

# New approaches towards GMO detection

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## 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.

## 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 losing specificity and overall performance

Dossier		Event	Maize	Oilseed rape	Probe	Reporter
1		Bt11 Maize	Bt11	T45		FAM
2		NK603 Maize	NK603	Ms8	TAMRA	FAM
3		GA21 Maize	GA21	Rf3	MACAGCAGGTGGGT	FAM
4		MON863 Maize	MON863	GT73	TCA	FAM
5		1507 Maize	1507	Rf1		FAM
6		T25 Maize	T25	Rf2		FAM
7		59122 Maize	59122	Ms1		FAM
8		H7-1 Sugar beet	H7-1 Sugar beet	Topas 19/2		FAM
9		MON810 Maize	MON810	Rice		FAM
10		281-24-236 Cotton	281-24-236 Cotton	LLRICE62	AGACACAA	FAM
11		3006-210-23 Cotton	3006-210-23 Cotton	LLRice601	CCCG	FAM
12		LLRICE62 Rice	LLRICE62	Bt63 Rice	CACCT	FAM
13		T45 oilseed rape	T45 oilseed rape	Sugar beet		FAM
14		EH92-527-1 Potato	EH92-527-1 Potato	H7-1 Sugar beet		FAM
15		Ms8 Oilseed rape	Ms8 Oilseed rape	Cotton	TTT	FAM
16		Rf3 Oilseed rape	Rf3 Oilseed rape	MON1445		FAM
17		GT73 (RT63) Rapeseed	GT73 (RT63) Rapeseed	MON88913	TAMRA	FAM
18		LLCotton25 Cotton	LLCotton25 Cotton	LLCotton25	TT	FAM
19		MON 531 Cotton	MON 531 Cotton	MON 531	C	FAM
20		A2704-12 Soybean	A2704-12 Soybean	281-24-236		FAM
21		MIR604 Maize	MIR604 Maize	3006-210-23		FAM
22		Rf1 Rapeseed	Rf1 Rapeseed	MON15985	B	FAM
23		Rf2 Rapeseed	Rf2 Rapeseed		TA	FAM
24		Ms1 Rapeseed	Ms1 Rapeseed		T	FAM
25		Topas 19/2 Rapeseed	Topas 19/2 Rapeseed		FT	FAM
26		MON1445 Cotton	MON1445 Cotton			FAM
27		Bt176 Maize	Bt176 Maize		TAMRA	FAM
28		MON15985 Cotton	MON15985 Cotton			FAM
29		40-3-2 Soybean	40-3-2 Soybean		GTAA	FAM
30		GA21 Maize	GA21 Maize			FAM
31		MON88017	MON88017		MGB	FAM
32		LY038 Maize	LY038 Maize		TAMRA	FAM
33		3272 Maize	3272 Maize			FAM
34		MON89788	MON89788		TAMRA	FAM
35		MON89034 Maize	MON89034 Maize			FAM
36		DP-356043 soybean	DP-356043 soybean		MGB	FAM
37		MON8913 cotton	MON8913 cotton		AGCAC	FAM
38		Rice GM events P35S-bar	Rice GM events P35S-bar			FAM
39		LLRice601 Rice	LLRice601 Rice		MGB	FAM
40		Bt63 Rice	Bt63 Rice		S	FAM
43		Bt10 Maize	Bt10 Maize		CTCGAG	FAM

## Plate layout:

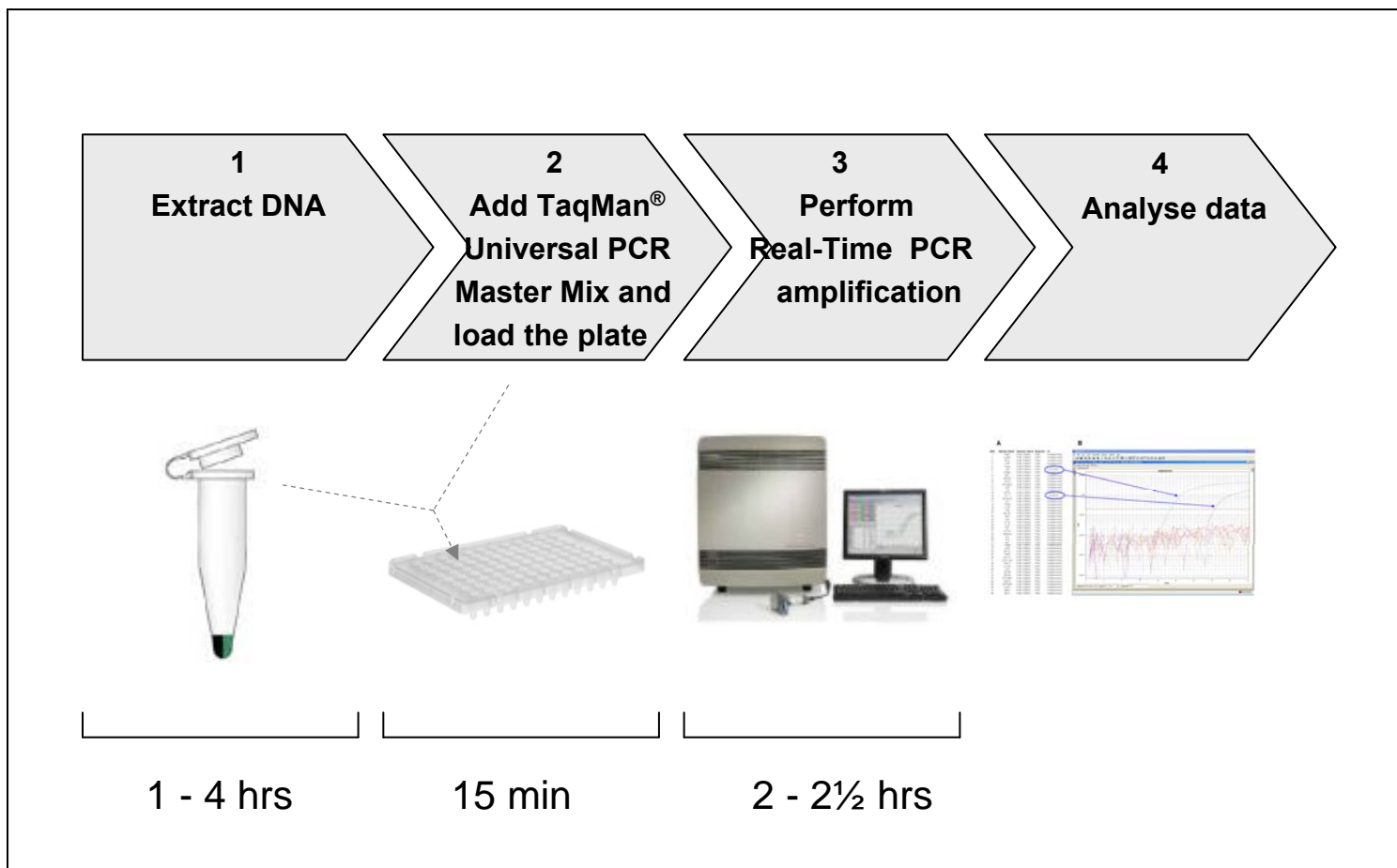
	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG Maize Ref	SAH7 Cotton Ref	PLD Rice Ref	CruA Oilseed Ref	Lectin Soybean Ref	GS Sugarbeet Ref	UGPase Potato Ref	Bt11 Maize	NK603 Maize	GA21 Maize Monsanto	MON863 Maize	1507 Maize
B	T25 Maize	59122 Maize	H7-1 Sugar beet	MON810 Maize	281-24-236 Cotton	3006-210-23 Cotton	LLRICE62 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
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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

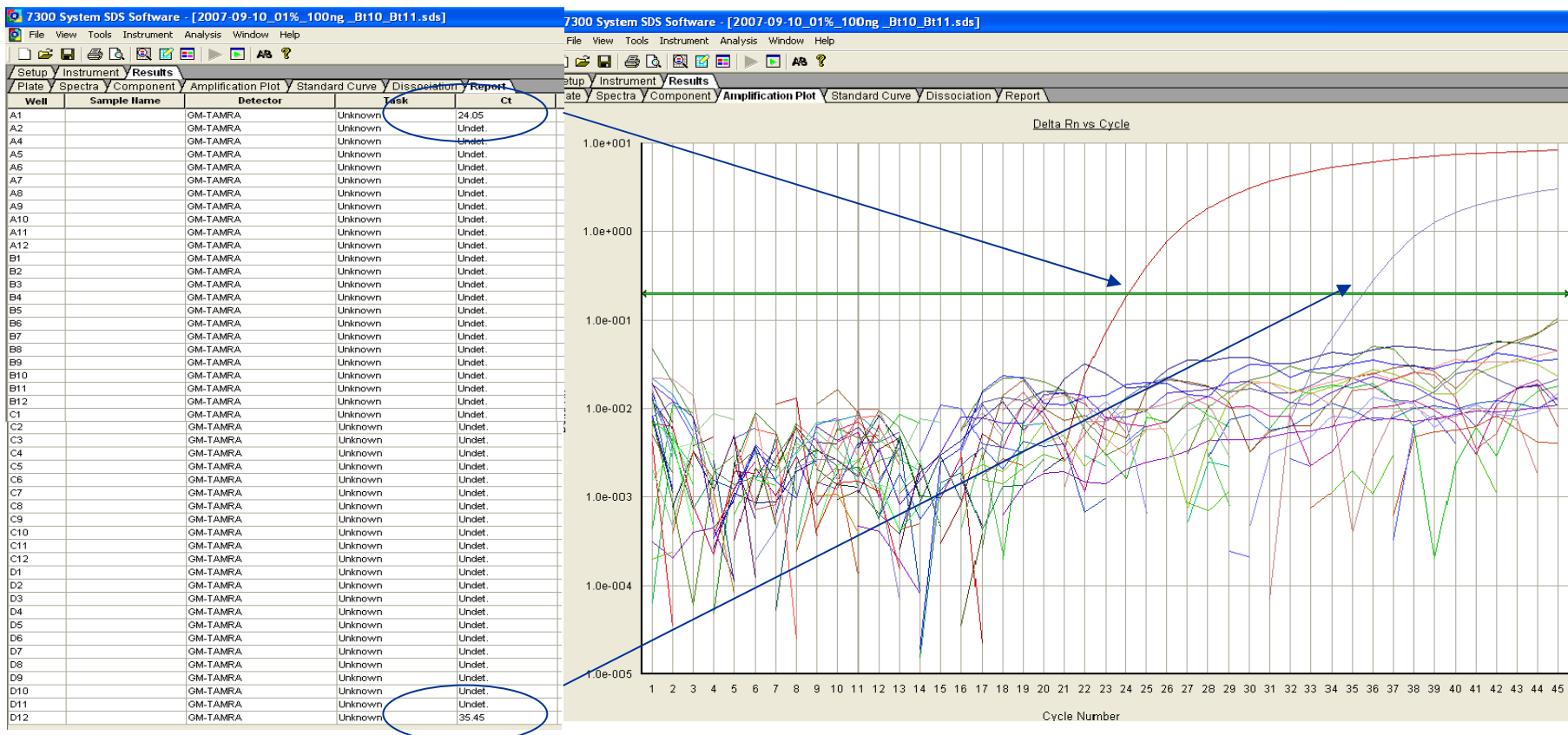


[illegible]

# Detection of maize event Bt-10

A1 = maize reference gene method

D12 = Bt-10 event-specific method

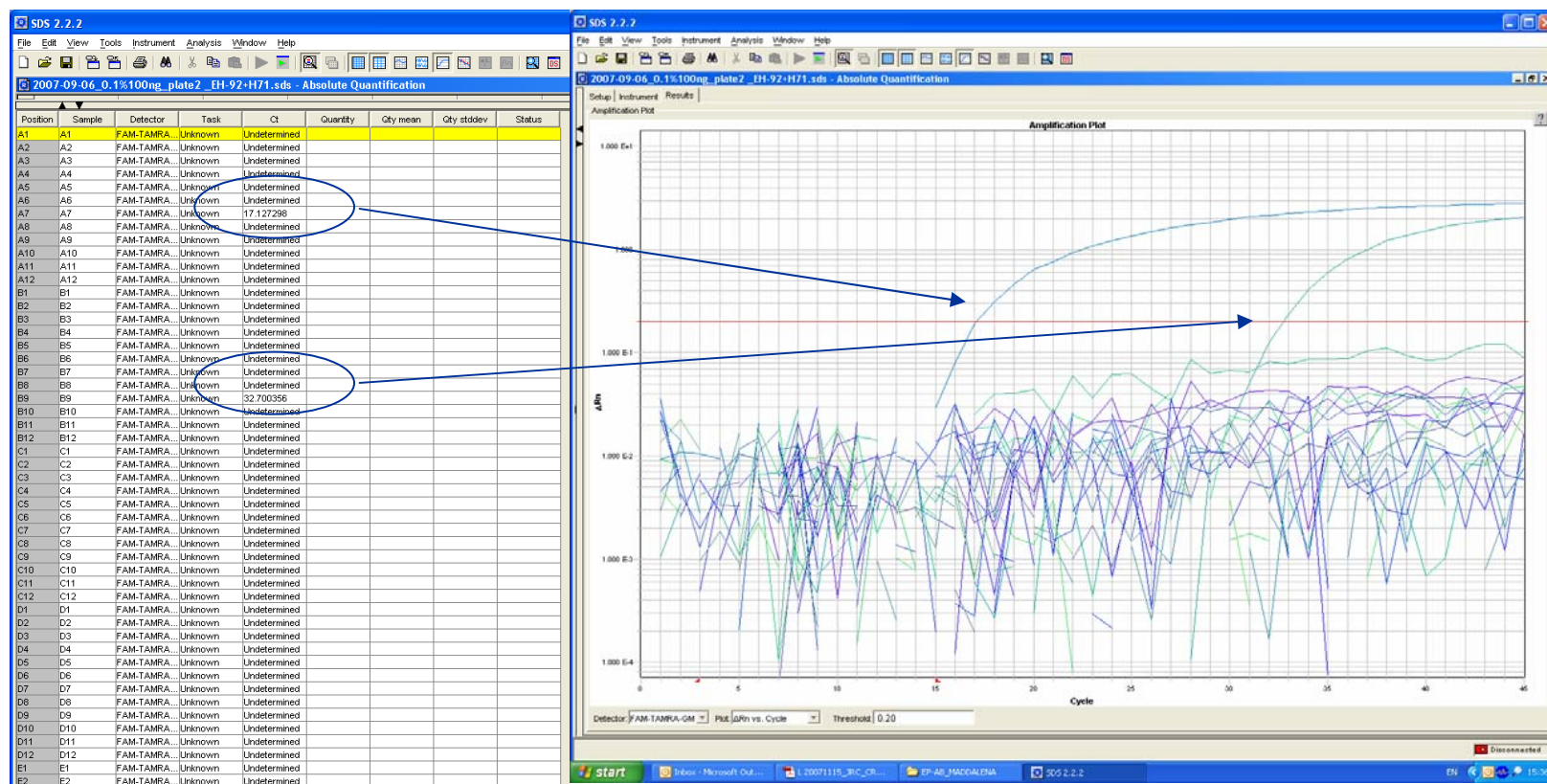




# Detection of potato event EH92-527-1

A7 = potato reference gene

B9 EH92-527-1 event-specific method

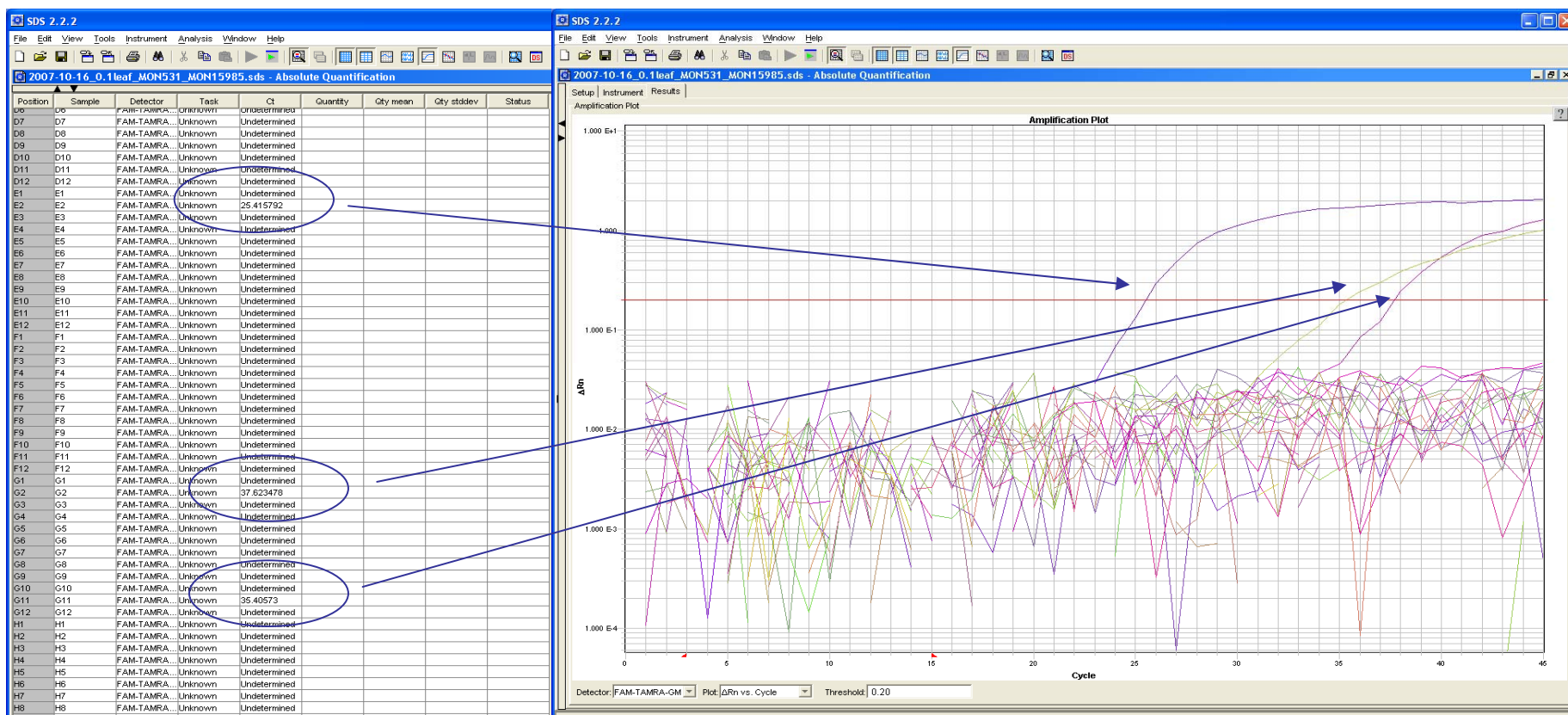


# Detection of cotton event MON15985

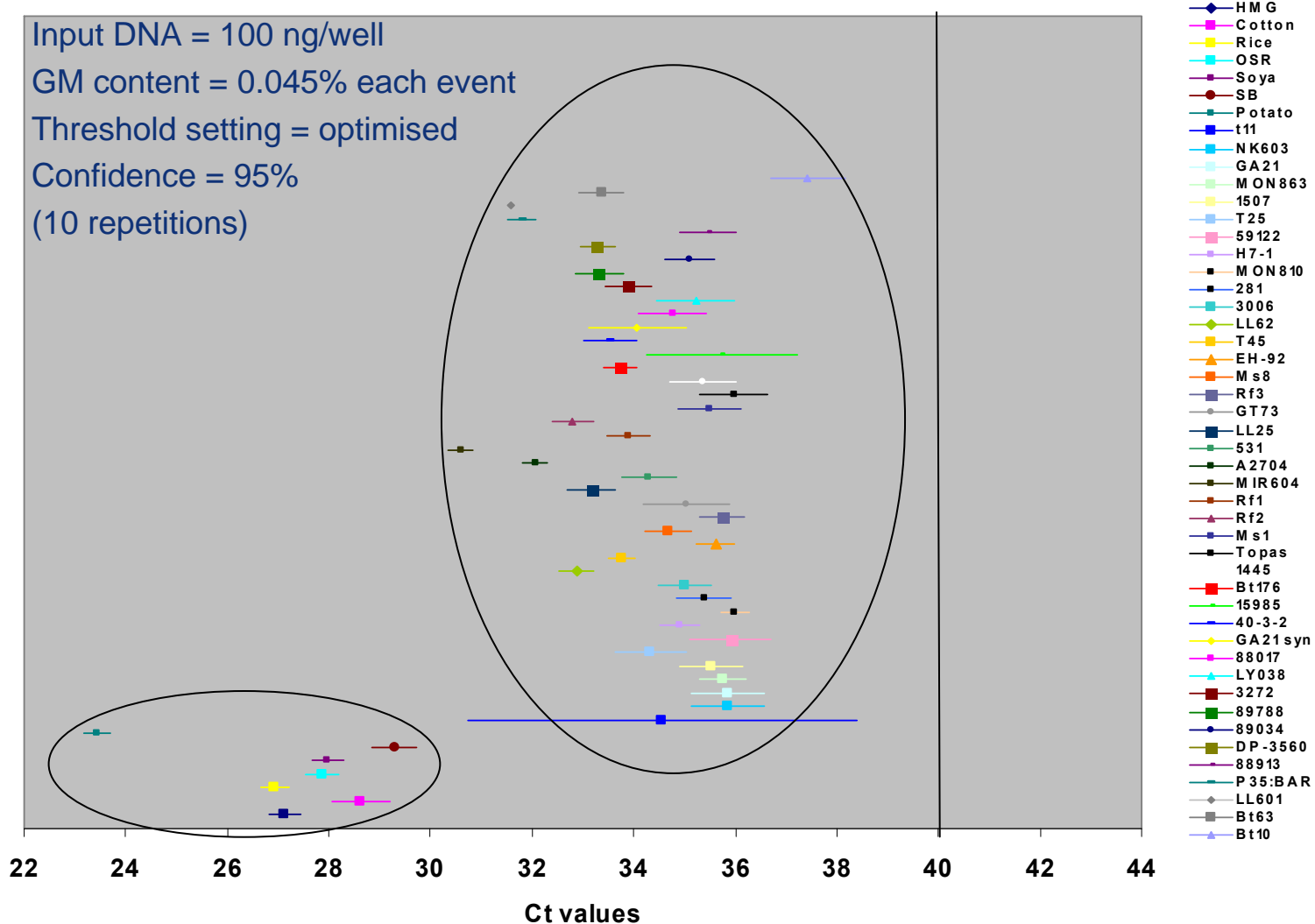
E2 = SAH7 cotton reference gene method

G2 = MON531 event-specific method

G11 = MON15985 event-specific method



# 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			
2	Cotton		24.87										24.717	24.824					
3	Rice																		
4	OSR																		
5	Soya			22.97															
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																		

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

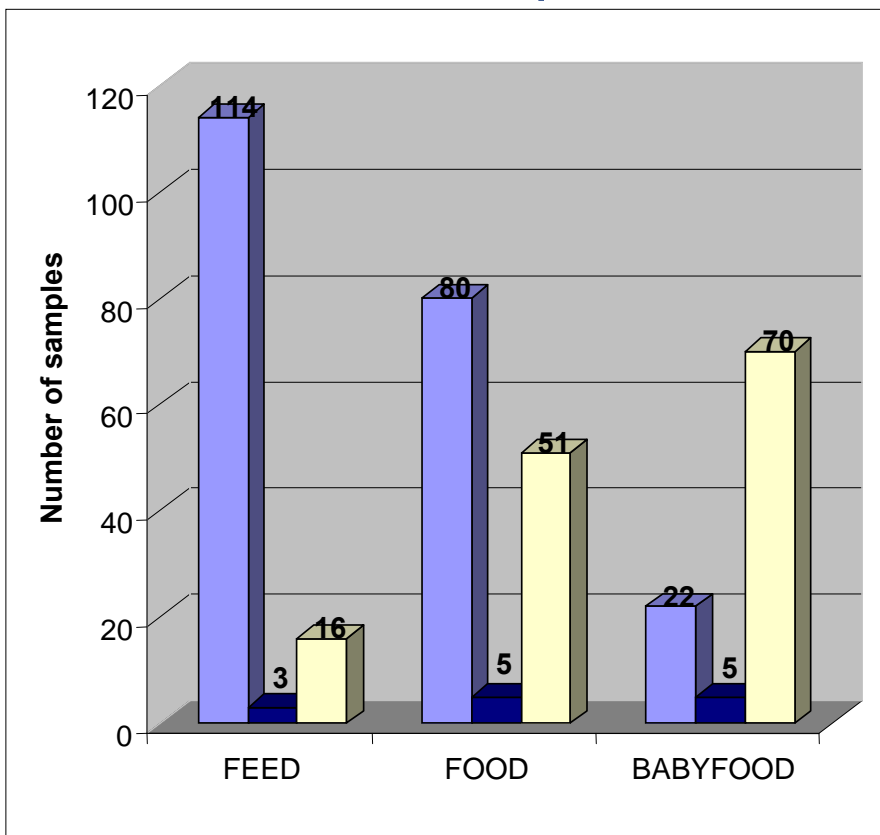
Indication of contamination of control sample of 3272 with traces of Bt10




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

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

37.74 38.625

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

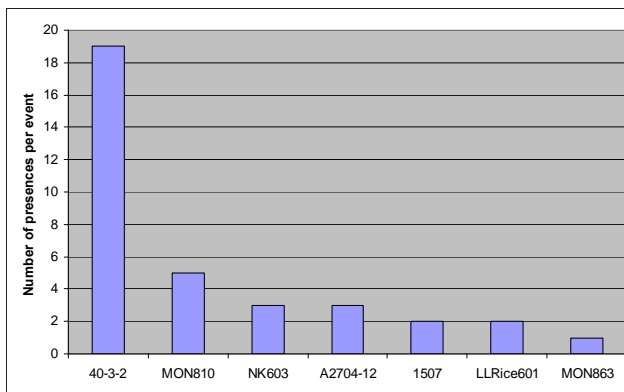
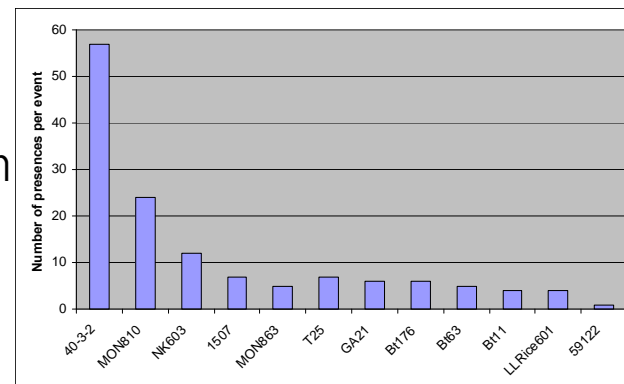
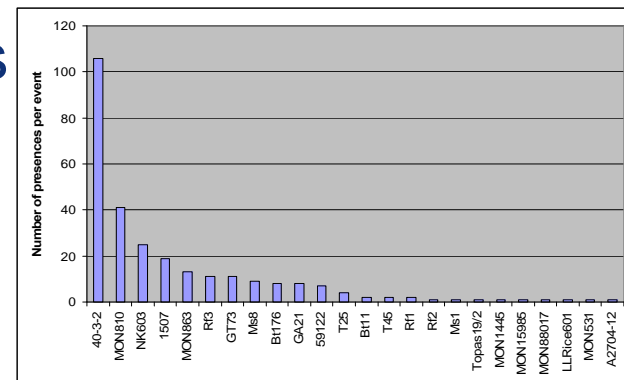


 GM-positive authorised in the EU  
 GM-positive not authorised in the EU  
 GM-free

- 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)



## 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)

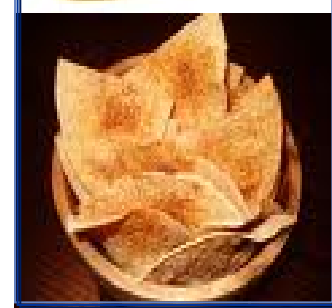
## Application III: Assessment of application on highly processed food

- 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

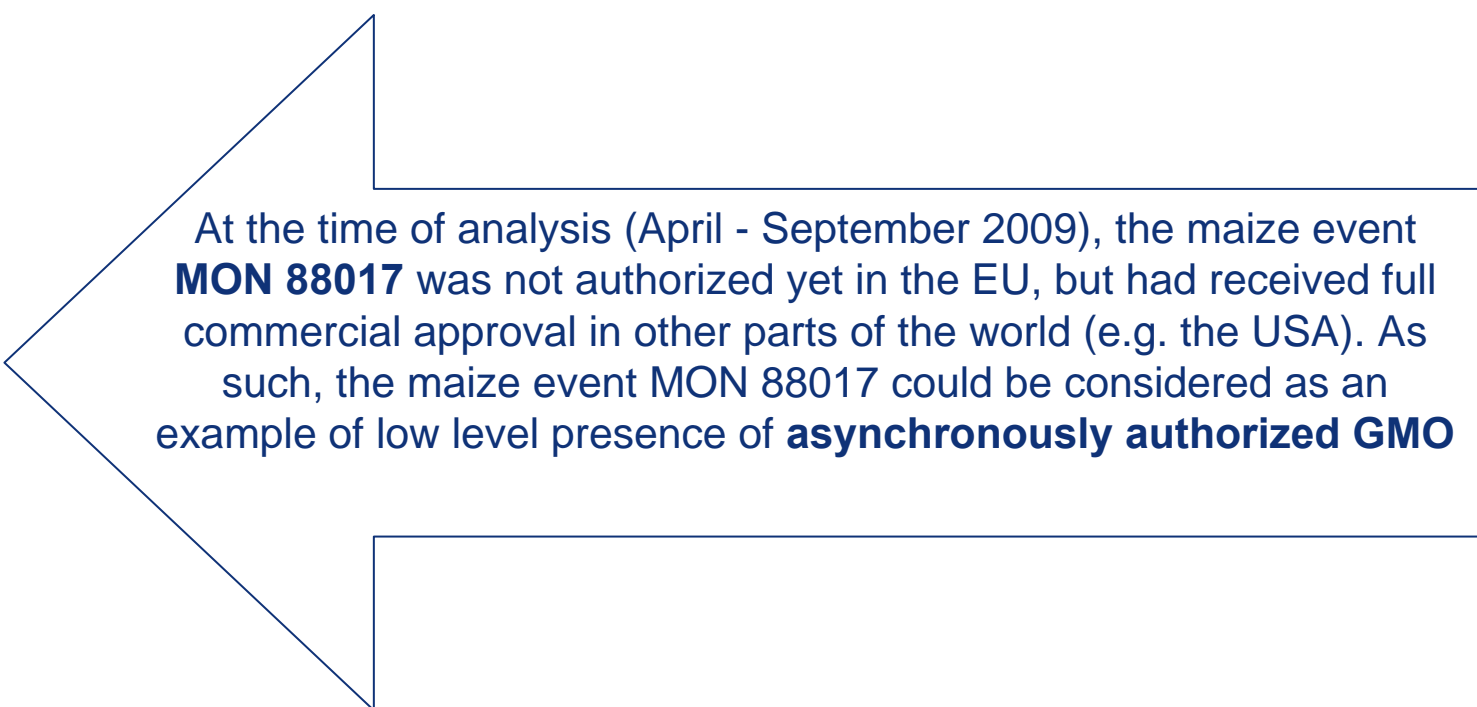
-The GM events retrieved were the following

- maize events:

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

- soybean event:

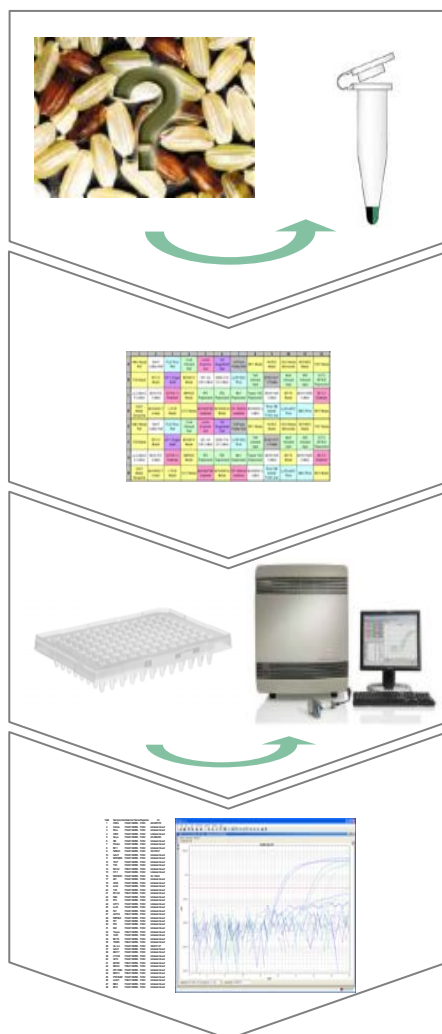
- GTS 40-3-2



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**

# General approach

1. **DNA extraction**
2. **Addition of PCR reagents & 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	GA21 Maize Monsanto	MON863 Maize	1507 Maize
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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
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# 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

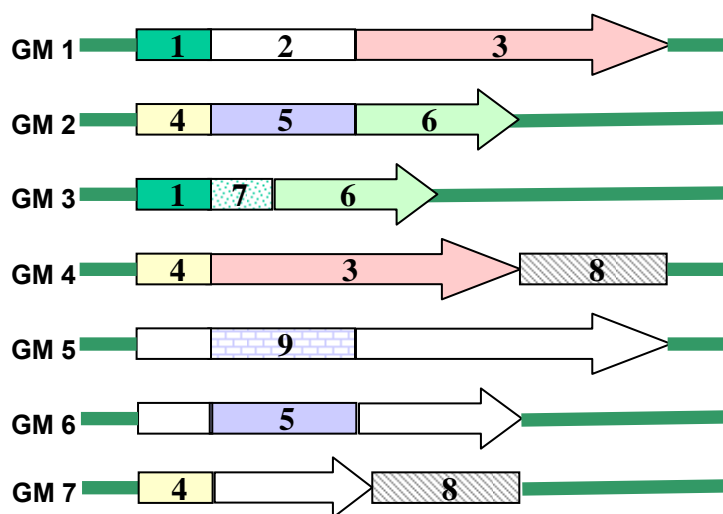
<i>well</i>	<i>RTi-PCR method</i>	<i>well</i>	<i>RTi-PCR method</i>
A1	HMG Maize Ref	B1	LY038
A2	HMG Maize Ref	B2	3272
A3	Bt11	B3	MON89034
A4	NK603	B4	98140
A5	GA21	B5	Lectin Soybean Ref
A6	MON863	B6	Lectin Soybean Ref
A7	DAS1507	B7	A2704-12
A8	T25	B8	40-3-2
A9	DAS59122	B9	MON89788
A10	MON810	B10	DP-356043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

	1	2	3	4	5	6	7	8	9	10	11	12
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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)									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 + ?

## Screening (applied to GMO):

A step in an analytical process enabling to demonstrate the presence of materials derived from Genetically Modified organisms (GMO) (ISO standards)

### 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

## What is a “matrix” (in mathematics):

A matrix is a **rectangular array of numbers** organized in rows and columns

5 x 6  
Matrix

$$\begin{pmatrix} a_{11} & a_{12} & a_{13} & a_{14} & a_{15} & a_{16} \\ a_{21} & ..... & & & & a_{26} \\ a_{31} & ..... & & & & a_{36} \\ a_{41} & ..... & & & & a_{46} \\ a_{51} & ..... & & & & a_{56} \end{pmatrix}$$

## 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**



## In the GMO Universe:

### A Table as a “practical” Matrix

	Target 1	Target 2	Target 3	Target 4	Target 5	Target 6
GMO-1	+	-	-	-	-	-
GMO-2	+	+	+	+	-	-
GMO-3	-	+	+		+	+
GMO-4	-	-	-	-	-	-
GMO-5	-	-	-	+	-	-

'+' : present

'-' : absent

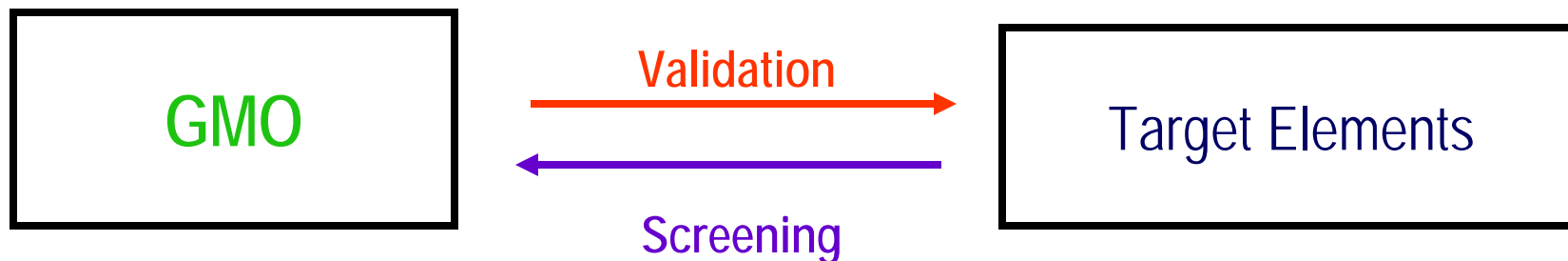
Species	Event	Productor	GRL of the published	p35S	INOS	35S	pNOS	rice actin	IGGS	npII	CP4 EPSPS	mEPSPS	PAT/pat	PAT/bar	barase	CryIAb	CryIAc	CryIF	Cry3Bb1
soybean	GTS 157	Monsanto	?	X							X								
soybean	A23112	Bayer CropScien	?	X															
soybean	A55127	Bayer CropScien	?	X		X							X						
soybean	A55127	Monsanto	?																
soybean	A55127	Monsanto	In Process																
soybean	35643-5	Pioneer Hi-Bred	In Process																
soybean	35643-1	Pioneer Hi-Bred	In Process																
maize	11	Zyngenta seeds	?	X	X														
maize	178	Zyngenta seeds	In Process	X									X			X			
maize	3N 810	Monsanto	In Process	X	X	X								X		X			
maize	A 21**	Monsanto	?		X							X							
maize	25	gEvo	?	X		X							X						
maize	3 603	Monsanto	?	X				X		X	X								
maize	MON 863	Monsanto	?	X	X					X								X	X
maize	CT507	cyogen/Pioneer	?	X		X							X						
maize	2A55122	Monsanto	?	X									X						
maize	NK 603 x MON810	Monsanto	?	X	X						X					X			
maize	T507 x NK603	Monsanto	?	X	X					X	X		X				X		
maize	MON 863 x NK 603	Monsanto	?	X	X			X		X	X								X
maize	GA 21 x MON 810	Monsanto	In Process	X	X											X			
maize	T25 x MON 810***			X	X	X							X			X			
maize	MON863 x MON810	Monsanto/Pioneer	?	X	X					X						X			X
maize	MON863 x MON810 x	Monsanto	?	X	X			X		X	X					X			X
maize	NK603	gEvo	?																
maize	MON 804	Sugenta	?	X	X														
maize	25x1501	X	?	X	X								X					X	
maize	3510		?													X			
maize	1507 x 5912	Sugenta	?																
maize	BP122x1507x NK603 Maize	Pioneer Hi-Bred	In Process																
maize	LY038		In Process																
maize	MON88017x MON810	Monsanto	In Process																
maize	MON88017x MON810	Monsanto	In Process																
maize	MON 89034	Monsanto	In Process																
maize	MON 89034 x NK603	Monsanto	In Process																
maize	MON 89034 x 89017	Monsanto	In Process																
maize	T272 maize	Sugenta Crop P	In Process																
canola	GT73	Monsanto	?								X								
canola	MS1vRF2/ MS1vRF2	Bayer CropScien	In Process		X			X		X				X	X				
canola	MS1vRF1/ MS1vRF1	Bayer CropScien	In Process		X			X		X				X	X				
canola	MS8vRF3/ MS8vRF3***	Bayer CropScier	?		X									X	X				
canola	TOPAS 19/2	Bayer CropScien	In Process	X		X		X		X			X						
canola	T45	Bayer CropScien	?	X									X						
canola	Falcon GS 4890***	Bayer CropScier	In Process	X									X						
canola	Pioneer 142	A-Evo		X									X						
canola	Canon GT106/AC			X		X							X						
canola	Quy226	Bayer CropScier	?		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	206210- 0381-24	Flow AgroScience	?	X	X														
cotton	COTTON2	Bayer CropScien	?	X															
cotton	BN 88913	Monsanto	In Process																
cotton	BN 88913	Monsanto	In Process																
cotton	BN 88913	Monsanto	In Process																
cotton	BN 88913	Monsanto	In Process																
cotton	BN 88913	Monsanto	In Process																
rice	LL 202	Aventis	?	X															
rice	LL 5601	Aventis	?	X											X				
rice	LL 5601	Aventis	?	X											X				
sugar beet	RUR1-14	KWS SAAT AG, N	?								X								

## Defining the set of targets applied for GMP screening with frequency table

Species	Event GMO	Producteur	CRL methods published?	p35S	tNOS	t35S	pNOS	rice actin	tOCS	nptII	CP4 EPSPS	mEPSPS	PAT/pat	PAT/ba	barnas	Cry1Ab	Cry1Ac	Cry1F	Cry3Bb1
soybean	GT40/3/2	Monsanto		X	X						X								
maize	Bt1	Syngenta seeds/Novartis		X	X								X			X			
maize	Bt176	Ciba-Geigy	In Process	X		X								X		X			
maize	MON 810	Monsanto	In Process	X	X											X			
maize	GA 21**, ***	Monsanto			X			X				X							
maize	25	AgrEvo		X		X							X						
maize	K 603	Monsanto		X	X			X		X	X								
maize	MON 863	Monsanto		X	X					X									X
maize	C1507	Mycogen/Pioneer		X		X							X					X	
maize	DAS59122	Monsanto		X									X						
maize	Bt10			X	X								X			X		X	
canola	GT73	Monsanto									X								
canola	MS1/RF2/MS1xRF2	ayer CropScience	In Process		X		X		X					X	X				
canola	MS1/RF1/MS1xRF1	ayer CropScience	In Process		X		X		X					X	X				
canola	MS8/RF3/MS8xRF3***	ayer CropScience			X									X	X				
canola	TOPAS 19/2	ayer CropScience	In Process	X		X	X		X	X			X						
canola	T45	ayer CropScience		X									X						
canola	Falcon GS 10/90***	ayer CropScience	In Process	X									X						
cotton	MON 1445***	Monsanto		X	X						X								
cotton	MON 531***	Monsanto															X		
cotton	MON 15985	Monsanto																	
rice	L RICE601			X										X					
rice	B 33			X				X								X			
sugar beet	RR H7-1	KWS SAAT AG. Monsanto									X								
				p35S	tNOS	t35S	pNOS	rice actin	tOCS	nptII	CP4 EPSPS	mEPSPS	pat	bar	barnase	Cry1Ab	Cry1Ac	Cry1F	Cry3Bb1
GMO event authorized in Europe (+Bt63, LL601 et Bt10)				24															
GMO with this trait				16	11	4	3	3	3	3	5	1	8	5	3	4	1	2	1
Trait "classement"				1 <sup>er</sup>	2 <sup>nd</sup>	6 <sup>ème</sup>	8 <sup>ème</sup>	8 <sup>ème</sup>	8 <sup>ème</sup>	8 <sup>ème</sup>	4 <sup>ème</sup>	14 <sup>ème</sup>	3 <sup>ème</sup>	4 <sup>ème</sup>	8 <sup>ème</sup>	6 <sup>ème</sup>	14 <sup>ème</sup>	13 <sup>ème</sup>	14 <sup>ème</sup>

The seven most represented elements in this GMO universe are p35S, tNOS, PAT/pat, PAT/bar, CP4-EPSPS, CryIAB and t35S.

## Matrix-based screening approach



Description of the relationships is represented by the “Matrix” format



## **GMO Screening approach:**

- analyte: **DNA**
- unit of measurement: **Haploid Genome Equivalent**
- detection technology: **PCR**
- reference material: genomic DNA (plasmids)
- critical parameters: ISO-standards\*

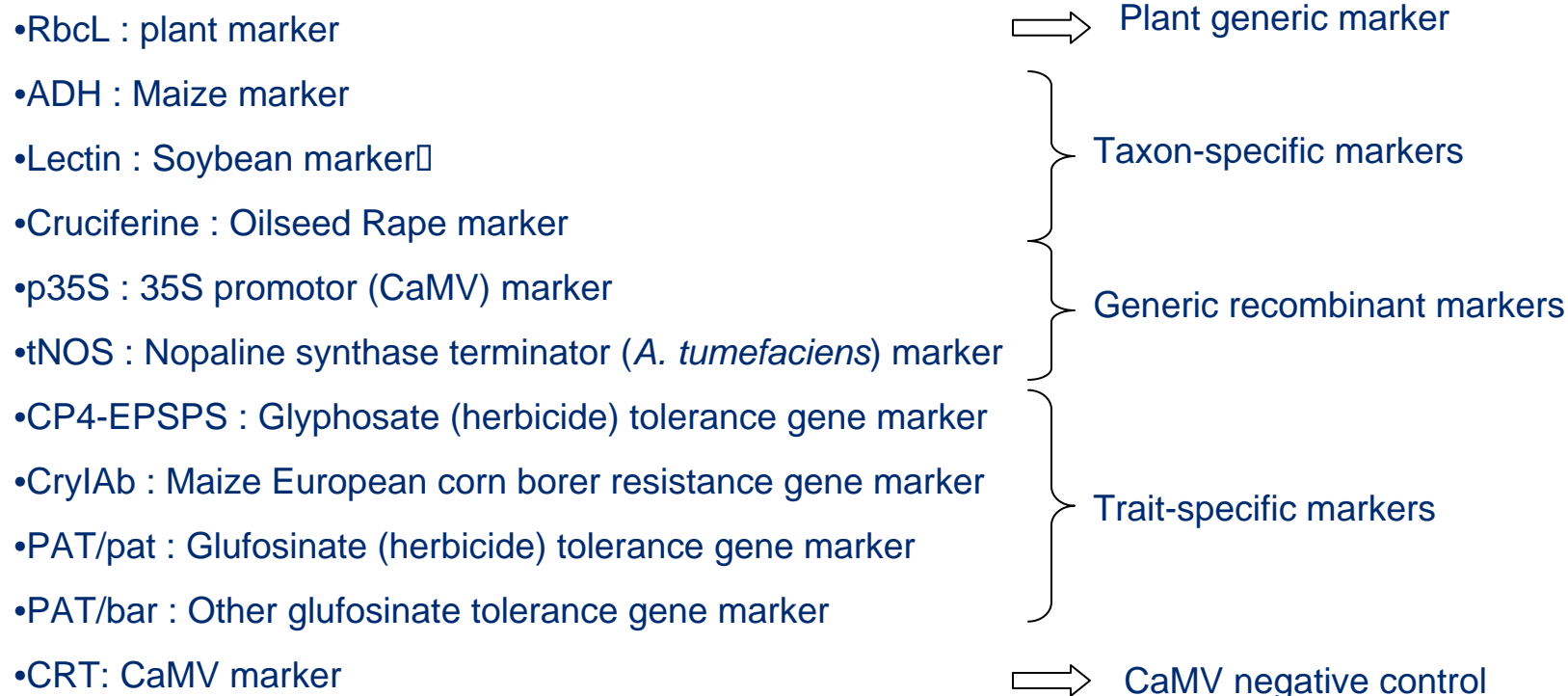
\* In the EU, also the guidelines of the European Network of GMO Laboratories (ENGL) are applied

# CoSYPS development (2005-2009)

## Strategy:

1. GMO universe definition in a matrix-format (June 2005, constant update)
2. Definition of screening elements
3. Unix Dbase of relevant recombinant DNA sequences
4. Develop uniform SYBR Q-PCR methods for screening  
(primers, PCR conditions, reference materials)
5. Common validation criteria of all SYBR Q-PCR methods (ISO 17025)
6. Development of a Mathematical Analysis tool
7. Development of integrated 96-well plate screening format and DSS

# CoSYPS for GM Soy, Maize, Rapeseed



*To date also SYBR®Green methods for rice, cotton, sugarbeet, potato, wheat and alfalfa*



## COSYPS markers in the EU GMO Universe

Featuring setup: 35S Promotor (CaMV), NOS terminator (Agrob), CP4-EPSPS, PAT/Pat, PAT/Bar and Cry1Ab

Esp_ce	Ev nement OGM	Producteur	CRL method published	p35S	tNOS	CP4 EPSPS	PAT/pat	PAT/bar	Cry1Ab
soybean	GTS 40/3/2	Monsanto	:	X	X	X			
maize	Bt 11	Syngenta seeds/Novartis	:	X	X		X		X
maize	Bt 176	Ciba-Geigy	:	X				X	X
maize	MON 810	Monsanto	:	X	X				X
maize	GA 21	Monsanto	:		X				
maize	T25	AgrEvo	:	X			X		
maize	NK 603	Monsanto	:	X	X	X			
maize	MON 863	Monsanto	:	X	X				
maize	TC1507	Mycogen/Pioneer	:	X			X		
maize	DAS59122	Monsanto	:	X			X		
maize	Bt10		:	X	X		X		X
canola	GT73	Monsanto	:			X			
canola	MS1/RF2/MS1xRF2	Bayer CropScience	:		X			X	
canola	MS1/RF1/MS1xRF1	Bayer CropScience	:		X			X	
canola	MS8/RF3/MS8xRF3	Bayer CropScience	:		X			X	
canola	TOPAS 19/2	Bayer CropScience	:	X			X		
canola	T45	Bayer CropScience	:	X			X		
canola	Falcon GS 40/90	Bayer CropScience	In Process	X			X		
cotton	MON 1445	Monsanto	:	X	X	X			
cotton	MON 531	Monsanto	:	X	X				X
rice	LLRICE601		:	X				X	
rice	Bt63		:		X				X
sugar beet	RUR H7-1	KWS SAAT AG. Monsanto	:			X			

⇒ All those GMO comprise at least one of the COSYPS markers

## Validation of SYBR®Green QPCR methods

All SYBR®Green PCR methods were validated “in house” (ISO standards).

Validation parameters:

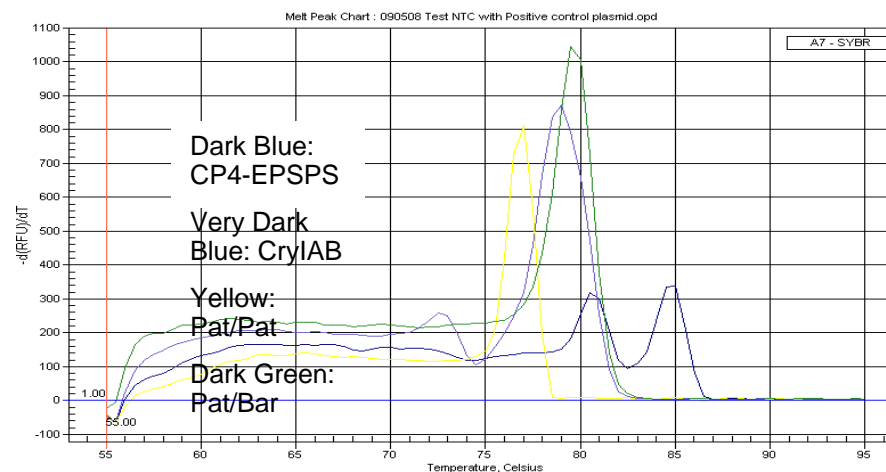
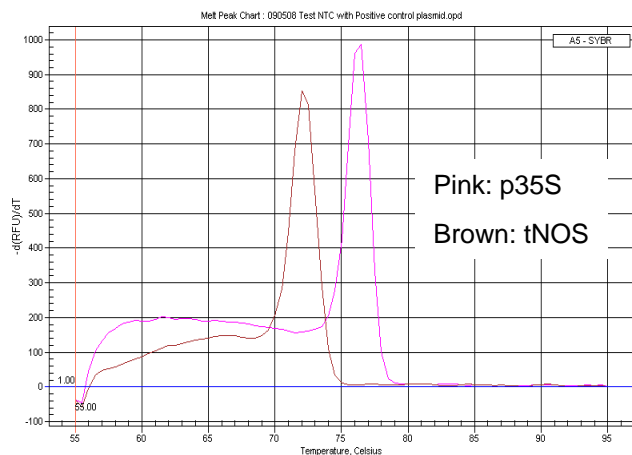
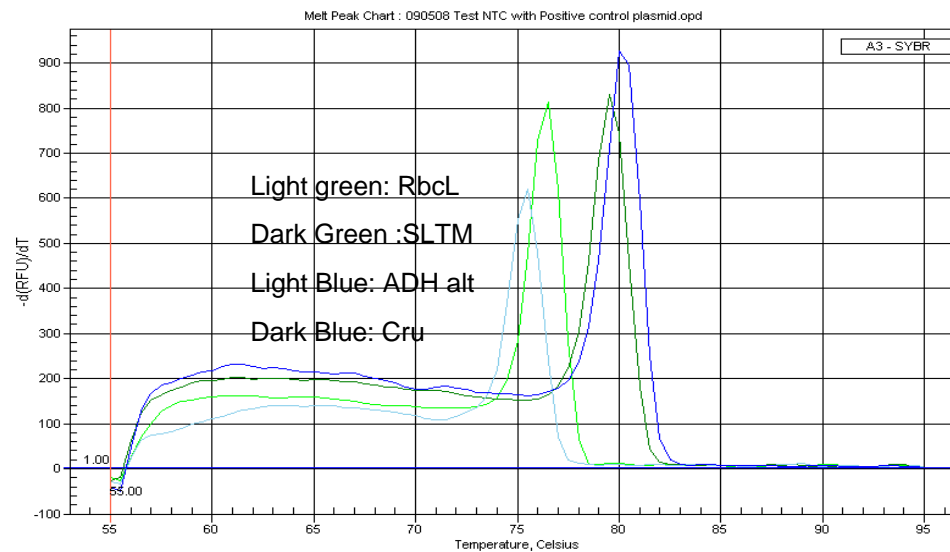
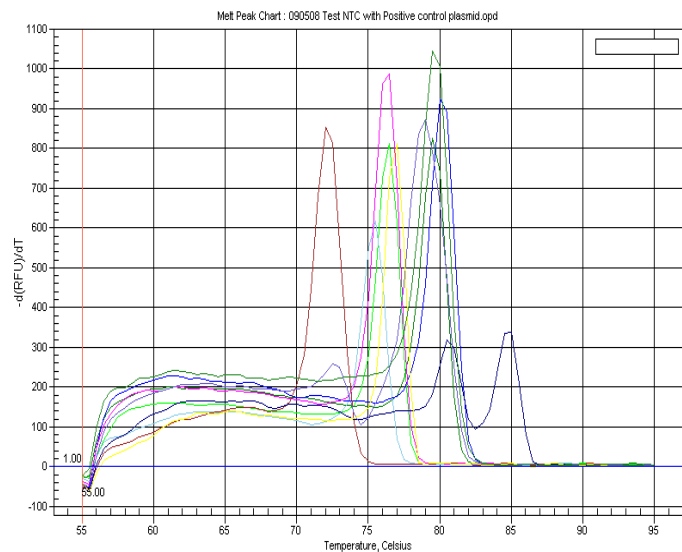
- Amplification of positive controls
- No amplification of negative controls
- One unique  $T_m$  with a clear dissociation peak
- One band on agarose gel at the expected size for positives
- Sequencing of the amplicon give the expected sequence
- No (or almost no) primer-dimer formation
- Identification of the limit of detection ( $LOD_6$ ) on dilution series with 6 repeats  
( $LOD_6$  is defined as the lowest DNA amount detectable 6/6 times)
- Robustness (by Proficiency tests)
- Repeatability (by RM testing)

## SYBRICONS: Single target plasmids (STP) as positive controls

13 STPs were constructed for this application as pENGL™-vectors

Name	LMBP number	Plasmid construction	insert	Primers used	insert Size
Sybricon009	LMBP 5459	pUC18 RbCl (OSR wt)	RbCl (OSR wt)	VPRBCP1 x VPRBCP2	95 bp
Sybricon021	LMBP 5836	pUC18 SLTM	Lectine	SLTM1 x SLTM 2	81 bp
Sybricon016	LMBP 5661	pUC18Adh short	ADH	Adh alt F x Adh alt R	84 bp
Sybricon013	LMBP 5589	pUC18 Cru 770	Cruciférine	Cru 770 F x Cru 770 R	85 bp
Sybricon017	LMBP 5662	pUC18 35S short	35S	35SN3F x 35SN3R	75 bp
Sybricon006	LMBP 5456	pUC18 tNOS	tNOS	tNOS_NN_Fwd x tNOS D REV	69 bp
Sybricon018	LMBP 5663	pUC18 CP4RRS-6	CP4RRS	CP4 Synthetic F x CP4 Synthetic R	108 bp
Sybricon019	LMBP 5664	pUC18 CP4GT73-8	CP4GT73	CP4 Synthetic F x CP4 Synthetic Rbis	108 bp
Sybricon004	LMBP 5454	pUC18 CryIAb-Bt/Cott-Bt11	CryIAb-Bt/Cott-Bt11	CryIAb_Bt.Cott_Fwd x CryIAb_Bt.Cott_Rev	73 bp
Sybricon020	LMBP 5693	pUC18 CryIAb-Bt/Cott-MON810	CryIAb-Bt/Cott-MON810	CryIAb_Bt.Cott_Fwd x CryIAb_Bt.Cott_Rev	73 bp
Sybricon005	LMBP 5455	pUC18 Pat/Pat N	Pat/Pat	Pat-Pat Fwd x Pat-Pat Rev	109 bp
Sybricon007	LMBP 5457	pUC18 Pat/Bar N	Pat/Bar	Pat-Bar Fwd x Pat-Bar Rev	69 bp
Sybricon021	LMBP xxxx	pUC18 CRT	CRT	CRT Fw x CRT Rev	87 bp

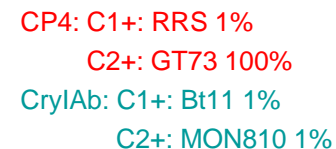
Average amplicon size is 84 bp ( $\pm$  15 bp)



## Melting curve analysis of the COSYPS SYBR®Green Plasmid mix

## Plasmid mix as positive control

## Agarose Gel analysis of the COSYPS SYBR®Green QPCR amplicons



## CoSYPS Mathematical Analysis Tool: a 'prime number' based modulator function

CoSYPS  $X_{\text{Prime}}$  numbers for the core screening elements applied in the EU-authorized plant GMO CoSYPS at IPH.

$X_{\text{Prime}}$	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

### Gödel Prime Product (GPP) :

Product of all Q-PCR results

- When an element is present: the score is set at the corresponding prime number for that element
- When an element is absent: the score is set at « 1 » for that element

## CoSYPS linear [1 x10] GM Event mathematical matrix

	Plant	Lect	ADH	p35S	Tnos	CP4	CryIaB	PAT	BAR	CRU	GPP
GTS40-3-2	3	5	1	13	17	19	1	1	1	1	62985
T25	3	1	7	13	1	1	1	29	1	1	7917
NK603	3	1	7	13	17	19	1	1	1	1	88179
GA21	3	1	7	1	17	1	1	1	1	1	357
Bt176	3	1	7	13	17	1	23	1	31	1	3309033
Bt11	3	1	7	13	1	1	23	29	1	1	182091
BT10	3	1	7	13	1	1	23	29	1	1	182091
MON810	3	1	7	13	17	1	23	1	1	1	106743
TC1507	3	1	7	13	1	1	23	29	1	1	182091
DAS59122	3	1	7	13	1	1	1	29	1	1	7917
MON863	3	1	7	13	17	1	1	1	1	1	4641
Topas19/2	3	1	1	13	17	1	1	29	1	11	211497
MS8/RF3	3	1	1	1	17	1	1	1	31	11	17391
MS1/RF1/RF2	3	1	1	1	17	1	1	1	31	11	17391
T45	3	1	1	13	1	1	1	29	1	11	12441
GT73	3	1	1	1	1	19	1	1	1	11	627
LL62 rice	3	1	1	13	1	1	1	1	31	1	1209
LL601	3	1	1	13	1	1	1	1	31	1	1209

GPP : Gödel Prime Product for an GM-event

## **COSYPS mathematical analysis algorithm**

Modulation function - Central theorem of Mathematics

$$\text{COSYPS } \text{GPP}_{\text{Sample}} / \text{GPP}_{\text{Event X}} = \text{R}$$

If  $R = 1$ , then only Event X is present,

If  $R = \text{integer number}$ , then Event X is present in addition to other events,

If  $R \neq \text{integer number}$ , then Event X is absent.



# COSYPS Decision Support System

## -Excel-based support

### - Integrates the decision values from the validation dossiers

- $T_m$ -values (identification of targets)
- LOD and  $LOQ_6$  ( $c_t$  cutoff value)
- Logical functions for “below LOD; below  $LOQ_6$ ; above  $LOQ_6$ )
- Matrix information on Presence/Absence of targets in Prime Numbers
- GPP modulation function for identification of GMO possibly present in sample
- Combinatory Matrix calculation for combined interpretation of Screening and identification results

INSTITUTE OF PUBLIC HEALTH  
Division Biosafety and Biotechnology  
Overview of all the results and matrix decision

## COSYPS MATRIX ANALYSIS AND EVENT DETERMINATION

SAMPLE ID:

Enforcement

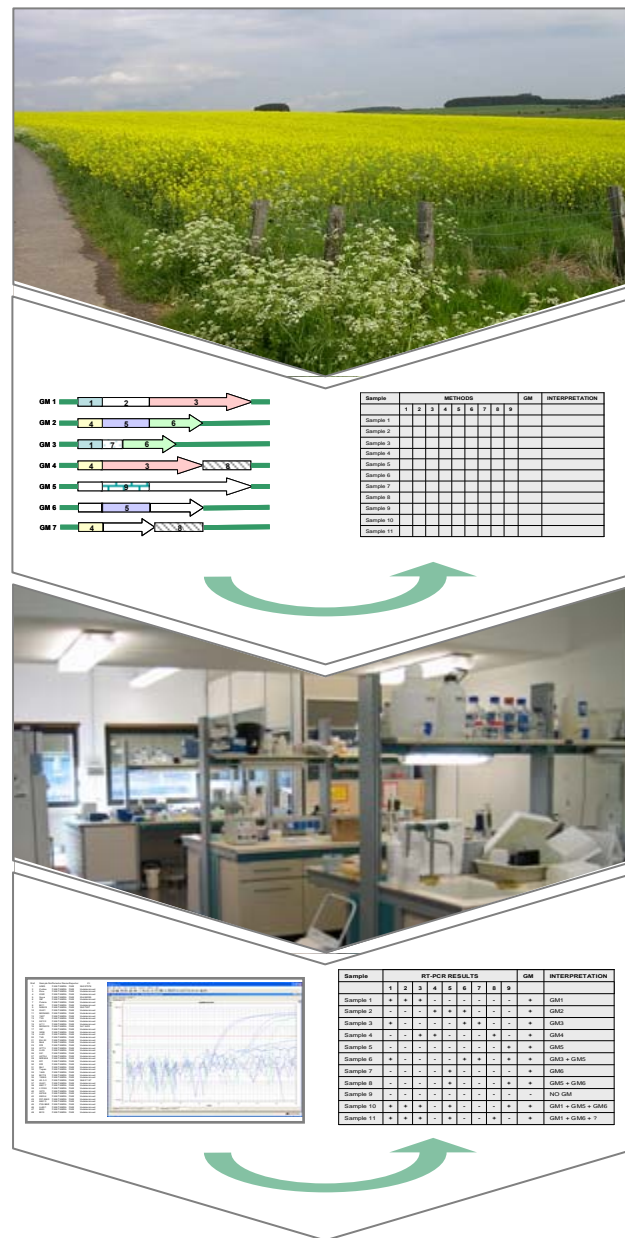
ANALYSIS										
			COSYPS RESULT							
Plant	3			1			3			
Lect	5			1			5			
ADH	7			1			7			
p35S	13			1			13			
Tnos	17			1			17			
CP4	19			1			19			
CryIAB	23			0			1			
PAT	29			0			1			
Bar	31			0			1			
CRU	11			0			1			
						Spr is	440895			
RESULT										
GTS40-3-2		Match	TRUE					1		
T25		Below LOD	0					0		
NK603		Match	TRUE					1		
GA21		Match	TRUE					1		
BT176		Below LOD	0					0		
Bt11		Below LOD	0					0		
Bt10		Below LOD	0					0		
MON810		Below LOD	0					0		
TC1507		Below LOD	0					0		
DAS59122		Below LOD	0					0		
MON863		Match	TRUE					1		
Topas 19/2		Below LOD	0					0		
MS8/RF3		Below LOD	0					0		
MS1/RF1/RF2		Below LOD	0					0		
T45		Below LOD	0					0		
GT73		Below LOD	0					0		
LL62 rice		Below LOD	0					0		
LL601		Below LOD	0					0		

Event	CaMV P35S A	CaMV P35S B	CaMV P35S C	CaMV P35S D	CaMV P35S E	FMV P35S	T-NOS A	T-NOS B	T-NOS C	T-NOS D	npII A	npII B	bar	CTP2-CP4EPSPS	Bt11	Bt176	T25	Bt63	GTS 40-3-2	Nema 282F	Pat/Bar	Pat/Pat	Cry/Ab	EPSPS-CP4-2	EPSPS-CP4-1	T-NOS E	P35Short
Prime Number	2	3	5	7	11	13	17	19	23	29	31	37	41	43	47	51	53	57	59	61	67	71	73	79	87	91	97
GT73 (RT73)	1	1	1	1	1	13	1	1	1	1	1	1	1	43	1	1	1	1	1	1	1	1	1	79	1	1	1
MS1	1	1	1	1	1	1	17	19	23	29	31	37	41	1	1	1	1	1	1	1	67	1	1	1	1	91	1
MS8	1	1	1	1	1	1	17	19	23	29	1	1	41	1	1	1	1	1	1	1	67	1	1	1	1	91	1
RF1	1	1	1	1	1	1	17	19	23	29	31	37	41	1	1	1	1	1	1	1	67	1	1	1	1	91	1
RF2	1	1	1	1	1	1	17	19	23	29	31	37	41	1	1	1	1	1	1	1	67	1	1	1	1	91	1
RF3	1	1	1	1	1	1	17	19	23	29	1	1	41	1	1	1	1	1	1	1	67	1	1	1	1	91	1
T45 (= HCN28 = ACS-BN008-2) (# Topas 19/2)	2	3	5	7	11	1	1	1	1	1	1	1	1	1	1	1	53	1	1	1	1	71	1	1	1	1	97
Topas 19/2 (HCN92)	2	3	5	7	11	1	1	1	1	1	31	37	1	1	1	1	53	1	1	1	1	71	1	1	1	1	97
59122	2	3	5	7	11	1	1	1	1	1	1	1	1	1	1	1	53	1	1	1	1	71	1	1	1	1	97
87460	1	3	5		11	1	17	19	23	29	31	37	1	1	1	1	1	1	1	1	1	1	1	1	1	91	97
88017	2	3	5	7	11	1	17	19	23	29	1	1	1	43	1	1	1	1	1	1	1	1	1	1	87	91	97
89034	2	3	5	7	11	13	17	19	23	29	1	1	1	1	1	1	1	1	1	1	1	1	73	1	1	91	97
176 (Bt 176) (b)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	51	1	1	1	1	1	1	1	1	1	1	1
Bt10	2	3	5	7	11	1	17	19	23	29	1	1	1	1	47	1	53	1	1	1	1	71	73	1	1	91	97
Bt11	2	3	5	7	11	1	17	19	23	29	1	1	1	1	47	1	53	1	1	1	1	71	73	1	1	91	97
Event 32	2	3	5	7	11	1	1	1	1	1	1	1	1	1	1	1	53	1	1	1	1	71	1	1	1	1	97
Event 3272	1	1	1	1	1	1	17	19	23	29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	91	1
Event 98140	1	1	1	1	11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GA21	1	1	1	1	1	1	17	19	23	29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	91	1
LY038	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MIR162	1	1	1	1	1	1	17	19	23	29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	91	1
MIR604	1	1	1	1	1	1	17	19	23	29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	91	1
MON810	2	3	5	7	11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	73	1	1	1	97	
Mon863	1	3	5	1	11	1	17	19	23	29	31	37	1	1	1	1	1	1	1	1	1	1	1	1	1	91	97
NK603	2	3	5	7	11	1	17	19	23	29	1	1	1	43	1	1	1	1	1	1	1	1	1	1	87	91	97
T25	2	3	5	7	11	1	1	1	1	1	1	1	1	1	1	1	53	1	1	1	1	71	1	1	1	97	
TC1507	2	3	5	7	11	1	1	1	1	1	1	1	1	1	1	1	53	1	1	1	1	71	1	1	1	97	
88913	1	1	1	1	11	13	1	1	1	1	1	1	1	43	1	1	1	1	1	1	1	1	1	79	1	1	1
1445, 1698	1	3	5	1	11	13	17	19	23	29	31	37	1	43	1	1	1	1	1	1	1	1	1	79	1	91	97
281-24-236	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	71	1	1	1	1	1	1
3006-210-23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	71	1	1	1	1	1	1
GHB614	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LLcotton25	2	3	5	7	11	1	17	19	23	29	1	1	41	1	1	1	1	1	1	1	67	1	1	1	1	91	1
MON 531	2	3	5	7	11	1	17	19	23	29	31	37	1	1	1	1	1	1	1	1	1	73	1	1	91	97	
EH92-527-1	1	1	1	1	1	1	17	19	23	29	31	37	1	1	1	1	1	1	1	1	1	1	1	1	1	91	1
Bt63 (Shanyou, JinYou)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	57	1	1	1	73	1	1	1	1	1
LLRICE601	1	1	1	1	1	1	1	1	1	1	1	1	41	1	1	1	1	1	1	1	67	1	1	1	1	1	97
LLRICE62	2	3	5	7	11	1	1	1	1	1	1	1	41	1	1	1	1	1	1	1	67	1	1	1	1	1	97
305423	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
356043	1	1	1	1	11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
A2704-12, A2704-21, A5547-35	2	3	5	7	11	1	1	1	1	1	1	1	1	1	1	1	53	1	1	1	1	71	1	1	1	1	97
A5547-127	2	3	5	7	11	1	1	1	1	1	1	1	1	1	1	1	53	1	1	1	1	71	1	1	1	1	97
GTS40-3-2	2	3	5	7	11	1	17	19	23	29	1	1	1	1	1	1	1	59	1	1	1	1	1	1	87	91	97
MON89788	1	1	1	1	1	13	1	1	1	1	1	1	1	43	1	1	1	1	1	1	1	1	79	1	1	1	1
BPS-CV127-9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H7-1, RUR H7	1	1	1	1	1	13	1	1	1	1	1	1	1	43	1	1	1	1	1	1	1	1	79	1	1	1	1
MON15985	2	3	5	7	11	1	17	19	23	29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	91	97
MON87705	1	1	1	1	1	13	1	1	1	1	1	1	1	43	1	1	1	1	1	1	1	1	79	1	1	1	1
FG72	1	1	1	1	1	1	17	19	23	29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	91	1
MON87701	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	73	1	1	1	1	1
AM04-1020	1	1	1	1	1	1	1	17	19	23	29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	91	1
Frequency table	19	22	22	19	25	7	24	24	24	24	9	9	8	8	2	1	10	1	1	0	8	12	7	6	3	24	22

7,18316E+39  
44161  
6,1772E+13  
53855294857  
6,1772E+13  
6,1772E+13  
53855294857  
843175410  
9,67122E+11  
843175410  
3,59906E+14  
1,64339E+16  
4,16888E+15  
51  
5,67164E+19  
5,67164E+19  
843175410  
19605131  
11  
19605131  
1  
19605131  
19605131  
16357110  
3,59906E+14  
1,64339E+16  
843175410  
843175410  
485771  
1,58938E+19  
71  
71  
1  
1,24406E+14  
3,67824E+17  
22487085257  
4161  
266459  
615520290  
1  
11  
843175410  
843175410  
2,25489E+16  
44161  
1  
44161  
4,39292E+12  
44161  
19605131  
73  
19605131

- 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.

**4.**  
**DSS - Level 3**  
**Data analysis**  
**&**  
**interpretation**



## **Towards Harmonization in GMO analysis:**

- Uniform GMO-Universe description (matrix)
- Equivalent validated GMO detection methods
- Effective, affordable GMO screening/ID platform

## 2011: the ‘GLOBAL Matrix’ initiative

- Extension of the scope of the EU-GMO matrix to Asia-Pacific, Africa and Latin America
- Compile information on globally commercialised GMO
- Genetic element based GMO description as a matrix table
- Control materials (seeds, tissue, powders, DNA...)
- FREE collaboration supported by a ‘Memorandum of Understanding’

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**Thank you!**

**Gracias!**

