

# Joint Research Centre (JRC)



*Matrix based approaches, decision-support systems  
and reference methods*

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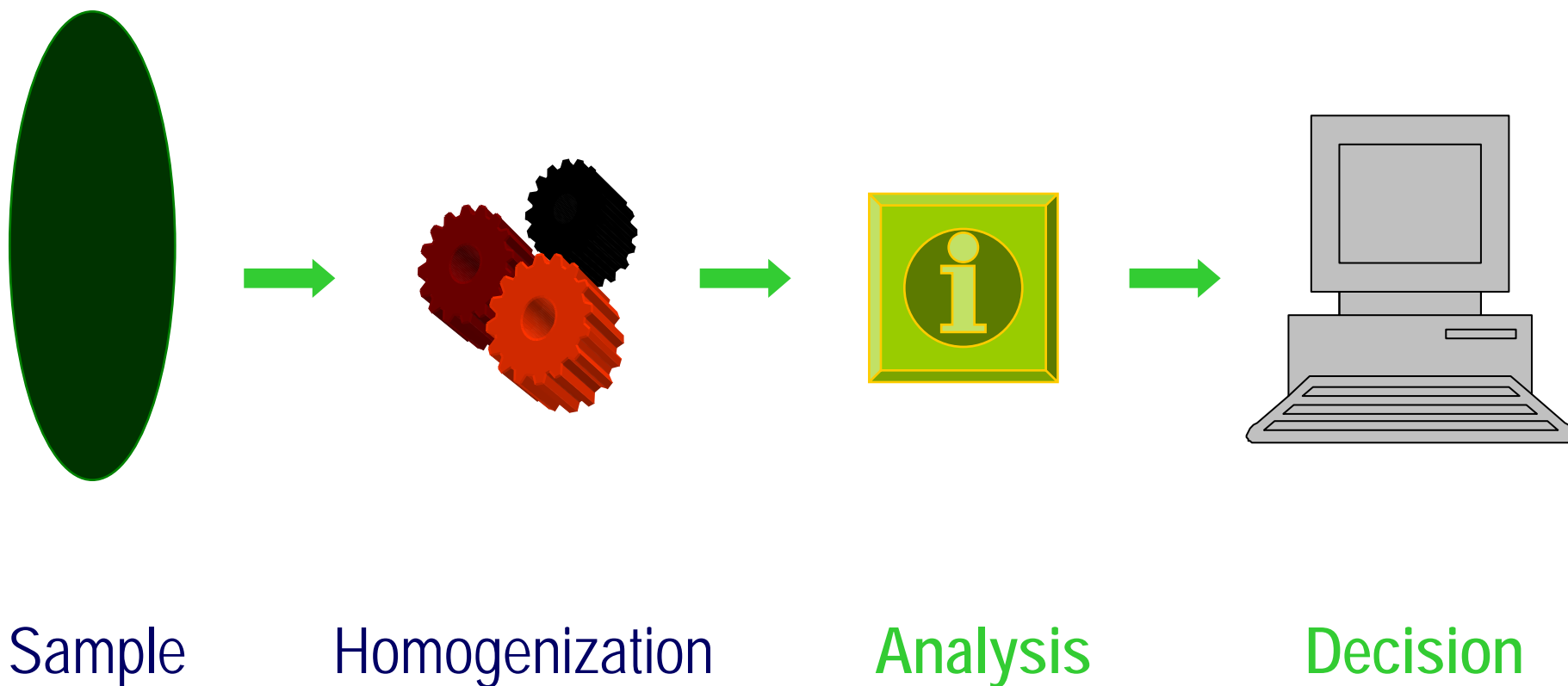
Molecular Biology and Genomics Unit

AGR 42006 - TAIEX - 28/29 October 2010

## *Overview of presentation*

- General principles in matrix-based screening approaches
- Case study: COSYPS analysis
- Decision Support System in GMO analysis
- Compendium of Reference Methods for GMO analysis

## *GMO analysis*



## ***Overview of presentation***

- **General principles in matrix-based screening approaches**
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## **Three key concepts in GMO screening analysis:**

***1: Universe***

***2: Matrix***

***3: Matrix-based screening approach***

## The concept “*Universe*”:

*Mathematical term:* “a class that contains (as elements) all the entities one wishes to consider in a given situation“

*Limited in time and space (e.g. legal framework of the EC in 2010)*

*⇒ limits are defined    ⇒ probabilities can be established*

## “Complexity of the GMO Universe” ( $\neq$ analysis)

### *Diversity*

(need to identify common features - coverage power)

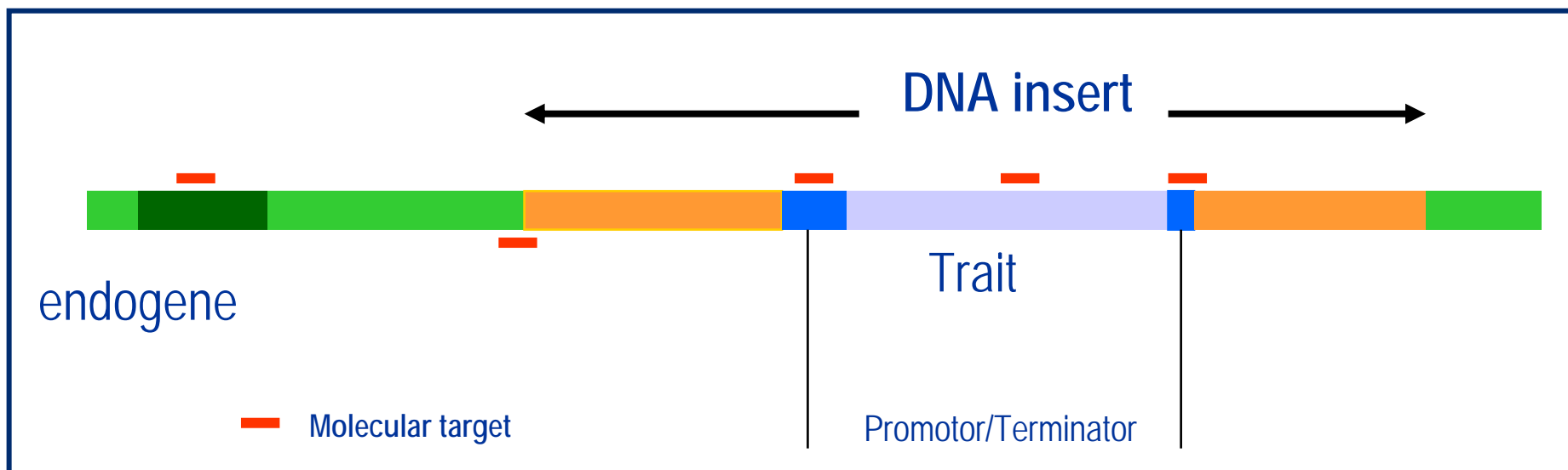
### *Similarity*

(need to identify **distinct** features - discriminatory power)

## “common/distinct features” for GMO analysis

In theory, any characteristic could serve

In the EU: **molecular targets** (DNA sequences)





# # GMO



## # target sequences

"[["  
 { } ⊂ ⇒ ⊆ ⊃ ⊄ ⊅ ⊆ ⊇ ⊈ ⊉ ⊊ ⊋ ⊌ ⊍ ⊎ ⊏ ⊐ ⊑ ⊒ ⊓ ⊔ ⊕ ⊖ ⊗ ⊘ ⊙ ⊚ ⊛ ⊜ ⊝ ⊞ ⊡ ⊢ ⊣ ⊤ ⊥ ⊦ ⊧ ⊨ ⊩ ⊪ ⊫ ⊬ ⊭ ⊮ ⊯ ⊰ ⊱ ⊲ ⊳ ⊴ ⊵ ⊶ ⊷ ⊸ ⊹ ⊺ ⊻ ⊼ ⊽ ⊾ ⊿ ⊺ ⊻ ⊼ ⊽ ⊾ ⊿

## What is a “MATRIX” ?



**Product/sample** designated as “**matrices**”



## Wikipedia : Science

- \* Extracellular matrix, any material part of a tissue that is not part of any cell
- \* Germinal matrix, an embryonic brain tissue
- \* Matrix (archaeology)
- \* Matrix (biology), the material between animal or plant cells or the material in the inner membrane of a mitochondrion
- \* **Matrix (chemical analysis), the remainder of the sample of which the analyte forms a part**
- \* Matrix (geology), the fine grains between larger grains in igneous or sedimentary rocks
- \* Matrix isolation, a continuous solid phase in which particles (precipitates, etc.) are embedded
- \* Nuclear matrix, an insoluble fraction of the cell nucleus
- \* Osteon or bone matrix, a form of connective tissue found in bone
- \* Position-specific scoring matrix, which represents a pattern or motif in biological sequences
- \* Similarity matrix, which scores the similarity between two data points

## A “matrix” in mathematics:

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

5 x 6  
Matrix

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

## 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

## 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**

[illegible]

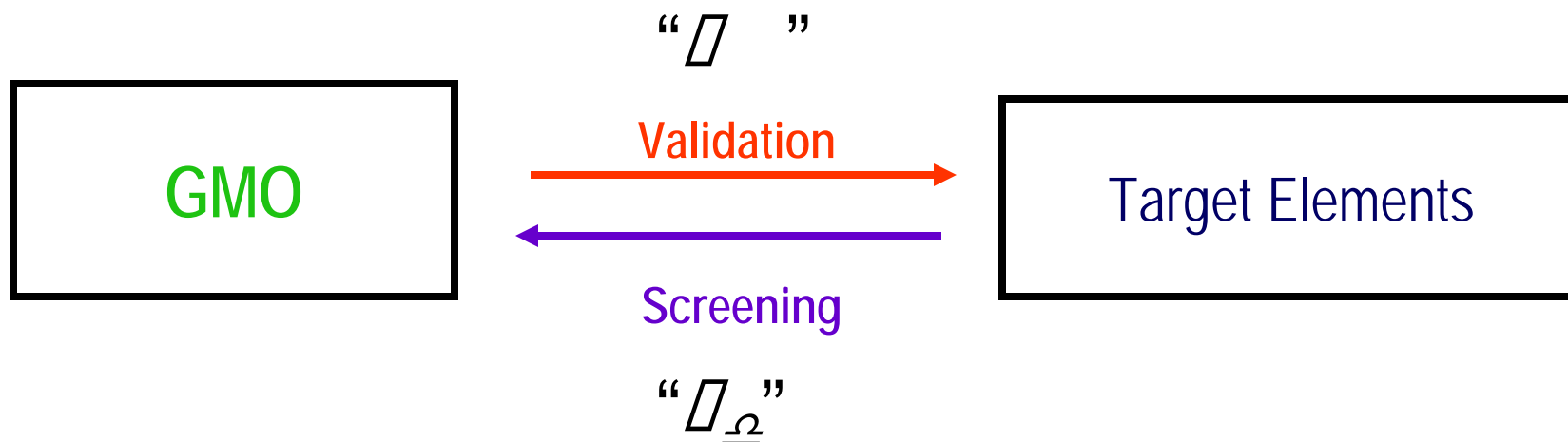
## Defining the set of targets applied for GMP screening with frequency table

Species	Event GMO	Producteur	CRL methods published?	p35S	tNOS	t35S	pNOS	rice actin	tOCS	npII	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	R R H7-1	KWS SAAT AG. Monsanto									X								
				p35S	tNOS	t35S	pNOS	rice actin	tOCS	npII	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

## Description of the GMO Universe:

# GMO



# target sequences

### Membership Function " $\mu$ "

$$\begin{aligned} \mu(x) &= \frac{1}{n} \sum_{i=1}^n \mu_i(x) \\ \mu_i(x) &= \frac{1}{m} \sum_{j=1}^m \mu_{ij}(x) \\ \mu_{ij}(x) &= \frac{1}{k} \sum_{l=1}^k \mu_{ijl}(x) \end{aligned}$$

### Detection Function " $\mu_d$ " :

$$\begin{aligned} \mu_d(x) &= \frac{1}{n} \sum_{i=1}^n \mu_{di}(x) \\ \mu_{di}(x) &= \frac{1}{m} \sum_{j=1}^m \mu_{dij}(x) \\ \mu_{dij}(x) &= \frac{1}{k} \sum_{l=1}^k \mu_{dijl}(x) \end{aligned}$$



## **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 EC, also the guidelines of the European Network of GMO Laboratories (ENGL) are applied

## ***Overview of presentation***

- General principles in matrix-based screening approaches
- **Case study: COSYPS analysis**
- Decision Support System in GMO analysis
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## COSYPS example

- COmbinatory SYbergreen Pcr Sscreening
- developed at Scientific Institute of Public Health (IPH (Be) (2005-2009)
- patented in 2007
- collaborative trial in 2009

## 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®Green Q-PCR methods for screening  
(primers, PCR conditions, reference materials)
5. Common validation criteria for all SYBR®Green Q-PCR methods
6. Development of a Mathematical Analysis tool
7. Development of integrated 96-well plate screening format and DSS

## Definition of Screening elements

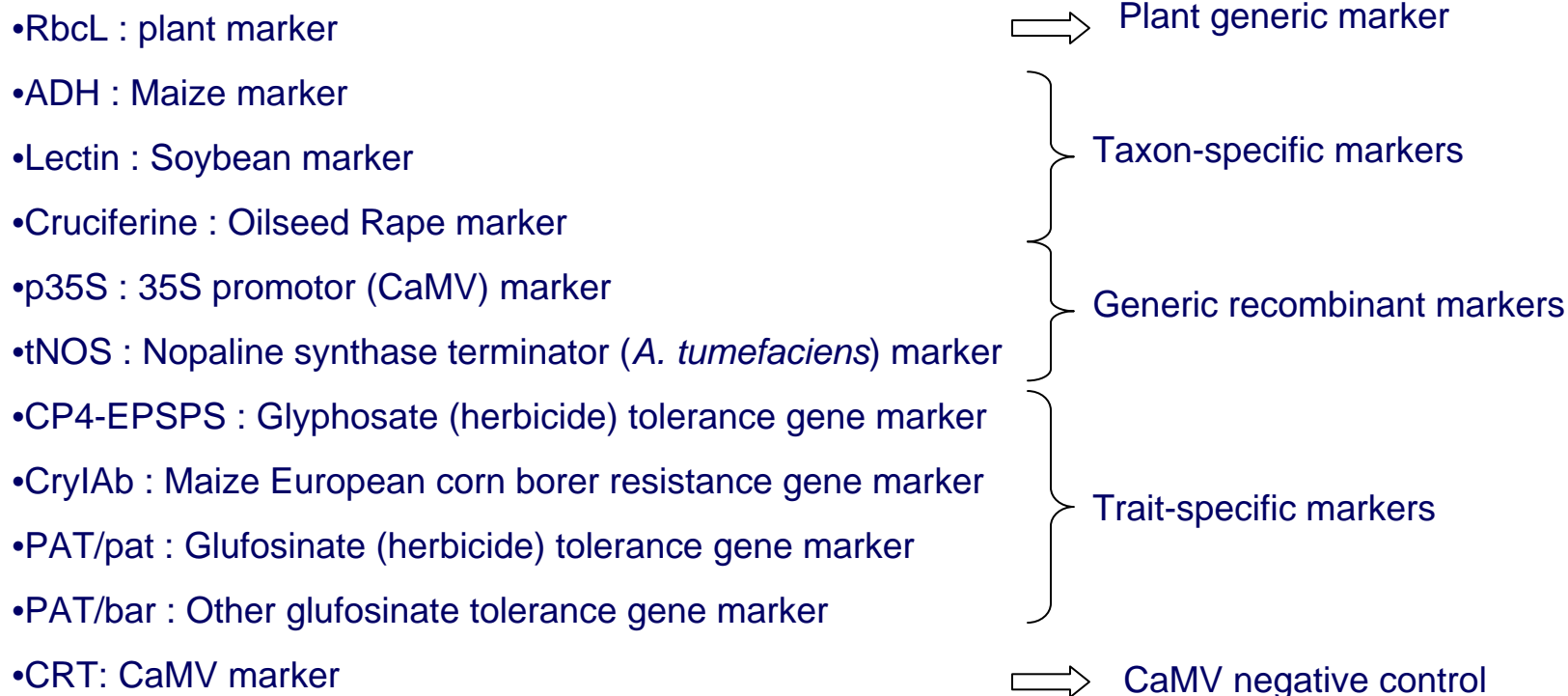
Linear [1 x N] matrix model for GM events

### Subclasses of constituents:

- **Kingdom** (e.g. plant, animals, bacteria,...)
  - **Taxon-/Species-specific** (e.g. maize, soy, oilseed rape, ...)
  - Construct-specific (inserted DNA sequence)
  - **Generic recombinant** (e.g. 35S promoter, T-Nos terminator, ...)
  - **Trait-specific** (e.g. CP4-EPSPS, PAT, BAR, CryIAb, ....)
  - Event-specific (plant/insert junctions, ...)
  - **Donor species markers** (e.g. RT CaMV)
- 
- Screening markers



## COSYPS for GM Soy, Maize, Rapeseed



*To date also SYBR®Green methods for rice, cotton, sugar beet and potato*

## COSYPS markers in the EU GMO Universe

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

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 GM markers

## COSYPS Q-PCR method parameters

### Standardized PCR running conditions

50 ng template DNA/reaction

260nM primer concentration (except for RBCL)

#### Standard PCR program

10'	95°C	1x	(hot start)
15"	95°C	40x	(denaturation)
1'	60°C		(elongation)
20'	60-95°	1x	(melting analysis)

## Validation of COSYPS SYBR®Green Q-PCR methods

All COSYPS methods were validated “in house” (ISO standards).

Validation parameters:

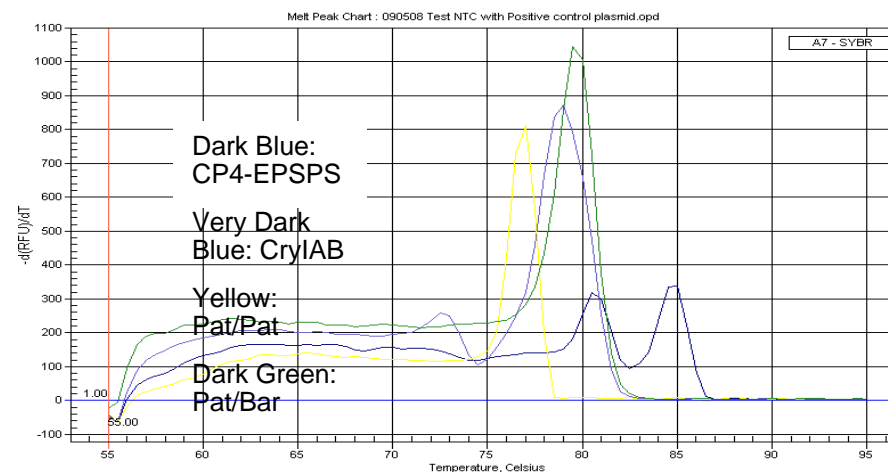
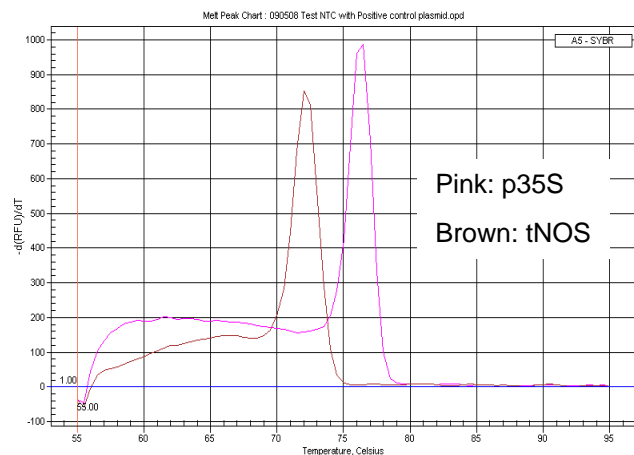
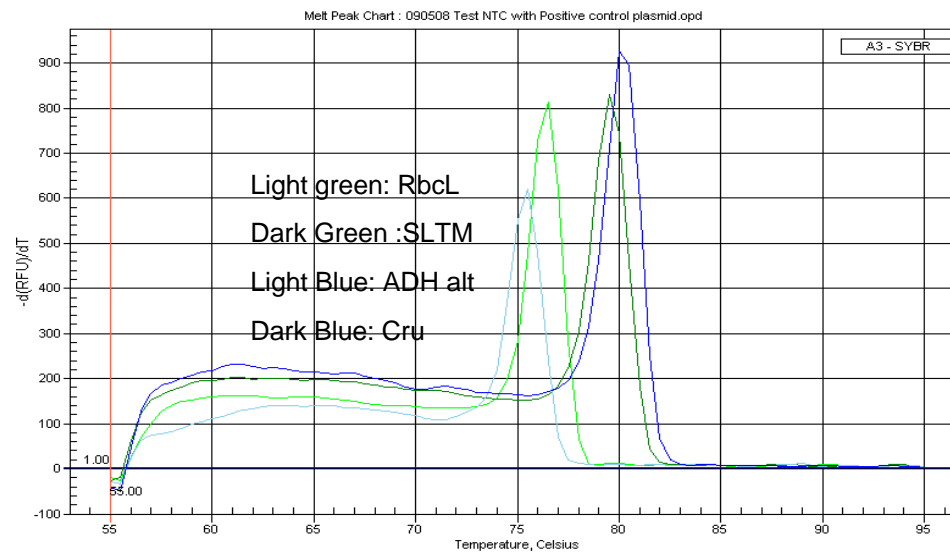
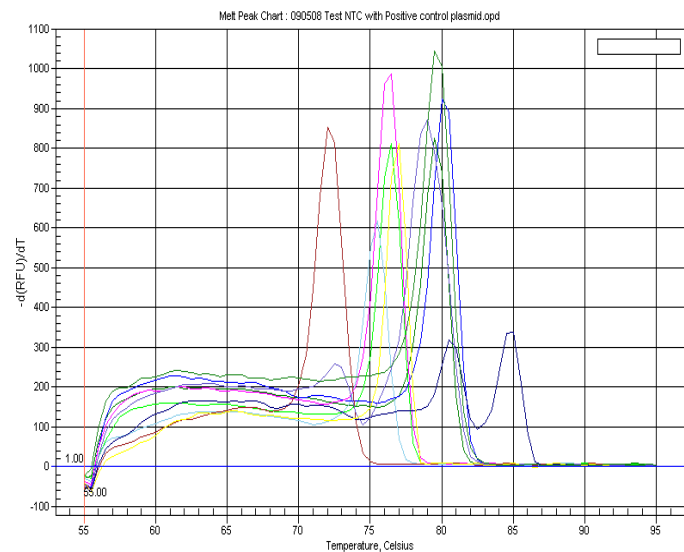
- Amplification of positive controls
- No amplification of negative controls
- One unique  $T_m$  with a clear dissociation peak
- One band on agarose gel at the expected size for positives
- Sequencing of the amplicon give the expected sequence
- No (or almost no) primer-dimer formation
- Identification 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 Reference Material 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

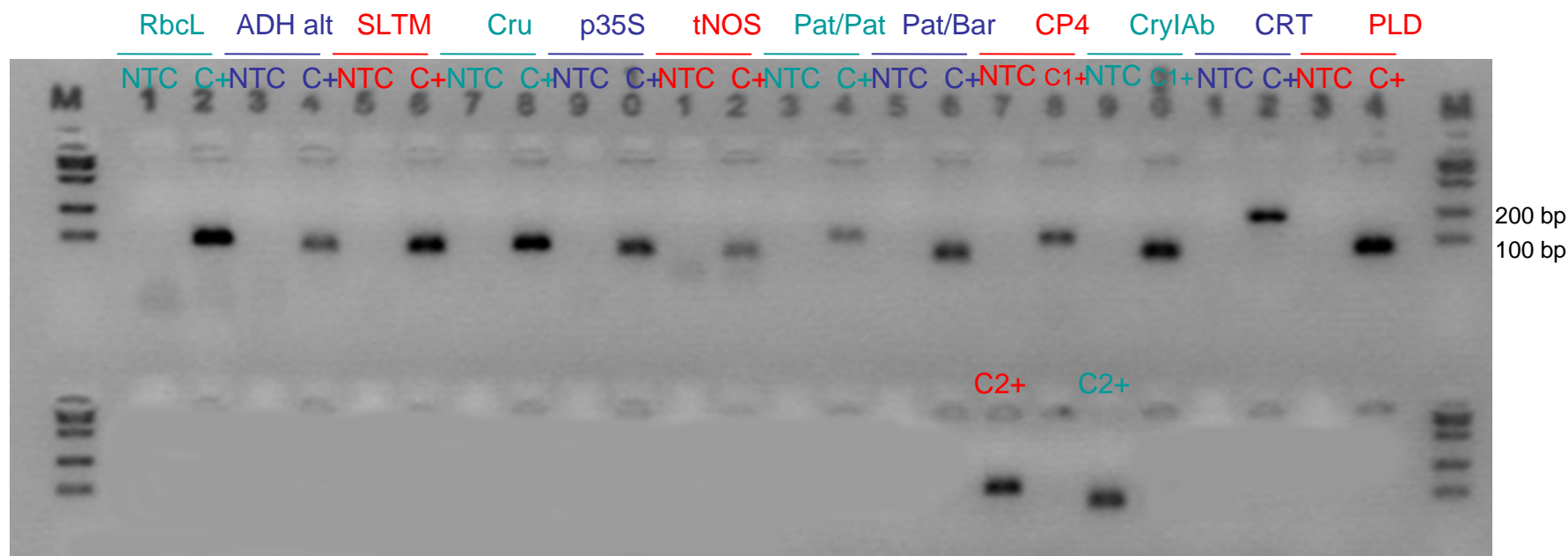
Average amplicon size is 84 bp ( $\pm 15$  bp)



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

## Plasmid mix as positive control

### Agarose Gel analysis of the COSYPS SYBR®Green Q-PCR amplicons



CP4: C1+: RRS 1%  
C2+: GT73 100%  
CryIAb: C1+: Bt11 1%  
C2+: MON810 1%

## Summary COSYPS: technical aspects

- SYBR®Green Q-PCR methods ( $T_m$  &  $C_t$  decision criteria)
- Tiered element organisation (kingdom, species, element)
- Genomic or plasmid DNA as reference materials
- Real time modus but also as qualitative approach applicable



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- ***Decision Support System in GMO analysis***
- Compendium of Reference Methods for GMO analysis

## Decision support systems for GMO analysis

- GMOTrack: a tool to identify optimal screening strategy (NIB/JRC)
- Prime Number algorithm for GMO tagging (IPH/JRC)
- Golden Standard GMO Matrix (GMOseek/JRC)
- Dropbox-based DSS (Waiblinger et al.) (boolean logics)
- eGMOLAN (IPH/JRC) (boolean, fuzzy logics, probabilistic heuristics)

# 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

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

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

## - Today: Excel–based support

Integrates the decision values from the validation dossiers

- $T_m$ -values (identification of targets)
- $LOD_6$  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
- Matricial calculation for combined interpretation of Screening and Identification results

## - Tomorrow : web application (eGMOLAN)

CONTROL ANALYSIS	Plant	SLTM (lectin)	ADH alt	CRU	p35S short	Tnos	CP4 synth	CryIAb	PAT	BAR	CaMV
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**Melting temperature** (green means corresponding to the amplicon)

Pos. Con.	Ct	21.74	21.83	22.46	20.68	30.16	29.19	22.16	29.47	30.10	29.23	25.00
	Tm	75.7	79.6	75.3	79.3	76.0	71.8	80.6	78.3	76.7	79.3	77.9
								84.2	79.6			
Conclude		2	2	2	2	2	2	2	2	2	2	2

<b>Conclusion (pos. controls)</b>	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
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Blanc Open	Ct	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
	Tm	75.70	70.50	70.50	69.00	69.00	69.00	69.00	70.20	74.40	69.60	69.00
	Conclude	0	0	0	0	0	0	0	0	0	0	0

<b>Conclusion (controls)</b>	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
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NTC run 1	Ct	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
	Tm	74.00	69.30	70.50	73.40	76.30	72.40	74.70	70.20	73.70	69.90	74.40
	Conclude	0	0	0	0	0	0	0	0	0	0	0

<b>Conclusion (controls)</b>	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
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<b>CONCLUSION</b>	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID
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COSYPS ANALYSIS	Plant	SLTM	ADH alt	CRU	p35S short	Tnos	CP4 synth	CryIAb	PAT	BAR	CaMV
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Run1	Ct	25	25	25.45	28.7922	32.8411	31.5409	31.1516	40	37.6524	33.2421	40
	Tm	76.0	79.6	75.7	79.6	76.3	72.1	84.5	72.8	77.0	79.6	70.2
	Conclude	3	3	3	3	3	3	2	0	1	2	0

Run2	Ct	25.2524	25.3654	25.79	29	33.0079	30.8442	31.0736	40	40	33.1206	40
	Tm	75.7	79.6	75.7	79.6	76.3	72.1	84.5	72.4	77.0	79.6	69.9
	Conclude	3	3	3	3	2	3	2	0	1	2	0

<b>Conclusion (2 runs)</b>	Quantifiable	Quantifiable	Quantifiable	Quantifiable	Identifiable	Quantifiable	Identifiable	Below LOD	Below LOD	Identifiable	Below LOD	Below LOD
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## 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		



## GM-EVENT IDENTIFICATION

Sample ID      Enforcement

### PART 1: COSYPS evaluation

	GTS40-3-2T25	NK603	GA21	Bt176	Bt11	BT10	MON810	TC1507	DAS59122	MON863	Topas19/2	MS8	RF3	MS1/RF1/MS2	GT73	LL62 rice	LL601
COSYPS Result	Match	Below LOD	Match	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD
Evaluation	Identify	No furth analysis	Identify	Identify	No furth analysis	No furth analysis	No furth analysis	No furth analysis	No furth analysis	No furth analysis	Identify	No furth analysis	No furth analysis	No furth analysis	No furth analysis	No furth analysis	No furth analysis

### PART 2: TAQMAN GM-EVENT IDENTIFICATION

First try have to be fill only when the first try was not correct. Always put the correct trial in the surrounded cells

Date	05/09/2008	05/09/2008	05/09/2008						05/09/2008								
first try date (not Ok)																	
Machine	7300-1	7300-2	7300-2						7300-2								
first try machine (not Ok)																	
Operator	EV	EV	EV						EV								
first try operator (not Ok)																	
Positive Control	29,47	32,00	35,00						31,00								
first try control (not Ok)																	
Run 1	34,05	45,00	45,00						45,00								
first try run 1 (not Ok)																	
	1	0	0						0								
Run 2	33,82	45,00	45,00						45,00								
first try run 2 (not Ok)																	
	1	0	0						0								
SUM	2	0	0						0								
ID results	< LOQ	Below LOD	Below LOD						Below LOD								

[illegible]

## Summary COSYPS: decision support aspects

- Unique Prime number identifiers
- Decision thresholds ( $LOQ_6$  and  $LOD_6$ )
- Integrates screening and identification data
- Excel-based support format

## ***Overview of presentation***

- General principles in matrix-based screening approaches
- Case study: COSYPS analysis
- Decision Support System in GMO analysis
- **Compendium of Reference Methods for GMO analysis**

## *Compendium of Reference Methods for GMO analysis*

- Aim:  
Provide an up-to-date reference for all validated methods for the detection of Genetically Modified Organisms (GMO)
- Legal framework:  
Regulation EC/2004/882  
Recommendation EC/2004/787
- Collaboration between the EURL-GMFF & ENGL

## **Scope:**

- GMO detection methods (plants, bacteria ...)
- ISO/IUPAC collaborative trial criteria
- DNA-based methods (cf. Recommendation EC/2004/787)

## Content:

### ***Chapter 1: Quantitative GMO detection PCR methods (48)***

*Maize quantitative PCR methods (20)*

*Soybean quantitative PCR methods (9)*

*Cotton quantitative PCR methods(8)*

*Oilseed rape quantitative PCR methods (4)*

*Potato quantitative PCR methods (1)*

*Rice quantitative PCR methods (1)*

*Sugar beet quantitative PCR methods (1)*

*Element- and Taxon-specific quantitative PCR methods (4)*

### ***Chapter 2: Qualitative GMO detection PCR methods(31)***

*Element-specific qualitative PCR methods (15)*

*Construct-specific qualitative PCR methods (8)*

*Event-specific qualitative PCR methods (2)*

*Taxon-specific qualitative PCR methods (6)*

## Quantitative PCR method for detection of maize event NK 603

### 1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of maize event NK 603 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan <sup>®</sup>
Compendium Reference	QT/ZM/008

### 2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	Maize flour
Materials used for calibration/controls	Certified Reference Material IRMM-415 (JRC-IRMM)
Tested GM events	
Event Name	NK 603
Unique Identifier	MON-00603-6
Crop Name	<i>Zea mays</i> L.

#### Collaborative Trial Description

The participants received 10 blind samples representing 5 GM levels, namely 0.1%, 0.49%, 0.98%, 1.96%, and 4.91% of maize event NK 603 in non-GM maize (w/w). In addition the laboratories received a calibration maize flour sample containing 4.91% of NK 603 maize in non-GM maize (IRMM-415), two negative DNA target controls consisting of maize event Bt 176 DNA and non-GM maize flour, reaction reagents, primers and probes for the *adh1* reference gene and the NK 603 specific system. For each unknown sample and for the calibration sample the laboratories performed an enhanced CTAB DNA extraction, a spectrophotometric quantification of the amount of DNA extracted, a real-time PCR monitor run (inhibition test) and a quantitative real-time PCR analysis. Samples were analyzed in parallel with both the reference and the transgenic specific system. The standard and control samples were analyzed in triplicates, the blind samples in quadruplicates. The two replicates for each GM level were analyzed in two separate runs.

#### Method Performance

LOD Relative	? 0.05%	LOD Absolute	not reported
LOQ Relative	0.1%	LOQ Absolute	not reported

#### Values determined in the collaborative trial

Test Level (%)	0.10%	0.49%	0.98%	2.0%	4.9%
Mean Value (%)	0.18%	0.85%	1.4%	2.2%	6.0%
RSD <sub>r</sub> (%)	24%	15%	17%	7.7%	22%
RSD <sub>x</sub> (%)	37%	34%	25%	26%	31%
Bias %	83.0%	73%	47%	14%	22%

	GM Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R <sup>2</sup>	not reported	not reported

#### Comment

The LOD and LOQ values were provided by the method developer and were not further assessed in the collaborative trial.

### 3. REFERENCES

Mazzara M, Paoletti C, Puimalainen J, Rasulo D, Van Den Eede G. Event-Specific Method for the Quantitation of Maize Line NK603 Using Real-Time PCR - Validation Report and Protocol. EUR 21825 EN. 2005. JRC32103 (ISBN 92-79-00106-X)

### 4. PRIMERS AND PROBES SEQUENCES

#### GM-target(s)

Primer Forward	5'-ATGAATGACCTCGAGTAAGCTTGTTAA-3'
Target element	Insert
Primer Reverse	5'-AAGAGATAACAGGATCCACTCAACACT-3'
Target element	3'-host genome
Amplicon length	108 bp
Probe	5'-FAM-TGGTACCACGCGACACACTTCCACTC-TAMRA-3'
Target element	DNA sequence in the 3' IBR

#### Taxon-target(s)

Primer Forward	5'-CCAGCCTCATGGCCAAAG-3'
Target element	<i>adh1</i>
Primer Reverse	5'-CCTTCTTGCGGCTTATCTG-3'
Target element	<i>adh1</i>
Amplicon length	70 bp
Probe	5'-FAM-CTTAGGGGCGAGACTCCCGTGTTCCCT-TAMRA-3'
Target element	alcohol dehydrogenase1 ( <i>adh1</i> ) gene

### 5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal	1x	TaqMan® Universal	1x
PCR Master Mix		PCR Master Mix	
Primer Fw	0,15 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,05 µmol/L	Probe	0,05 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 300	Template DNA	maximum 300
Final Volume	50 µL	Final Volume	50 µL

### 6. AMPLIFICATION CONDITIONS

#### GM-target(s) and taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45



## **Foreseen distribution:**

- Official presentation: 9 November 2010
- Compendium booklet distributed at ENGL meeting 9/10 November 2010
- Laboratory version (plastified sheets) available by end 2010
- Compendium document available as PDF file
- Web documentation

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**Thank you for your attention**