III. Building a screening strategy: combine PSP with a Decision Support System



Sample 1 rep 1	Sample 1 rep 2	Sample 2 rep 1	Sample 2 rep 2	Positive Control	Negative Control
hmg p35s	hmg p35s	hmg p35s	hmg p35s	hmg p35s	hmg p35s
lec tNOS	lec tNOS	lec tNOS	lec tNOS	lec tNOS	lec tNOS
CruA CTP2-CP4EPSPS	CruA CTP2-CP4EPSPS	CruA CTP2-CP4EPSPS	CruA CTP2-CP4EPSPS	CruA CTP2-CP4EPSPS	CruA CTP2-CP4EPSPS
sah7 pat	sah7 pat	sah7 pat	sah7 pat	sah7 pat	sah7 pat
ugg bar	ugg bar	ugg bar	ugg bar	ugg bar	ugg bar
pld Cry1Ab/Ac	pld Cry1Ab/Ac	pld Cry1Ab/Ac	pld Cry1Ab/Ac	pld Cry1Ab/Ac	pld Cry1Ab/Ac
gs CV 127	gs CV 127	gs CV 127	gs CV 127	gs CV 127	gs CV 127
DAS 40278 DP 305423	DAS 40278 DP 305423	DAS 40278 DP 305423	DAS 40278 DP 305423	DAS 40278 DP 305423	DAS 40278 DP 305423

+

JRC GMO-Matrix³

Web!

Best identification strategy!

II. Assay standardization for use on PSP (and re-assessment of method performance)

The following events match the selected pattern:

	CaMV P-35S	T-nos	CTP2-CP4 EPSPS	pat	bar	cry1Ab/Ac	DAS-40278-9	CV127	DP-305423-1
Corn (Zea mays):									
GMO Event NK603 Maize (MON-00603-6)	2	2	2	0	0	0	0	0	0
GMO Event MON88017 Maize (MON-88017-3)	2	2	2	0	0	0	0	0	0

The following events can also be present:

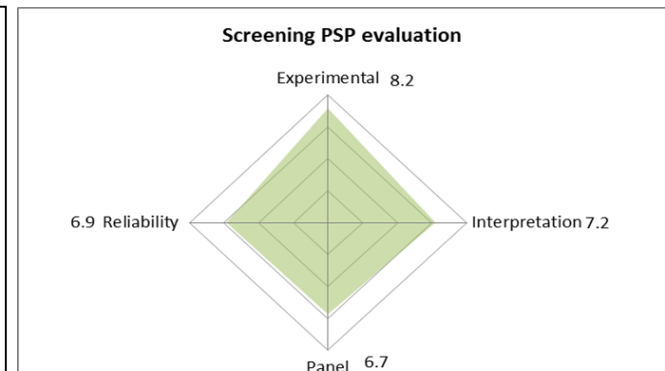
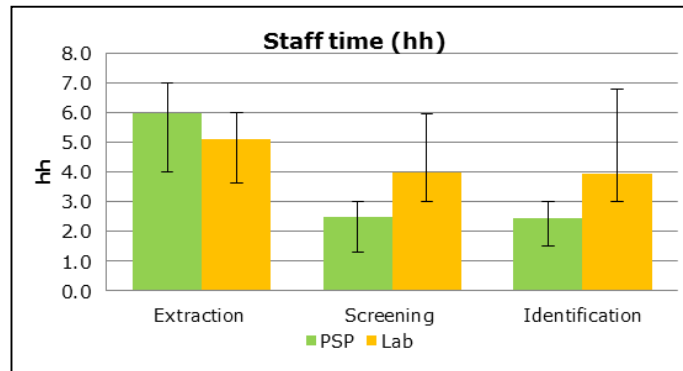
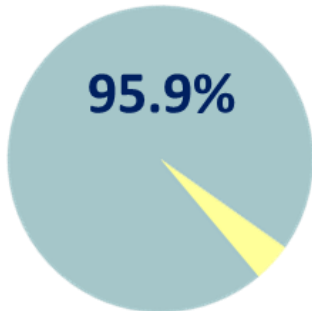
	CaMV P-35S	T-nos	CTP2-CP4 EPSPS	pat	bar	cry1Ab/Ac	DAS-40278-9	CV127	DP-305423-1
Corn (Zea mays):									
GMO Event 98140 Maize (DP-098140-6)	2	0	0	0	0	0	0	0	0
GMO Event GA21 Maize (MON-00021-9)	0	2	0	0	0	0	0	0	0

PSPs perform well and are functional

Pilot Study (20 EU NRLs, 135 real-life samples) - workflow

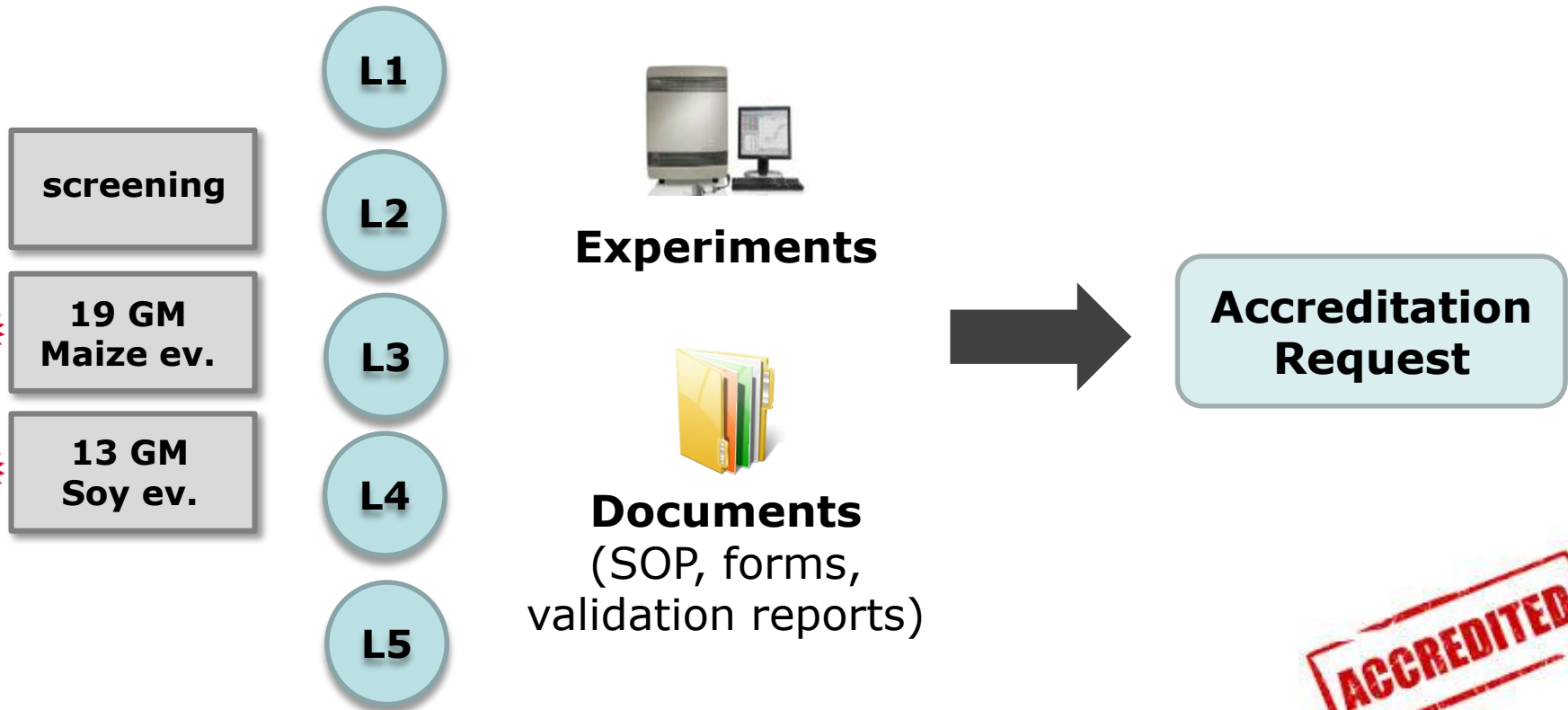


Performance of the strategy (PSP + JRC GMO-Matrix)



Proof of concept study

Integration of PSPs in the quality system of official testing laboratories working under ISO17025 accreditation



Semi-quantitative use of ePSP

Eur Food Res Technol
DOI 10.1007/s00217-011-1615-5

ORIGINAL PAPER

Applicability of the “Real-Time PCR-Based Ready-to-Use Multi-Target Analytical System for GMO Detection” in processed maize matrices

Linda Kluga · Silvia Folloni · Marc Van den Bulcke ·
Guy Van den Eede · Maddalena Querci

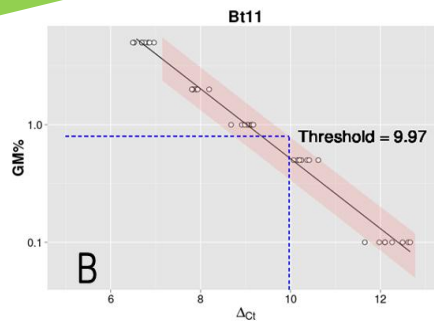
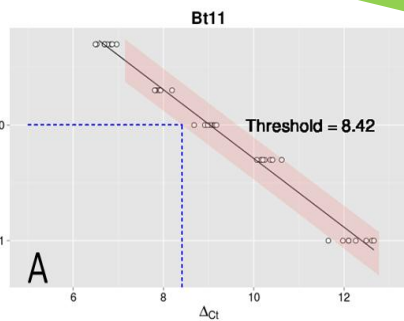
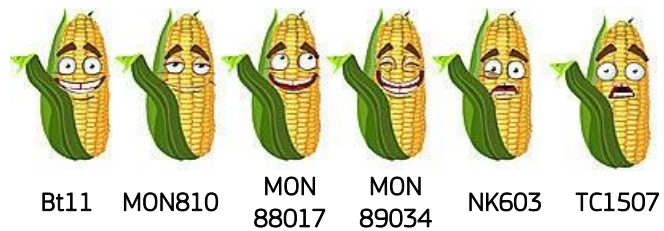
Table 5 Theoretic correlation between the ΔC_t , the dilution factor and the GM%

ΔC_t	Dilution	GM%
1	2	50.00
2	4	25.00
3	8	12.50
4	16	6.25
5	32	3.13
6	64	1.56
7	128	0.78
8	256	0.39
9	512	0.20
10	1024	0.10
11	2048	0.05
12	4096	0.02
13	8192	0.01
14	16384	0.01



$$\Delta Cq = Cq_{GM} - Cq_{Txn} \approx \%GM$$

Estimation and assessment of ΔCq Thresholds



Below 0.9% Above

Assessment on simulated samples



GM%	<0.9%	Quant.	>0.9%
0.1	99.1	0.9	0.0
0.5	58.2	41.1	0.7
1	10.5	83.2	6.3
2	0.0	17.5	82.5
5	0.0	0.7	99.3

Thank you for your attention!

