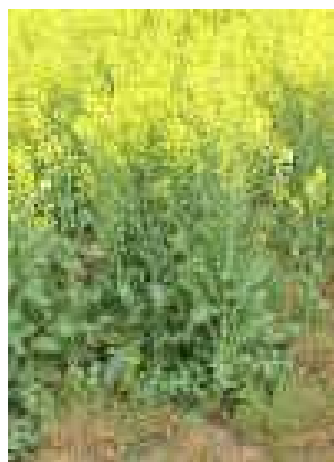
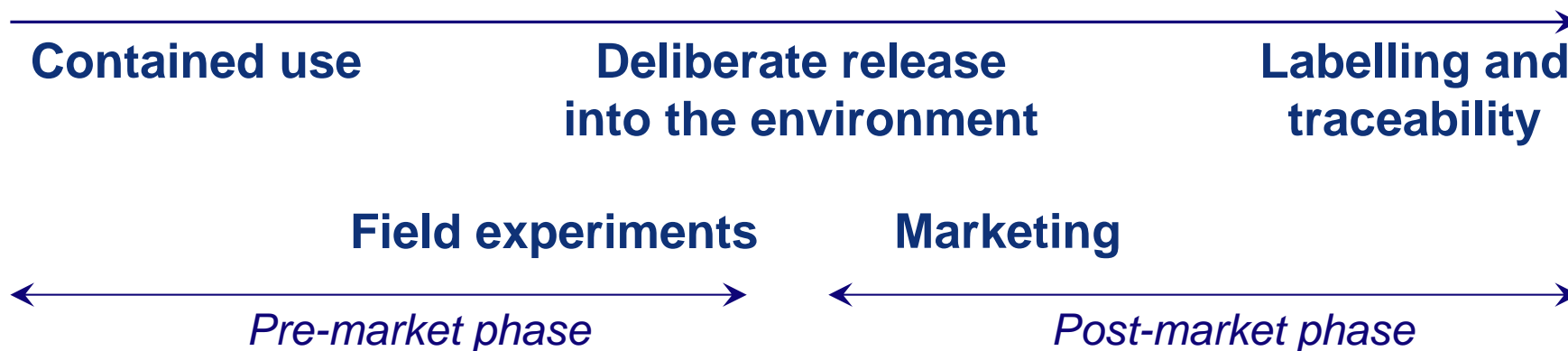


Overview on GMO detection approaches

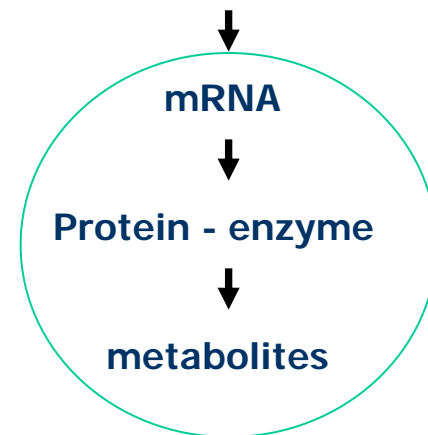
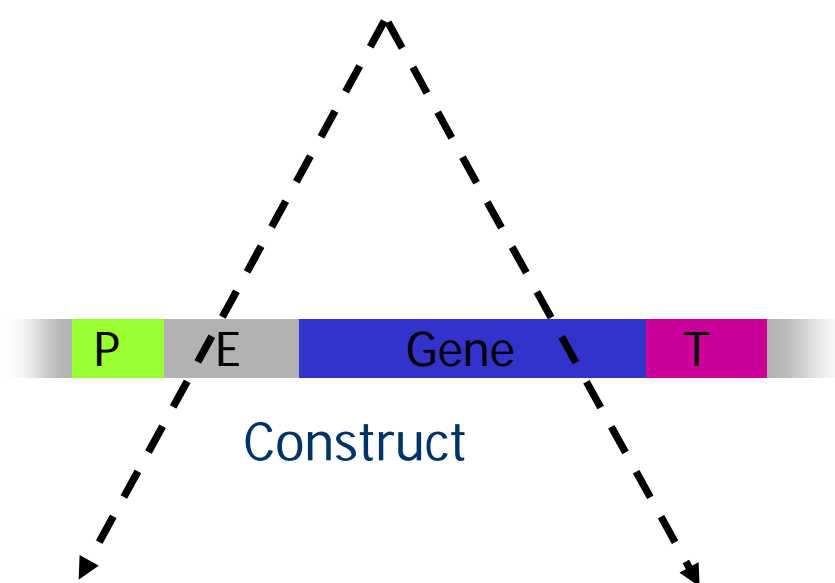
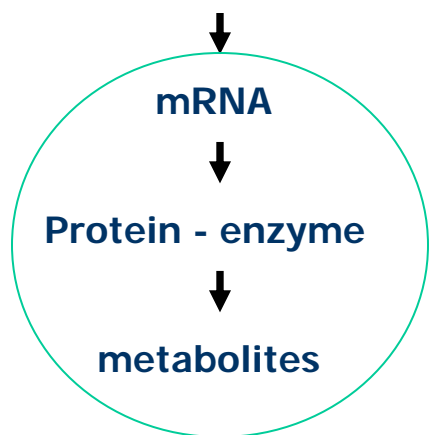
Maddalena QUERCI

Molecular Biology & Genomics Unit
Institute for Health and Consumer Protection (IHCP)
European Commission Joint Research Centre

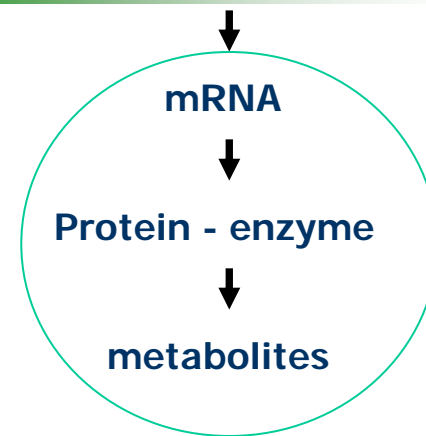
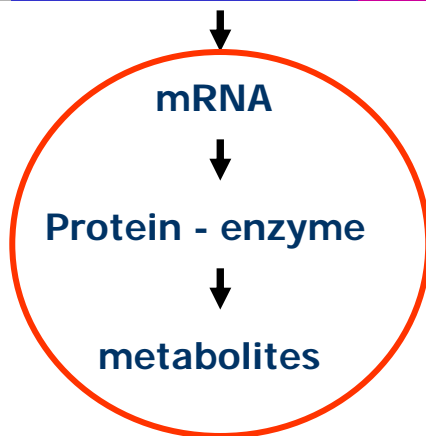
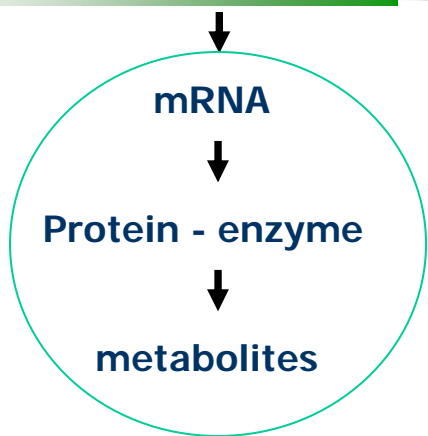




WT



GMO



How to Know if a Product is Genetically Modified ?



Phenotype identification

→ Most genetically modified market products appear identical to traditional counterparts.

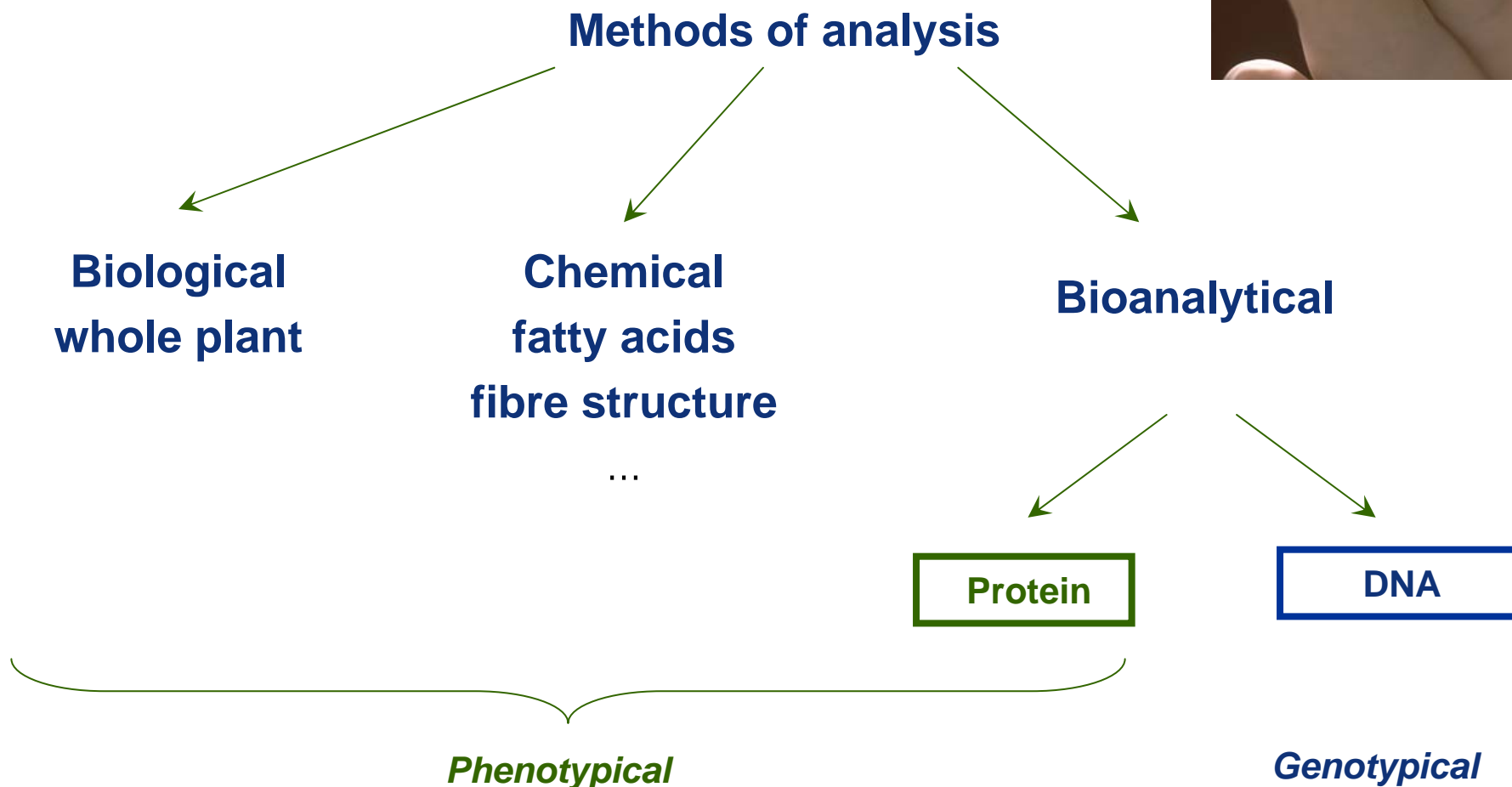
DNA-based methods

→ Genetic differences can always be found in the DNA sequences, provided that the genetic changes made to produce the GM product are known.

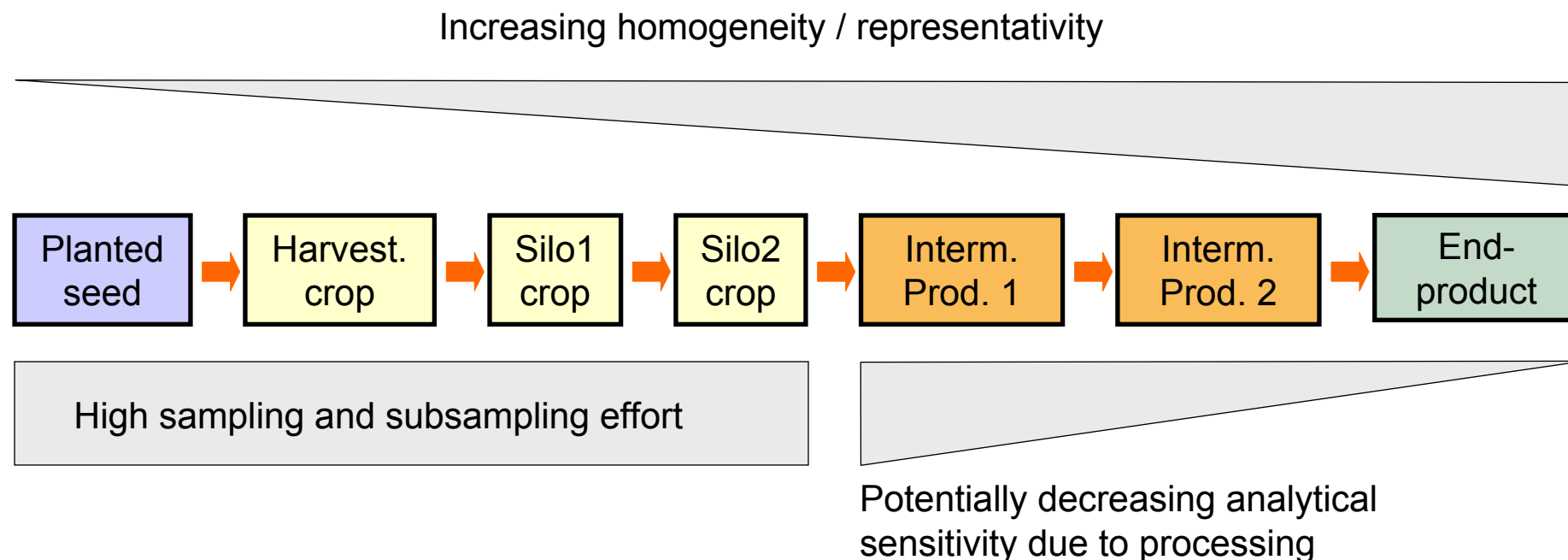
Protein-based methods

→ Plant transformation generally leads to the presence of a new protein which can be detected using immunological methods.

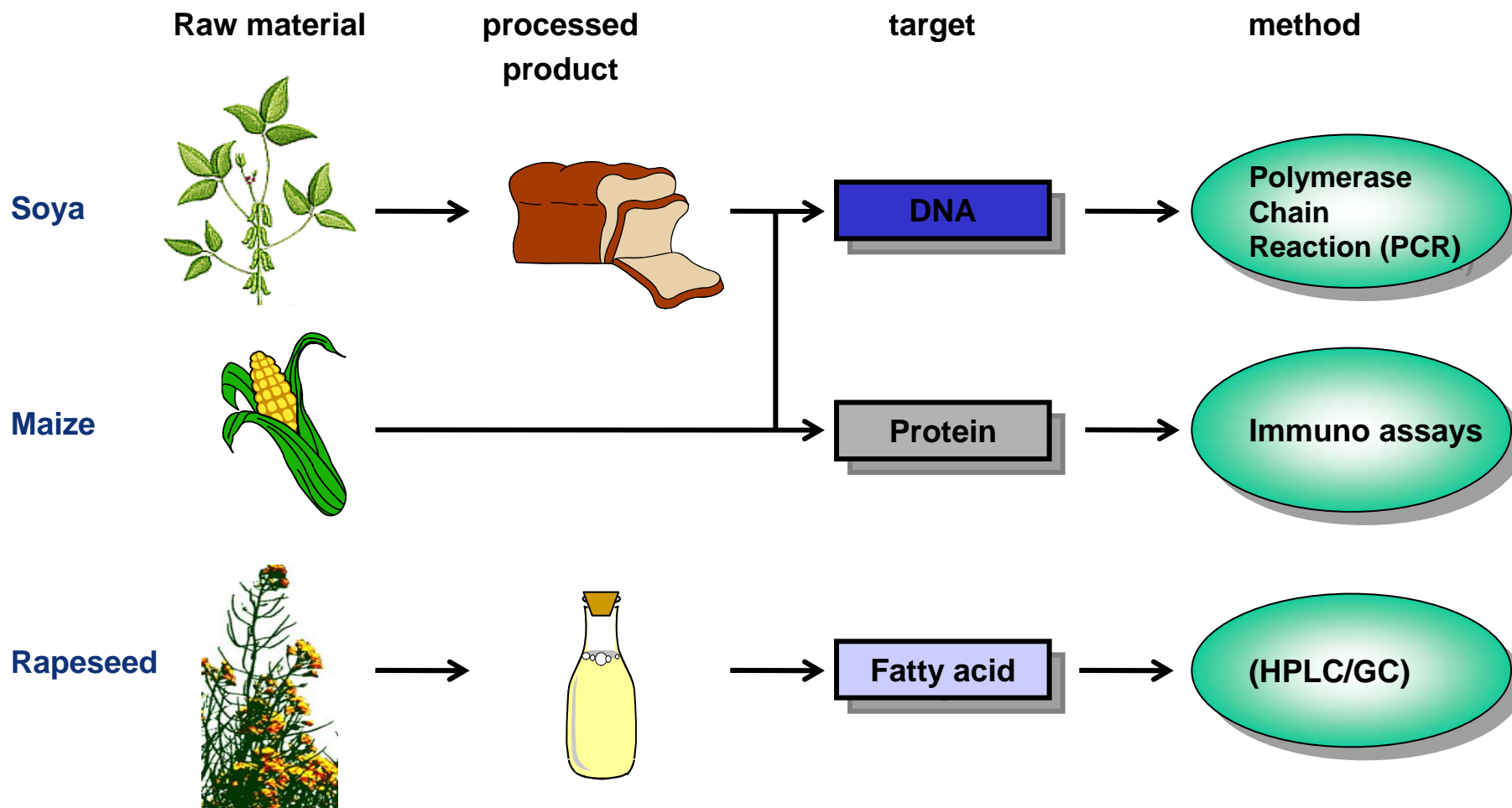
Which method to select ?



Experience along the production chain

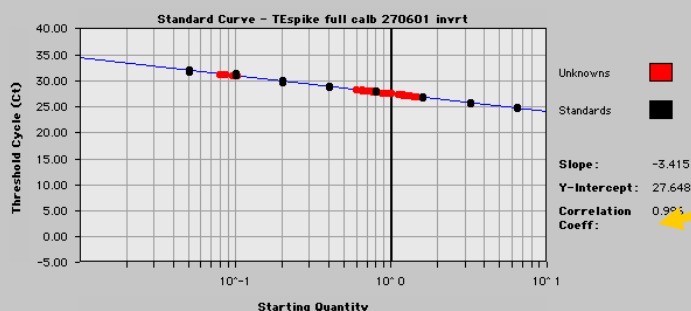
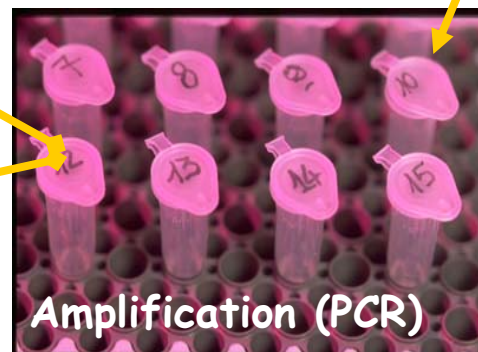
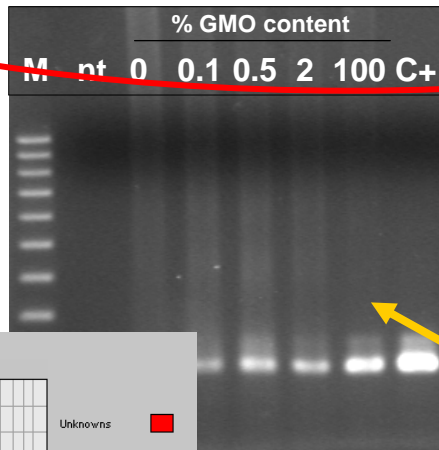


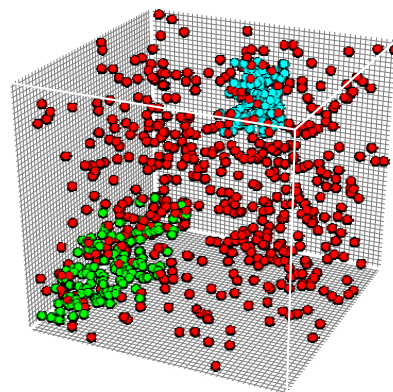
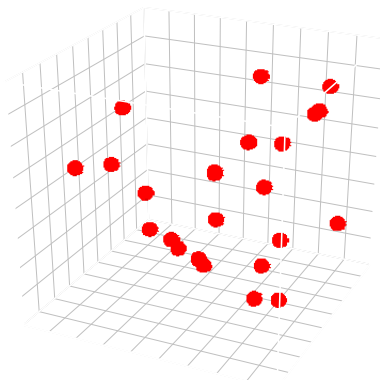
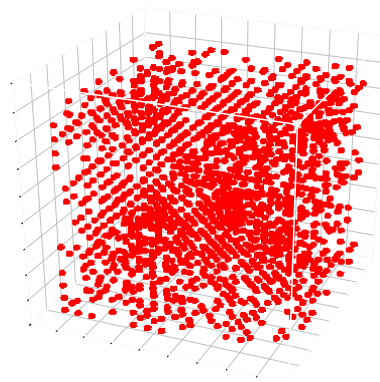
Samples properties and detection purpose





Sampling





Lot properties
- i.e. the distribution of
a contaminant in a
bulk mass - greatly
affect the
effectiveness of
sampling procedures.

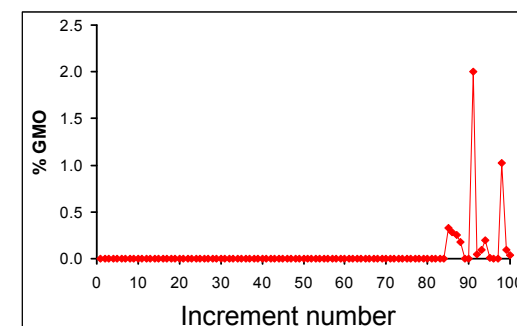
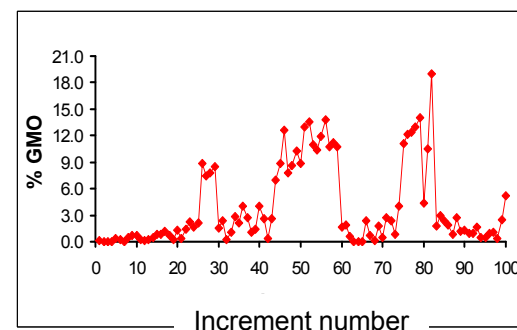
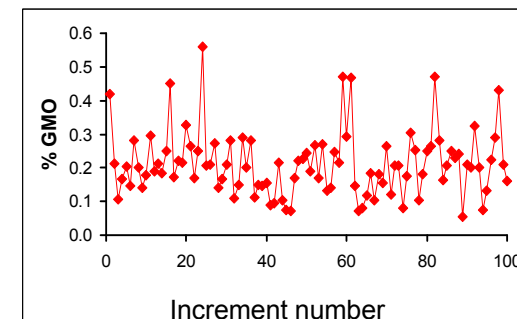
KeLDA (Kernel Lot Distribution Assessment) Project

Aim: to assess the distribution of GM contaminations in grain lots
imported within EU Member States

Participants: ENGL Labs - 10 Member States

Project Structure:

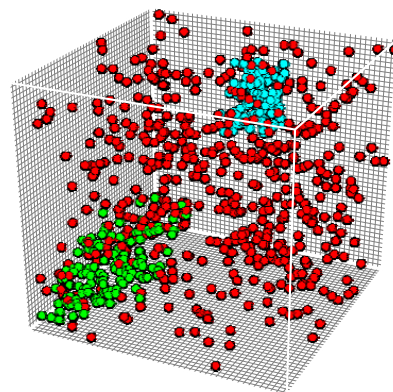
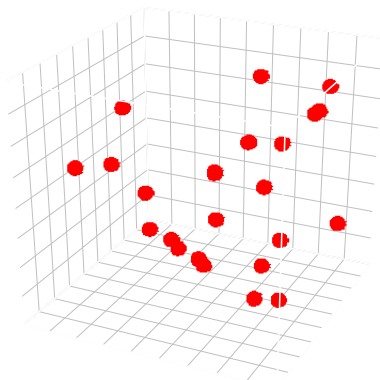
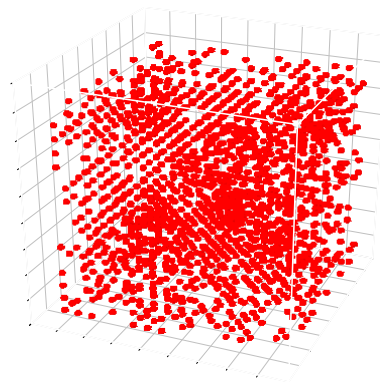
- 17 soybean lots likely to contain GM material
- Systematic sampling (ISO 6644) at each MS entrance point
- Sampling period = total off-loading time/100
- 100 samples/lot
- 3000 grains / sample (~ 0.5Kg)



Recommendation 2004/787/EC ...on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1830/2003

Based on statistical model and real data





Recommendation
2004/787/EC
provides a sampling
protocol to estimate
lot GMO content,
without imposing any
distribution

Recommended approach for foodstuff sampling

Commission Recommendation of 4 October 2004 on sampling and detection of GMOs and material produced from GMOs as or in products in the context of Regulation (EC) 1830/2003 [2004/787/EC]

- **Lots of bulk agricultural commodities (e.g. grains)**
 - **EC Recommendation 787/2004**
- **Lots of NON packed food products (e.g. flour)**
 - **EC Recommendation 787/2004**
- **Lots of packed food products (e.g. cookies)**
 - **ISO standard 2859 (1985)**

Take-home message

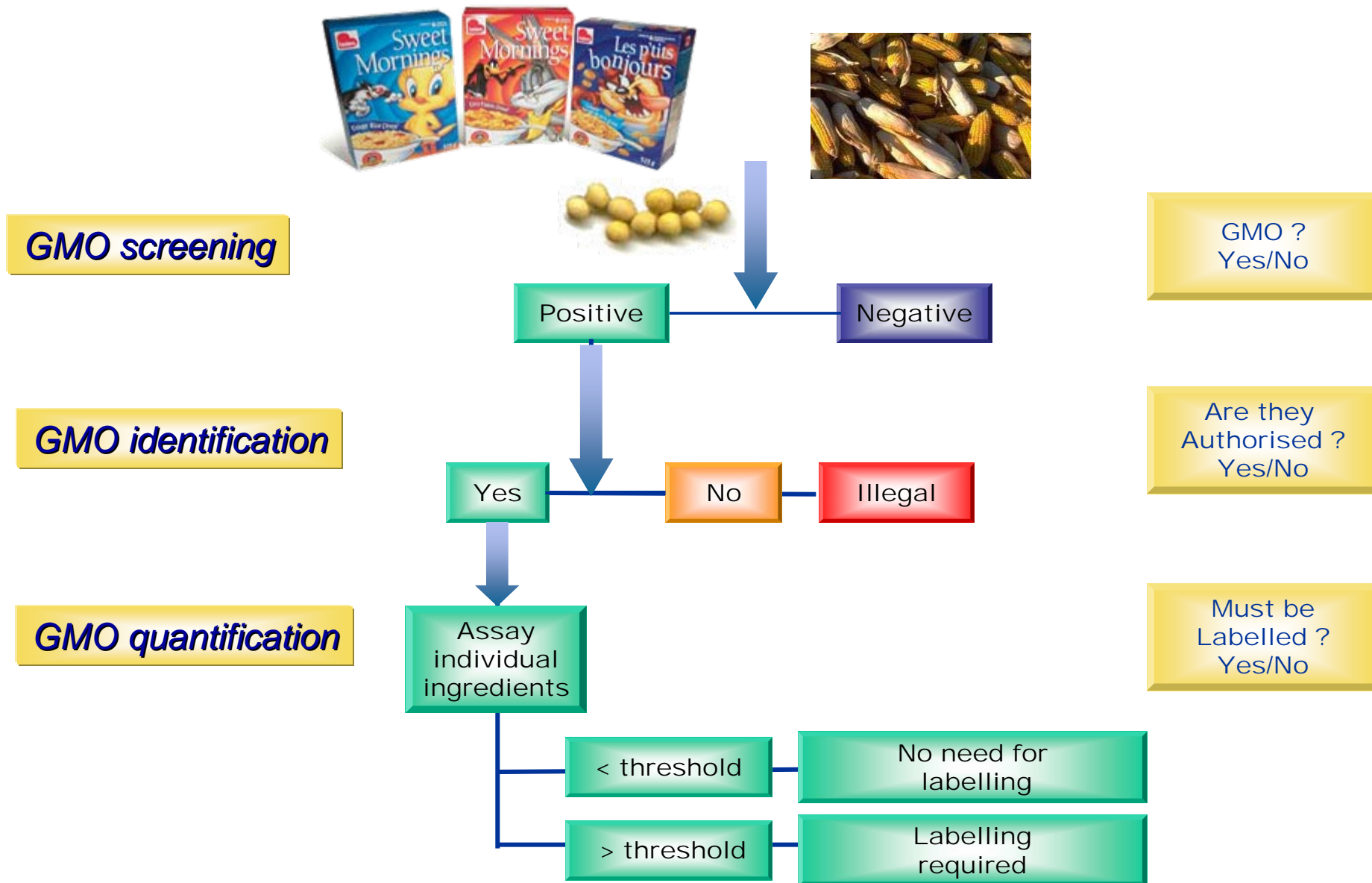
- Correct sampling is NOT common practice
- Correct sampling is expensive and time consuming

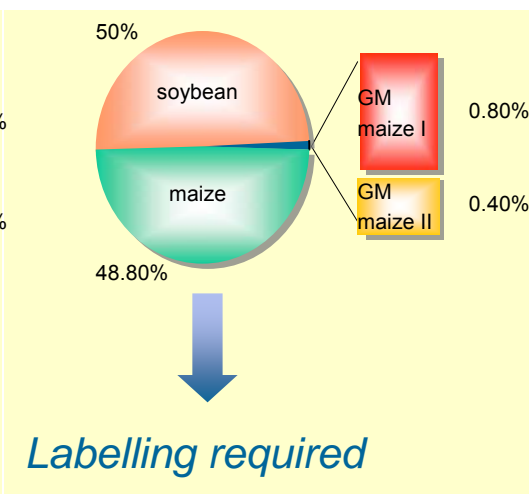
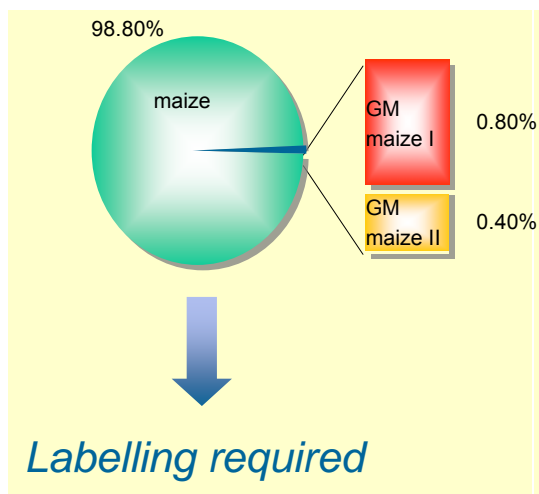
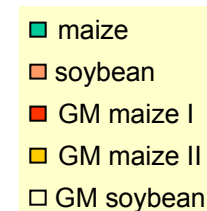
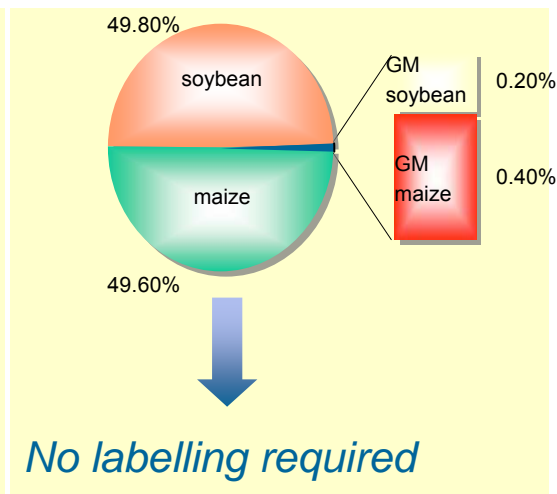
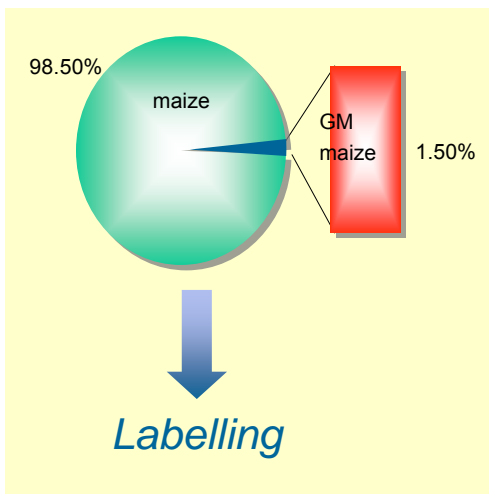
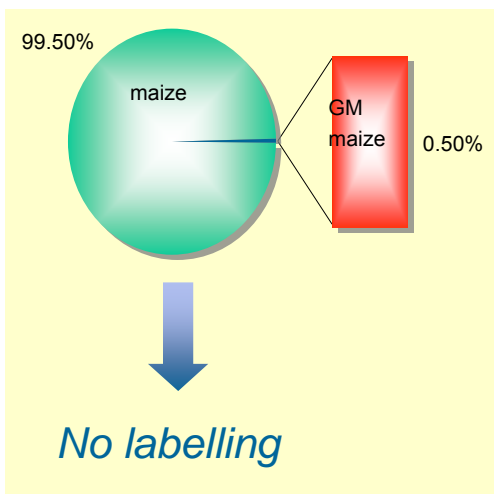
BUT

If sample is taken in a incorrect way
(not representative)



analytical result can NOT be extrapolated to the whole lot



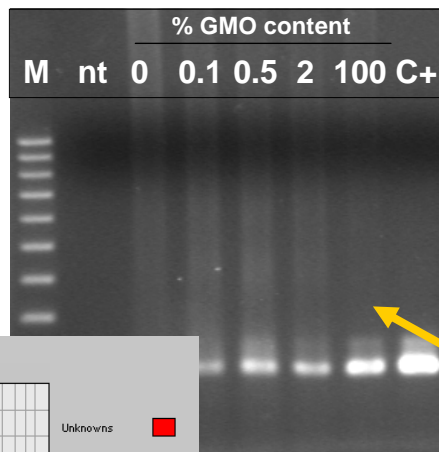
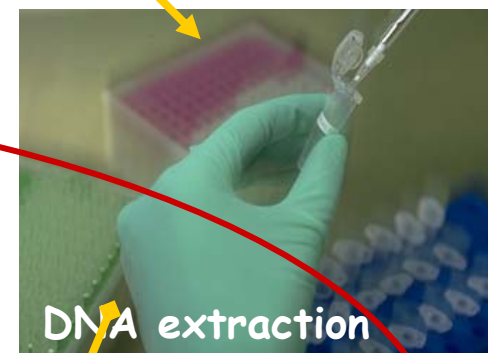


How much "maize" is present?

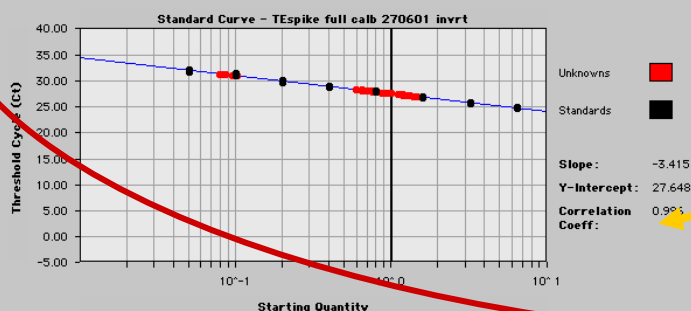
How much "GM-maize 1" is present?

How much "GM-maize 2" is present?

Make calculations



Analytical



- **Characterization**

To elucidate molecular structure of transgene events at the locus of insertion

- **Screening**

To detect genetic elements of the T-DNA insert (CaMV 35S promoter; 3' nos terminator,...)

- **Detection**

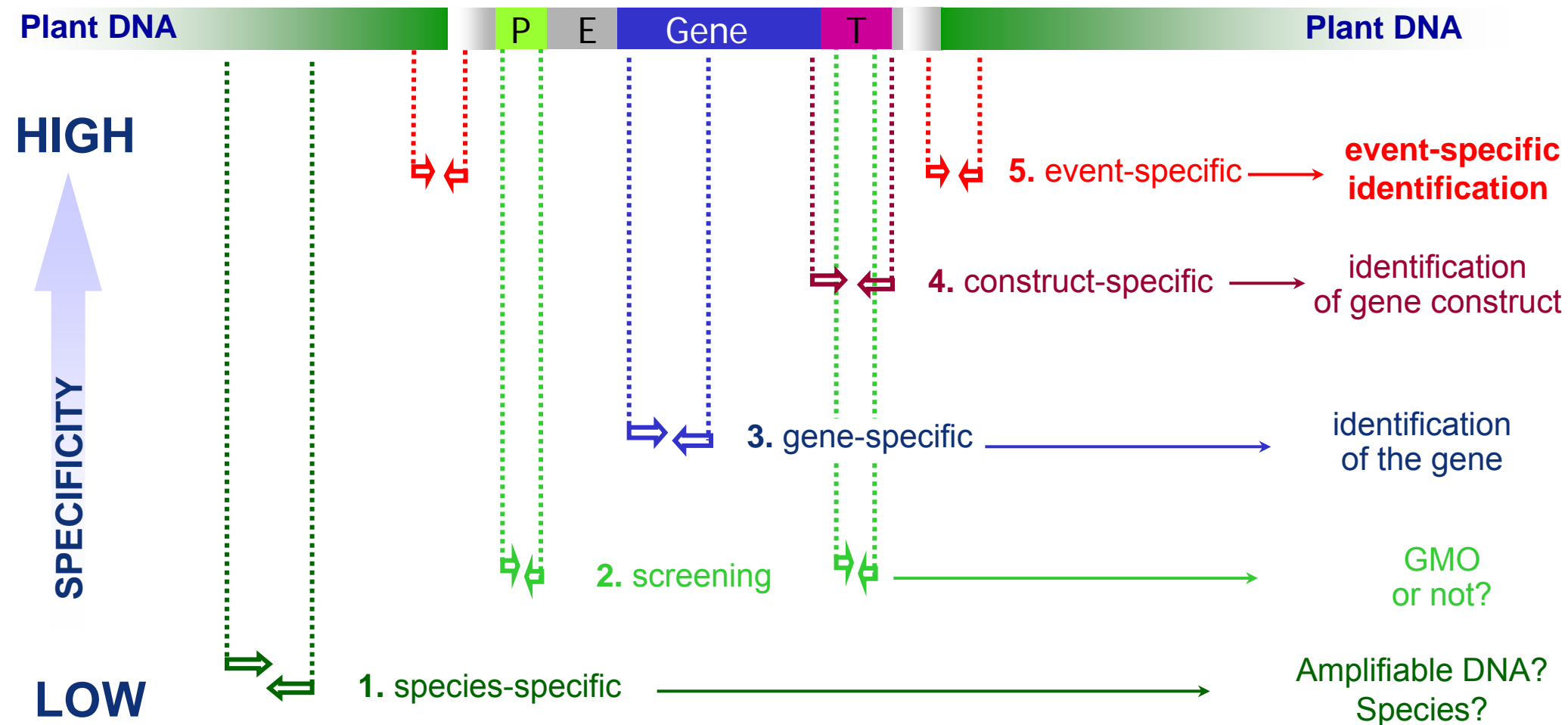
To confirm the presence/absence of a GMO

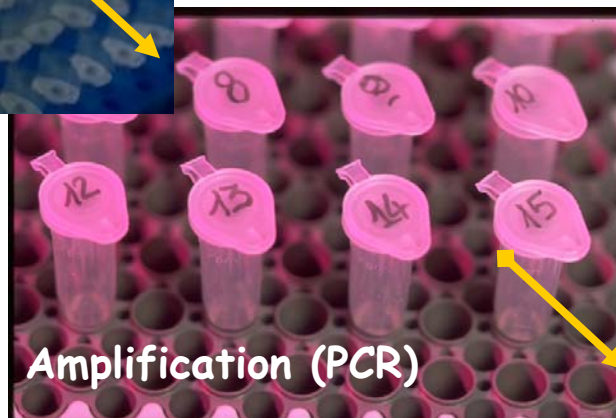
- **Identification**

To confirm the presence of genetic elements which are unique for a specific transgenic event - which GMO is/are present

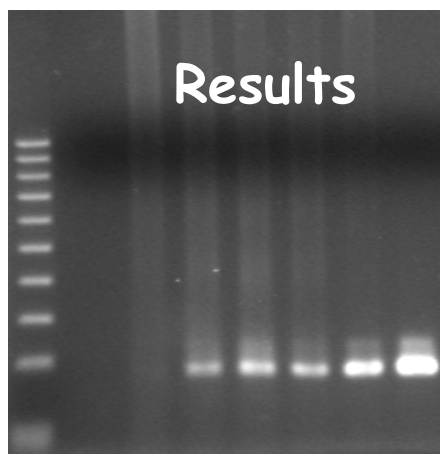
- **Quantification**

Estimation of the GMO content





% GMO content							
M	nt	0	0.1	0.5	2	100	C+



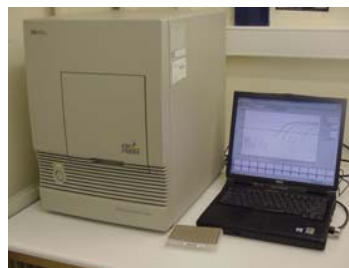
base
pairs
(bp)

500
400
300
200
100

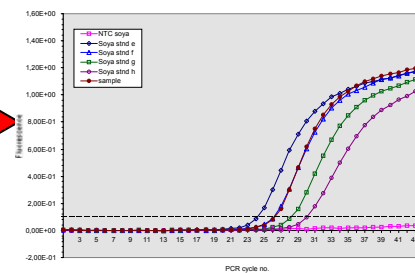


Wild type

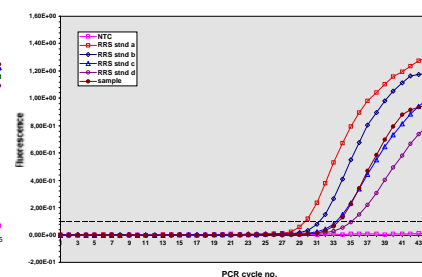
GMO



Target taxon specific

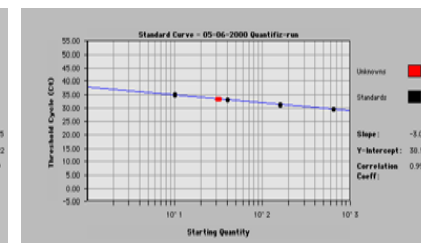
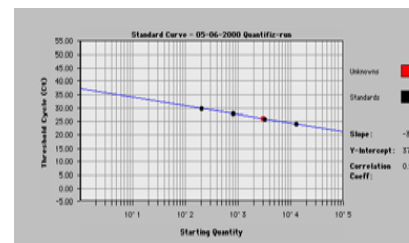


GM specific



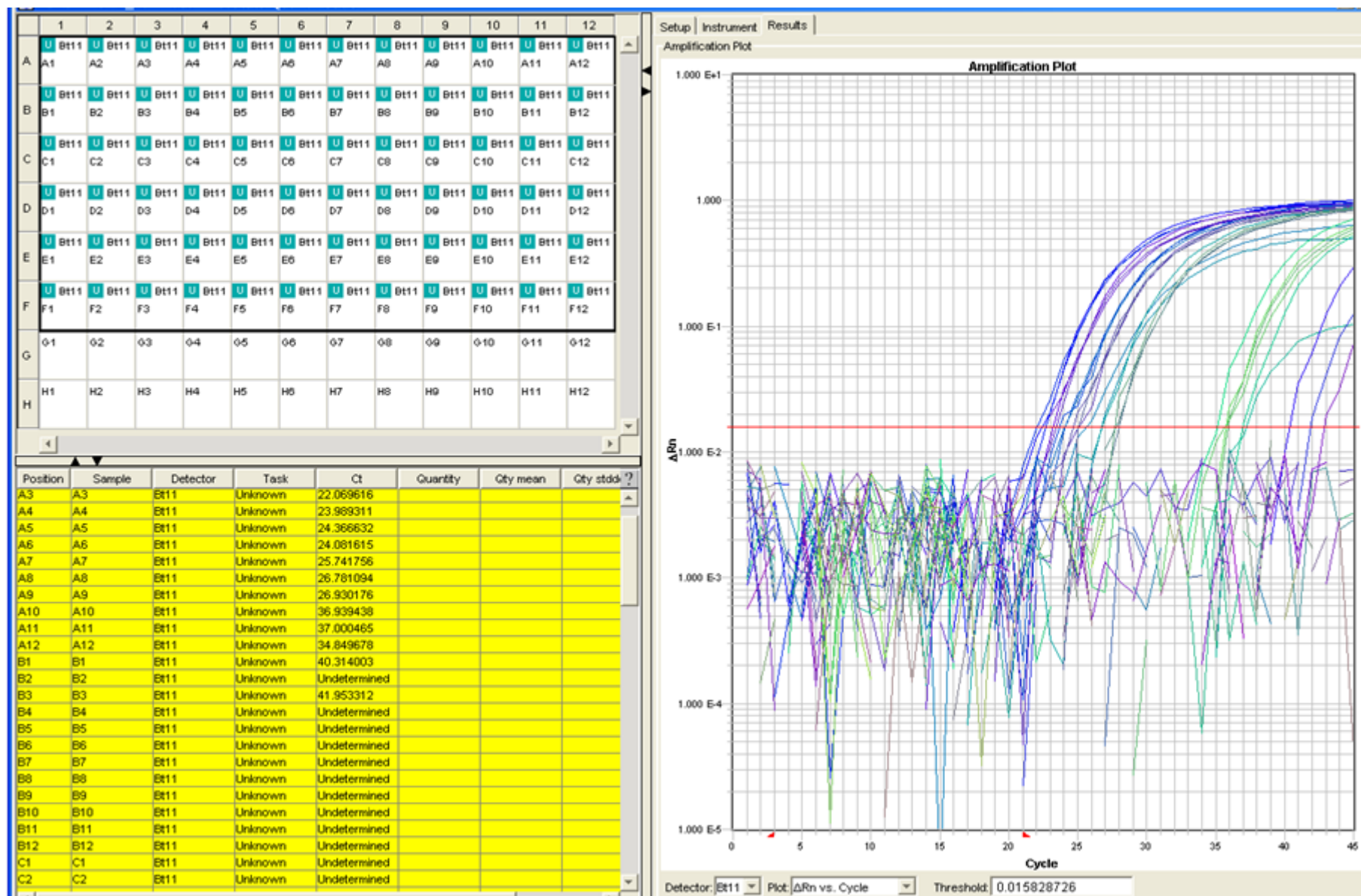
1.
Sample preparation,
and
DNA extraction

2.
DNA amplification
in
real-time PCR machine

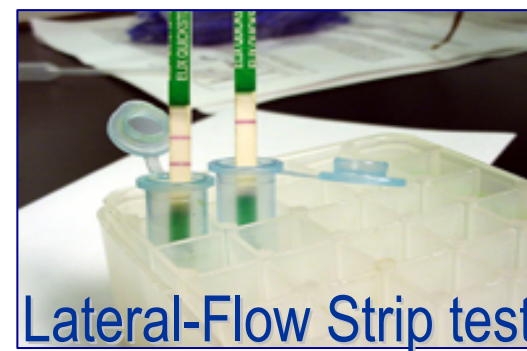
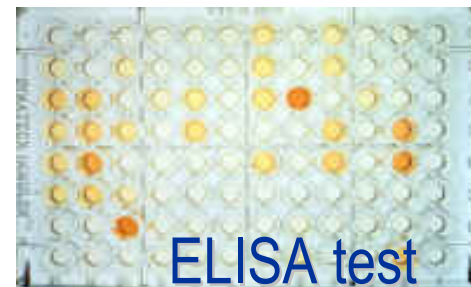
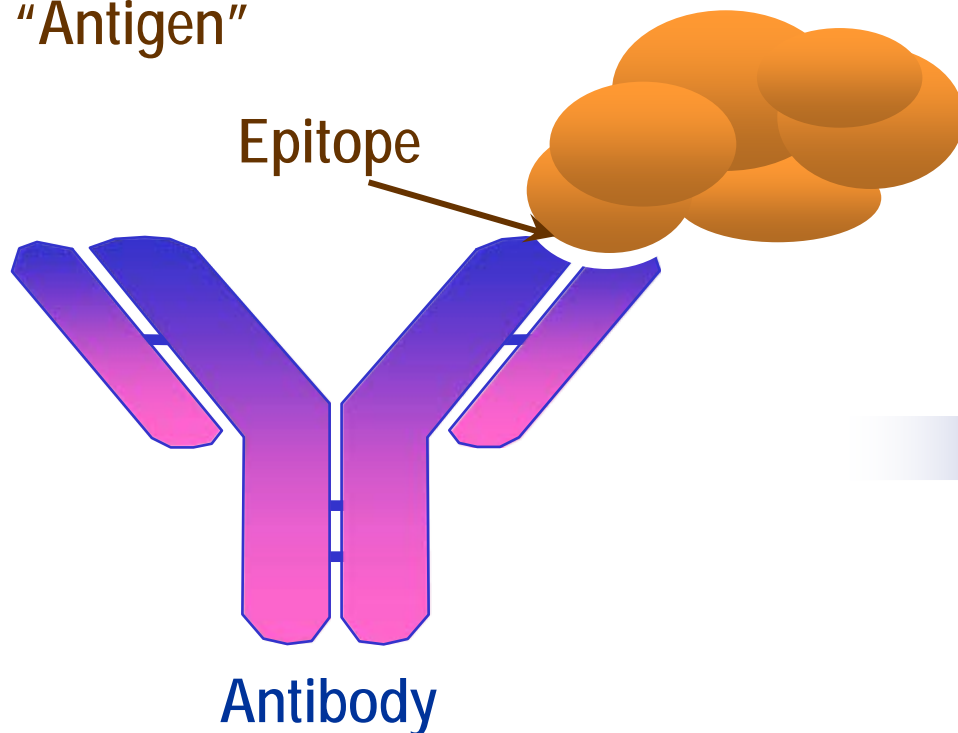


$$\frac{\text{GM}}{\text{endogenous}} \times 100$$

3.
Interpretation of result



- 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!**



- 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)

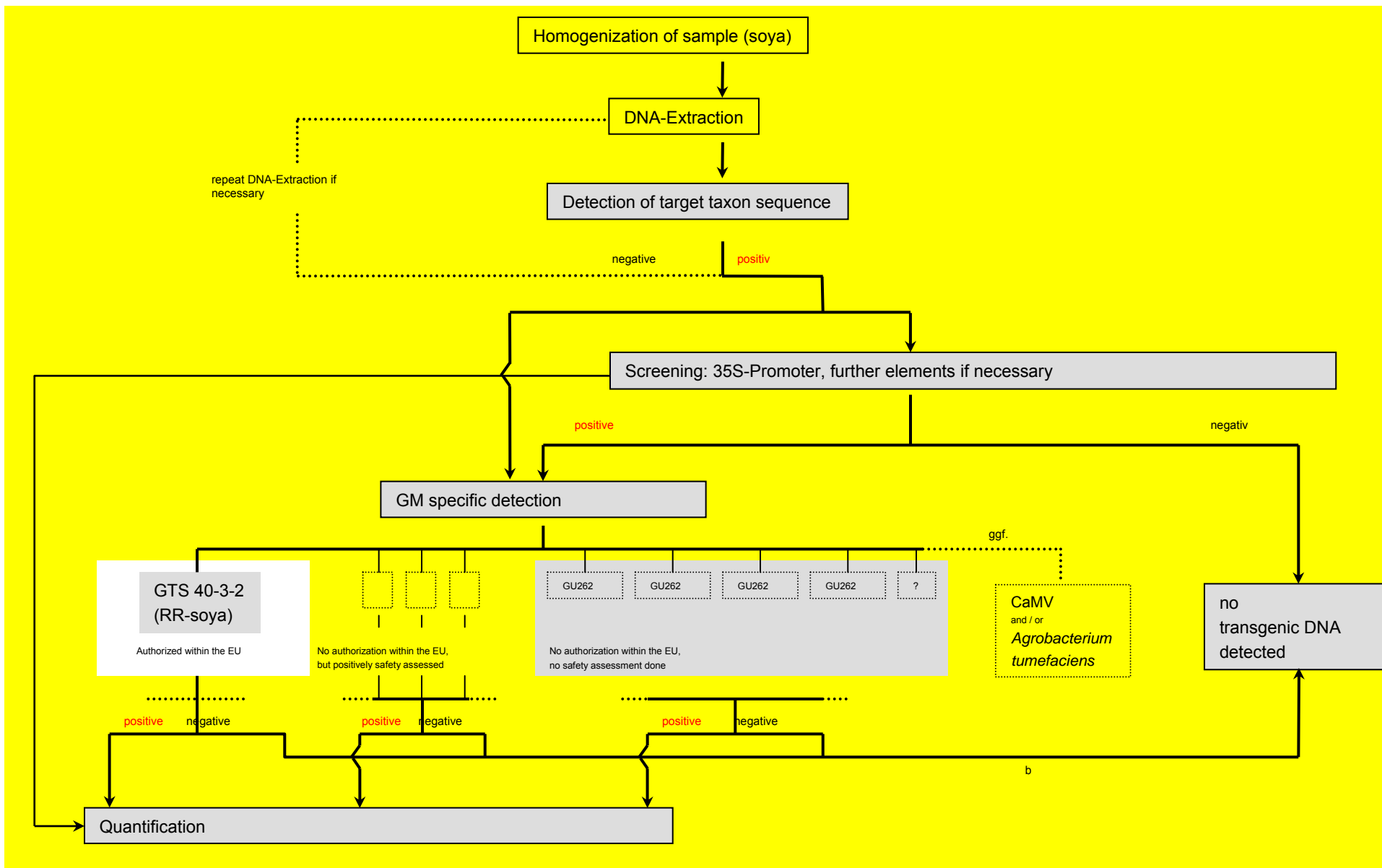
Current context

- 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.

Table 17: Events in commercial GM crops and in pipelines worldwide, by crop

Crop	Commercial in 2008	Commercial pipeline	Regulatory pipeline	Advanced de- velopment	Total by 2015*
Soybeans	1	2	4	10	17
Maize	9	3	5	7	24
Rapeseed	4	0	1	5	10
Cotton	12	1	5	9	27
Rice	0	1	4	10	15
Potatoes	0	0	3	5	8
Other crops	7	0	2	14	23
All crops	33	7	24	61	124

(Stein & Rodríguez-Cerezo, 2009)



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 tools able to overcome the difficulties of applying a complex analytical procedure, often exceeding laboratories' capabilities.

The JRC is presently involved in two novel approaches, both based upon the use of ready-to-use pre-spotted plates:

- ✓ The use of event-specific methods known to the EURL-GMFF;
- ✓ The accurate combination of screening methods targeting elements common to groups of GMOs.

The strategy:

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

Targets:

- 7 plant species
- 39 GM events
(+ stack events derived from them)

Methods:

all methods submitted to the CRL-GMFF for validation, represented once, including methods for emergency cases, e.g. Bt-10 maize and LL601 rice

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

The challenge:

- **source:** 48 real-time PCR methods with individual characteristics, reaction conditions, cycling setting and efficiency

- **output:** unique system in which all methods work with a unique set of conditions without losing specificity and overall performance

Event or	Event	Species	Primer F	Primer R	Probe	Probe	Reporte
1	Bt11 Maize	Maize	GCGGACCCCTATTGTTTA	TCCAGATCCCTCCATGAG	AAATCATTCAMATATGATCCGCTCA	TAMRA	FAM
2	NR603 Maize	Maize	ATGATGACCTCGAGTAGCTGTAA	AAGAGATAACAGATCCATCAACACT	TGCTACACGCGACACACTCCACTC	TAMRA	FAM
3	GA21 Maize	Maize	CTTATCGTATGCTATTTCGACCTTAGA	TGGCTCGGATCCCTCT	CATATACTAACTATCTATCTCTTCGACAGCAGGTGGT	TAMRA	FAM
4	MON883 Maize	Maize	GTAGGATCGGAAGCTGGGTAC	TGTTACGGCTCAATGCTGACCT	TGACACGCCATCGACACAGTAGGGTCA	TAMRA	FAM
5	1507 Maize	Maize	TAGTCTCGGCGAGATGG	CTTTGCGAGATCAAGGG	TAACTCAAGGCCCTCACTCGG	TAMRA	FAM
6	T25 Maize	Maize	ACAAGGCTGTGGTGTCCAC	GACATGATCTCTCTCCACGG	TCATTGAGTGGTGGTCCGATTGTGG	TAMRA	FAM
7	59122 Maize	Maize	GSGATAGCAGTAAGAGGGCTC	CCTTAATCTCCGCTCATGATGAG	TTTAACTGAGGCGGGGAAAGACAA	TAMRA	FAM
8	HT-1 Sugar beet	Sugar beet	TGGGATCTGGTGGCTCTAAGT	AATGCTGCTAAATCTGAG	AAGGCGGGAACGACATCT	TAMRA	FAM
9	MON810 Maize	Maize	TGGAAGGACGAGGACTCTAAGT	GCCACCTCTCTTTCCACTATCTT	AACATCTCTTCATCTGCCGAGC	TAMRA	FAM
10	201-24-236 Cotton	Cotton	CTCATTCGCTGATCATGTAGATTTC	GGACATCTCTGGGCTTTGTG	TTGGGTTATTAAGTCAGATTAGAGGGAGACAA	TAMRA	FAM
11	3006-210-23 Cotton	Cotton	AAATATTACAATGATGAGTATGATG	ACTCTTTCTTTCTCCATATGACC	TACTCAATTGCTGATCATGATGATTCCGG	TAMRA	FAM
12	LLRICE82 Rice	Rice	AGCTGGGCTATAGCGAAGAGG	TGCTACGGGCTGACGCTCTA	GGACCGATTATTATATCTTTAGTCACCT	TAMRA	FAM
13	T45 oilseed rape	Rape	CAATGGACACAGAAATTAGC	GACTCTGATGAACTGTTGGC	TAGAGGACCTAACGACACTCGCGT	TAMRA	FAM
14	EH92-027-1 Potato	Potato	GTGTCAACACATTTACAGCA	TCCCTAATCTCCGCTCATGA	AGATTGTGTTCCGCGCTTCAGTT	TAMRA	FAM
15	Mid Oilseed rape	Rape	GTTAGAAGAAAGTAAACATTTATAGCGGG	GGAGGGGTGTTTGTGTTATC	AATATATGACGAGTCCCGGGAAATTC	TAMRA	FAM
16	R10 Oilseed rape	Rape	AGATTTAGCATGATGACATCAGCA	CATAAGGAGATGGAGACTTAG	GGCACCTCTATCGACCTAAGGCCCA	TAMRA	FAM
17	GT73 (R763) Rapeseed	Rape	CCATATTGACCATCATCTCATGTCT	GCCTATACGAAGGCGAAGAAAGGA	TTCCCGGACATGATGATCTCTCTT	TAMRA	FAM
18	LLCotton25 Cotton	Cotton	CAGATTTTGTGGGATTTGGAATTC	CAAGCAATTAATCTGAG	CTTACAGTACTCGGGGTGGAGCGG	TAMRA	FAM
19	MON 531 Cotton	Cotton	TCCCATTCGAGTTTCTCAGGT	AMCCATCCGACCCCACTGA	TTGTCCCTCCACTCTCTCTC	TAMRA	FAM
20	A2704-12 Soybean	Soy	GCAGAAAGCGGTGACTCTCT	ATTCAAGGCTCGGCACTGTT	CGGCTCTCCGATCGCCCTCC	TAMRA	FAM
21	MR804 Maize	Maize	GCGGCGCAATCAACAG	GGTCTAAGCTGACTCCCTAATCT	AGGGGGAAGCGCAATCTGATG	TAMRA	FAM
22	R11 Rapeseed	Rape	CTAAGGSGGTGAGATGTAGC	CGGCGCTCACTTTTGGTGTG	CTCATCTATCTCCACCGCTGACGCTCA	TAMRA	FAM
23	R12 Rapeseed	Rape	GGGTGACACATATAGSAGS	GGGCGTCGACCGGCGTGG	GACCGGCGCAATTTGGCTTAGGGT	TAMRA	FAM
24	Mx1 Rapeseed	Rape	AGCGTCGCGCATCTACATT	CTAGATCGGAAGCTGAAGATGG	CTATTGCTGTGCTACCTAGCGGACTT	TAMRA	FAM
25	Topas 1902 Rapeseed	Rape	GTTCGGGTCTGTGAGTCC	CGACCGGCGCTGATATAGA	TCCCGGCTCATCGGGG	TAMRA	FAM
26	MON1445 Cotton	Cotton	GGAGTAGAGGATCAGATAAACAC	ATCGACCTGAGGCCAAGCT	ATCAGATTGTGTTCCGCGCTTGAGTT	TAMRA	FAM
27	Bt176 Maize	Maize	GGCGGTGAGGAGCTGTT	GGGAGAGAGCTACATGTTTCTAA	AGCAACGAGTGGGCGACACC	TAMRA	FAM
28	MON1985 Cotton	Cotton	GTACTAGATCGGGGATATCC	AAGGTTGCTAATGATGGGA	CGGCTAGAGTATGAGTCTGCTGCTGAA	TAMRA	FAM
29	40-3-2 Soybean	Soy	TTTCATCAAAATAGATCATACATACAGTT	GGCATTTGTAGAGGCCACTT	GGTTTTCATTTGGG	MBB	FAM
30	GA21 Maize	Maize	CGTATGCTATTTCGACCTTAGAACCA	GGGATCTCTCTGGGGT	TTTCTCAAGCAGGCTGGGTCCGGG	TAMRA	FAM
31	MON8817		GAGGAGACCTCGAGAGGCT	TCCGAGTGAACATCCA	TCCCGGCTTCGATTAAACAGAGTGGGG	TAMRA	FAM
32	LY038 Maize	Maize	TGGGTTGAGTCTCGGATGTT	AGGAATGATATCAAGCTTATCGA	CGAGCGGAGTTATGGGTCGACGG	TAMRA	FAM
33	3272 Maize	Maize	TGATCAGACAGATTCTTTTATGG	GGTTTCCCGGCTCAAGTTA	ACTGCTGACCGCGGCAACACTG	TAMRA	FAM
34	MON89788		TCCCGCTCTAGCGCTCAAT	TGAGCAGGACCTCGAGAA	CTGAGGCGGGAAGGACACTCTG	TAMRA	FAM
35	MON8904 Maize	Maize	TTCTCATATGACCATCATCTCAT	GGGTATCTAATACGGTGGTTTAA	ATCCCGGAATTTGTT	MBB	FAM
36	DP-368043 soybean	Soy	GTGCAATAGGCTAGGTTTACGAAAA	TTTGATATCTTGAGATAGACGAGAGTGT	CTCTAGAGATCGCTCAACATGGTGGAGCAC	TAMRA	FAM
37	MON8913 cotton	Cotton	GGCTTGGCTACCTTAGAGAGTCT	CAATTAACCTAATAGTAGGCCAATATAC	AACTACTAGTTGTGGTACTACT	MBB	FAM
38	Rice GM events P35S-bar	Rice	TATCTCTGGAGAGCCCTCC	ATGTGGGCGGGGCGTGTCTG	TCTATATAGGAGGTTGATTTTATT	MBB	FAM
39	LLRice01 Rice	Rice	TCTAGATCCGAAAGCAGATGT	GGAGGGCGCGGAGTGT	CGACCTCCCAACATAAAGCGCGCTG	TAMRA	FAM
40	BH3 Rice	Rice	GACTCTCGAGTATATGACAGA	AGCTGGTACCTCGACTTATTCAG	TGAGTTCACTGAGTACTGCAACACTGAG	TAMRA	FAM
43	Bt10 Maize	Maize	CACACAGGAGATTATAGGGTACTCA	ACACGGAATGTTGAATCACTCT	AATACCGCTGATTAATGCTTCA	MBB	FAM

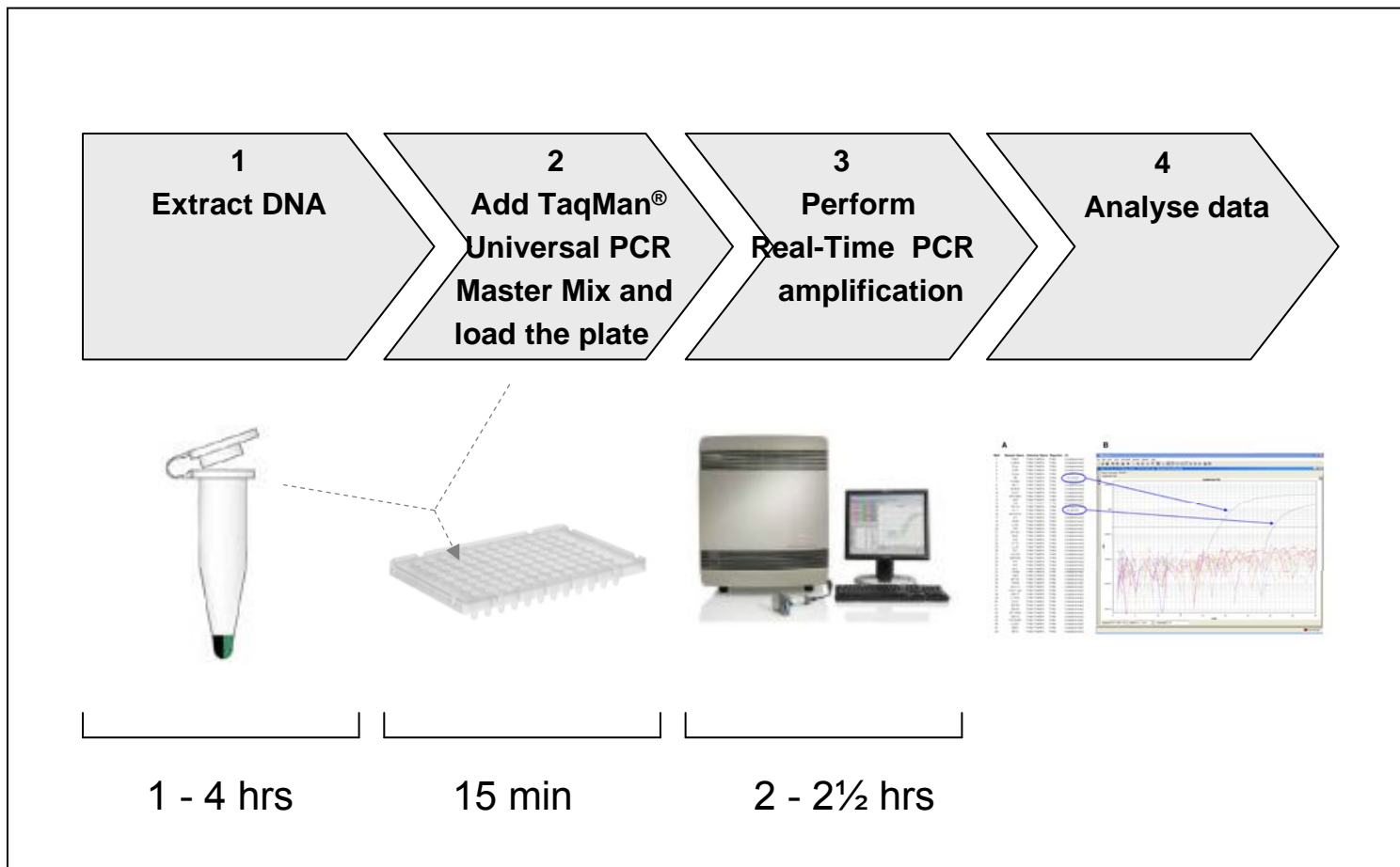
Plate layout:

	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	Bt11 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 Rapeseed	MON1445 Cotton	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	Bt11 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

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



Interpretation of results

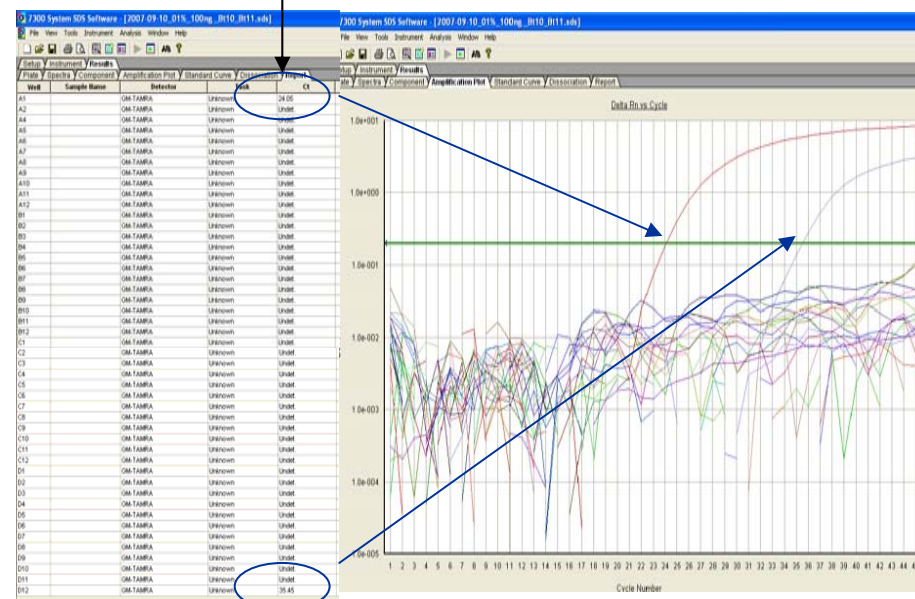
	1	2	3	4	5	6	7	8	9	10	11	12
A	hmg Maize Ref	B4H7 cotton Ref	FLD Rice Ref	CruK Oilseed Ref	Lectin Soybean Ref	GS Sugarcane Ref	UOPase Potato Ref	Bt11 Maize	Nb803 Maize	GA21 Maize Monsanto	MON883 Maize	1507 Maize
B	T25 Maize	S9122 Maize	HT-1 Sugar beet	MON810 Maize	201-24-23 Cotton	3008-210-23 Cotton	LLRICE62 Rice	T45 oilseed rape	EH92-527-1 Potato	M88 Oilseed rape	RTD Oilseed rape	GT73 (BT63) Rapeseed
C	LLCotton2 S Cotton	MON531 Cotton	A2704-12 Soybean	MR604 Maize	RM Rapeseed	R2 Rapeseed	M81 Rapeseed	Topas 192 Rapeseed	MON1445 Cotton	BT176 Maize	MON15905 Cotton	40-3-2 Soybean
D	GA21 Maize Syngenta	MON88017 Maize	LY038 Maize	3272 Maize	MON89788 soybean	MON89034 Maize	CP-35043 soybean	MON89913 cotton	Rice OM events P350-bar	LLRice601 Rice	BE3 Rice	BT10 Maize
E	H810 Maize Ref	B4H7 Cotton Ref	FLD Rice Ref	CruK Oilseed Ref	Lectin Soybean Ref	GS Sugarcane Ref	UOPase Potato Ref	Bt11 Maize	Nb803 Maize	GA21 Maize Monsanto	MON883 Maize	1507 Maize
F	T25 Maize	S9122 Maize	HT-1 Sugar beet	MON810 Maize	201-24-23 Cotton	3008-210-23 Cotton	LLRICE62 Rice	T45 oilseed rape	EH92-527-1 Potato	M88 Oilseed rape	RTD Oilseed rape	GT73 (BT63) Rapeseed
G	LLCotton2 S Cotton	MON531 Cotton	A2704-12 Soybean	MR604 Maize	RM Rapeseed	R2 Rapeseed	M81 Rapeseed	Topas 192 Rapeseed	MON1445 Cotton	BT176 Maize	MON15905 Cotton	40-3-2 Soybean
H	GA21 Maize Syngenta	MON88017 Maize	LY038 Maize	3272 Maize	MON89788 soybean	MON89034 Maize	CP-35043 soybean	MON89913 cotton	Rice OM events P350-bar	LLRice601 Rice	BE3 Rice	BT10 Maize

Sample 1

Sample 2

A1 = hmg maize reference method

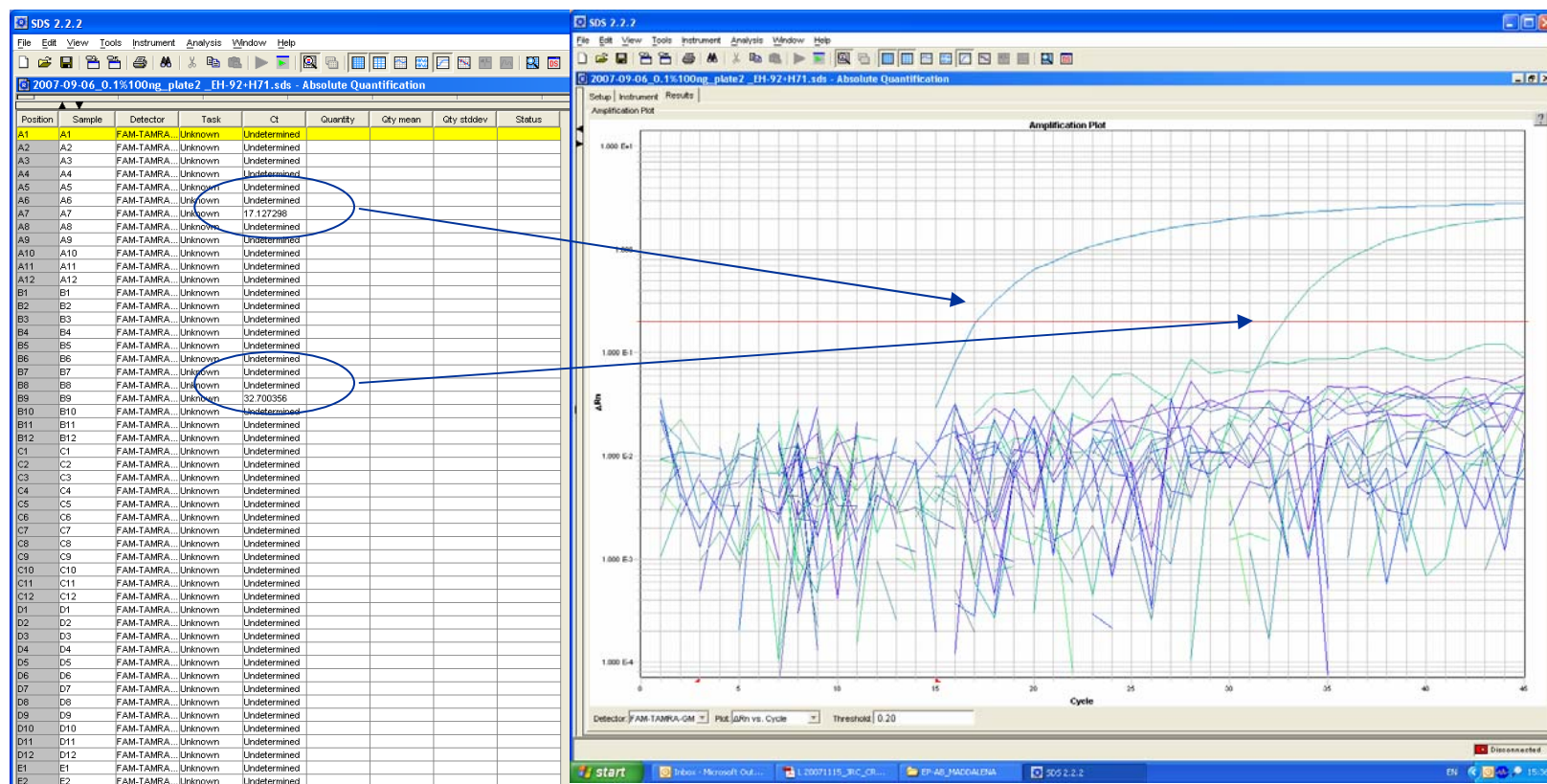
D12 = Bt10
event-specific method



Detection of potato event EH92-527-1

A7 = potato reference gene

B9 EH92-527-1 event-specific method

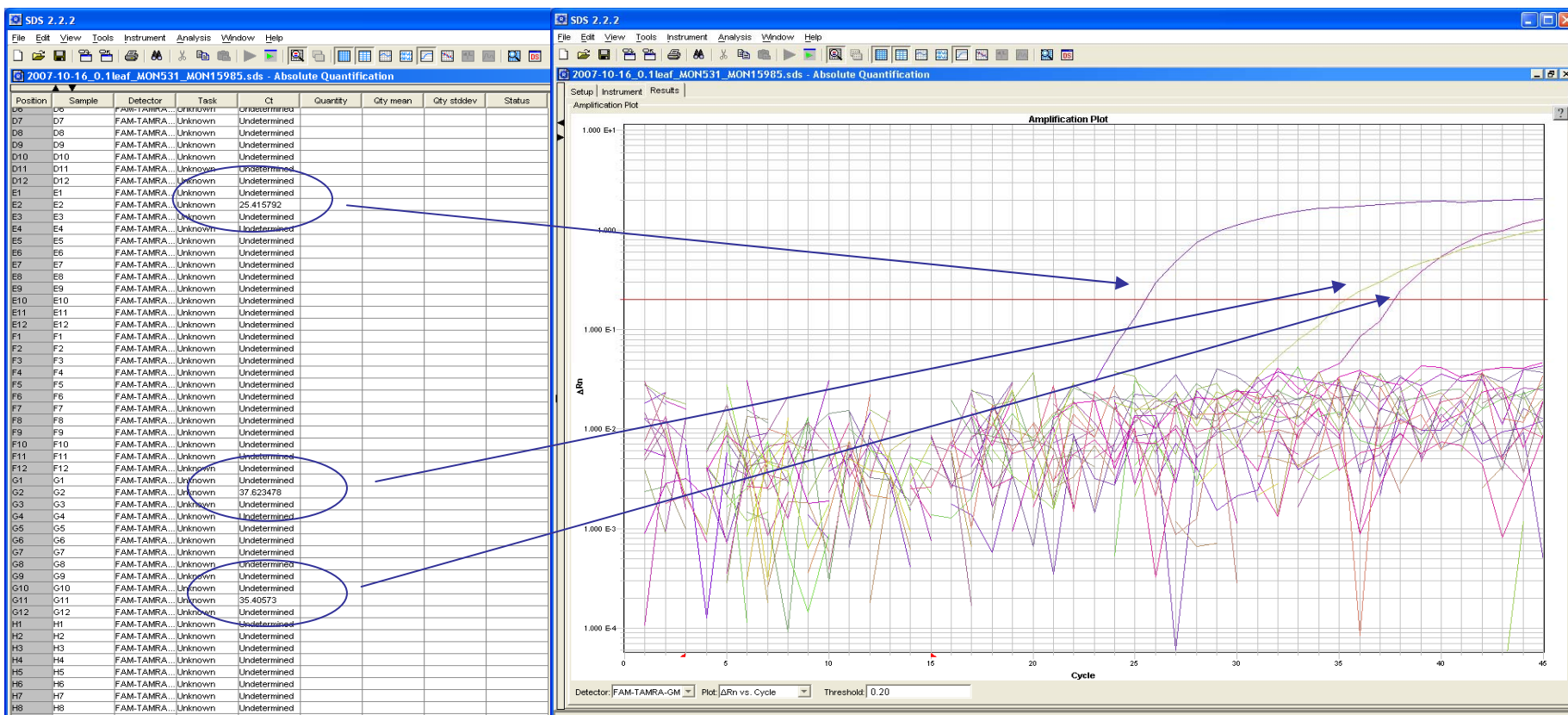


Detection of cotton event MON15985

E2 = SAH7 cotton reference gene method

G2 = MON531 event-specific method

G11 = MON15985 event-specific method



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

Plate layout

<i>well</i>	<i>RTi-PCR method</i>	<i>well</i>	<i>RTi-PCR method</i>
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

	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



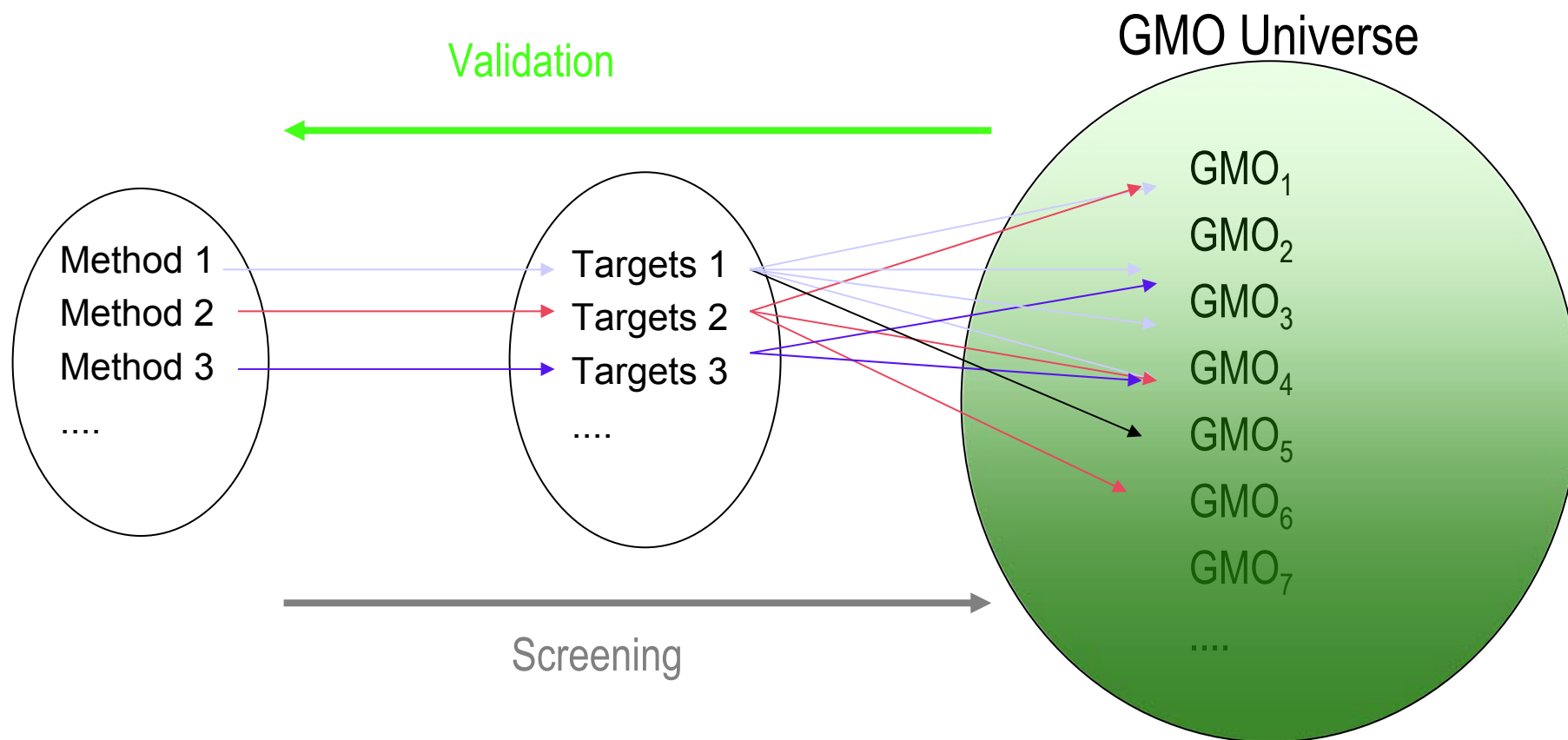
Ready-to-use pre-spotted plate/strip systems in response to the different needs of GMO analysis:

Screening formulation based on matrix approach

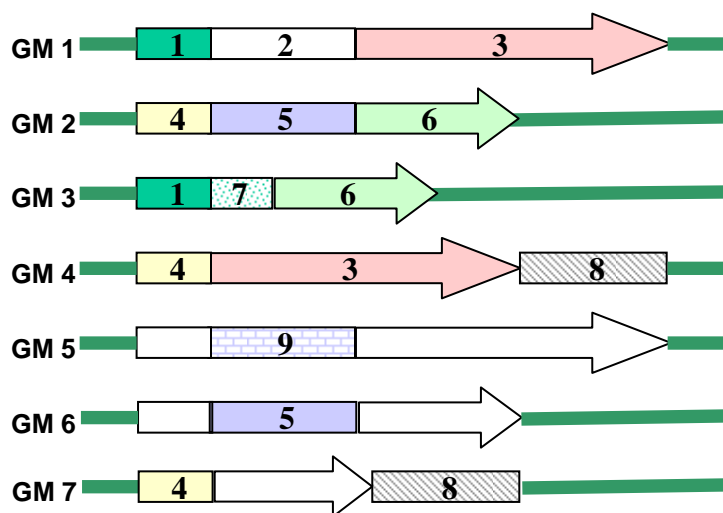
Requirements to apply screening in GMO analysis:

- 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

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



Combination of screening methods targeting common GM elements



Sample	RESULTS (1 method = 1 element)									GM	Interpretation
	METHOD n.										
	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 + ?

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	Debate (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			

Species	Event #	Productor	GRL # published	p3SS	tNOS	t3SS	pNOS	rice actin	tOCS	nptII	CPL EPSPS	mEPSPS	PAT/pat	PAT/bar	barnase	Cry1Ab	CryIac	Cry1F	Cry3Bb1
soybean	GT37 x 372	Monsanto	-	X							X								
soybean	A 27 x 12	Bayer CropScienc	-	X		X													
soybean	A 68 x 127*	Bayer CropScienc	-	X		X							X						
soybean	MON 90303*	Monsanto	-																
soybean	MON 89788	Monsanto	In Process																
soybean	MON 89543-5	Pioneer Hi-Bred	In Process																
soybean	MON 89543-1	Pioneer Hi-Bred	In Process																
maize	MON 111	Syngenta seeds/	-	X												X			
maize	MON 176	Zea GenZ	In Process	X		X		X					X				X		
maize	MON 810	Monsanto	In Process	X		X								X			X		
maize	A 21** ***	Monsanto	-		X							X							
maize	MON 803	DuPont	-	X		X		X					X						
maize	KR 603	Monsanto	-	X		X				X	X								
maize	MON 863	Monsanto	-	X		X				X								X	X
maize	CT507	cogen/Pioneer	-	X		X		X						X					
maize	MON 8122	Monsanto	-	X										X					
maize	KR 603 x MON 810	Monsanto	-	X		X					X					X			
maize	MON 1507 x NK603	Monsanto	-	X		X		X		X	X			X				X	
maize	MON 863 x NK 603	Monsanto	-	X		X				X	X		X					X	X
maize	GA 21 x MON 810	Monsanto	In Process	X		X										X			
maize	T25 x MON 810***	Monsanto	-	X		X		X					X			X			
maize	MON 863 x MON 810	Monsanto/Pioneer	-	X		X				X						X			X
maize	MON 863 x MON 810 x NK603	Monsanto	-	X		X		X		X	X					X			X
maize	MON 803	Syngenta	-		X														
maize	GBA 507	Monsanto	-	X		X													
maize	BT10	Monsanto	-		X								X			X		X	
maize	1507 x 50122	Syngenta	-																
maize	BP122x1507x NK603 Maize	Pioneer Hi-Bred	In Process																
maize	LVO38	Rohm & Haas LLC	In Process																
maize	MON 8907X MON 810	Monsanto	In Process																
maize	MON 8907X MON 810	Monsanto	In Process																
maize	MON 89034 MON 810	Monsanto	In Process																
maize	MON 89034 x NK603	Monsanto	In Process																
maize	MON 89034 x 89017	Monsanto	In Process																
maize	CT22 Maize	Syngenta Crop Pr	In Process																
canola	GT73	Monsanto	-								X								
canola	MS1vRF2/ MS1vRF2	Bayer CropScienc	In Process		X		X		X					X		X			
canola	MS1vRF1/ MS1vRF1	Bayer CropScienc	In Process				X		X						X		X		
canola	MS&RF3/ MS&RF3***	Bayer CropScienc	-		X									X		X			
canola	TOPAS 19/2	Bayer CropScienc	In Process	X			X		X	X			X						
canola	T45	Bayer CropScienc	-	X									X						
canola	Falcon GS 4090**	Bayer CropScienc	In Process	X									X						
canola	Monstar 142	A&Evo					X						X						
canola	Falcon 05006 AC			X			X						X						
canola	Qw425	Bayer CropScienc	?		X														
cotton	MON 1445***	Monsanto	-	X		X					X								
cotton	MON 531***	Monsanto	-														X		
cotton	MON 531 x MON 1445	Monsanto	In Process																
cotton	MON 15985	Monsanto	-																
cotton	MON 15985 MON 1445	Monsanto	In Process																
cotton	MON 210- 9281-54	Flow AgroScience	-	X		X													
cotton	COTTON2	Bayer CropScienc	-	X															
cotton	PI 88913	Monsanto	In Process																
cotton	PI 88913	Monsanto	In Process																
cotton	MON 15			X															
rice	LL 5602	Aventis	-	X															
rice	LL 5601			X										X					
rice				X				X								X			
sugar beet	RUR1 x 11	KWS SAAT AG. I	-								X								

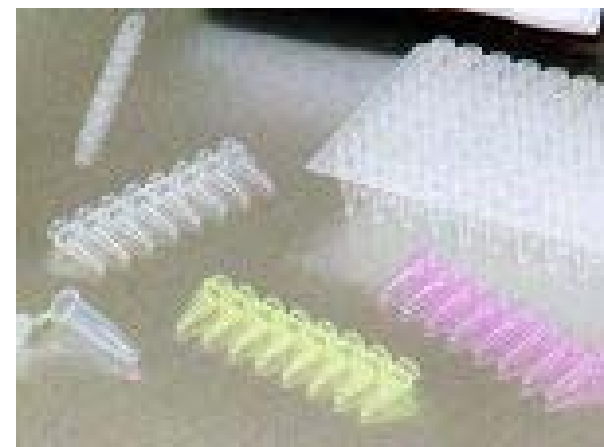
Possible elements/targets for 'screening' pre-spotted plates/strips

Soybean	Cotton	Maize	Rapeseed	Potato	Sugar beet	Rice
Lectin	sah7	hmg	CruA	UGPase	GS	PLD
P35S	P35S	P35S	P35S	P35S	P35S	P35S
T35S	T-nos	T-nos	T-nos	T-nos	CP4EPSPS	T-nos
T-nos	CP4EPSPS	Event 98140	CP4EPSPS
CP4EPSPS	pat			
Event 305423	nptII					
Event 356043	Event CBH614					
Event CV127	...					

→ screening elements, marker genes

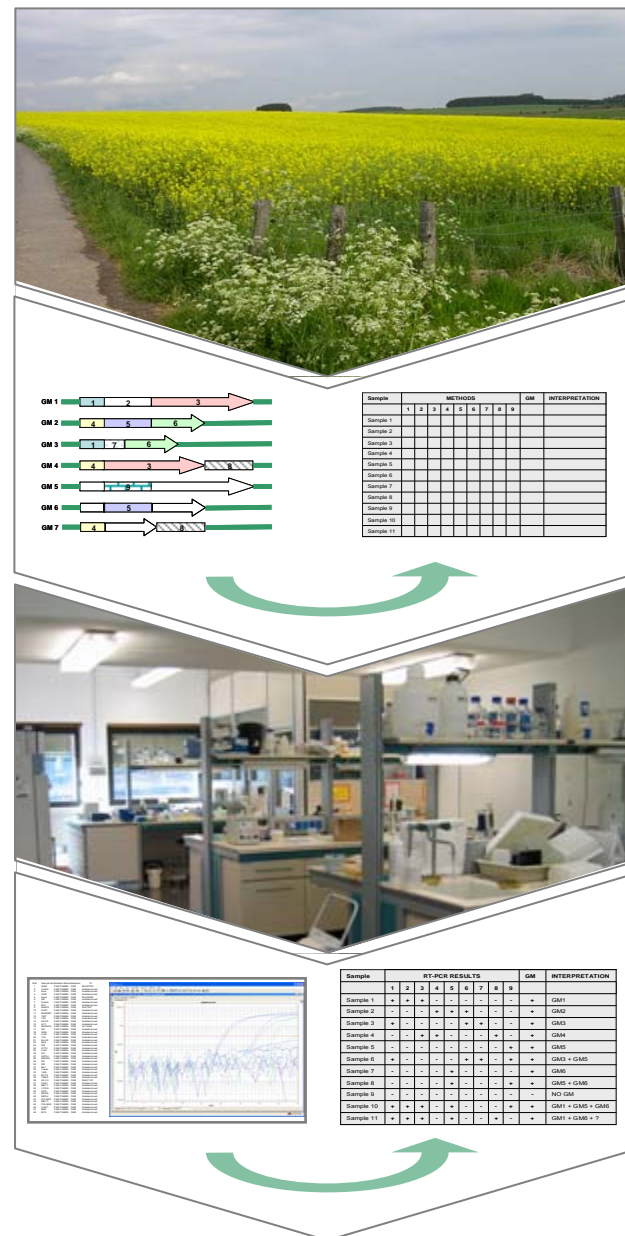
→ event-specific methods where needed

→ validated screening methods



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


4.
DSS - Level 3
Data analysis
&
interpretation



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 several events/targets 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!

