From analytical methods for labelling towards analytical methods for control:

Multi-target detection approaches

IHCP - Institute for Health and Consumer Protection

Ispra - Italy

http://ihcp.jrc.ec.europa.eu/

http://www.jrc.ec.europa.eu/

Context of the work

- Worldwide adoption and use of GMOs is rapidly increasing
- 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;
    - Traceability from the point of production or import down to the table and vice versa (from farm to fork);
    - Post-market monitoring requirements;
    - GMO control based on combination of screening + event-specific detection methods;
    - Increasing number of GMOs already approved or 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.

Rationale

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	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	

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 loosing specificity and overall performance

Dossier	Event	Species	Primer F	Primer R	Probe	Probe	Reporter
1	Bt11 Maize	Maize	GCGGAACCCCTATTTGTTTA	TCCAAGAATCCCTCCATGAG	AAATACATTCAAATATGTATCCGCTCA	TAMRA	FAM
2	NK603 Maize	Maize	ATGAATGACCTCGAGTAAGCTTGTTAA	AAGAGATAACAGGATCCACTCAAACACT	TGGTACCACGCGACACACTTCCACTC	TAMRA	FAM
3	GA21 Maize	Maize	CTTATCGTTATGCTATTTGCAACTTTAGA	TGGCTCGCGATCCTCCT	CATATACTAACTCATATCTCTTTCTCAACAGCAGGTGGGT	TAMRA	FAM
4	MON863 Maize	Maize	GTAGGATCGGAAAGCTTGGTAC	TGTTACGGCCTAAATGCTGAACT	TGAACACCCATCCGAACAAGTAGGGTCA	TAMRA	FAM
5	1507 Maize	Maize	TAGTCTTCGGCCAGAATGG	CTTTGCCAAGATCAAGCG	TAACTCAAGGCCCTCACTCCG	TAMRA	FAM
6	T25 Maize	Maize	ACAAGCGTGTCGTGCTCCAC	GACATGATACTCCTTCCACCG	TCATTGAGTCGTTCCGCCATTGTCG	TAMRA	FAM
7	59122 Maize	Maize	GGGATAAGCAAGTAAAAGCGCTC	CCTTAATTCTCCGCTCATGATCAG	TTTAAACTGAAGGCGGGAAACGACAA	TAMRA	FAM
8	H7-1 Sugar beet	Sugar beet	TGGGATCTGGGTGGCTCTAACT	AATGCTGCTAAATCCTGAG	AAGGCGGGAAACGACAATCT	TAMRA	FAM
9	MON810 Maize	Maize	TCGAAGGACGAAGGACTCTAACGT	GCCACCTTCCTTTTCCACTATCTT	AACATCCTTTGCCATTGCCCAGC	TAMRA	FAM
10	281-24-236 Cotton	Cotton	CTCATTGCTGATCCATGTAGATTTC	GGACAATGCTGGGCTTTGTG	TTGGGTTAATAAAGTCAGATTAGAGGGAGACAA	TAMRA	FAM
11	3006-210-23 Cotton	Cotton	AAATATTAACAATGCATTGAGTATGATG	ACTCTTTCTTTTTCTCCATATTGACC	TACTCATTGCTGATCCATGTAGATTTCCCG	TAMRA	FAM
12	LLRICE62 Rice	Rice	AGCTGGCGTAATAGCGAAGAGG	TGCTAACGGGTGCATCGTCTA	CGCACCGATTATTTATACTTTTAGTCCACCT	TAMRA	FAM
13	T45 oilseed rape	Rape	CAATGGACACATGAATTATGC	GACTCTGTATGAACTGTTCGC	TAGAGGACCTAACAGAACTCGCCGT	TAMRA	FAM
14	EH92-527-1 Potato	Potato	GTGTCAAAACACAATTTACAGCA	TCCCTTAATTCTCCGCTCATGA	AGATTGTCGTTTCCCGCCTTCAGTT	TAMRA	FAM
15	Ms8 Oilseed rape	Rape	GTTAGAAAAAGTAAACAATTAATATAGCCGG	GGAGGGTGTTTTTGGTTATC	AATATAATCGACGGATCCCCGGGAATTC	TAMRA	FAM
16	Rf3 Oilseed rape	Rape	AGCATTTAGCATGTACCATCAGACA	CATAAAGGAAGATGGAGACTTGAG	CGCACGCTTATCGACCATAAGCCCA	TAMRA	FAM
17	GT73 (RT63) Rapeseed	Rape	CCATATTGACCATCATACTCATTGCT	GCTTATACGAAGGCAAGAAAAGGA	TTCCCGGACATGAAGATCATCCTCCTT	TAMRA	FAM
18	LLCotton25 Cotton	Cotton	CAGATTTTTGTGGGATTGGAATTC	CAAGGAACTATTCAACTGAG	CTTAACAGTACTCGGCCGTCGACCGC	TAMRA	FAM
19	MON 531 Cotton	Cotton	TCCCATTCGAGTTTCTCACGT	AACCAATGCCACCCCACTGA	TTGTCCCTCCACTTCTTCTC	TAMRA	FAM
20	A2704-12 Soybean	Soy	GCAAAAAAGCGGTTAGCTCCT	ATTCAGGCTGCGCAACTGTT	CGGTCCTCCGATCGCCCTTCC	TAMRA	FAM
21	MIR604 Maize	Maize	GCGCACGCAATTCAACAG	GGTCATAACGTGACTCCCTTAATTCT	AGGCGGGAAACGACAATCTGATCATG	TAMRA	FAM
22	Rf1 Rapeseed	Rape	CTAAGGGAGGTCAAGATGTAGC	CGGGCCTAACTTTTGGTGTG	CTCATCATCCTCACCCAGTCAGCATCA	TAMRA	FAM
23	Rf2 Rapeseed	Rape	GGGTGAGACAATATATCGACG	GGGCATCGCACCGGTGAG	CACCGGCCAAATTCGCTCTTAGCCGT	TAMRA	FAM
24	Ms1 Rapeseed	Rape	ACGCTGCGGACATCTACATT	CTAGATCGGAAGCTGAAGATGG	CTCATTGCTGATCCACCTAGCCGACTT	TAMRA	FAM
25	Topas 19/2 Rapeseed	Rape	GTTGCGGTTCTGTCAGTTCC	CGACCGGCGCTGATATATGA	TCCCGCGTCATCGGCGG	TAMRA	FAM
26	MON1445 Cotton	Cotton	GGAGTAAGACGATTCAGATCAAACAC	ATCGACCTGCAGCCCAAGCT	ATCAGATTGTCGTTTCCCGCCTTCAGTTT	TAMRA	FAM
27	Bt176 Maize	Maize	GGCCGTGAACGAGCTGTT	GGGAAGAAGCCTACATGTTTTCTAA	AGCAACCAGATCGGCCGACACC	TAMRA	FAM
28	MON15985 Cotton	Cotton	GTTACTAGATCGGGGATATCC	AAGGTTGCTAAATGGATGGGA	CCGCTCTAGAACTAGTGGATCTGCACTGAA	TAMRA	FAM
29	40-3-2 Soybean	Soy	TTCATTCAAAATAAGATCATACATACAGGTT	GGCATTTGTAGGAGCCACCTT	CCTTTTCCATTTGGG	MGB	FAM
30	GA21 Maize	Maize	CGTTATGCTATTTGCAACTTTAGAACA	GCGATCCTCCTCGCGTT	TTTCTCAACAGCAGGTGGGTCCGGGT	TAMRA	FAM
31	MON88017		GAGCAGGACCTGCAGAAGCT	TCCGGAGTTGACCATCCA	TCCCGCCTTCAGTTTAAACAGAGTCGGGT	TAMRA	FAM
32	LY038 Maize	Maize	TGGGTTCAGTCTGCGAATGTT	AGGAATTCGATATCAAGCTTATCGA	CGAGCGGAGTTTATGGGTCGACGG	TAMRA	FAM
33	3272 Maize	Maize	TCATCAGACCAGATTCTCTTTTATGG	CGTTTCCCGCCTTCAGTTTA	ACTGCTGACGCGGCCAAACACTG	TAMRA	FAM
34	MON89788		TCCCGCTCTAGCGCTTCAAT	TCGAGCAGGACCTGCAGAA	CTGAAGGCGGGAAACGACAATCTG	TAMRA	FAM
35	MON89034 Maize	Maize	TTCTCCATATTGACCATCATACTCATT	CGGTATCTATAATACCGTGGTTTTTAAA	ATCCCCGGAAATTATGTT	MGB	FAM
36	DP-356043 soybean	Soy	GTCGAATAGGCTAGGTTTACGAAAAA	TTTGATATTCTTGGAGTAGACGAGAGTGT	CTCTAGAGATCCGTCAACATGGTGGAGCAC	TAMRA	FAM
37	MON88913 cotton	Cotton	GGCTTTGGCTACCTTAAGAGAGTC	CAAATTACCCATTAAGTAGCCAAATTAC	AACTATCAGTGTTTGACTACAT	MGB	FAM
38	Rice GM events P35S::bar	Rice	TATCCTTCGCAAGACCCTTCC	ATGTCGGCCGGGCGTCGTTCTG	TCTATATAAGGAAGTTCATTTCATT	MGB	FAM
39	LLRice601 Rice	Rice	TCTAGGATCCGAAGCAGATCGT	GGAGGGCGCGGAGTGT	CCACCTCCCAACAATAAAAGCGCCTG	TAMRA	FAM
40	Bt63 Rice	Rice	GACTGCTGGAGTGATTATCGACAGA	AGCTCGGTACCTCGACTTATTCAG	TCGAGTTCATTCCAGTTACTGCAACACTCGAG	TAMRA	FAM
43	Bt10 Maize	Maize	CACACAGGAGATTATTATAGGGTTACTCA	ACACGGAAATGTTGAATACTCATACTCT	AATAACCCTGATAAATGCTTCA	MGB	FAM

Plate layout:

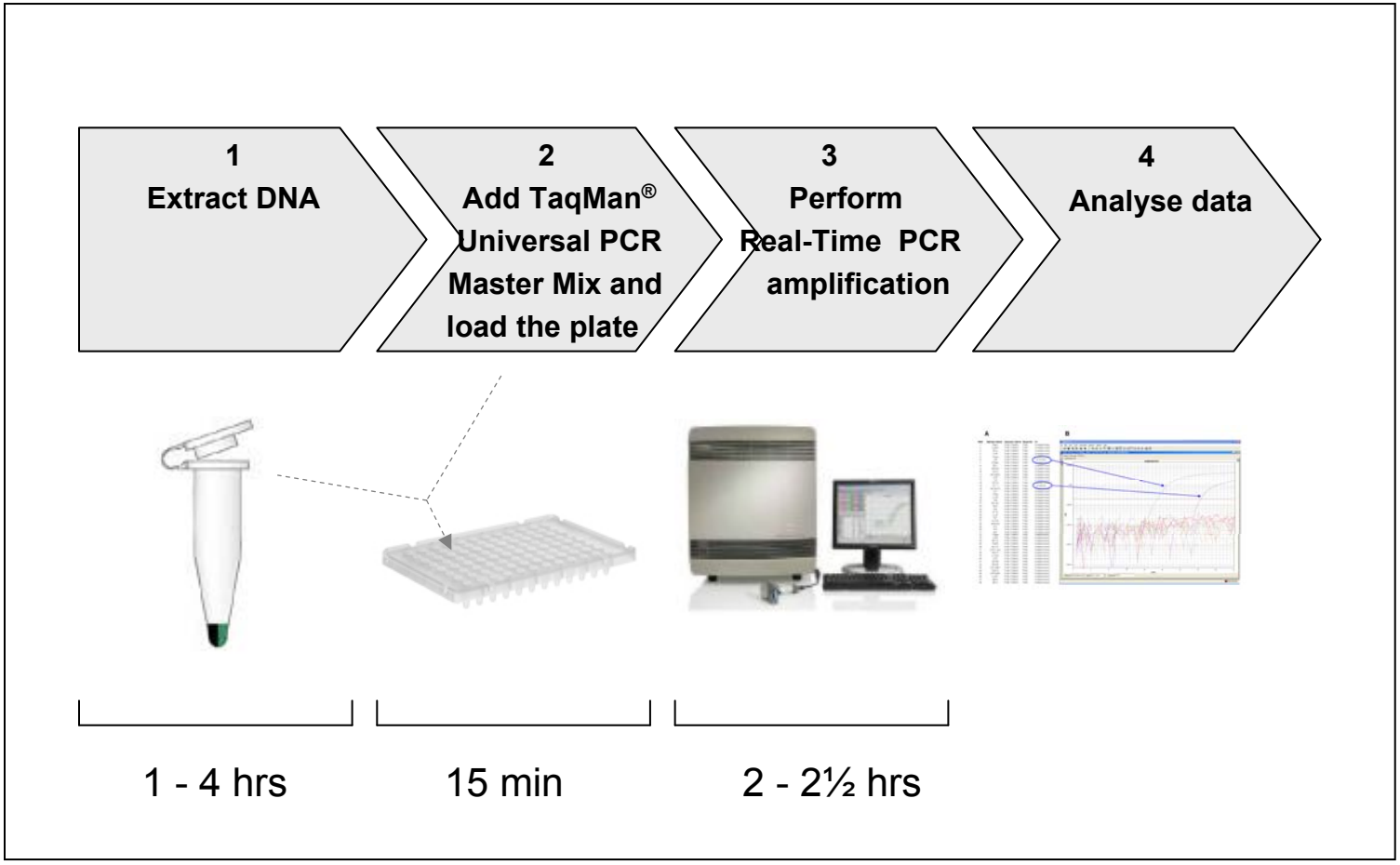
	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG Maize Ref	SAH7 Cotton Ref	PLD Rice Ref	CruA Oilseed Ref	Lectin Soybean Ref	GS Sugarbeet Ref	UGPase Potato Ref	Bt11 Maize	NK603 Maize	GA21Maize Monsanto	MON863 Maize	1507 Maize
B	T25 Maize	59122 Maize	H7-1 Sugar beet	MON810 Maize	281-24-236 Cotton	3006-210-23 Cotton	LLRICE62 Rice	T45 oilseed rape	EH92-527-1 Potato	Ms8 Oilseed rape	Rf3 Oilseed rape	GT73 (RT63) Rapeseed
C	LLCotton25 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	GA21Maize 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	LLCotton25 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

Advantages of the system: reduced workload and time saving

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



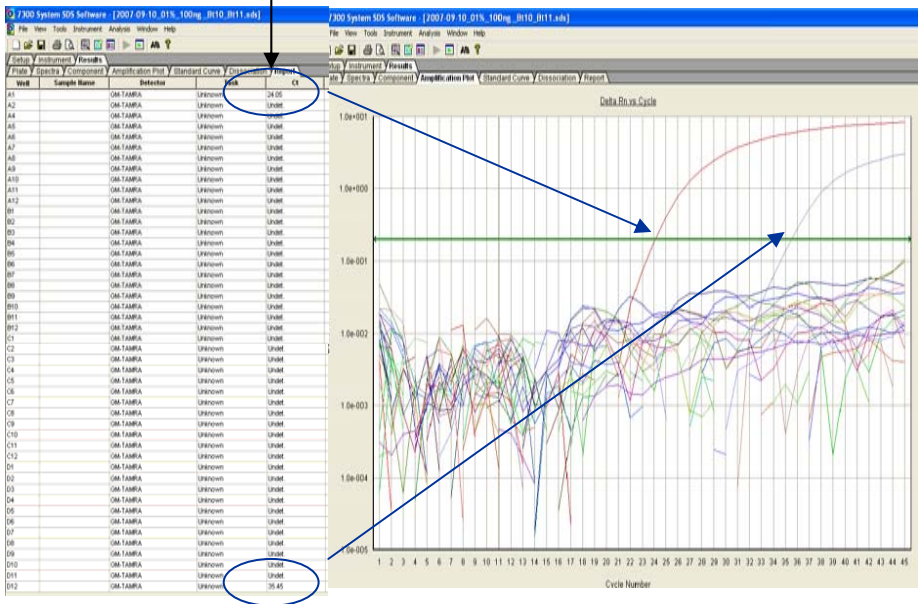
Interpretation of results

Sample 1

Sample 2

hmg maize reference method

Bt10
event-specific method

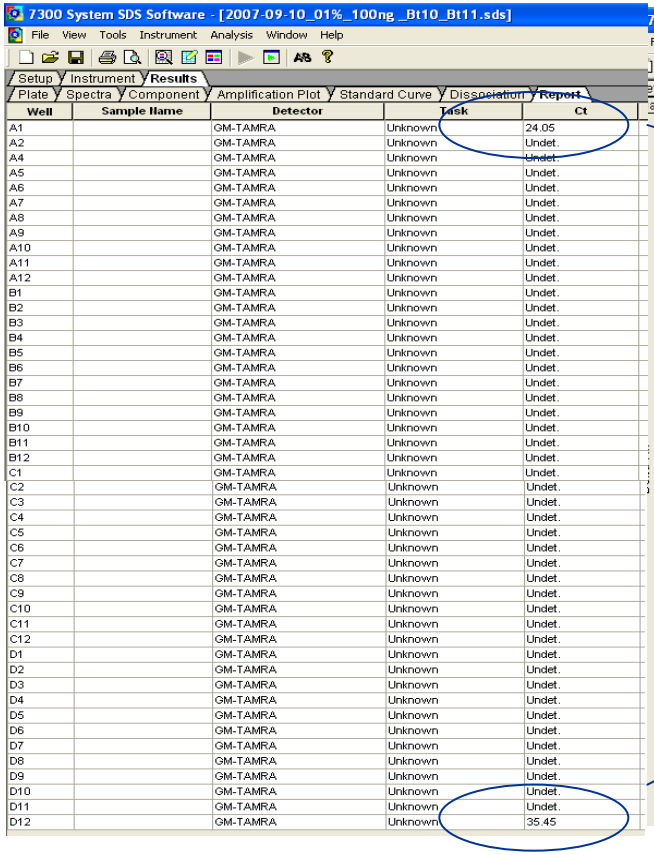


Specificity

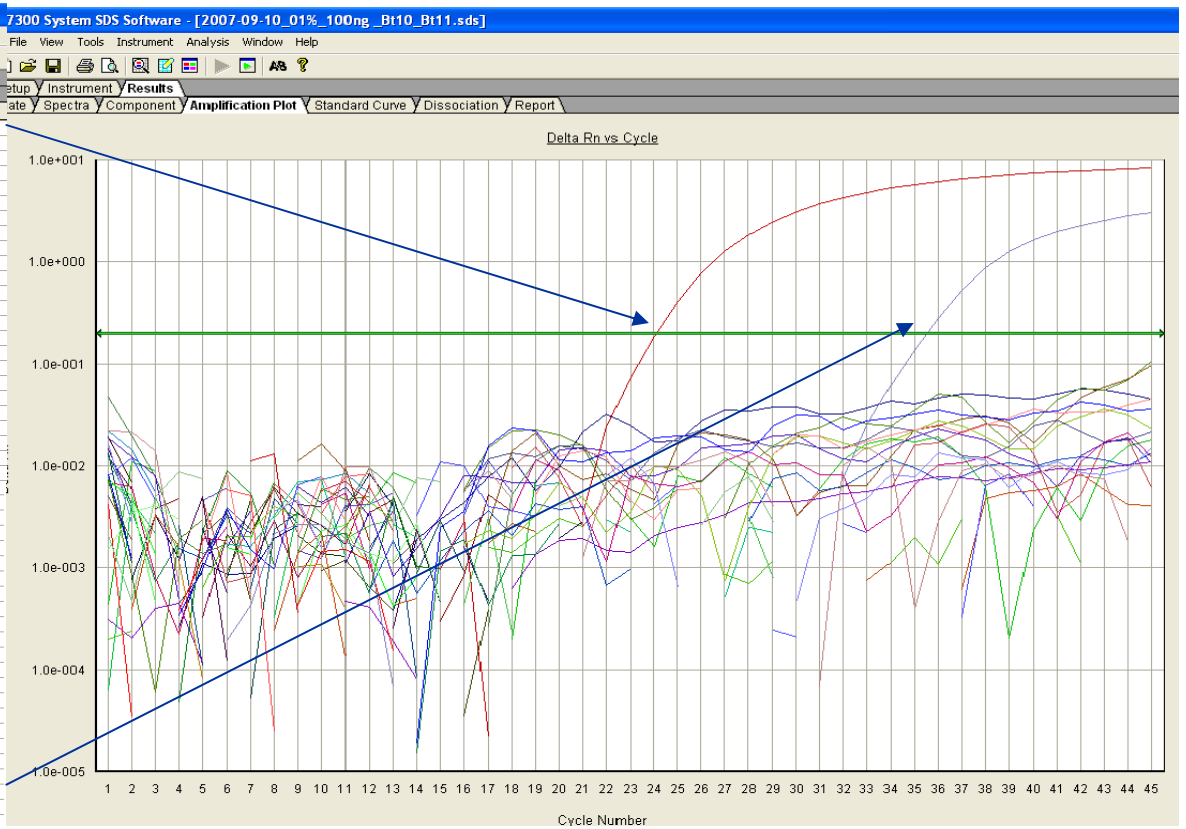
Type of detection	Methods	WILD TYPE: Maize	Cotton	Rice	Rapeseed	Soybean	Sugar beet	Potato	SAMPLES / GM EVENTS: Bt11 Maize	NK603 Maize	GA21Maize Monsanto	MON863 Maize	1507 Maize	T25 Maize	59122 Maize	H7-1 Sugar beet	MON810 Maize	281-24-236X3006-210-23 Cotton	LLRICE62 Rice	T45 Rapeseed	EH92-527-1 Potato	Ms8 Rapeseed	Rf3 Rapeseed	GT73 (RT63) Rapeseed	LLCotton25 Cotton	MON 531 Cotton	A2704-12 Soybean	MIR604 Maize	Rf1 Rapeseed	Rf2 Rapeseed	Ms1 Rapeseed	Topas 19/2 Rapeseed	MON 1445 Cotton	Bt176 Maize	MON15985 Cotton	40-3-2 Soybean	GA21 Maize Syngenta	MON 88017 Maize	LY038 Maize	3272 Maize
Taxon specific	**SAH7 Cotton Ref**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	CruA Rapeseed R				+															+		+	+	+				+	+	+	+									
	Lectin Soybean R					+																					+									+					
	GS Sugar beet R						+									+																									
	UGPase Potato R							+													+																			
Event specific	Bt11 Maize								+																															
	NK603 Maize									+																															
	GA21 Maize Method 1										+																											+			
	MON863 Maize											+																													
	1507 Maize												+																												
	T25 Maize													+																											
	59122 Maize														+																										
	281-24-236 Cotton															+																									
	3006-210-23 Cotton																+																									
	T45 Rapeseed																			+																					
	EH92-527-1 Potato																				+																				
	Ms8 Rapeseed																					+																			
	Rf3 Rapeseed																						+																		
	GT73 Rapeseed																							+																	
	MON 531 Cotton																								+																
	MIR604 Maize																									+															
	Rf1 Rapeseed																											+													
	Rf2 Rapeseed																												+												
	Ms1 Rapeseed																													+											
	Topas 19/2 Rapeseed																														+										
	MON 1445 Cotton																															+									
	MON15985 Cotton																																+								
	GA21 Maize Method 2										+																											+			
	MON 88017 Maize																																						+		
	LY038 Maize																																							+	
	3272 Maize																																								+
	MON 89788 Soybean																																								
	MON 89034 Maize																																								
	DP-356043-5 Soybean																																								
	MON 88913 Cotton																																								
Construct specific	Rice P35S::bar																			+																					
Event specific	LLRice601 Rice																																								
	Bt63 Rice																																								
	Bt10 Maize																																								

Detection of maize event Bt-10

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

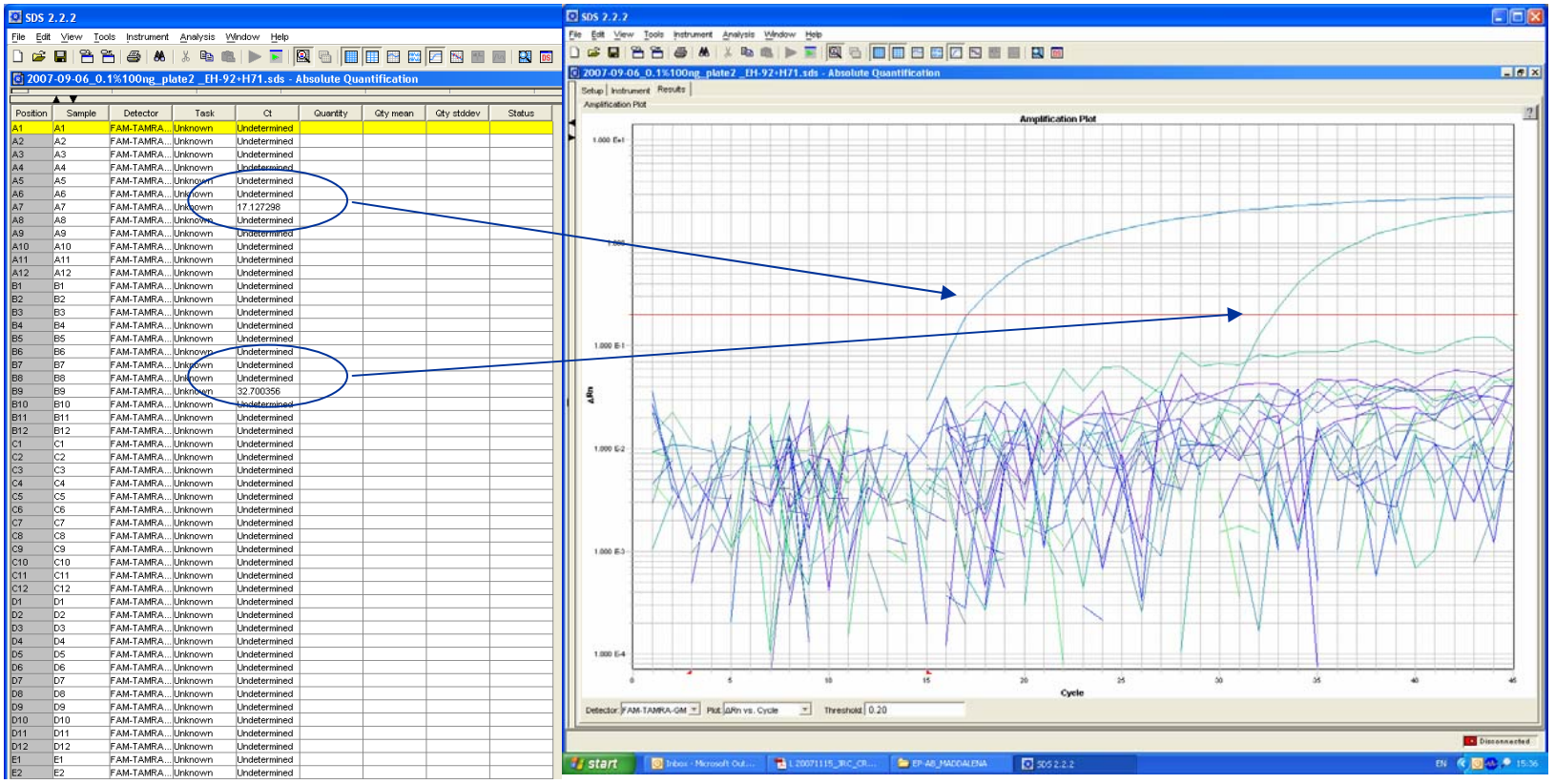


Well	Sample Name	Detector	Task	Ct
A1		GM-TAMRA	Unknown	24.05
A2		GM-TAMRA	Unknown	Undet.
A4		GM-TAMRA	Unknown	Undet.
A5		GM-TAMRA	Unknown	Undet.
A6		GM-TAMRA	Unknown	Undet.
A7		GM-TAMRA	Unknown	Undet.
A8		GM-TAMRA	Unknown	Undet.
A9		GM-TAMRA	Unknown	Undet.
A10		GM-TAMRA	Unknown	Undet.
A11		GM-TAMRA	Unknown	Undet.
A12		GM-TAMRA	Unknown	Undet.
B1		GM-TAMRA	Unknown	Undet.
B2		GM-TAMRA	Unknown	Undet.
B3		GM-TAMRA	Unknown	Undet.
B4		GM-TAMRA	Unknown	Undet.
B5		GM-TAMRA	Unknown	Undet.
B6		GM-TAMRA	Unknown	Undet.
B7		GM-TAMRA	Unknown	Undet.
B8		GM-TAMRA	Unknown	Undet.
B9		GM-TAMRA	Unknown	Undet.
B10		GM-TAMRA	Unknown	Undet.
B11		GM-TAMRA	Unknown	Undet.
B12		GM-TAMRA	Unknown	Undet.
C1		GM-TAMRA	Unknown	Undet.
C2		GM-TAMRA	Unknown	Undet.
C3		GM-TAMRA	Unknown	Undet.
C4		GM-TAMRA	Unknown	Undet.
C5		GM-TAMRA	Unknown	Undet.
C6		GM-TAMRA	Unknown	Undet.
C7		GM-TAMRA	Unknown	Undet.
C8		GM-TAMRA	Unknown	Undet.
C9		GM-TAMRA	Unknown	Undet.
C10		GM-TAMRA	Unknown	Undet.
C11		GM-TAMRA	Unknown	Undet.
C12		GM-TAMRA	Unknown	Undet.
D1		GM-TAMRA	Unknown	Undet.
D2		GM-TAMRA	Unknown	Undet.
D3		GM-TAMRA	Unknown	Undet.
D4		GM-TAMRA	Unknown	Undet.
D5		GM-TAMRA	Unknown	Undet.
D6		GM-TAMRA	Unknown	Undet.
D7		GM-TAMRA	Unknown	Undet.
D8		GM-TAMRA	Unknown	Undet.
D9		GM-TAMRA	Unknown	Undet.
D10		GM-TAMRA	Unknown	Undet.
D11		GM-TAMRA	Unknown	Undet.
D12		GM-TAMRA	Unknown	35.45



Detection of potato event EH92-527-1

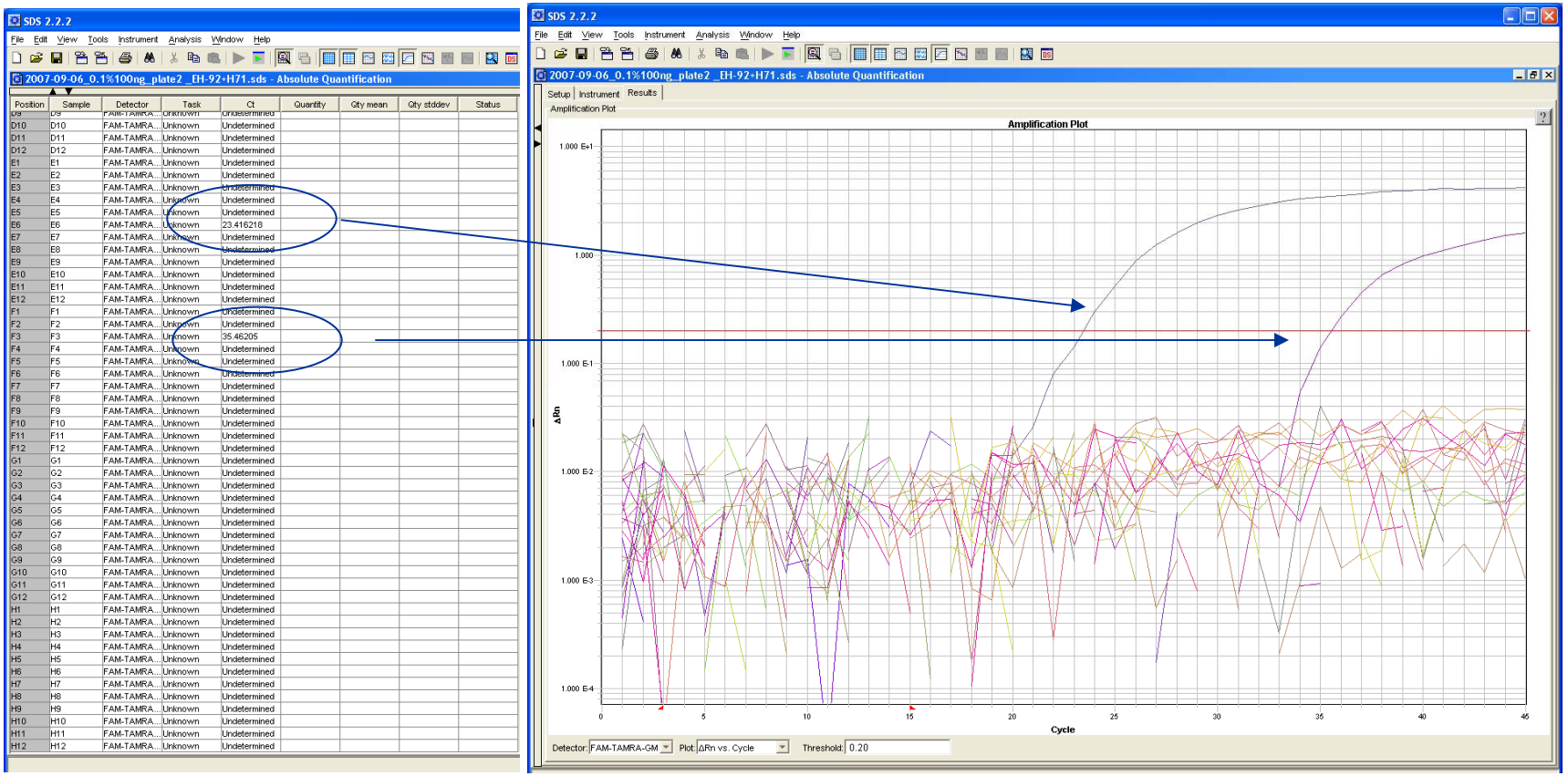
A7 = potato reference gene
B9 EH92-527-1 event-specific method



Detection of Sugar Beet event H7-1

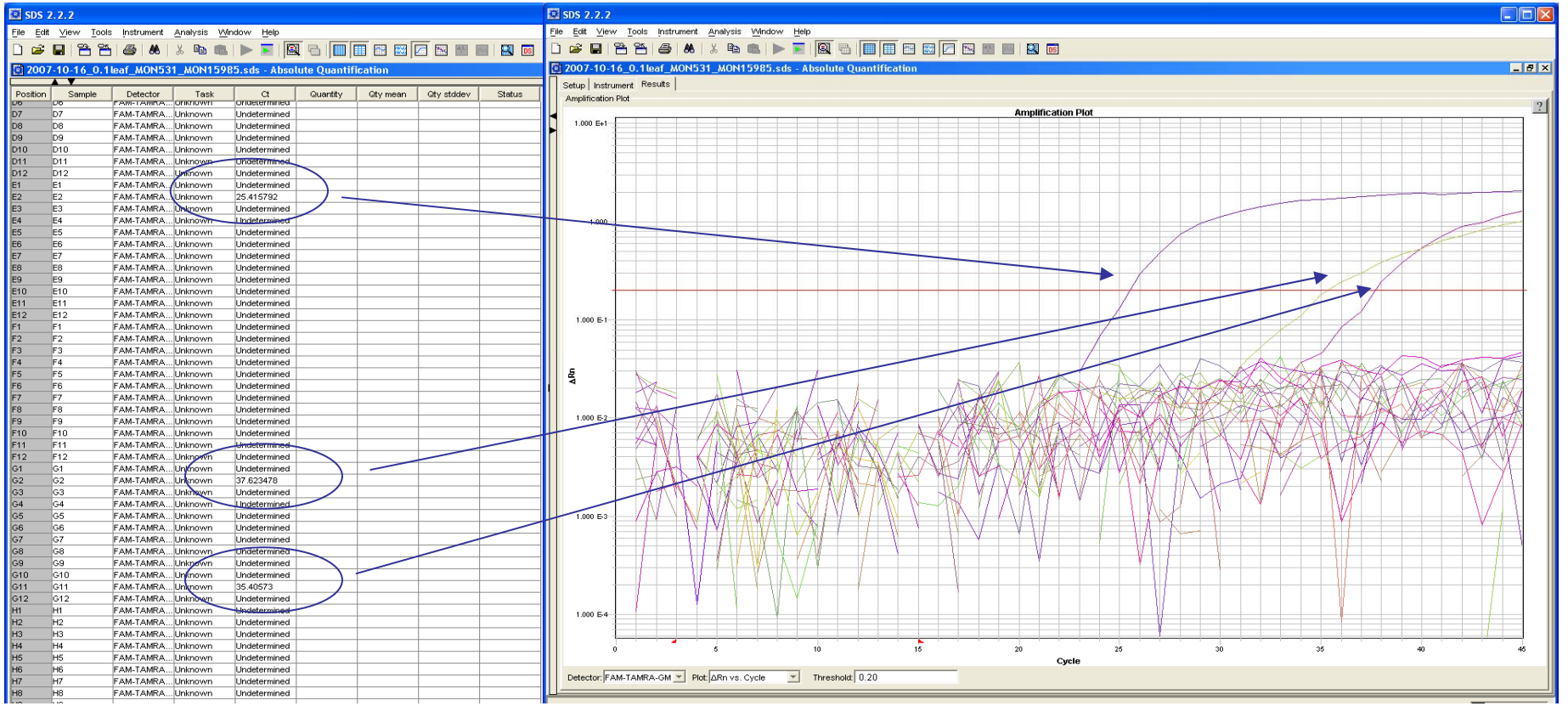
E6 = Sugar Beet reference gene

F3 = H7-1 event-specific method

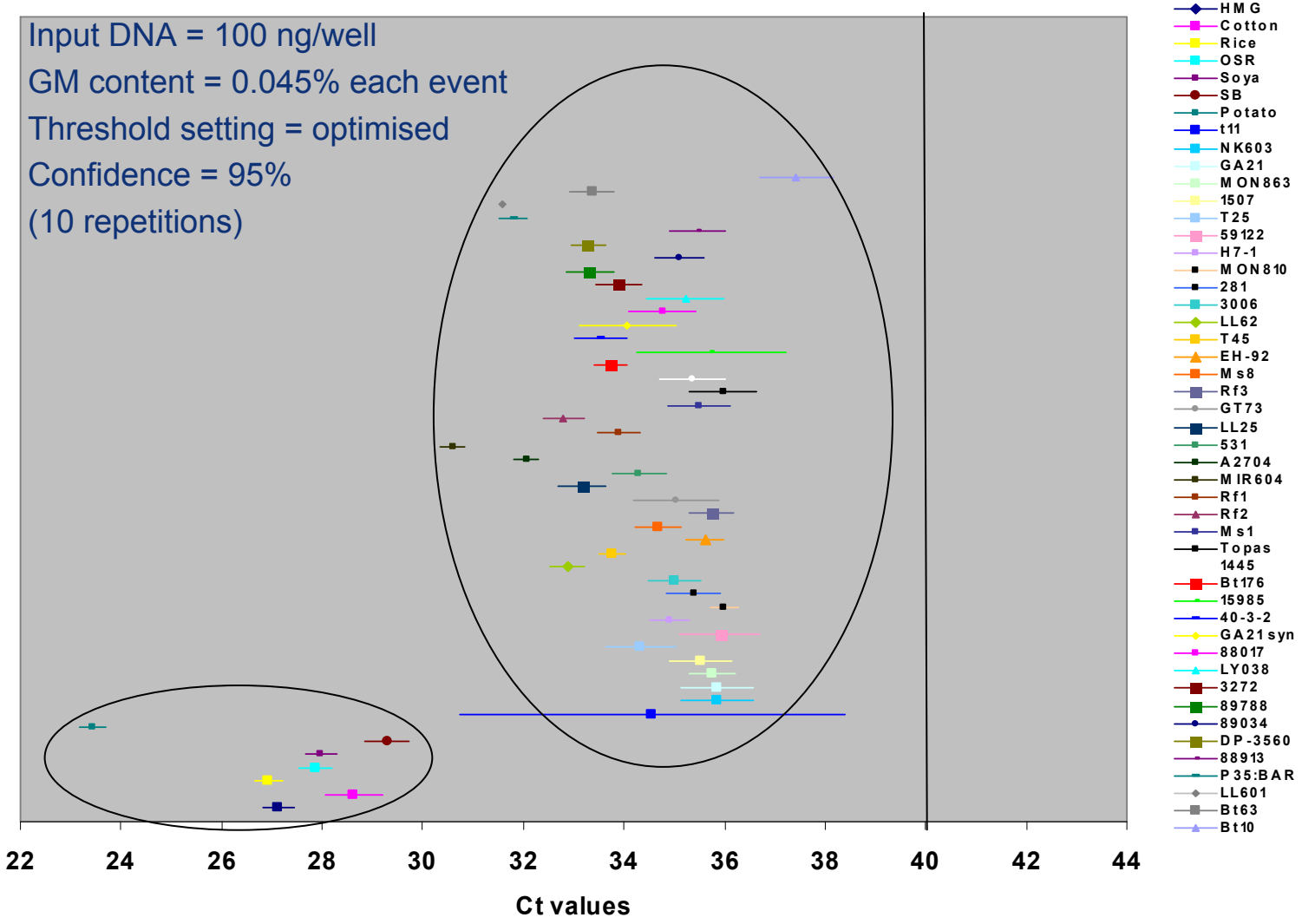


Detection of cotton event MON15985

E2 = SAH7 cotton reference gene method
G2 = MON531 event-specific method
G11 = MON15985 event-specific method



Performance



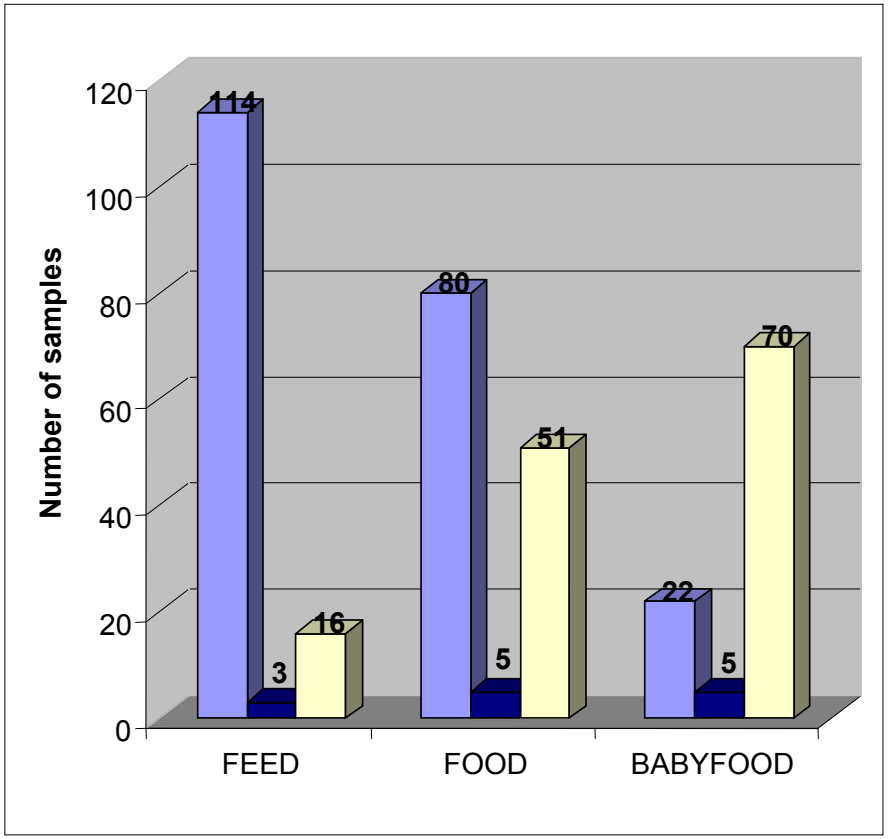
Application I: Verification of purity of control samples, provided by the applicants to the EURL-GMFF

		HMG	Cotton	Soya	SB	Bt11		NK603		GA21		MON863		1507		T25		15985		40-3-2		88017		LY038		3272		89788		89034	
1	HMG	24.28				24.34	24.57	25	24.49	25.19	24.55	24.729	24.93	24.66	24.48	25.23	25.08					25.04	24.64	25.174	24.91	24.95	25.058			25.161	25.15
2	Cotton		24.87															24.717	24.824												
3	Rice																														
4	OSR																														
5	Soya			22.97																23	22.78							23.66	23.51		
6	SB				22.94																										
7	Potato																														
8	Bt11					35.39	29.01																								
9	NK603							26.79	26.92																						
10	GA21									28.92	28.73																				
11	MON863											26.046	25.96																		
12	1507													24.96	24.5																
13	T25															25.99	25.79														
14	59122																														
15	H7-1																														
16	MON810													34.96	35																
17	281																														
18	3006																														
19	LL62																														
20	T45																														
21	EH-92																														
22	Ms8																														
23	Rf3																														
24	GT73																														
25	LL25																														
26	531																														
27	A2704																														
28	MIR604																														
29	Rf1																														
30	Rf2																														
31	Ms1																														
32	Topas																														
33	1445																														
34	Bt176																														
35	15985																	23.856	24.063												
36	40-3-2																			23.09	23.07										
37	GA21 syn									26.41	26.23																				
38	88017																					25.33	25.33								
39	LY038																							25.381	25.403						
40	3272																									24.51	24.836				
41	89788																											23.62	23.55		
42	89034																													25.887	25.89
43	DP-3560																														
44	88913																														
45	P35:BAR																														
46	LL601																														
47	Bt63																														
48	Bt10																									37.74	38.525				

Indication of contamination of control sample of TC1507 with traces of MON810

Indication of contamination of control sample of 3272 with traces of Bt10

Application II: survey on the presence of GMOs in food/feed samples in the EU – ENGL, 2008

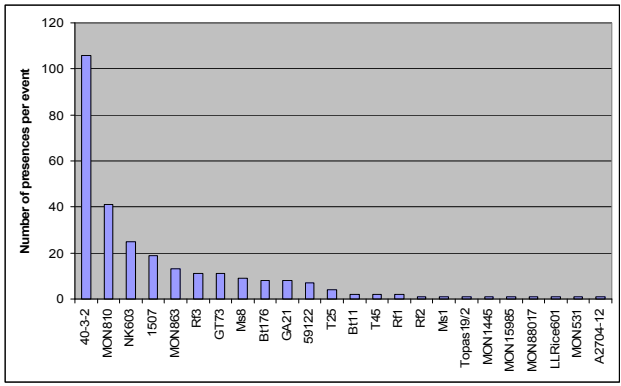


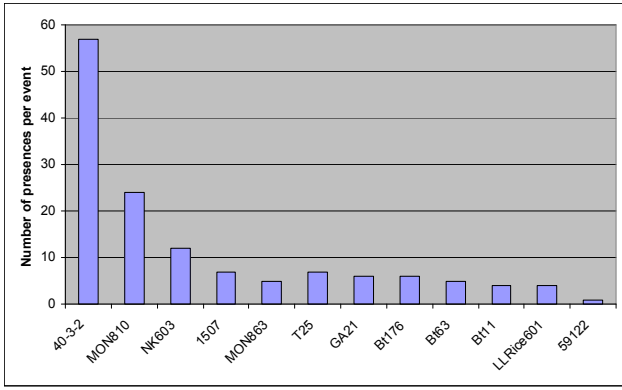
Results from a total of 366 samples randomly taken from the market in 21 EU countries

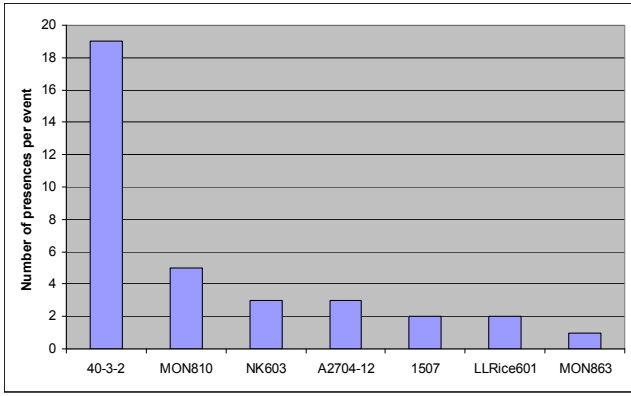
Positives*:

Feed: 88% **(117/133)**
Food: 62% **(85/136)**
Babyfood: 28% **(27/97)**

*** positives (not quantified) / total**







System transferability

- 7900HT Real-Time PCR System [Applied Biosystems]
- 7300/7500 Real-Time PCR Systems [Applied Biosystems]
- ABI PRISM® 7000/7700 SDS [Applied Biosystems]
- iCycler iQ Real-Time PCR Detection System [Bio-Rad]
- MX3000 (Stratagene)

Application III: Assessment of application on highly processed food

- Target product group - commercial maize chips (mainly tortilla-type)

- Sampling strategy – random purchase from supermarkets in different EU countries

  - 64 samples from 10 countries collected
  - None of the products were labeled for the presence of GMOs

Application III: Assessment of application on highly processed food

- Sampling
  - 1g x 2 from each bag (the analysis of each sample was performed in duplicate)

- DNA extraction and quantification
  - CTAB-based method modified for highly processed matrices
  - DNA concentration determined using PicoGreen dsDNA Assay kit

- GMO detection using ready-to-use pre-spotted plates

- Quantification
  - MON810 maize – most frequently found event in samples
  - Quantification of MON 810 maize was carried out by using the MON 810 5' event-specific/*hmg*-taxon gene method validated by the EURL-GMFF

Application III: Assessment of application on highly processed food

- All samples tested positive for **maize** (*hmg)*,
- 1/3 of samples also tested positive for **soybean** (*lec),*
- 1 sample positive for **rice** (*pld)* (traces)

25% of samples = **no GMO** presence detected,
75% of samples = **positive** for one or more **GM** events.




Application III: Assessment of application on highly processed food

- **The GM events retrieved were the following**

maize events:

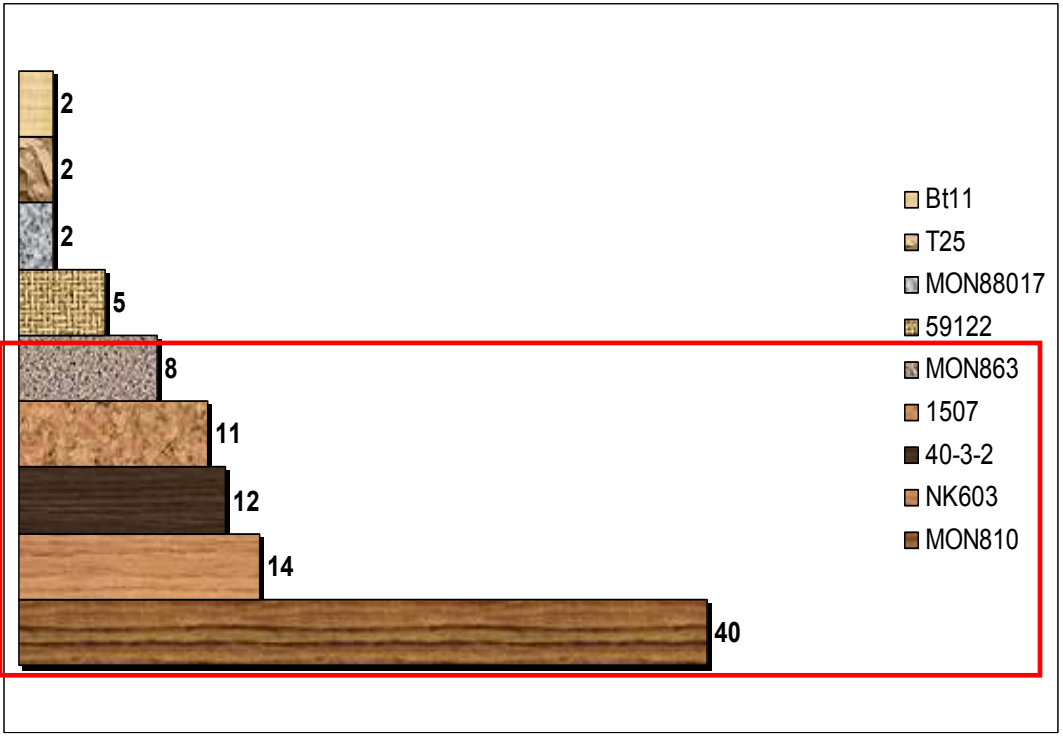
- MON 810
- NK 603
- TC1507
- MON 863
- 59122
- MON 88017
- T25
- Bt11

soybean event:

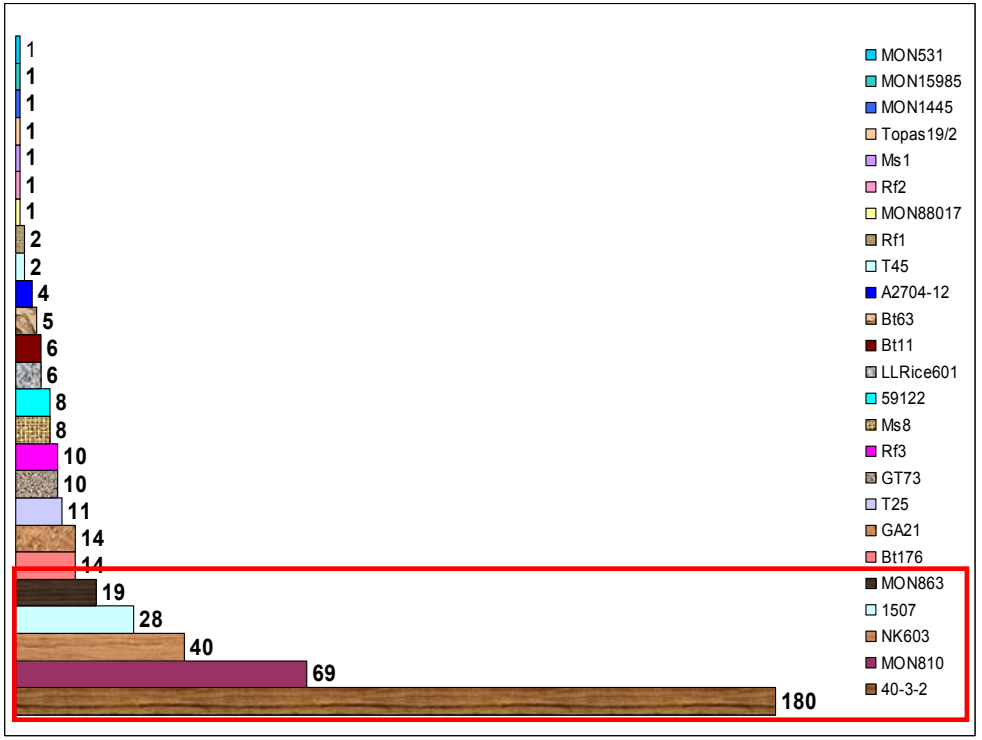
- GTS 40-3-2

At the time of analysis (April - September 2009), the maize event **MON 88017** was not authorized yet in the EU, but had received full commercial approval in other parts of the world (e.g. the USA). As such, the maize event MON 88017 could be considered as an example of low level presence of **asynchronously authorized GMO**

Application III: Assessment of application on highly processed food



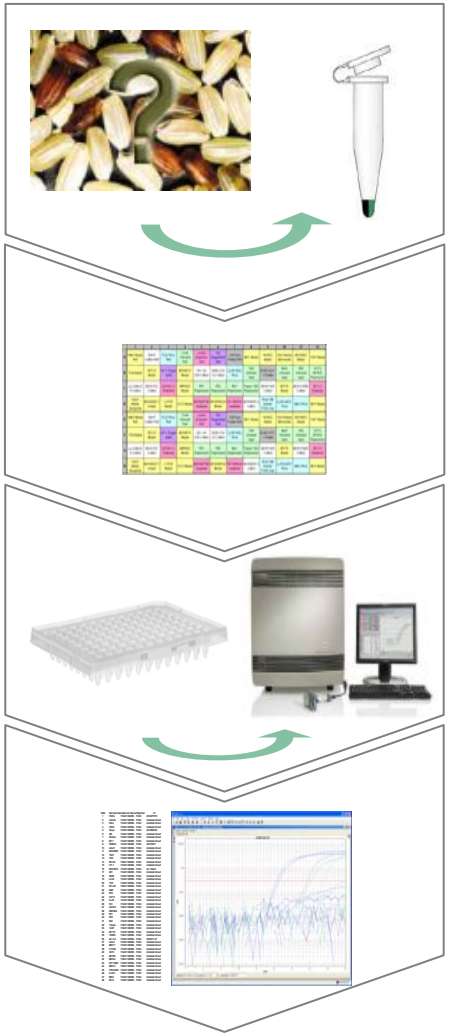
Results from robustness study of the detection of GMO using ready-to-use pre-spotted plates on highly processed food samples– IHCP MBG Unit, 2009



Results of a survey on the presence of GMOs in food and feed samples present on the European territory – ENGLnet laboratories, 2008

General approach

1. DNA extraction
2. Addition of PCR regents & plate loading
3. RTi-PCR amplification
4. Data interpretation



	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	GA21Maize 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	LLCotton25 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	GA21Maize 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	LLCotton25 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

	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG Maize Ref	Bt11 Maize	NK603 Maize	GA21 Maize	MON863 Maize	1507 Maize	T25 Maize	59122 Maize	MON810 Maize	MIR604 Maize	MON88017 Maize	LY038 Maize
B	3272 Maize	MON89034 Maize	98140 Maize	Bt176 Maize	SAH7 Cotton Ref	281-24-236 Cotton	3006-210-23 Cotton	LLCotton25 Cotton	MON 531 Cotton	MON1445 Cotton	MON15985 Cotton	MON88913 Cotton
C	Lectin Soybean Ref	A2704-12 Soybean	40-3-2 Soybean	MON89788 Soybean	DP-356043 Soybean	DP-305423 Soybean	A5547-127 Soybean	CruA Oilseed rape Ref	T45 Oilseed rape	Ms8 Oilseed rape	Rf3 Oilseed rape	GT73 Oilseed rape
D	Rf1 Oilseed rape	Rf2 Oilseed rape	Ms1 Oilseed rape	Topas 19/2 Oilseed rape	PLD Rice Ref	LLRICE62 Rice	LLRice601 Rice	Bt63 Rice	GS Sugarbeet Ref	H7-1 Sugarbeet	UGPase Potato Ref	EH92-527-1 Potato
E	HMG Maize Ref	Bt11 Maize	NK603 Maize	GA21 Maize	MON863 Maize	1507 Maize	T25 Maize	59122 Maize	MON810 Maize	MIR604 Maize	MON88017 Maize	LY038 Maize
F	3272 Maize	MON89034 Maize	98140 Maize	Bt176 Maize	SAH7 Cotton Ref	281-24-236 Cotton	3006-210-23 Cotton	LLCotton25 Cotton	MON 531 Cotton	MON1445 Cotton	MON15985 Cotton	MON88913 Cotton
G	Lectin Soybean Ref	A2704-12 Soybean	40-3-2 Soybean	MON89788 Soybean	DP-356043 Soybean	DP-305423 Soybean	A5547-127 Soybean	CruA Oilseed rape Ref	T45 Oilseed rape	Ms8 Oilseed rape	Rf3 Oilseed rape	GT73 Oilseed rape
H	Rf1 Oilseed rape	Rf2 Oilseed rape	Ms1 Oilseed rape	Topas 19/2 Oilseed rape	PLD Rice Ref	LLRICE62 Rice	LLRice601 Rice	Bt63 Rice	GS Sugarbeet Ref	H7-1 Sugarbeet	UGPase Potato Ref	EH92-527-1 Potato

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

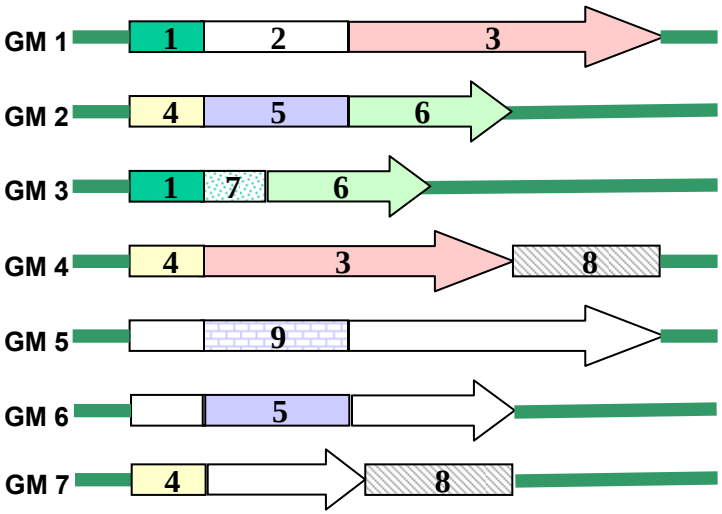
well	RTi-PCR method	well	RTi-PCR method
A1	HMG Maize Ref	B1	LY038
A2	HMG Maize Ref	B2	3272
A3	Bt11	B3	MON89034
A4	NK603	B4	98140
A5	GA21	B5	Lectin Soybean Ref
A6	MON863	B6	Lectin Soybean Ref
A7	DAS1507	B7	A2704-12
A8	T25	B8	40-3-2
A9	DAS59122	B9	MON89788
A10	MON810	B10	DP-356043
A11	MIR604	B11	DP-305423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



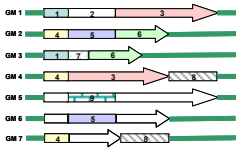
Sample	RESULTS (1 method = 1 element) METHOD n.									GM	Interpretation
	1	2	3	4	5	6	7	8	9		
Sample 1	+	+	+	-	-	-	-	-	-	+	GM 1
Sample 2	-	-	-	+	+	+	-	-	-	+	GM 2
Sample 3	+	-	-	-	-	+	+	-	-	+	GM 3
Sample 4	-	-	+	+	-	-	-	+	-	+	GM 4
Sample 5	-	-	-	-	-	-	-	-	+	+	GM 5
Sample 6	+	-	-	-	-	+	+	-	+	+	GM 3 + 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.

1.
Field or sample to be tested

2.
DSS - Level 1 Matrix approach



Sample	METHODS									GM	INTERPRETATION
	1	2	3	4	5	6	7	8	9		
Sample 1											
Sample 2											
Sample 3											
Sample 4											
Sample 5											
Sample 6											
Sample 7											
Sample 8											
Sample 9											
Sample 10											
Sample 11											

3.
DSS - Level 2 Application of analytical method(s)

4.
DSS - Level 3 Data analysis & interpretation

Sample	RT-PCR RESULTS									GM	INTERPRETATION
	1	2	3	4	5	6	7	8	9		
Sample 1	+	+	+	-	-	-	-	-	-	+	GM1
Sample 2	-	-	-	+	+	+	-	-	-	+	GM2
Sample 3	+	-	-	-	-	+	+	-	-	+	GM3
Sample 4	-	-	-	-	-	-	-	-	+	+	GM4
Sample 5	-	-	-	+	-	-	-	-	+	+	GM5
Sample 6	+	-	-	-	-	+	+	-	+	+	GM3 + GM5
Sample 7	-	-	-	-	+	-	-	-	+	+	GM6
Sample 8	-	-	-	-	+	-	-	-	+	+	GM5 + GM6
Sample 9	-	-	-	-	-	-	-	-	-	-	NO GM
Sample 10	+	+	+	-	+	-	-	-	+	+	GM1 + GM5 + GM6
Sample 11	+	+	+	-	+	-	-	+	-	+	GM1 + GM6 + ?

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

In-house production of ready-to-use pre-spotted RT-PCR plates for GMO detection

Plate design

	1	2	3	4	5	6	7	8	9	10	11	12
A	hmg	Bt11	NK603	GA21	MON863	TC1507	hmg	Bt11	NK603	GA21	MON863	TC1507
B	T25	59122	MIR604	88017	LY038	3272	T25	59122	MIR604	88017	LY038	3272
C	hmg	Bt11	NK603	GA21	MON863	TC1507	hmg	Bt11	NK603	GA21	MON863	TC1507
D	T25	59122	MIR604	88017	LY038	3272	T25	59122	MIR604	88017	LY038	3272
E	hmg	Bt11	NK603	GA21	MON863	TC1507	hmg	Bt11	NK603	GA21	MON863	TC1507
F	T25	59122	MIR604	88017	LY038	3272	T25	59122	MIR604	88017	LY038	3272
G	hmg	Bt11	NK603	GA21	MON863	TC1507	hmg	Bt11	NK603	GA21	MON863	TC1507
H	T25	59122	MIR604	88017	LY038	3272	T25	59122	MIR604	88017	LY038	3272

In-house production of ready-to-use pre-spotted RT-PCR plates for GMO detection

Calibration of the automated liquid handling system

Adaptation for DNA spotting on plates to be used for RT-PCR

Prototype pre-spotted plates production

In-house production of ready-to-use pre-spotted RT-PCR plates for GMO detection

Mean Ct values (in-house *vs.* outsourced production)

Target	In-house results		Querci et al. 2009	
	Mean Ct /6 plates	st.dev.	Mean Ct / 10 rep.	st.dev.
HMG	26,27	0,23	25,94	0,20
Bt 11	34,11	0,32	39,06*	3,06*
NK 603	37,20	0,48	35,85	0,45
GA 21	33,56	0,35	33,17	0,49
MON 863	35,27	0,31	34,78	0,38
TC 1507	35,10	0,36	34,34	0,50
T 25	33,55	0,40	33,69	0,59
59122	35,56	0,38	34,83	0,37
MIR 604	30,34	0,26	29,50	0,27
MON 88017	34,37	0,31	33,95	0,35
LY038	34,63	0,38	34,31	0,35
3272	32,81	0,28	32,60	0,33

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 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.

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!

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