Overview on technical aspects of GMO detection approaches

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Operation of GMO labs to comply with EU legislation

GMO detection (screening)

Positive

Negative

GMO ? Yes/No

GMO identification

Yes

No

Illegal

Are they Authorised ? Yes/No

GMO quantification

Assay individual ingredients

Must be Labelled ? Yes/No

< threshold

No need for labelling

> threshold

Labelling required

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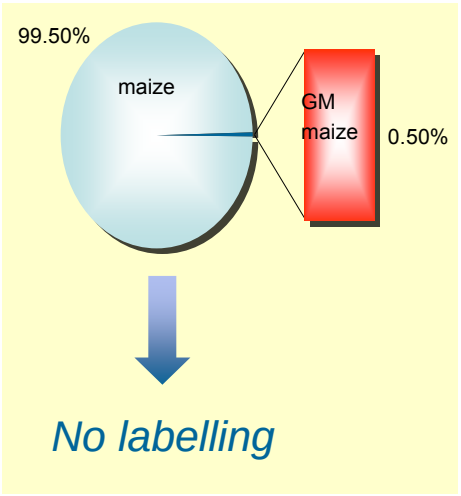
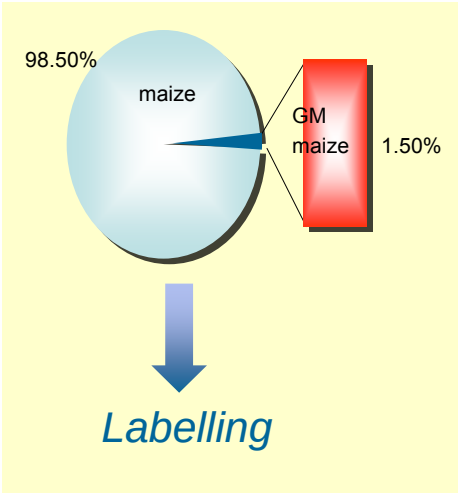
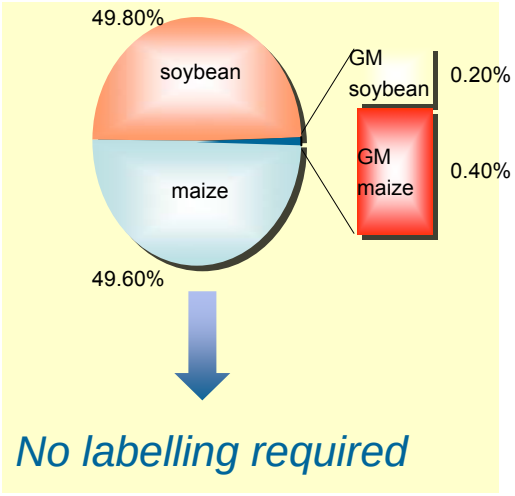
Quantification of GMOs and labelling



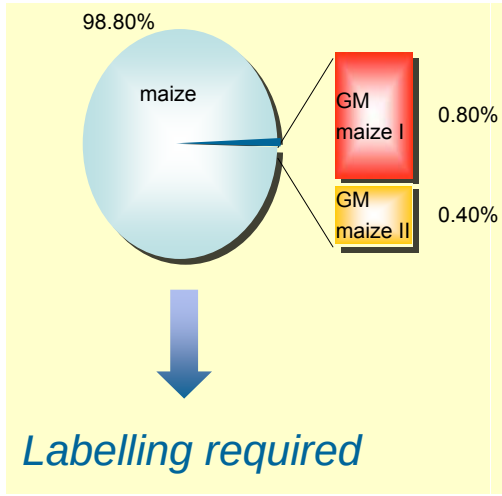
No labelling



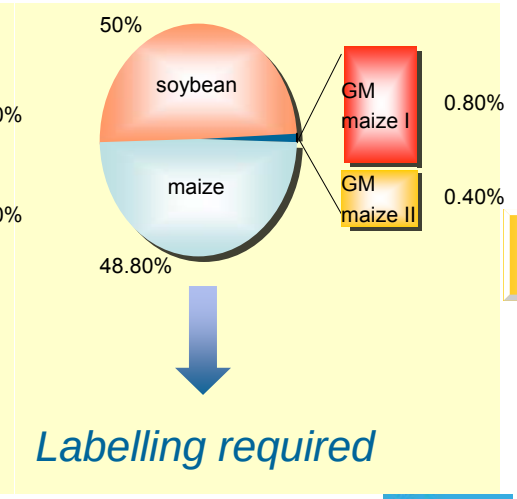
Labelling



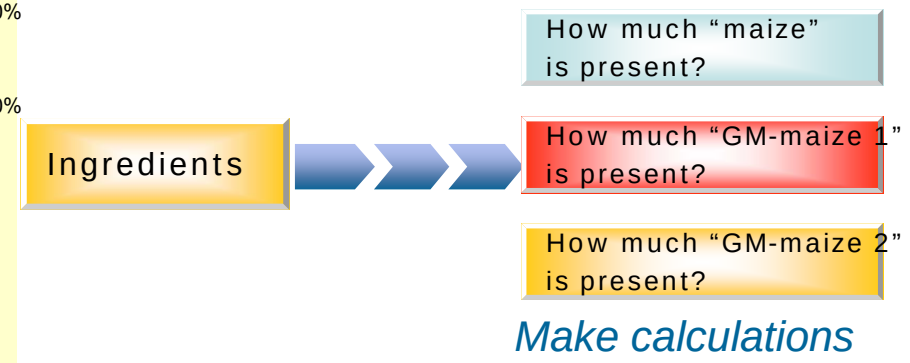
No labelling required



Labelling required



Labelling required



Make calculations

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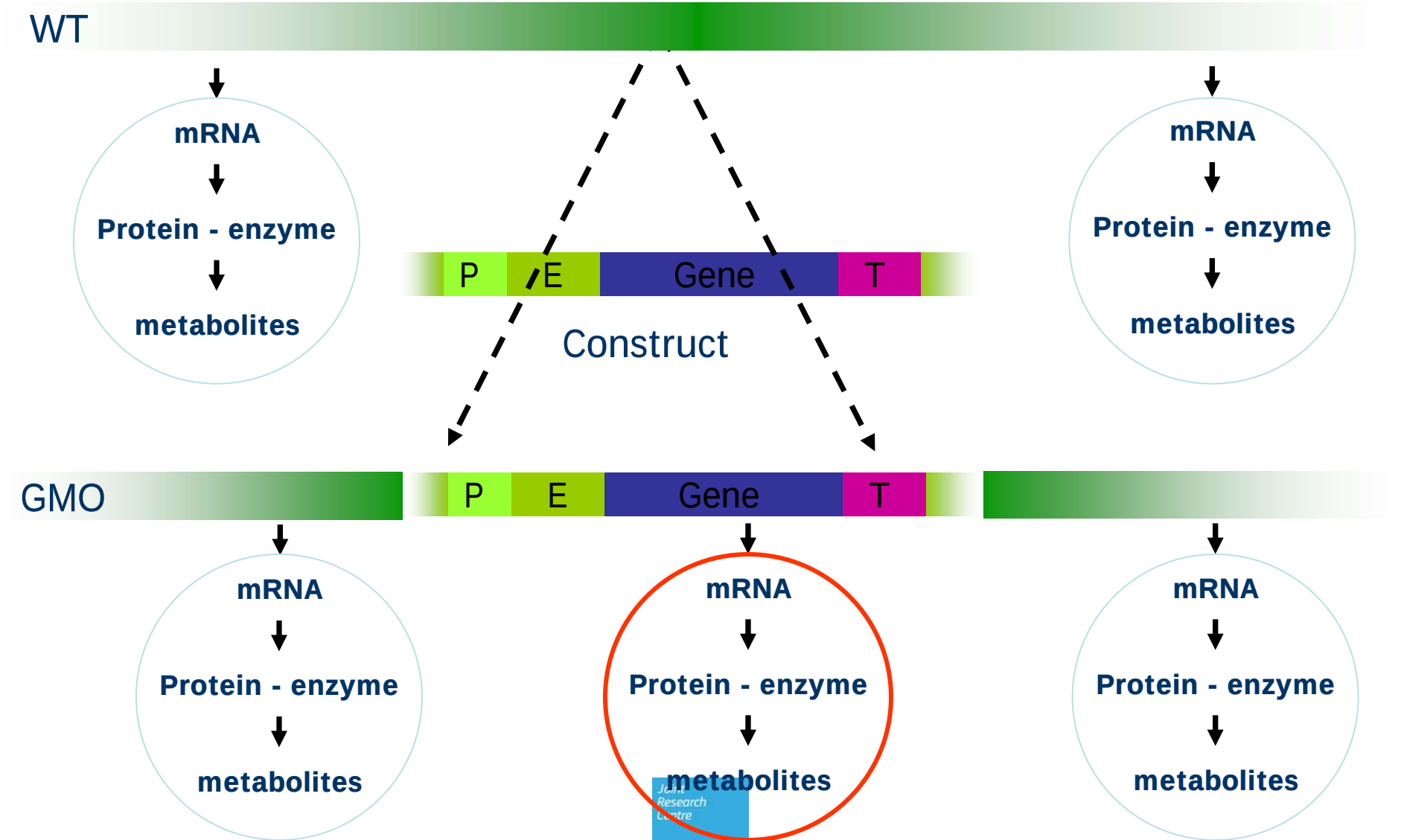
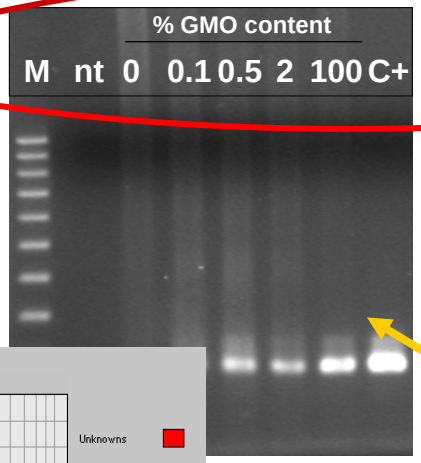
How to Know if a Product is Genetically Modified ?

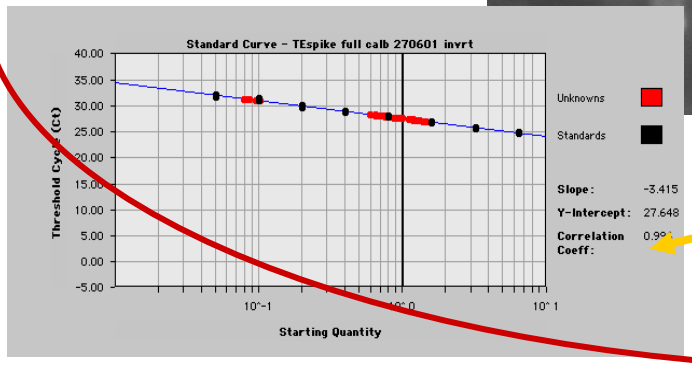


Steps in DNA based GMO analysis and source of errors

Sampling

Analytical





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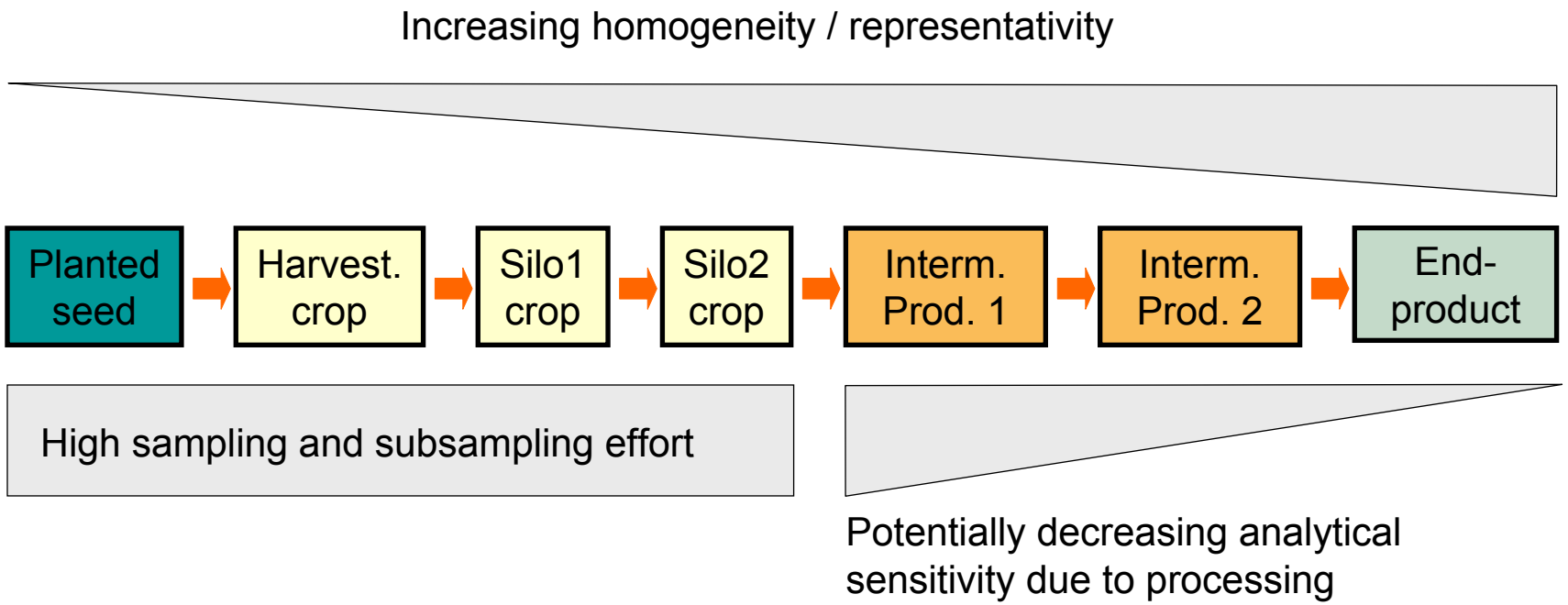
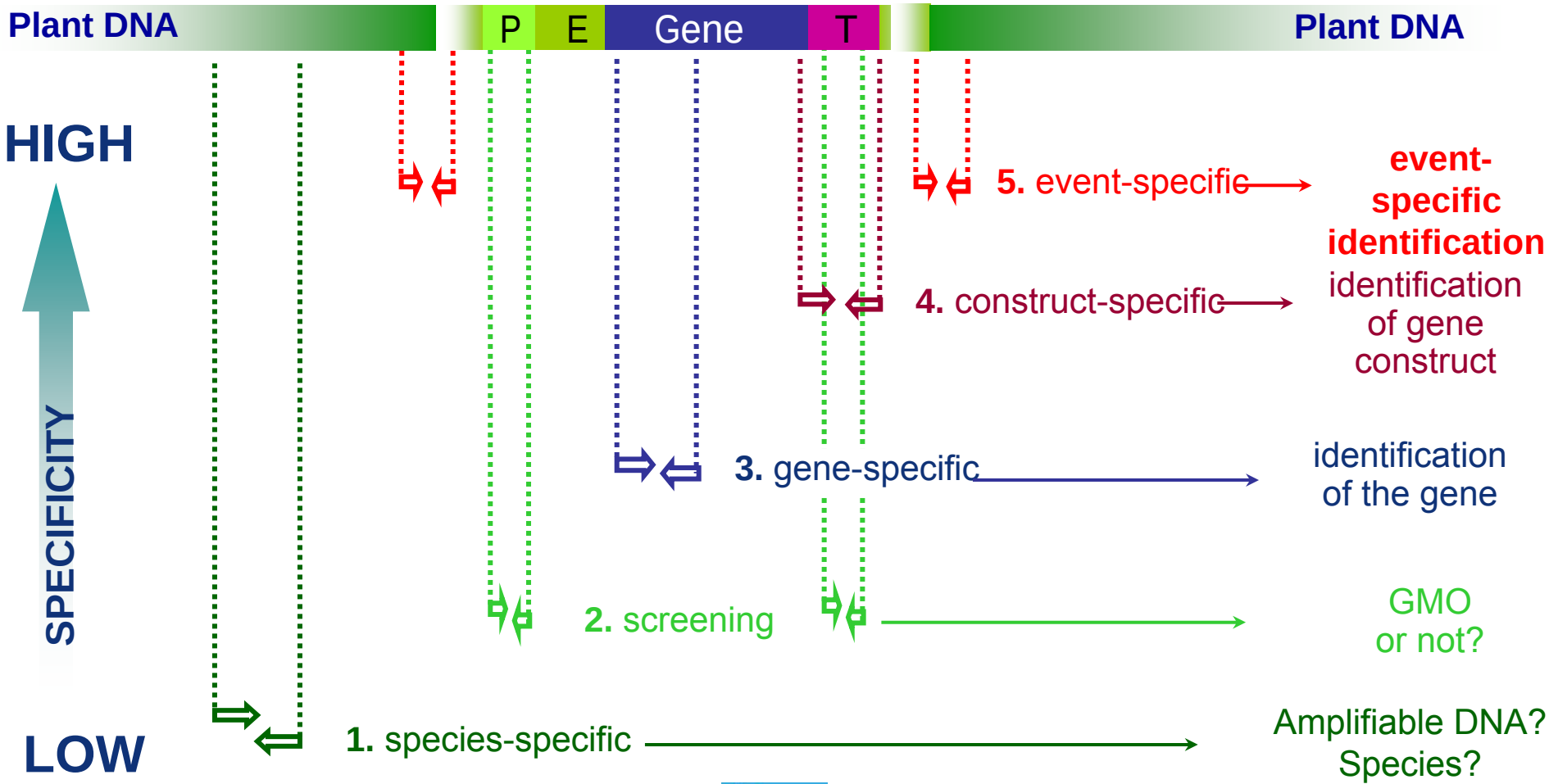
Detection strategies, which method to select?

Experience along the production chain



Detection strategies, which target to select?



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Strategies for GMO testing

Step	Purpose	Suitable methods
Screening	Broad screening for revealing the (eventual) presence of GMOs. The test provides information on presence/absense. It also serves the purpose of reducing the number of samples that need further testing.This step does not allow knowing which GMO is/are present.	Methods targeting (regulatory) elements most commonly used (Element-specific; modification-specific methods). Today more methods need to be used/combined to have 100% coverage.
Identification	Allows the identification of the GMO(s) present in the tested sample. Used to identify approved GMOs and to exclude non-approved GMOs	Methods allowing the unequivocal identification of the GM events. The highest specificity is obtained using „event-specific“ methods = methods targeting the specific region, in the genome, in which the foreign DNA is integrated. This region is unique for each event.
Quantification	Allows definying how much GM material is present in the sample. Applied to check for labelling requirements	Event-specific methods (EURL-GMFF validated)

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Pros and cons - DNA based methods

Genetic modifications = DNA modifications

DNA stable and inheritable

DNA traceable unit for all purposes

- Matrix limitations may apply

Sensitive, fit for identification and quantification

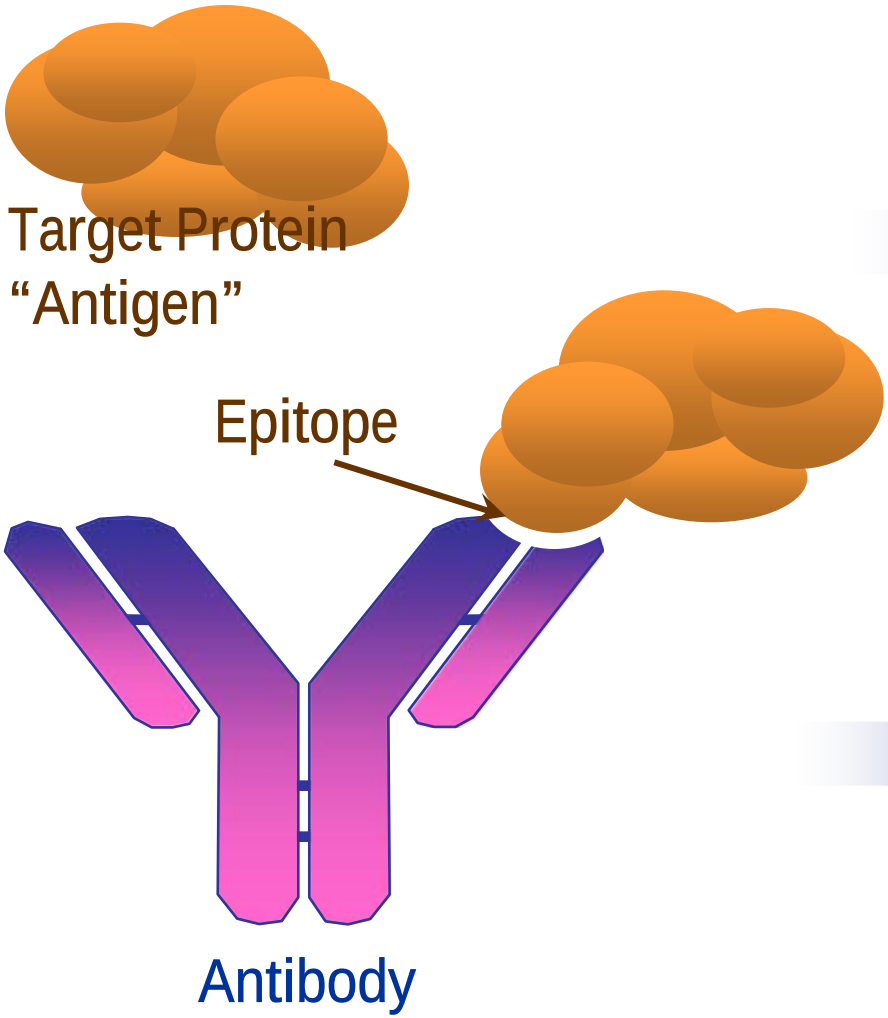
Costs:

- Efficient screening (multiple targets and GMOs)
- Expensive identification and quantification
- Equipment, reference material, skilled staff

Limited coverage, although superior to protein

- Can only detect what we have methods for!

GMO analysis by immuno assays



Pros and cons - protein based methods

Advantages/benefits:

- Speed
- Cost
- Practicability and easy transferibility
- Low risk of false positives (carry over)
- Well established in the food industry

Drawbacks:

- Matrix limitations and sensitivity
- Coverage low (methods only for few GMOs)
- Low fitness for Qn analysis
- Limited identification (no event identification except for ’unique’ traits)

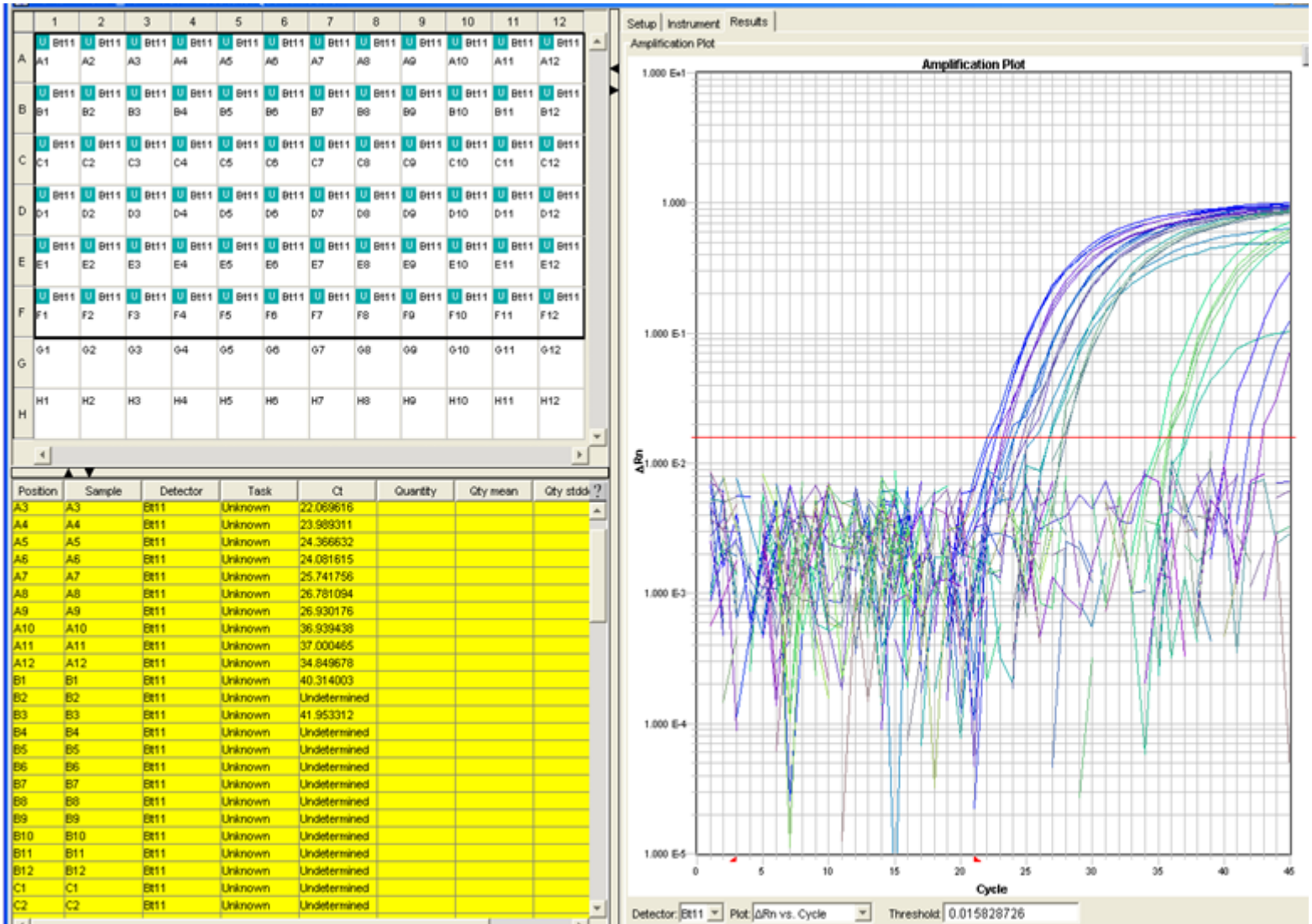
Present context

- Worldwide adoption and use of GMOs is rapidly increasing (acreage, countries);
- Constant rise in GMO complexity, number of traits and events;
- In the EU:
    - Mandatory labelling of GMOs and derived food/feed products (if above 0.9%) requires event-specific methods;
    - GMO control based on combination of screening + event-specific detection methods;
    - Increasing number of GMOs under approval;
    - Asynchronous approval process complicates the analytical procedure.

↑ Higher number of methods to be applied for full product characterisation.

↑ Increased time and cost of analysis/sample.

Endpoint RT-PCR for qualitative PCR detection



Position	Sample	Detector	Task	Ct	Quantity	Qty mean	Qty stddev
A3	A3	Bt11	Unknown	22.069616			
A4	A4	Bt11	Unknown	23.989311			
A5	A5	Bt11	Unknown	24.366632			
A6	A6	Bt11	Unknown	24.081615			
A7	A7	Bt11	Unknown	25.741756			
A8	A8	Bt11	Unknown	26.781094			
A9	A9	Bt11	Unknown	26.930176			
A10	A10	Bt11	Unknown	36.939438			
A11	A11	Bt11	Unknown	37.000465			
A12	A12	Bt11	Unknown	34.849678			
B1	B1	Bt11	Unknown	40.314003			
B2	B2	Bt11	Unknown	Undetermined			
B3	B3	Bt11	Unknown	41.953312			
B4	B4	Bt11	Unknown	Undetermined			
B5	B5	Bt11	Unknown	Undetermined			
B6	B6	Bt11	Unknown	Undetermined			
B7	B7	Bt11	Unknown	Undetermined			
B8	B8	Bt11	Unknown	Undetermined			
B9	B9	Bt11	Unknown	Undetermined			
B10	B10	Bt11	Unknown	Undetermined			
B11	B11	Bt11	Unknown	Undetermined			
B12	B12	Bt11	Unknown	Undetermined			
C1	C1	Bt11	Unknown	Undetermined			
C2	C2	Bt11	Unknown	Undetermined			

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

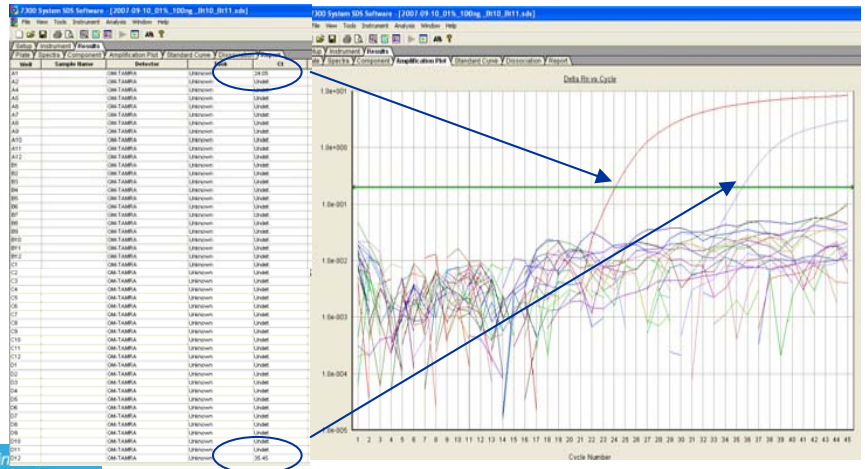
Pre-spotted plates

	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG Maize Ref	SAH7 Cotton Ref	PLD Rice Ref	CruA Oilseed Ref	Lectin Soybean Ref	GS Sugarbeet Ref	UGPase Potato Ref	t11 Maize	NK603 Maize	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	t11 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

Targets: 7 plant species
39 GM events



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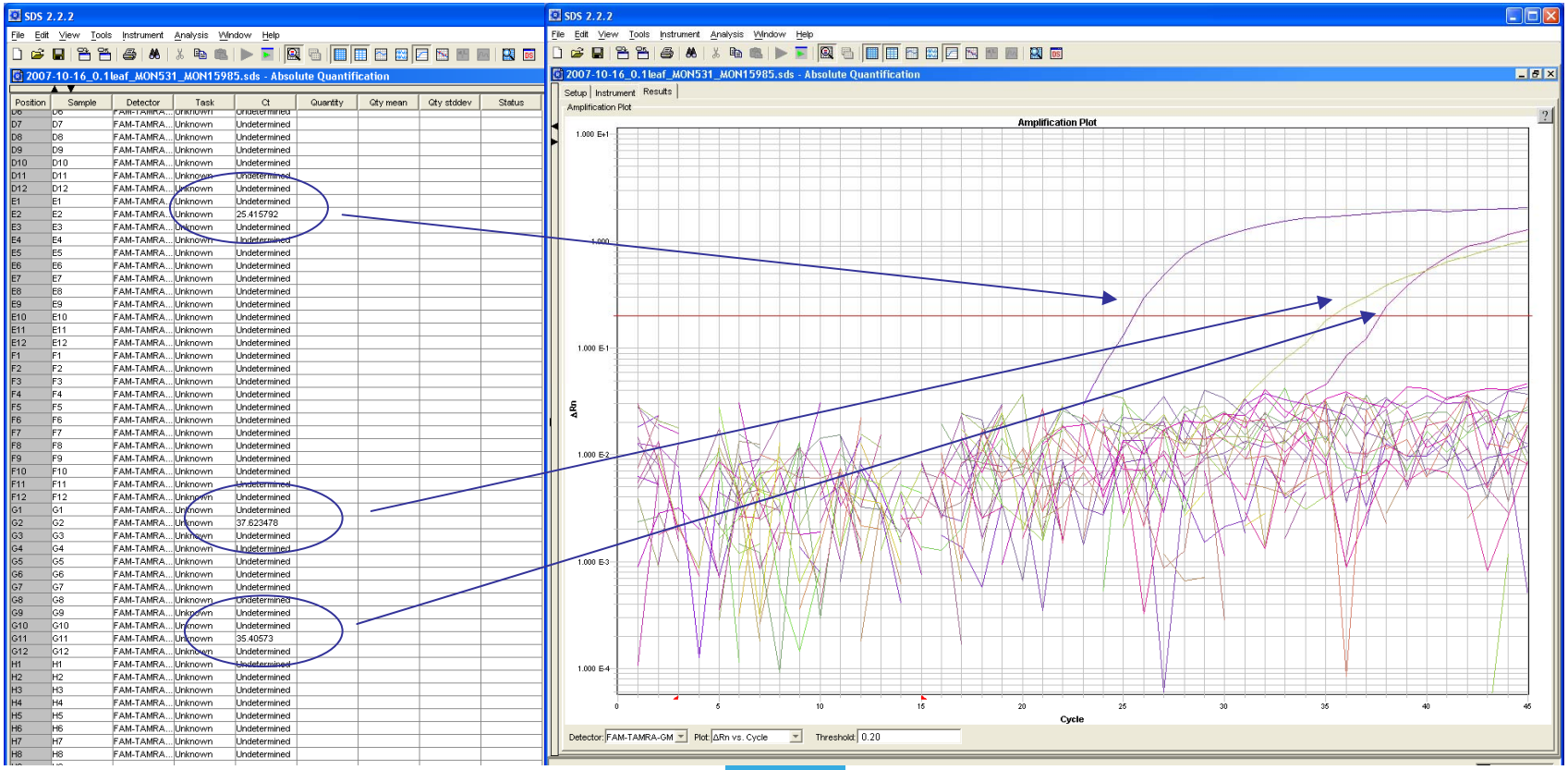
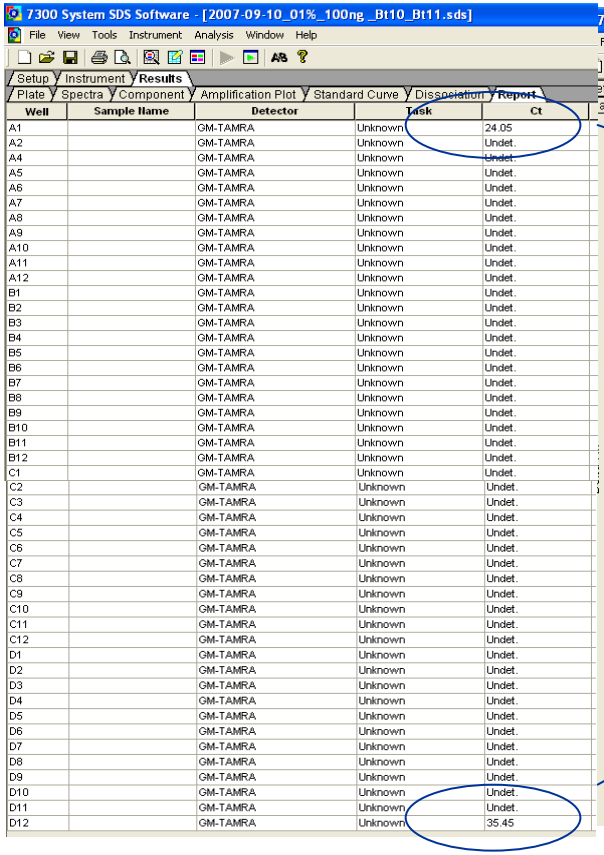
Detection of cotton event MON15985

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

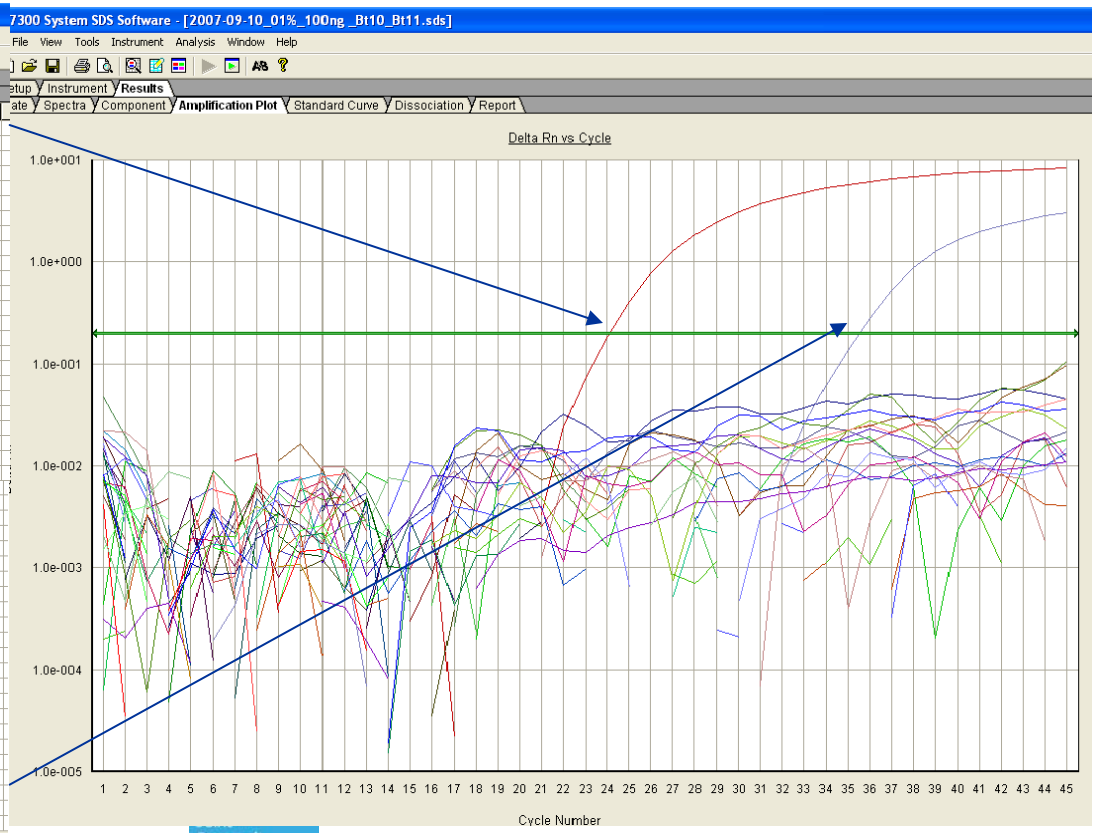


Detection of maize event Bt-10

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



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



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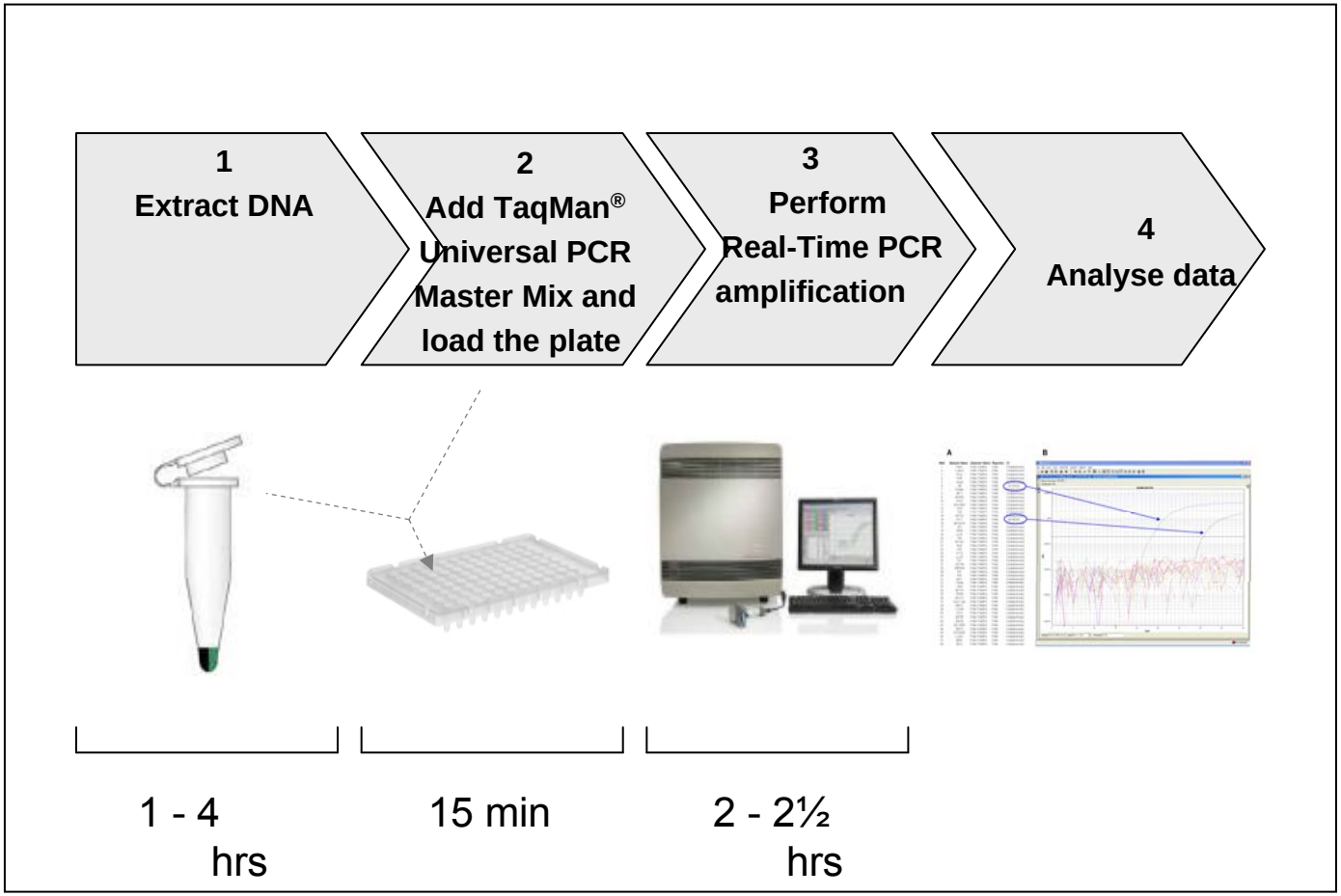
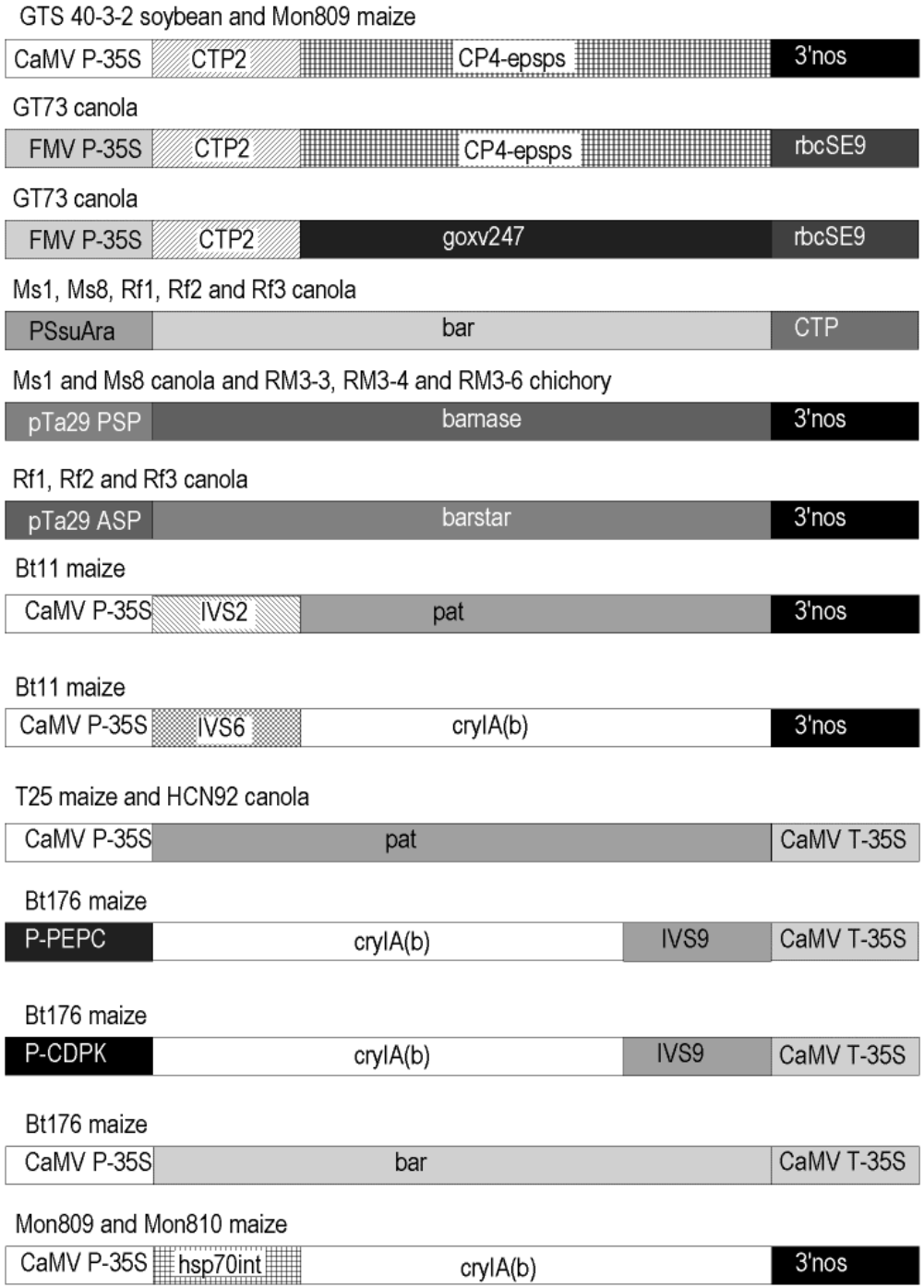
Workflow and approximate timing for GMO analysis using the ready-to-use multi-target analytical system



Some commercial GM corn events and detectability by P35S and t-nos screening tests

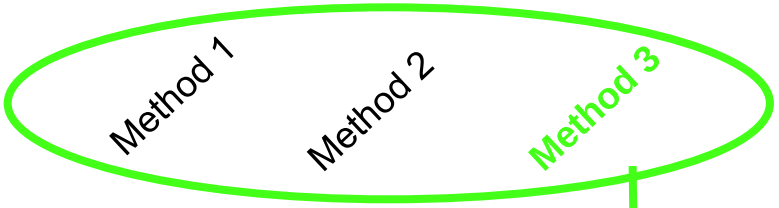
	P35S	T-nos
Bt11	Y	Y
MON810	Y	NO
T25	Y	NO
Bt176	Y	NO
NK603	Y	Y
GA21	NO	Y
MON863	Y	Y
CBH351	Y	Y
TC1507	Y	NO

GM commercial events



Matrix approach

The matrix is a simple table including the relationships between the **targets** and the **methods** within a **screening set up**



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

+': present

-': absent

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Matrix approach

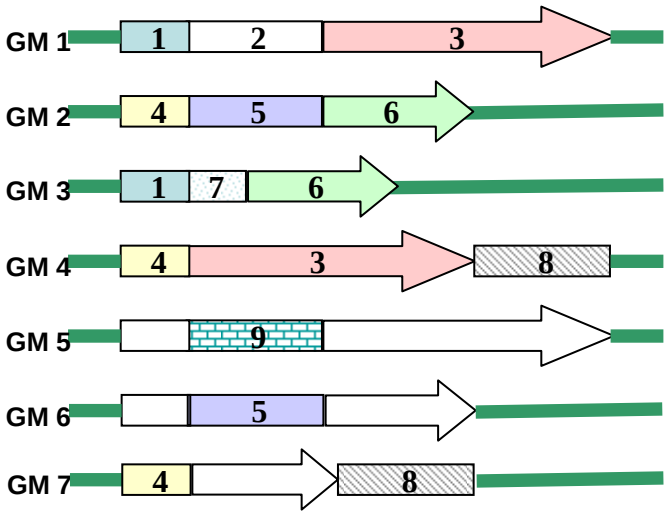
DATA

- **GMO lines**
- **Species (knowledge on sample characteristic)**
- **Assays (methods): screening, construct specific, event specific**

CHARACTERISTIS

- **Flexible**
  - **New GMOs can be added**
  - **New assays can be added**
  - **New species can be added**

Matrix approach: Combination of screening methods targeting common GM elements



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 + ?

Matrix approach

-Matrix approach dev by Waiblinger et al allows more than 90 % of analyses as screening PCRs

-P35S and T-nos mostly used PCRs

- P35S: to date at least 46 events
- T-nos: to date at least 35 events

Screening for further target sequences useful:

→ GM plants without P35S or T-nos

→ additional information before identification of the event

Matrix approach - Screening use in daily work

- screening for
  P35S, T-nos, bar, CTP2-EPSPS, P35S-pat,
  detects all events e.g. listed in AGBIOS database
- (one exception: LY038)

→ a maximum of five real-time PCRs are necessary
(less, if multiplex PCR is performed)

screening for five or more elements reduces number of possible events that have to be considered for further identification

Targets and official methods for screening

5 targets selected for screening

Sequence for screening	official real-time PCR method available? (interlaboratory tested)
P35S	yes (duplex)
T-nos	yes (duplex)
bar	yes
CTP2-CP4EPSPS	yes
P35S-pat	yes

Waiblinger et al.(2008) Dtsch Lebensm Rundsch 104(6):261–264

Combination of screening methods targeting common GM elements

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

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

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Thank you!