

Overview on GMO detection approaches



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- **GMO producers**
 - To assure purity and segregation of products
 - To trace genetic modification in breeding
- **Food & feed industry, seed companies**
 - To assure purity and segregation of products
 - To assure compliance with legislation
- **Competent (enforcement) authorities**
 - Product control, compliance with legislation
 - To be able to retrieve specific products
 - E.g. if marketing permission withdrawn
- **Laboratories**
 - To provide analytical services to customers

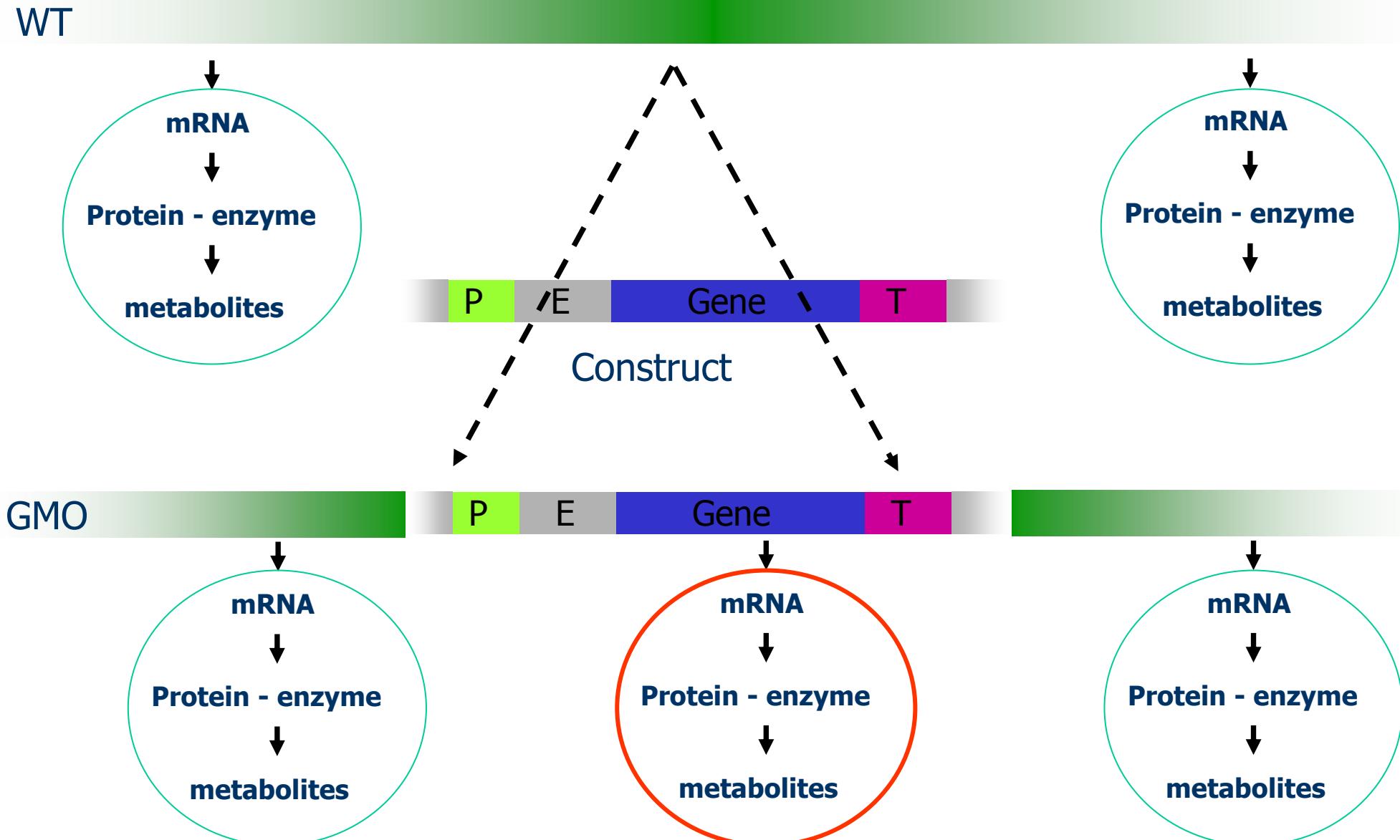
Why to analyse GMOs?

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How to Know if a Product is Genetically Modified ?

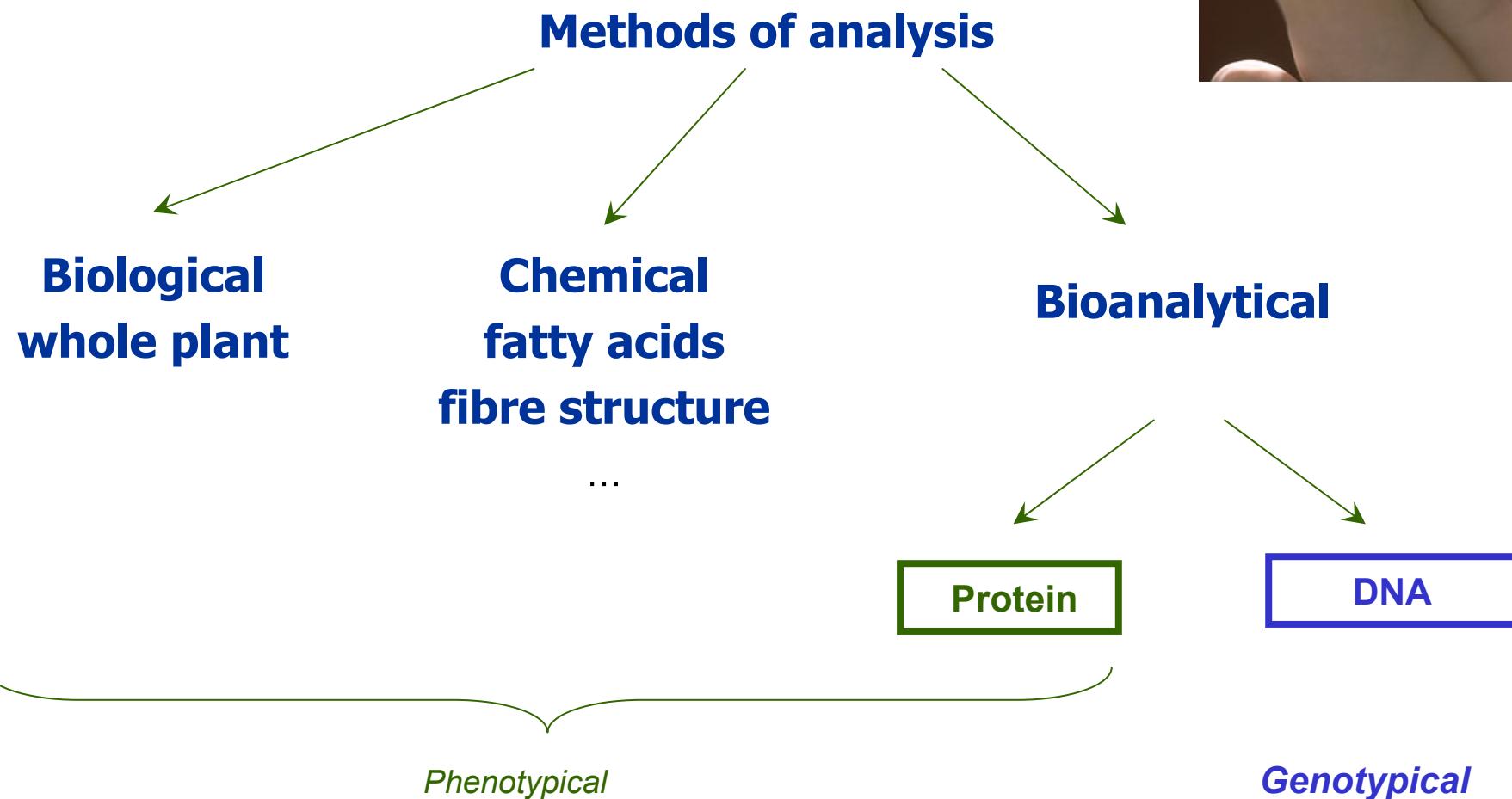


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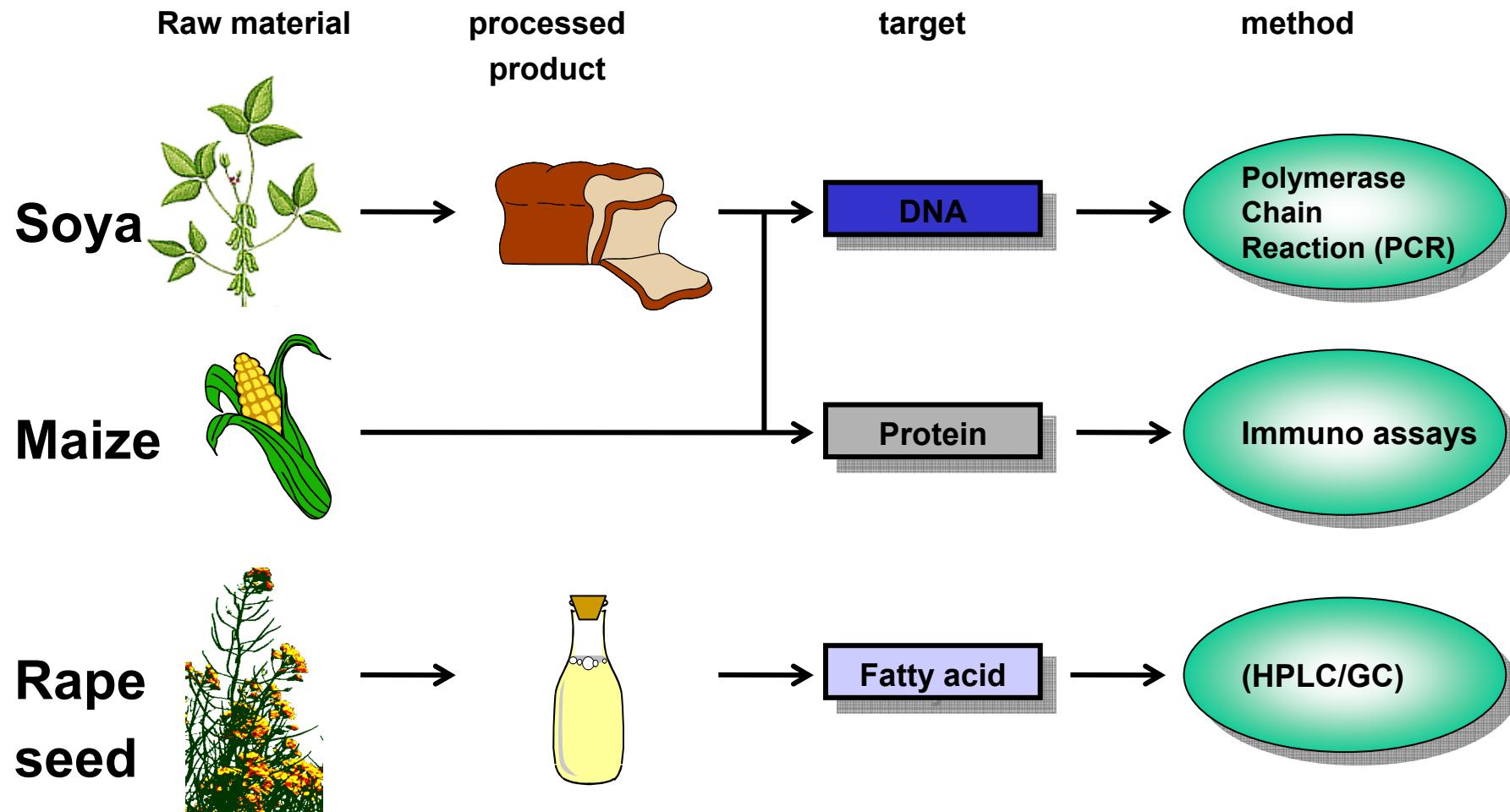
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Which method to select ?



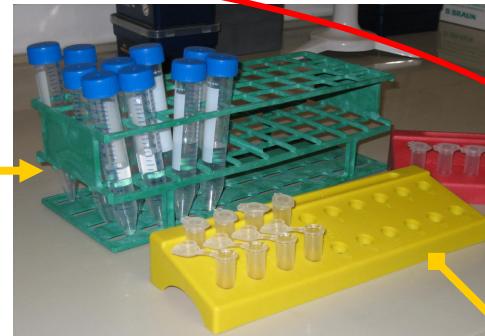
Detection strategies



Steps in DNA based GMO analysis

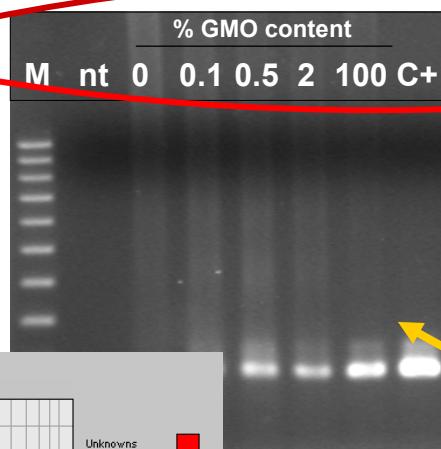
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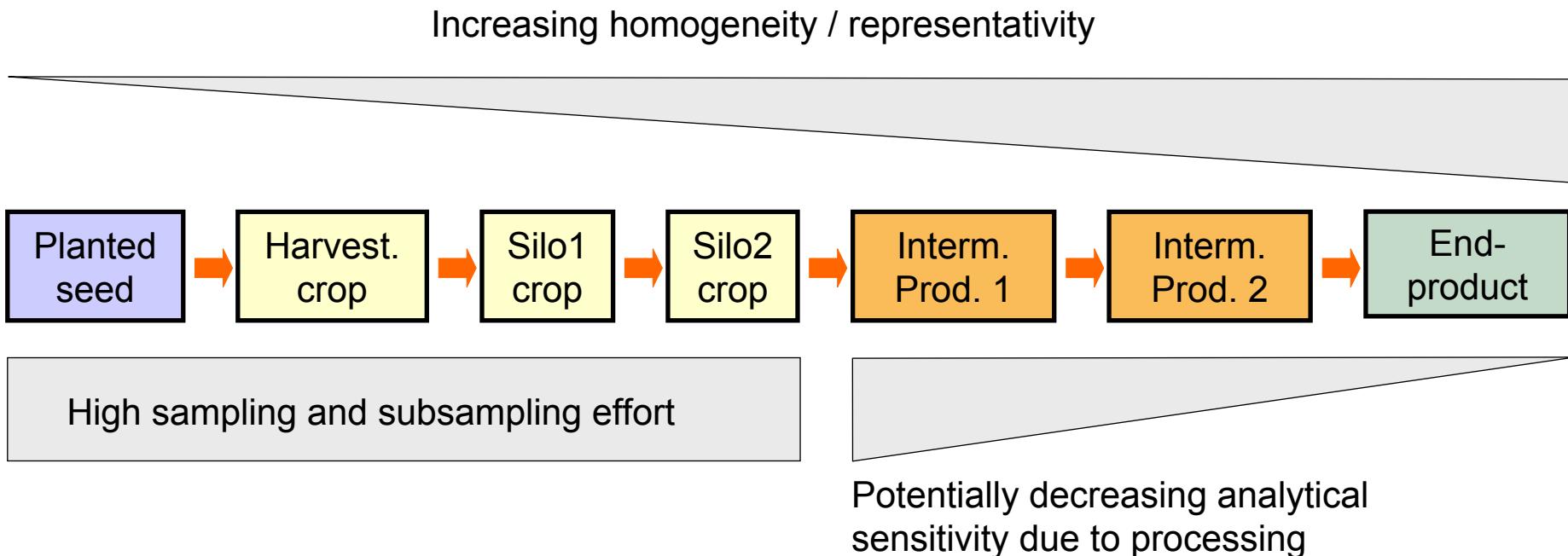
Sampling

Analytical

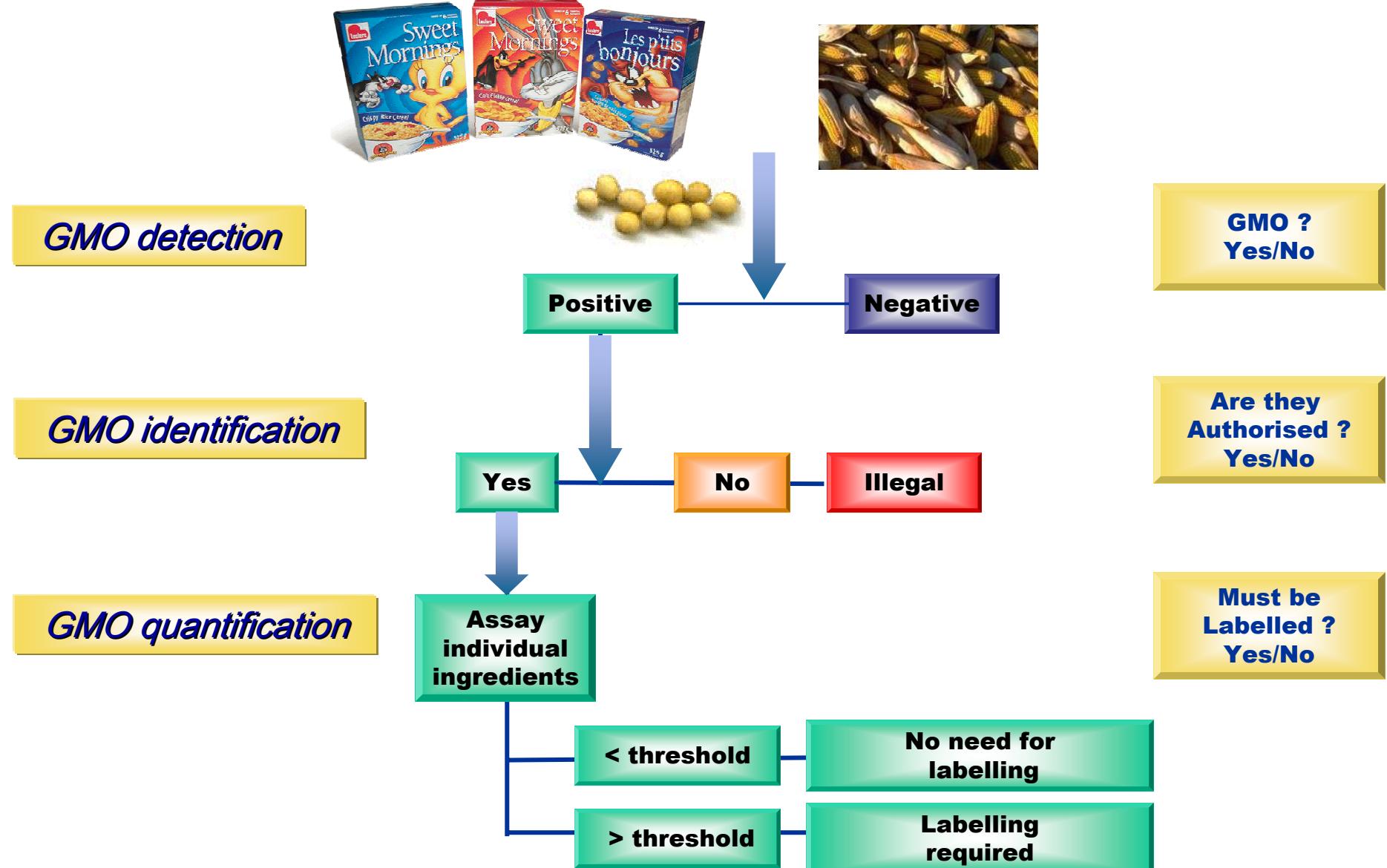


Amplification (PCR)

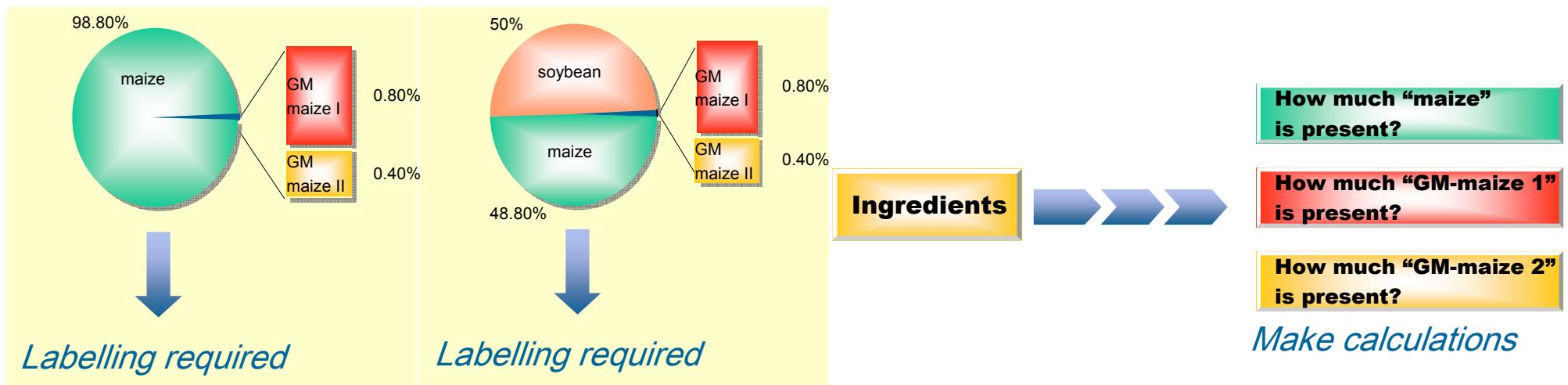
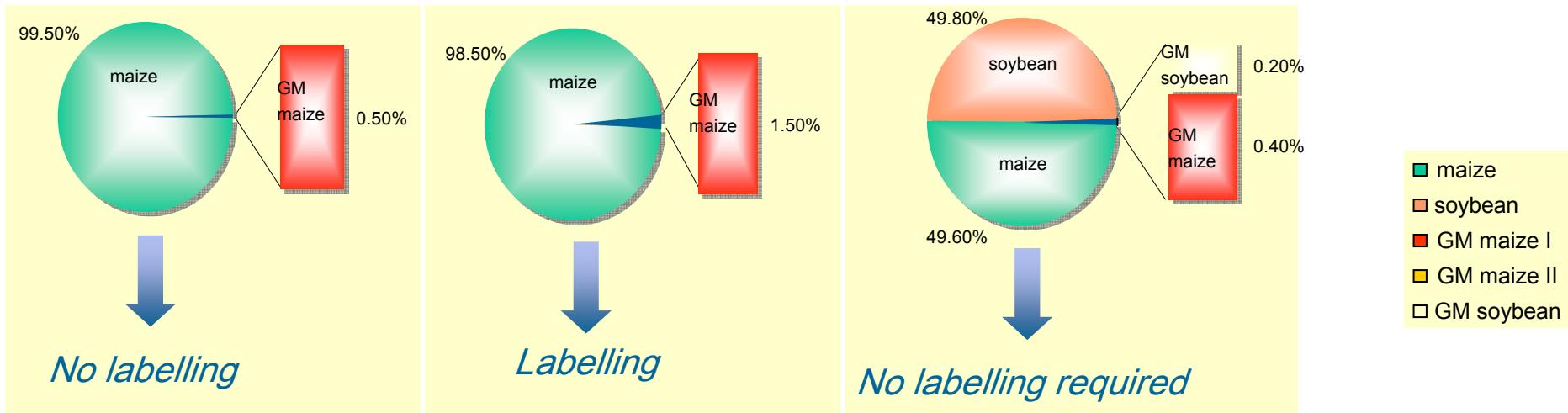
Experience along the production chain



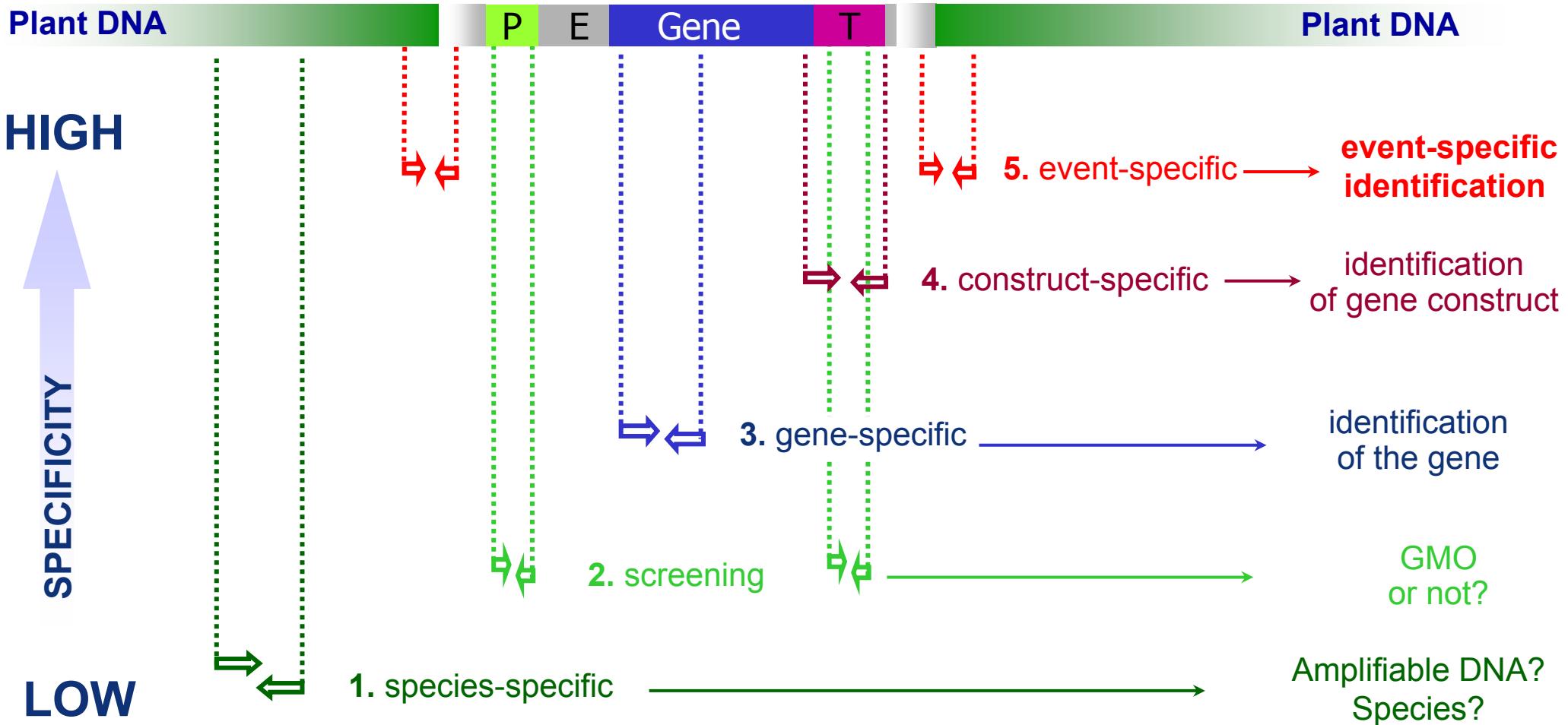
Operation of GMO laboratories to comply with EU legislation



Quantification of GMOs and labelling



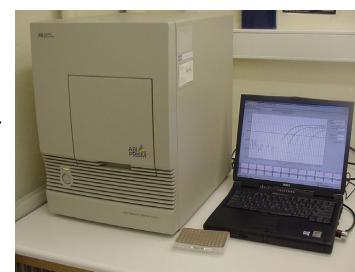
Detection strategies, which target to select?



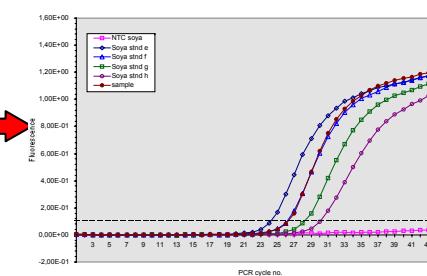
GMO quantification by RT-PCR

Wild type

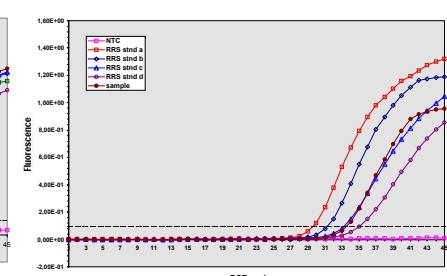
GMO



Target taxon specific

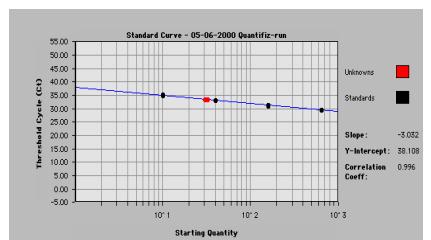
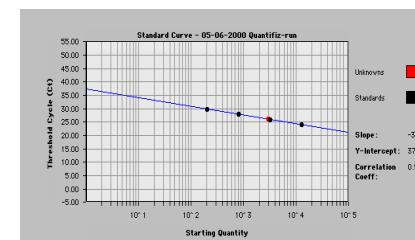


GM specific



1.
Sample preparation,
and
DNA extraction

2.
DNA amplification
in
real-time PCR machine



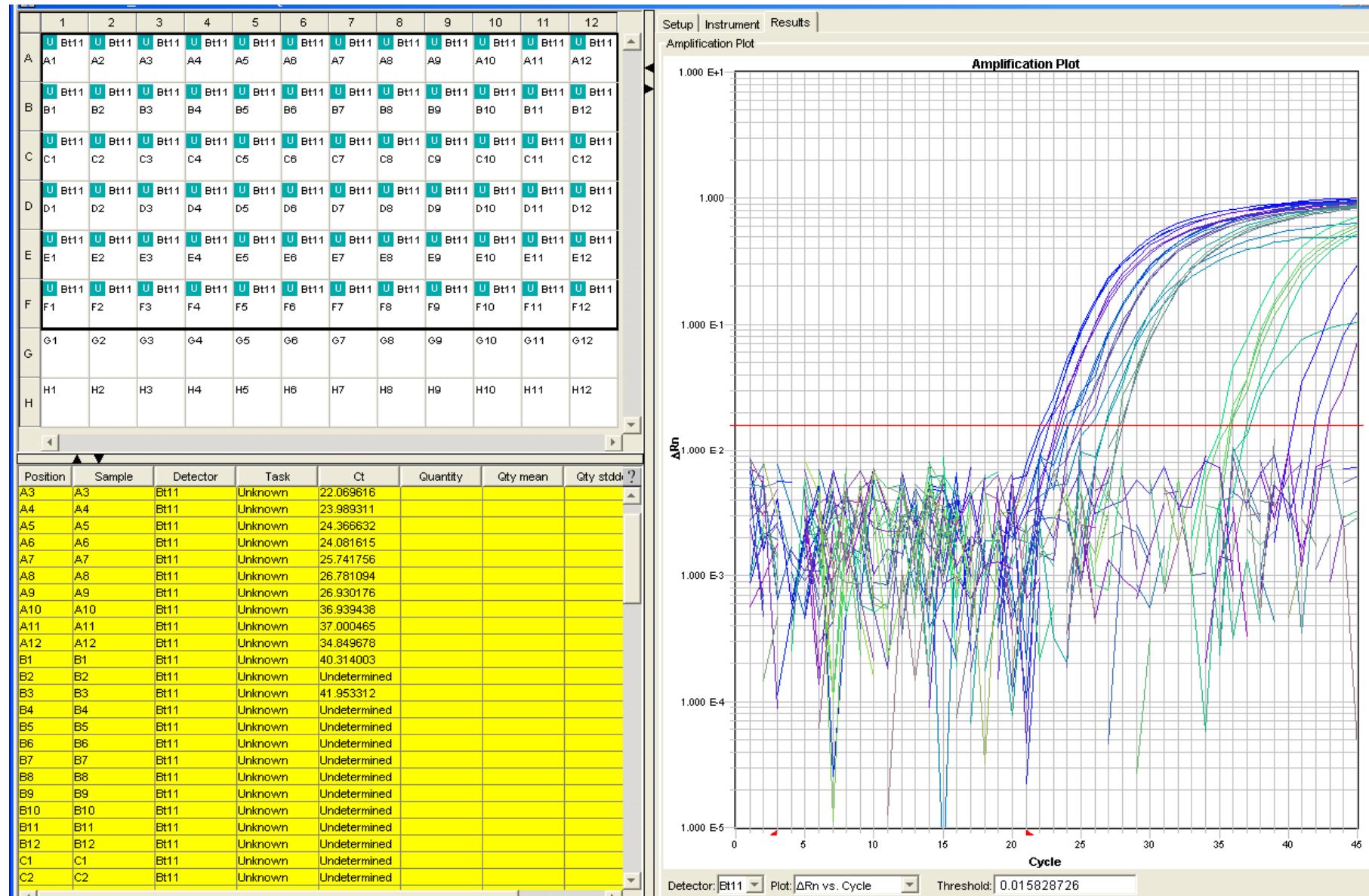
GM
endogenous $\times 100$

3.
Interpretation of result

Qualitative PCR: endpoint RT-PCR detection

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Pros and cons - DNA methods

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- Genetic modifications = DNA modifications
- DNA stable and inheritable
- DNA traceable unit for all purposes
 - Matrix limitations may apply
- Sensitive, fit for identification and quantification
- Costs:
 - Efficient screening (multiple targets and GMOs)
 - Expensive identification and quantification
 - Equipment, reference material, skilled staff
- Limited coverage, although superior to protein
 - Can only detect what we have methods for!

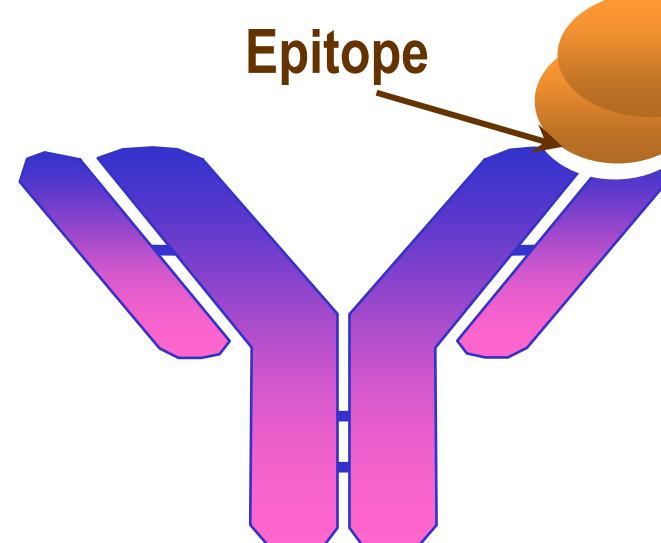
GMO analysis by immuno assays

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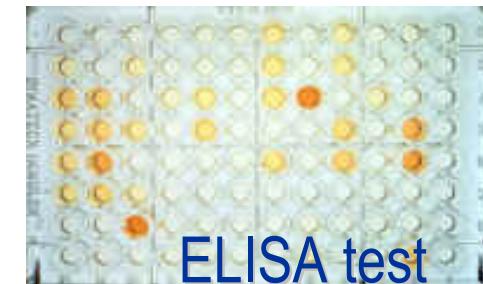


Target Protein
“Antigen”



Epitope

Antibody



ELISA test



Reverse ELISA



Lateral-Flow Strip test

- Advantages/benefits:
 - Speed
 - Cost
 - Practicability and easy transferability
 - Low risk of false positives (carry over)
 - Well established in the food industry
- Drawbacks:
 - Matrix limitations and sensitivity
 - Coverage low (methods only for few GMOs)
 - Low fitness for Qn analysis
 - Limited identification (no event identification except for 'unique' traits)

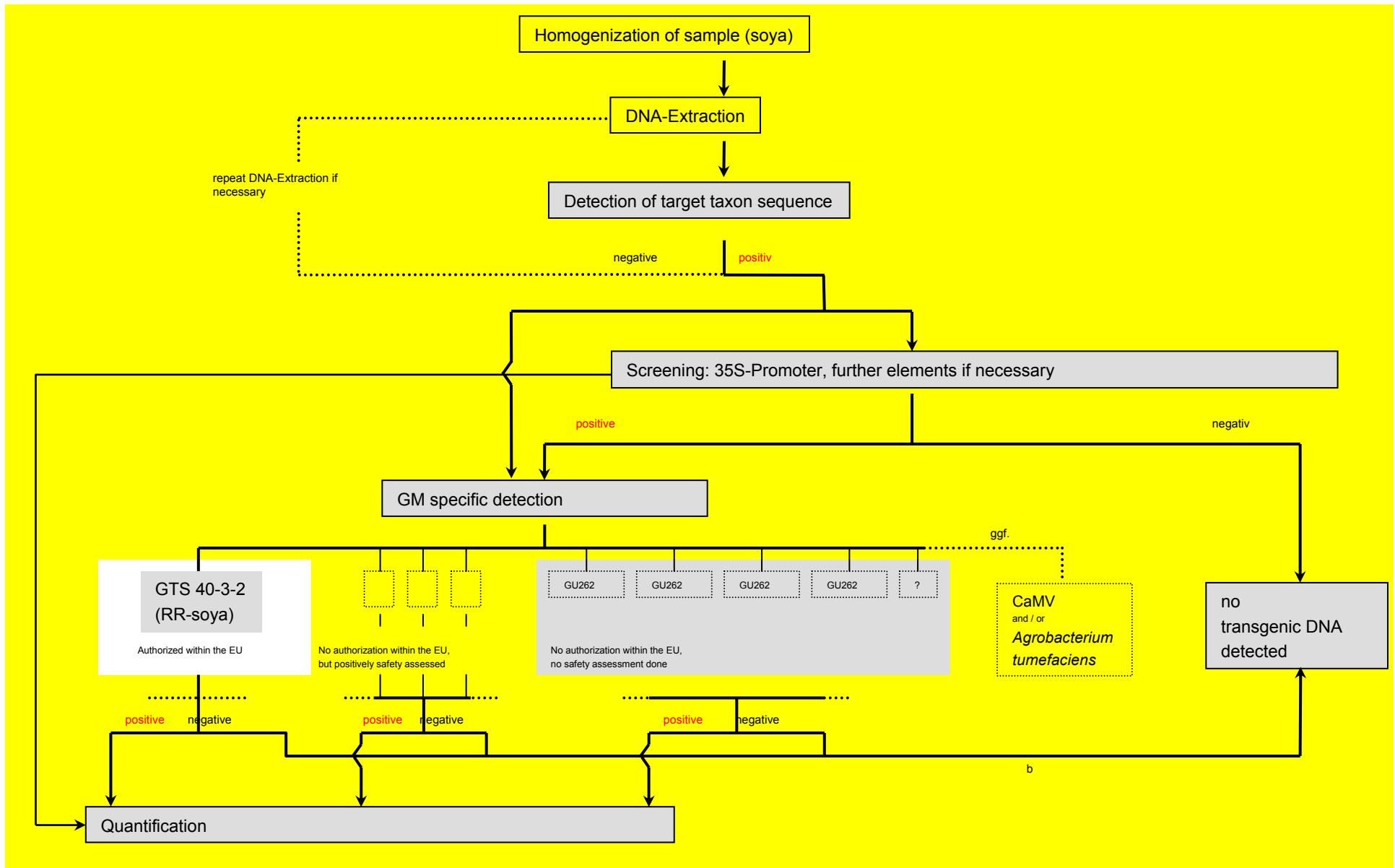
Present context

- Worldwide adoption and use of GMOs is rapidly increasing (acreage, countries);
 - Constant rise in GMO complexity, number of traits and events;
 - In the EU:
 - Mandatory labelling of GMOs and derived food/feed products (if above 0.9%) requires event-specific methods;
 - Post-market monitoring requirements;
 - GMO control based on combination of screening + event-specific detection methods;
 - Increasing number of GMOs under approval;
 - Asynchronous approval process complicates the analytical procedure.
- ↑ Higher number of methods to be applied for full product characterisation.
- ↑ Increased time and cost of analysis/sample.

Analysis of soybean samples

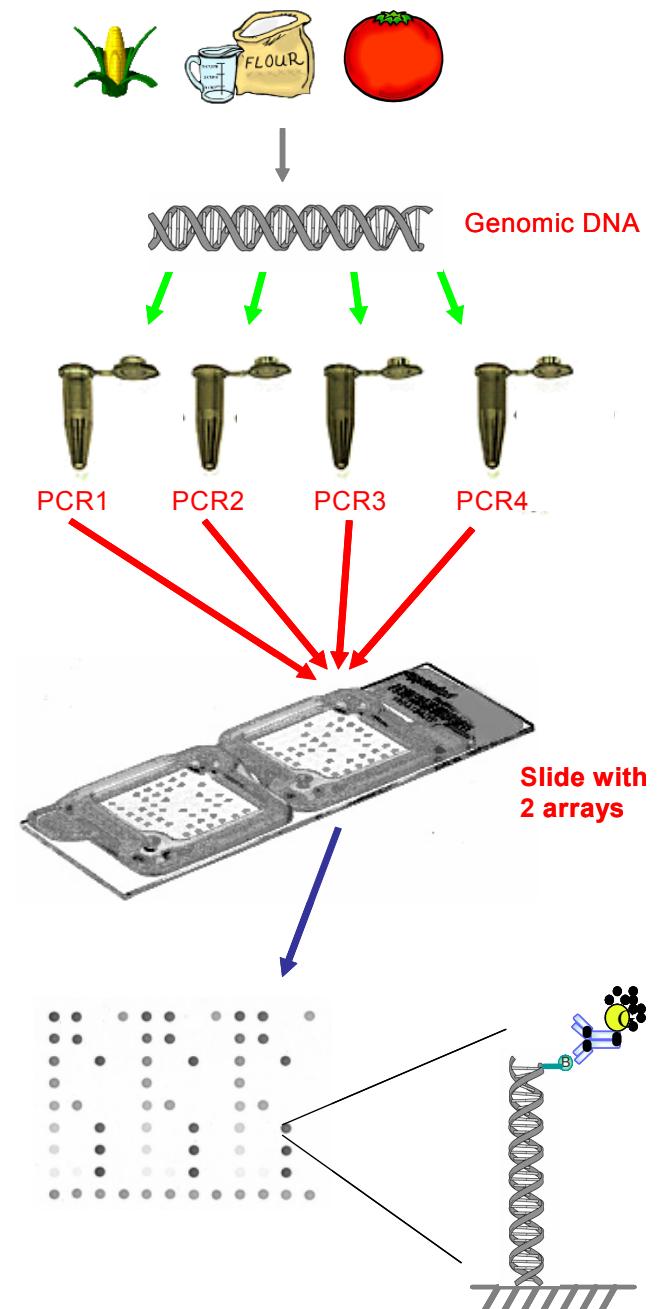
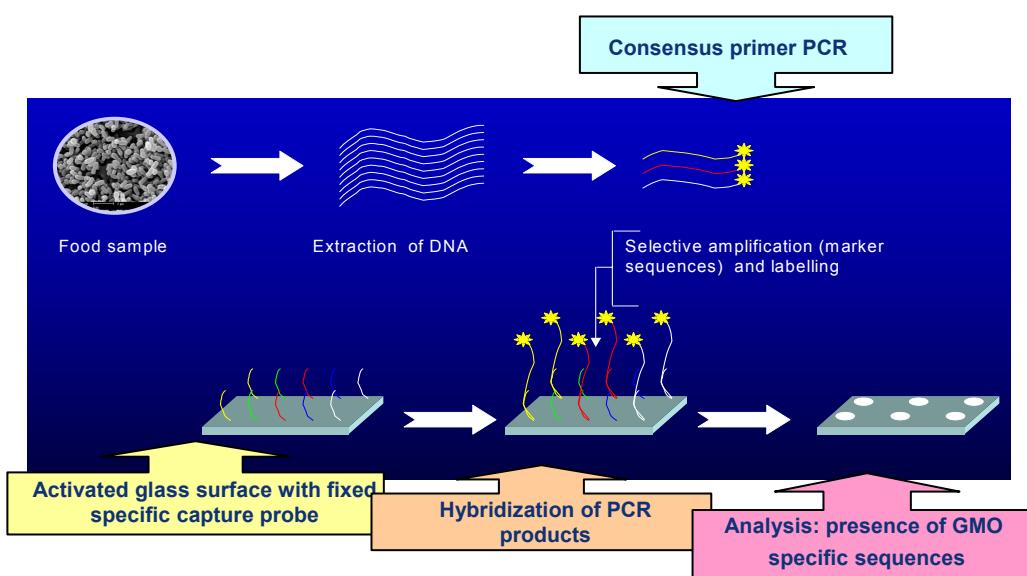
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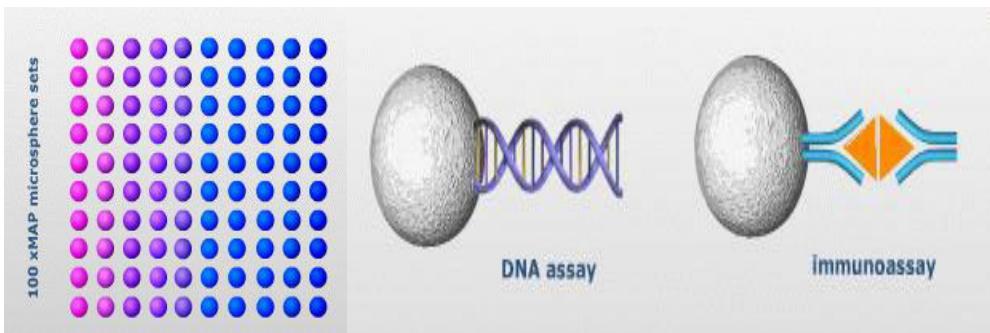
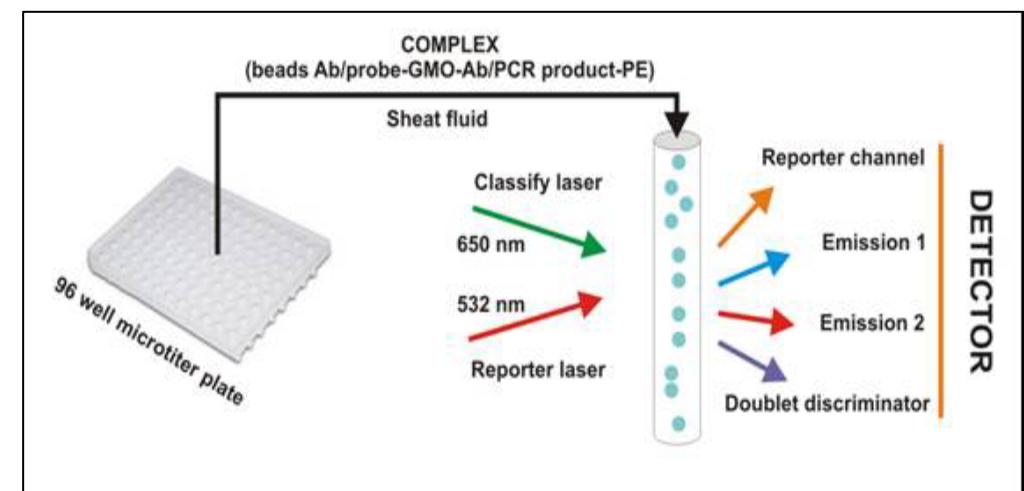
Next generation approaches for GMO analysis: Development and validation of high-throughput / multi-target detection methods

Multiplex DNA micro-array



Next generation approaches for GMO analysis: Development and validation of high-throughput / multi-target detection methods

Micro-sphere based assays
(xMAP/Luminex technology)



Within the present context....

The only way to foster appropriate testing and to guarantee proper GMO control in the EU is to facilitate the work of enforcement laboratories.

This can be achieved by developing and providing them tools able to overcome the difficulties of applying a complex analytical procedure, often exceeding laboratories' capabilities.

Next generation approaches for GMO analysis: Development and validation of high-throughput / multi-target detection methods

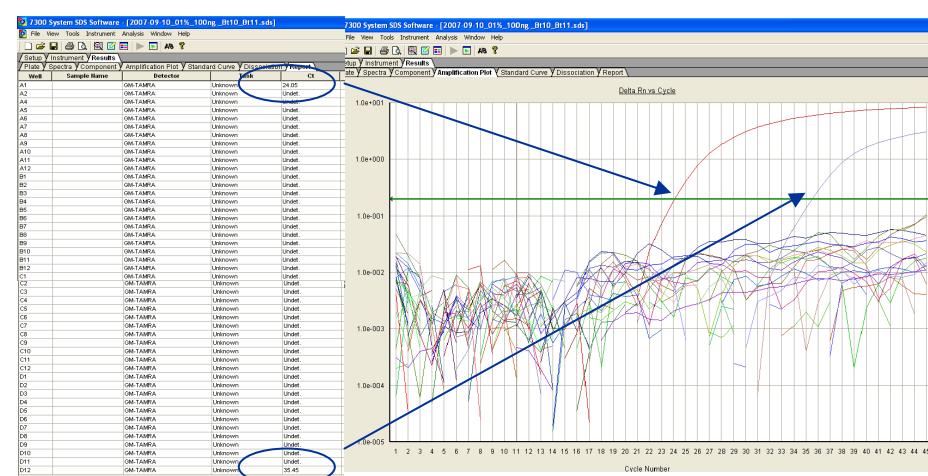
**Real-Time PCR based ready-to-use
multi-target analytical system
for the detection of EU authorised
and unauthorised
GM events**

Targets: 7 plant species
39 GM events

	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG Maize Ref	SAH7 Cotton Ref	PLD Rice Ref	CruA Oilseed Ref	Lectin Soybean Ref	GS Sugarbeet Ref	UGPase Potato Ref	t11 Maize	NK603 Maize	GA21 Maize Monsanto	MON863 Maize	1507 Maize
B	T25 Maize	59122 Maize	H7-1 Sugar beet	MON810 Maize	281-24-236 Cotton	3006-210-23 Cotton	LLRICE62 Rice	T45 oilseed rape	EH92-527-1 Potato	Ms8 Oilseed rape	Rf3 Oilseed rape	GT73 (RT63) Rapeseed
C	LLCotton2 5 Cotton	MON 531 Cotton	A2704-12 Soybean	MIR604 Maize	Rf1 Rapeseed	Rf2 Rapeseed	Ms1 Rapeseed	Topas 19/2 Cotton	MON1445 Maize	Bt176 Maize	MON15985 Cotton	40-3-2 Soybean
D	GA21 Maize Syngenta	MON88017 maize	LY038 Maize	3272 Maize	MON89788 soybean	MON89034 Maize	DP-356043 soybean	MON88913 cotton	Rice GM events P35S:bar	LLRice601 Rice	Bt63 Rice	Bt10 Maize
E	HMG Maize Ref	SAH7 Cotton Ref	PLD Rice Ref	CruA Oilseed Ref	Lectin Soybean Ref	GS Sugarbeet Ref	UGPase Potato Ref	t11 Maize	NK603 Maize	GA21 Maize Monsanto	MON863 Maize	1507 Maize
F	T25 Maize	59122 Maize	H7-1 Sugar beet	MON810 Maize	281-24-236 Cotton	3006-210-23 Cotton	LLRICE62 Rice	T45 oilseed rape	EH92-527-1 Potato	Ms8 Oilseed rape	Rf3 Oilseed rape	GT73 (RT63) Rapeseed
G	LLCotton2 5 Cotton	MON 531 Cotton	A2704-12 Soybean	MIR604 Maize	Rf1 Rapeseed	Rf2 Rapeseed	Ms1 Rapeseed	Topas 19/2 Rapeseed	MON1445 Cotton	Bt176 Maize	MON15985 Cotton	40-3-2 Soybean
H	GA21 Maize Syngenta	MON88017 maize	LY038 Maize	3272 Maize	MON89788 soybean	MON89034 Maize	DP-356043 soybean	MON88913 cotton	Rice GM events P35S:bar	LLRice601 Rice	Bt63 Rice	Bt10 Maize

Sample 1

Sample 2



Real-time PCR based ready-to-use multi-target analytical system for GMO analysis

Pre-spotted plates

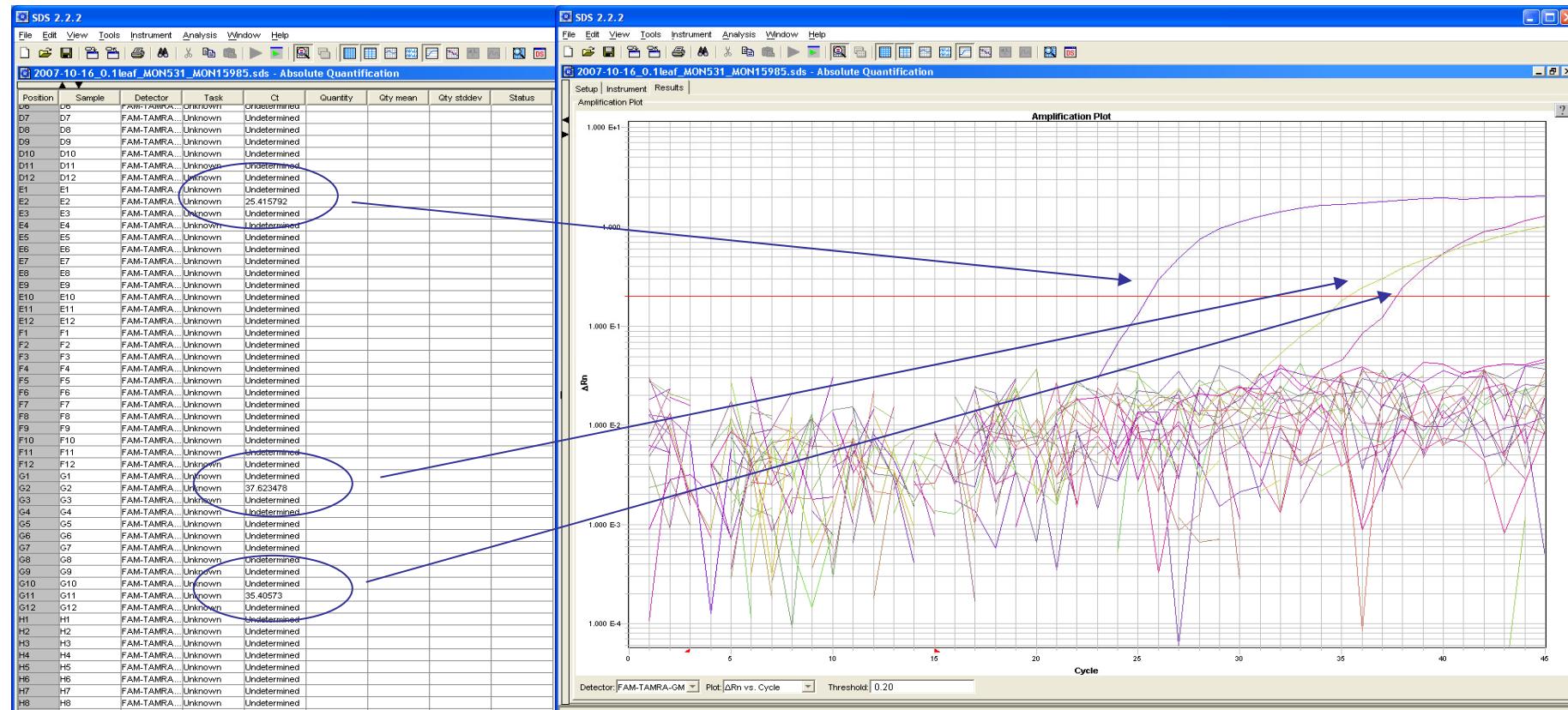
- **source:** 48 real-time PCR methods validated by the CRL-GMFF
 - **output:** unique system in which all methods work with a unique set of conditions without loosing specificity and overall performance

Detection of cotton event MON15985

E2 = SAH7 cotton reference gene method

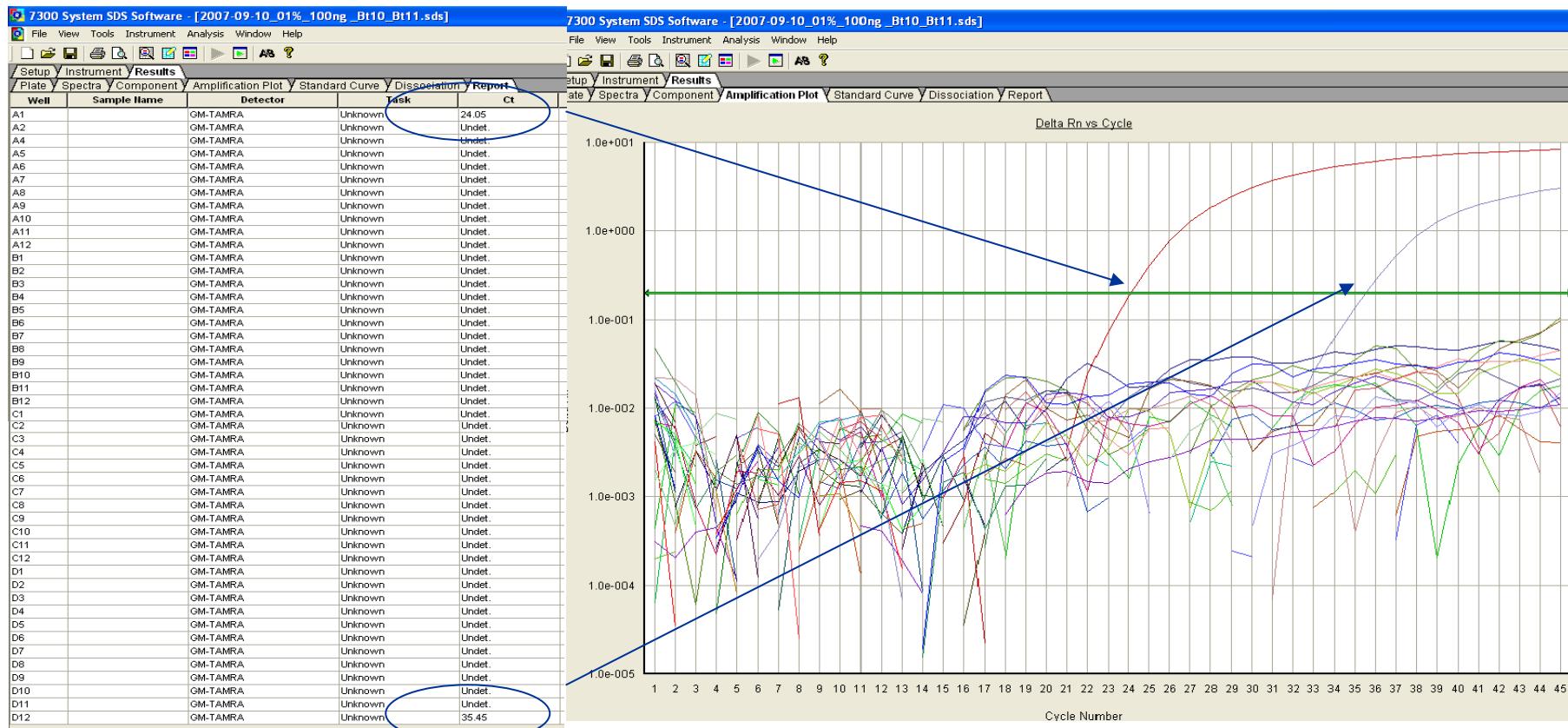
G2 = MON531 event-specific method

G11 = MON15985 event-specific method



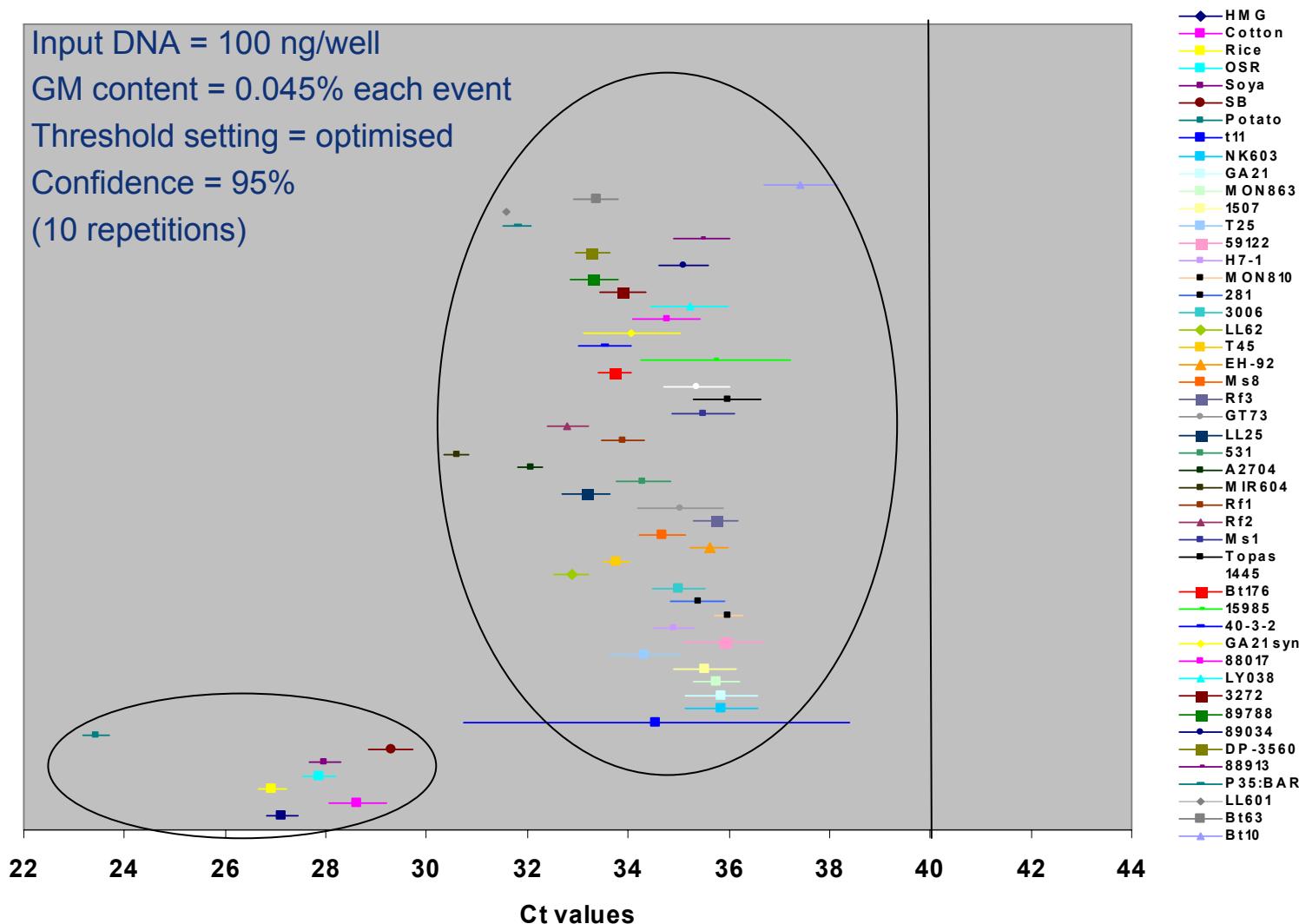
Detection of maize event Bt-10

A1 = maize reference gene method
 D12 = Bt-10 event-specific method

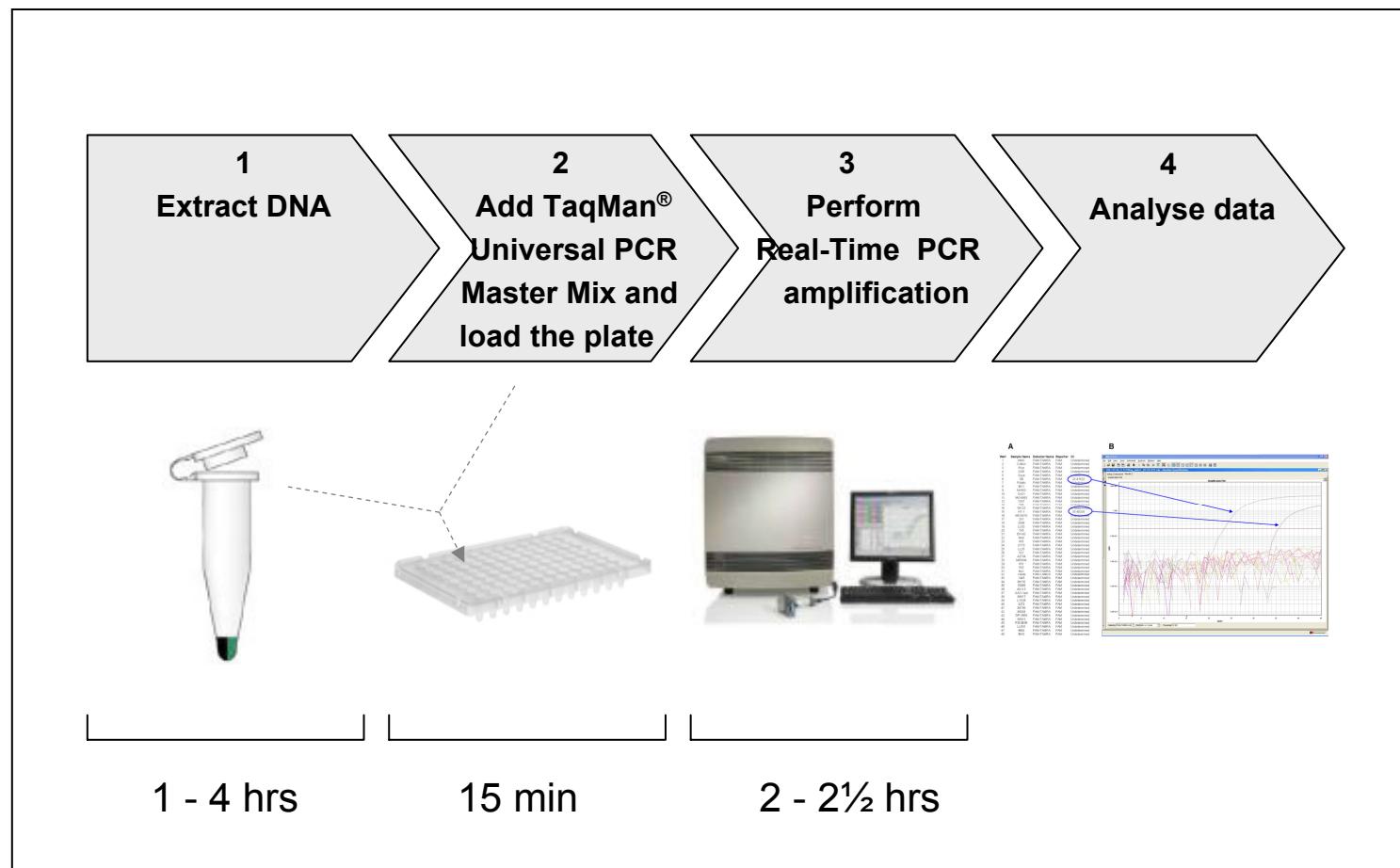


Performance of event-specific plates: specificity

Performance of event-specific plates: sensitivity



Workflow and approximate timing for GMO analysis using the ready-to-use multi-target analytical system



Ready-to-use pre-spotted plate/strip systems in response to the different needs of GMO analysis:

Crop-specific formulation (for commodities testing)

Maize and soybean events detected

well	RTi-PCR method	well	RTi-PCR method
A1	HMG Maize Ref	B1	LY038
A2	HMG Maize Ref	B2	3272
A3	Bt11	B3	MON89034
A4	NK603	B4	98140
A5	GA21	B5	Lectin Soybean Ref
A6	MON863	B6	Lectin Soybean Ref
A7	DAS1507	B7	A2704-12
A8	T25	B8	40-3-2
A9	DAS59122	B9	MON89788
A10	MON810	B10	DP-356043
A11	MIR604	B11	DP-305423
A12	MON88017	B12	A5547-127

Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG Maize Ref	HMG Maize Ref	Bt11 Maize	NK603 Maize	GA21 Maize	MON863 Maize	DAS1507 Maize	T25 Maize	DAS59122 Maize	MON810 Maize	MIR604 Maize	MON88017 Maize
B	LY038 Maize	3272 Maize	MON89034 Maize	98140 Maize	Lectin Soybean Ref	Lectin Soybean Ref	A2704-12 Soybean	40-3-2 Soybean	MON89788 Soybean	DP-356043 Soybean	DP-305423 Soybean	A5547-127 Soybean
C	HMG Maize Ref	HMG Maize Ref	Bt11 Maize	NK603 Maize	GA21 Maize	MON863 Maize	DAS1507 Maize	T25 Maize	DAS59122 Maize	MON810 Maize	MIR604 Maize	MON88017 Maize
D	LY038 Maize	3272 Maize	MON89034 Maize	98140 Maize	Lectin Soybean Ref	Lectin Soybean Ref	A2704-12 Soybean	40-3-2 Soybean	MON89788 Soybean	DP-356043 Soybean	DP-305423 Soybean	A5547-127 Soybean
E	HMG Maize Ref	HMG Maize Ref	Bt11 Maize	NK603 Maize	GA21 Maize	MON863 Maize	DAS1507 Maize	T25 Maize	DAS59122 Maize	MON810 Maize	MIR604 Maize	MON88017 Maize
F	LY038 Maize	3272 Maize	MON89034 Maize	98140 Maize	Lectin Soybean Ref	Lectin Soybean Ref	A2704-12 Soybean	40-3-2 Soybean	MON89788 Soybean	DP-356043 Soybean	DP-305423 Soybean	A5547-127 Soybean
G	HMG Maize Ref	HMG Maize Ref	Bt11 Maize	NK603 Maize	GA21 Maize	MON863 Maize	DAS1507 Maize	T25 Maize	DAS59122 Maize	MON810 Maize	MIR604 Maize	MON88017 Maize
H	LY038 Maize	3272 Maize	MON89034 Maize	98140 Maize	Lectin Soybean Ref	Lectin Soybean Ref	A2704-12 Soybean	40-3-2 Soybean	MON89788 Soybean	DP-356043 Soybean	DP-305423 Soybean	A5547-127 Soybean



Ready-to-use pre-spotted plate/strip systems in response to the different needs of GMO analysis:

Screening formulation based on matrix approach

Requirements to apply screening in GMO analysis:

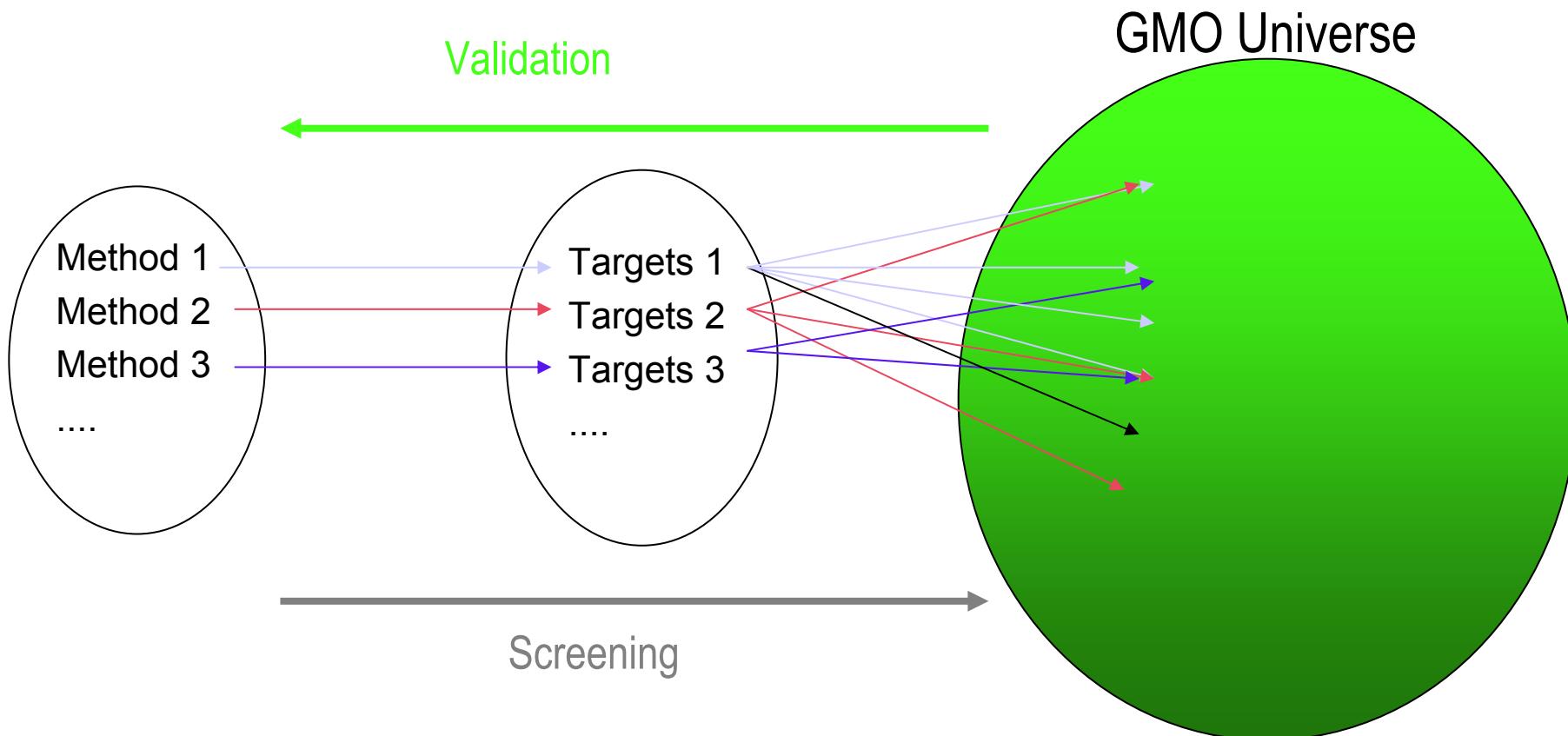
- defined **analyte** type (DNA)
- defined **GMO Universe** (e.g. EU authorized GMO for food and feed use)
- defined **targets** in the GMO of this Universe
- validated **methods** to demonstrate the presence of these targets

Matrix approach in GMO screening

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Relationship between the “GMO Universe”, the targets and the methods



Ready-to-use pre-spotted plate/strip systems in response to the different needs of GMO analysis:

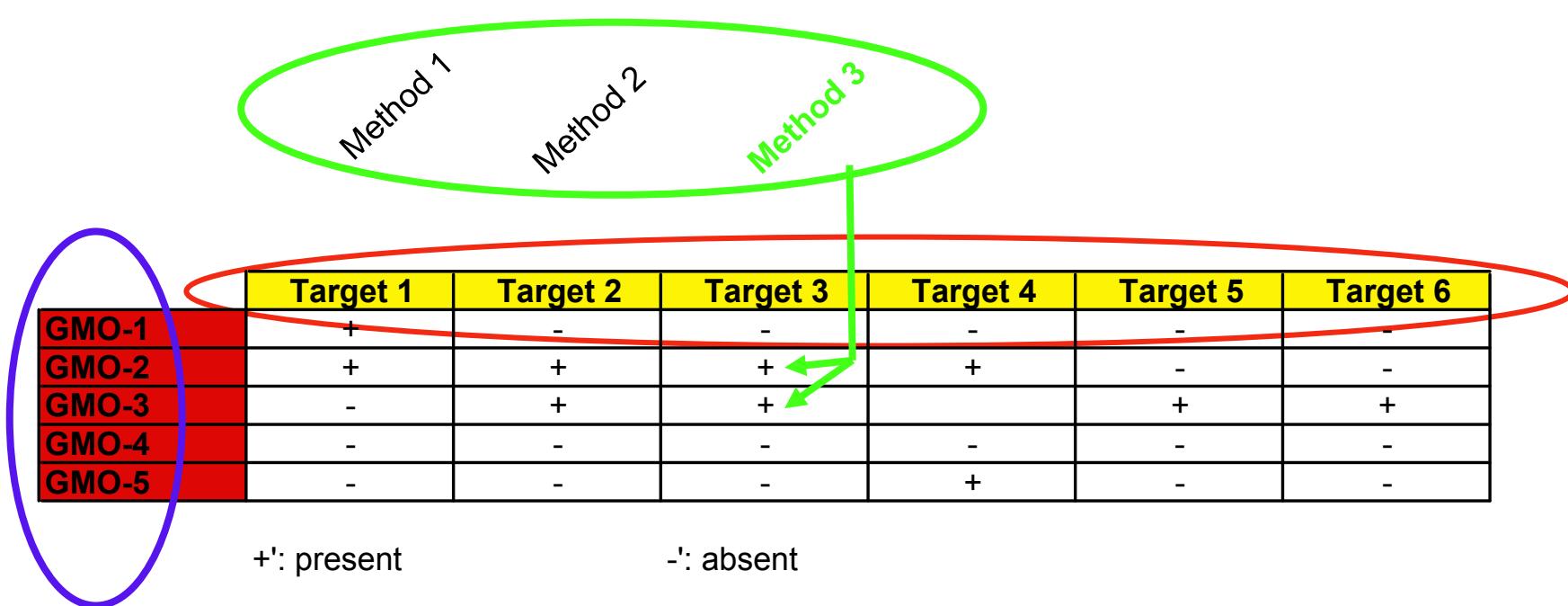
Screening formulation based on matrix approach

What is a “GMO Matrix” (for screening purposes)

- 1) A description of the set of GMO in form of a (Excel) table
- 2) Each GMO is described as a combination of genetic elements which are the targets for the screening (such as the p35S, tNOS, CryIAb,)
- 3) A (mathematical) “matrix” form wherein the **relation** between targets and GMO is represented
 - the (**molecular**) **targets** are listed as **columns**
 - the **GMO** are represented as **rows**

Screening formulation based on pre-spotted plates and Matrix approach

The matrix representation facilitates to describe the relationships between the “**GMO Universe**”, the **targets** and the **methods** within a **screening set up**



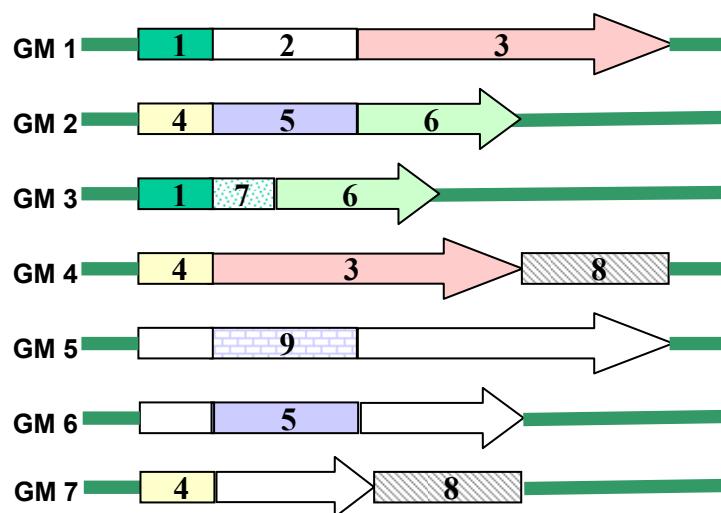
COSYPS: Combinatory SYBR® Green Q-PCR Screening

Semi-quantitative detection system
using a set of SYBR® Green RT-PCR
methods coupled to a software based
application for data interpretation

X _{Prime}	PCR Test	Core element class	Primer Reference
3	RBCI	Plant	Debode (pers. Comm.), 2004
5	Lectin	Species (soya)	Terry and Harris, 2002
7	Alcohol dehydrogenase	Species (maize)	SBB/ISP
11	Cruciferine	Species (Oilseed rape)	SBB/ISP
13	CaMV p35S	Generic (promotor)	SBB/ISP
17	Agrobacterium T-NOS	Generic (terminator)	SBB/ISP
19	CP4-EPSPS	Trait (herbicide res.)	SBB/ISP
23	CryIAb	Trait (insect res.)	SBB/ISP
29	PAT/pat	Trait (herbicide res.)	SBB/ISP
31	PAT/bar	Trait (herbicide res.)	SBB/ISP

GMO	p35S	tNOS	CP4 EPSPS	PAT/pat	PAT/bar	Cry1Ab
GTS 40/3/2	X	X	X			
Bt 11	X	X		X		X
Bt 176	X				X	X
MON 810	X	X				X
GA 21		X				
T25	X			X		
NK 603	X	X	X			
MON 863	X	X				
TC1507	X			X		
DAS59122	X			X		
Bt10	X	X		X		X
GT73			X			
MS1/RF2/ MS1xRF2		X			X	
MS1/RF1/ MS1xRF1		X			X	
MS8/RF3/ MS8xRF3		X			X	
TOPAS 19/2	X			X		
T45	X			X		
Falcon GS 40/90	X			X		
MON 1445	X	X	X			
MON 531	X	X				X
LLRICE601	X				X	
Bt63		X				X
RUR H7-1			X			

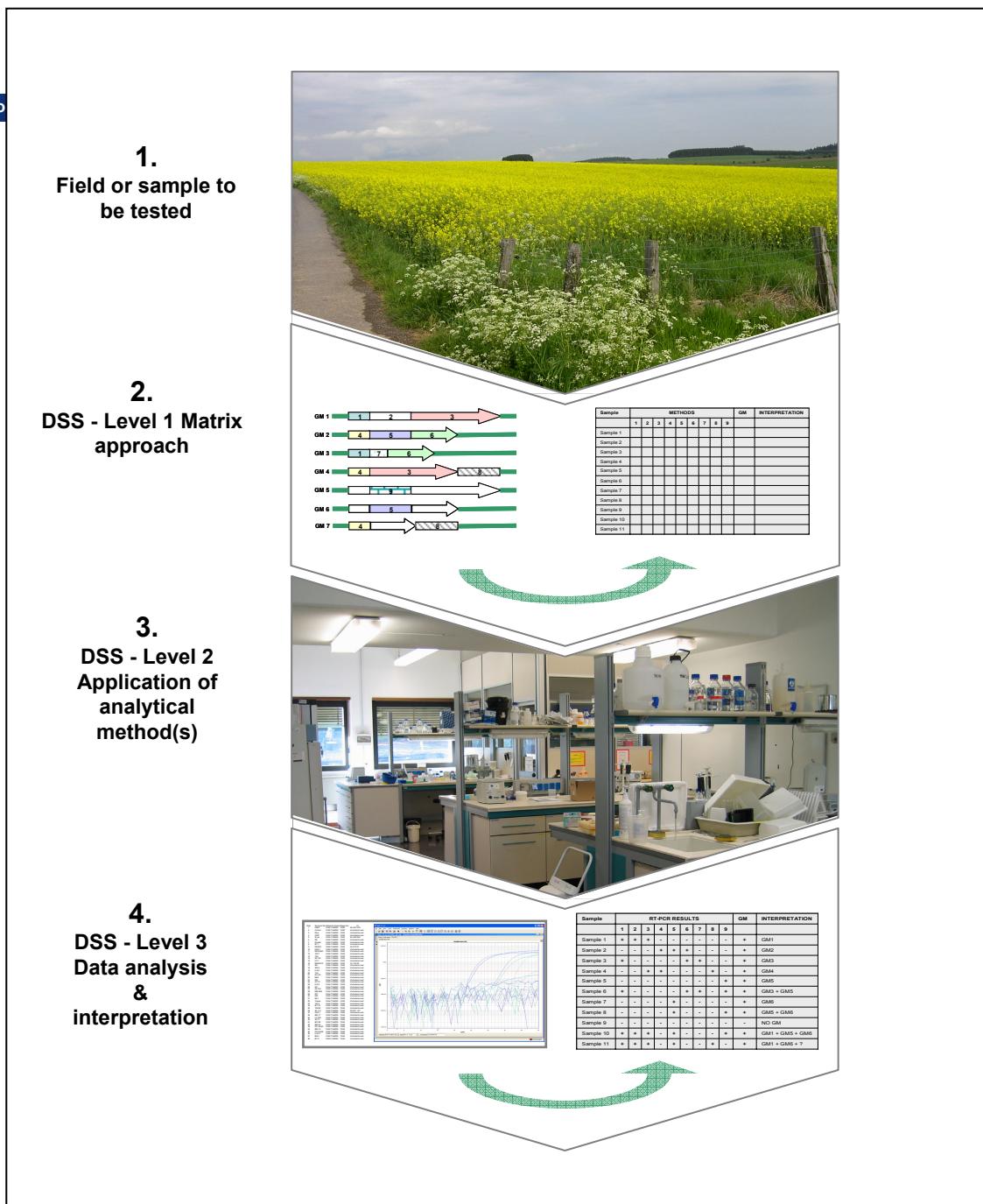
Combination of screening methods targeting common GM elements



Sample	RESULTS (1 method = 1 element)									GM	Interpretation
	1	2	3	4	5	6	7	8	9		
Sample 1	+	+	+	-	-	-	-	-	-	+	GM 1
Sample 2	-	-	-	+	+	+	-	-	-	+	GM 2
Sample 3	+	-	-	-	-	+	+	-	-	+	GM 3
Sample 4	-	-	+	+	-	-	-	+	-	+	GM 4
Sample 5	-	-	-	-	-	-	-	-	+	+	GM 5
Sample 6	+	-	-	-	-	+	+	-	+	+	GM 3 + GM 5
Sample 7	-	-	-	-	+	-	-	-	-	+	GM 6
Sample 8	-	-	-	-	+	-	-	-	+	+	GM 5 + GM 6
Sample 9	-	-	-	-	-	-	-	-	-	-	NO GM
Sample 10	+	+	+	-	+	-	-	-	+	+	GM 1 + GM 5 + GM 6
Sample 11	+	+	+	-	+	-	-	+	-	+	GM 1 + GM 6 + ?

Decision Support Systems in GMO analysis using a matrix-based approach in combination with ready-to- use multi-target analytical system.

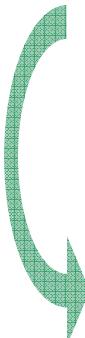
- 1) Sample definition,
 - 2) Establishment of a GMO matrix and decision on optimal analysis strategy (screening & identification),
 - 3) RTi-PCR amplification using ready-to-use pre-spotted plates, and
 - 4) Combined interpretation of the analytical results.



Future technological impact

The ‘ready-to-use multi-target analytical system’ based on pre-spotted plates has demonstrated a great potential for increasing harmonisation in GMO testing:

- Tool to test all (several) many events at once (need to 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.



The combination of this system with the matrix-based screening approach, integrated into a Decision Support System allows to tackle the current complexity and will foster harmonisation GMO analysis

Thank you!

Gracias por su atención!

Muito obrigada!

