



Setting-up a GMO detection laboratory

Success factors to reliable performance

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- **Introduction (the basics, the criteria, the differing situations ...)**
- **Relevant dimensions**
- **Quality dimensions**
- **Challenges in practice and conclusions**

- ISO 17025 (general management principles and prerequisites)
- ISO 21569:2005, ISO 21570:2005...
(general and specific GMO testing prerequisites)
- CRL list of methods and guidance documents for method validation
- Proficiency testing schemes

Three main dimensions

- Specific Customer requirements
- Cost efficiency
- Quality

- Inhouse-, private service- or public lab (what to confirm) ?
- Throughput, environment & premises
(Sample numbers, interfering activities ...)
- Dominating matrices and species or broad range of matrices
- Area (low or high GMO incidence)
- Technology (protein based, gel/PCR, fluorescent PCR, quantitative PCR ...?)

- Gel based assays vs qualitative realtime Assays,
- Qualitative versus quantitative assays
- Event versus screening assays
- IT infrastructure (documentation effort) and tools (e.g. valid. Excel sheets)
- Automation of sample preparation or PCR setup
- Multiplexing

- Calculation of control parameters
- No Template Controls
- Positive Controls
- p-35S negative, t-nos positive sample
- Negative sample
- Negative sample
- p-35S positive, t-nos positive sample
- Inhibited sample
- p-35S positive, t-nos negative sample
- Negative Sample

35S				NOS				IPC			
	Ct	dRn	Out		Ct	dRn	Out		Ct	dRn	Out
Control	31,7	0,78	0	Control	32,1	0,82	0	Control	32,5	0,81	0
SD	0,35	0,05		SD	0,35	0,04		SD	0,29	0,02	
Delta	6	0,7		Delta	6	0,7		Delta	4	0,5	
CuIDT	32,7	0,16		CuIDT	38,1	0,17		CuIDT	36,5	0,30	
	Ct	neg	pos		Ct	neg	pos		Ct	neg	pos
NTC	45,0	N/A	N/A	NTC	45,0	N/A	N/A	Pos Control	32,8		
Extr Control	N/A	N/A	N/A	Extr Control	N/A	N/A	N/A	Extr Control	N/A		
Threshold	0,03			Threshold	0,04			Threshold	0,04		

Well	Temp	35S	Ct	dRn	Result	NOS	Ct	dRn	Result	IPC	Ct	dRn	Total		
A1	N	35S	45,0	0,00	neg	-	NOS	45,0	0,00	neg	-	IPC	32,6	0,50	valid
A2	N	35S	45,0	0,00	neg	-	NOS	45,0	0,00	neg	-	IPC	32,6	0,50	valid
A3	N	35S	45,0	0,00	neg	-	NOS	45,0	0,00	neg	-	IPC	32,9	0,56	valid
A4	N	35S	45,0	0,00	neg	-	NOS	45,0	0,00	neg	-	IPC	32,0	0,54	valid
A5	N	35S	45,0	0,00	neg	-	NOS	45,0	0,00	neg	-	IPC	32,6	0,50	valid
A6	N	35S	45,0	0,00	neg	-	NOS	45,0	0,00	neg	-	IPC	32,2	0,53	valid
A7	P	35S	32,0	0,74	pos	*	NOS	32,1	0,62	pos	*	IPC	33,0	0,56	valid
A8	P	35S	31,9	0,85	pos	*	NOS	32,2	0,63	pos	*	IPC	32,8	0,59	valid
A9	P	35S	32,0	0,73	pos	*	NOS	32,5	0,64	pos	*	IPC	32,0	0,59	valid
A10	P	35S	32,1	0,85	pos	*	NOS	31,5	0,67	pos	*	IPC	32,7	0,67	valid
A11	U	35S	45,0	0,00	neg	-	NOS	35,1	0,43	pos	*	IPC	34,4	0,40	valid
A12	U	35S	45,0	0,00	neg	-	NOS	38,8	0,32	pos	*	IPC	34,4	0,43	valid
B1	U	35S	45,0	0,00	neg	-	NOS	45,0	0,00	neg	-	IPC	34,2	0,43	valid
B2	U	35S	45,0	0,00	neg	-	NOS	45,0	0,00	neg	-	IPC	34,2	0,42	valid
B3	U	35S	45,0	0,00	neg	-	NOS	45,0	0,00	neg	-	IPC	34,6	0,51	valid
B4	U	35S	45,0	0,00	neg	-	NOS	45,0	0,00	neg	-	IPC	35,0	0,51	valid
B5	U	35S	34,4	0,66	pos	*	NOS	34,7	0,56	pos	*	IPC	32,5	0,70	valid
B6	U	35S	34,3	0,66	pos	*	NOS	33,9	0,59	pos	*	IPC	32,3	0,71	valid
B7	U	35S	45,0	0,00	inhib	!	NOS	45,0	0,00	inhib	!	IPC	45,0	0,12	inhib
B8	U	35S	45,0	0,01	inhib	!	NOS	45,0	0,01	inhib	!	IPC	45,0	0,02	inhib
B9	U	35S	36,9	0,40	pos	*	NOS	45,0	0,01	neg	-	IPC	34,0	0,56	valid
B10	U	35S	36,7	0,40	pos	*	NOS	45,0	0,02	neg	-	IPC	33,7	0,51	valid
B11	U	35S	45,0	0,01	neg	-	NOS	45,0	0,02	neg	-	IPC	32,5	0,54	valid
B12	U	35S	45,0	0,03	neg	-	NOS	45,0	0,00	neg	-	IPC	32,9	0,59	valid

Examples:

- Short delivery times
- Need to detect „unknown GMO“ (enhanced screening)
- Electronic data exchange (EOL)
- Complex analytical strategies, confirmations etc.
dependent on screening results

→ Finding the right solution

commercial
planting

(Asynchronous)
approvals

field testing

species

**GMO testing
Strategy**

time pressure

sample type

cross-
contamination

specific
requirements
(e.g. organic)



UP-to date information

- Customer consultancy to apply the right systems the right analytical strategy to be cost efficient
- Cover all relevant GMO (may depend on area/approval situation, origin of product etc.)

Requires

- Suitable method portfolio of validated methods
- Knowing what GMO are relevant (commercially and other)
- Knowing how a given GMO is detectable (analytical screening strategy)
- Knowing about up-coming GMO

Contamination control

- Specifically trained staff (from cleaning personnel to labmanager)
- Specific training aspects (grinding, pipetting, disposing ...)
- Forward flow „Y“ principle
- Internal „traffic“ and air flows
- Number and location of work areas



Process architecture and IT (LIMS)

- To avoid human error
- To improve documentation

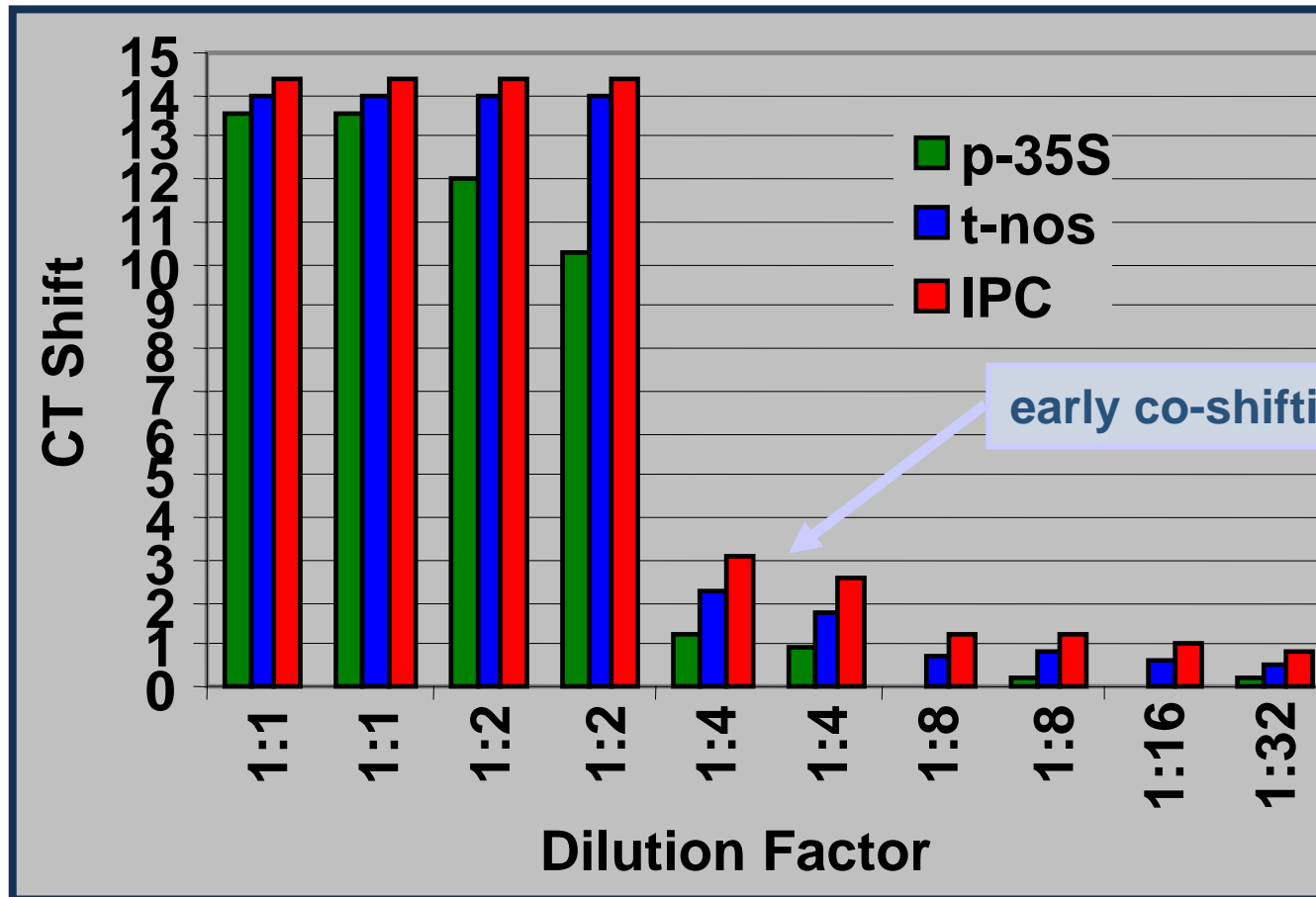
INHOUSE Method Validation

Why? Different equipment, supplies etc ...

- Avoid „nice weather conditions“ (e.g. using CRM´s only) during inhouse validation
- Include robustness aspects (e.g. thermocycler specifics)
- Challenge the methods by using low spiking levels
- New GMO may need revalidation of screening systems (are they detected?)
- Needs suitable reference materials and adequate quality controls in validation.

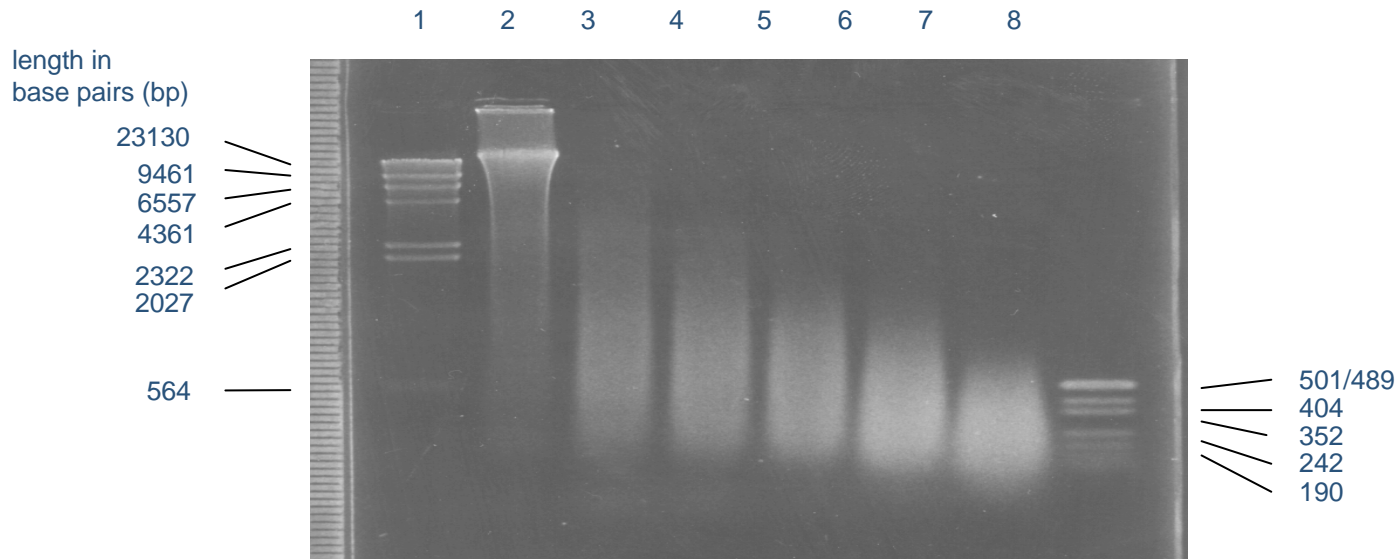
Example Robustness: IPC reliability

Ct shifts with dilution series of inhibitor-spiked DNA preparations



Analytical controls

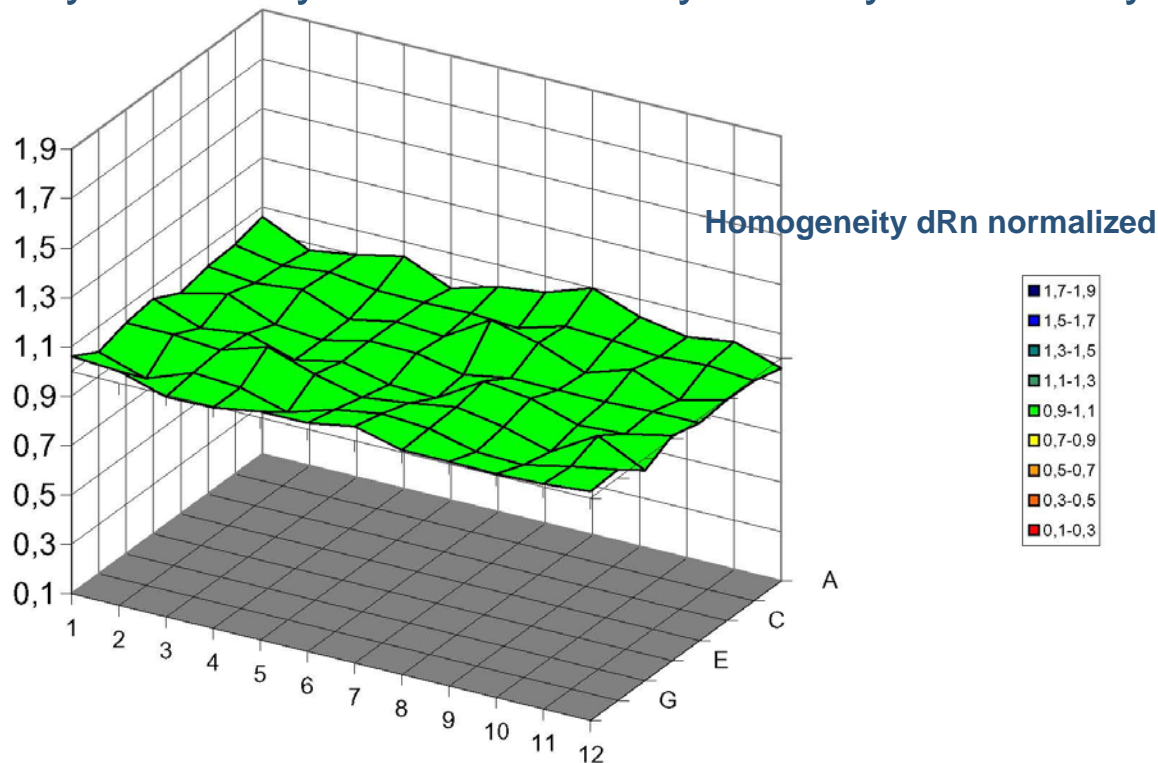
- DNA amount (misleading results of OD, fragmentation)
- Positive control (should include extraction process)
- Negative control (enough in number as compared to sample numbers)
- Inhibition control: Prefer low level spiked or quantitative IPC controls



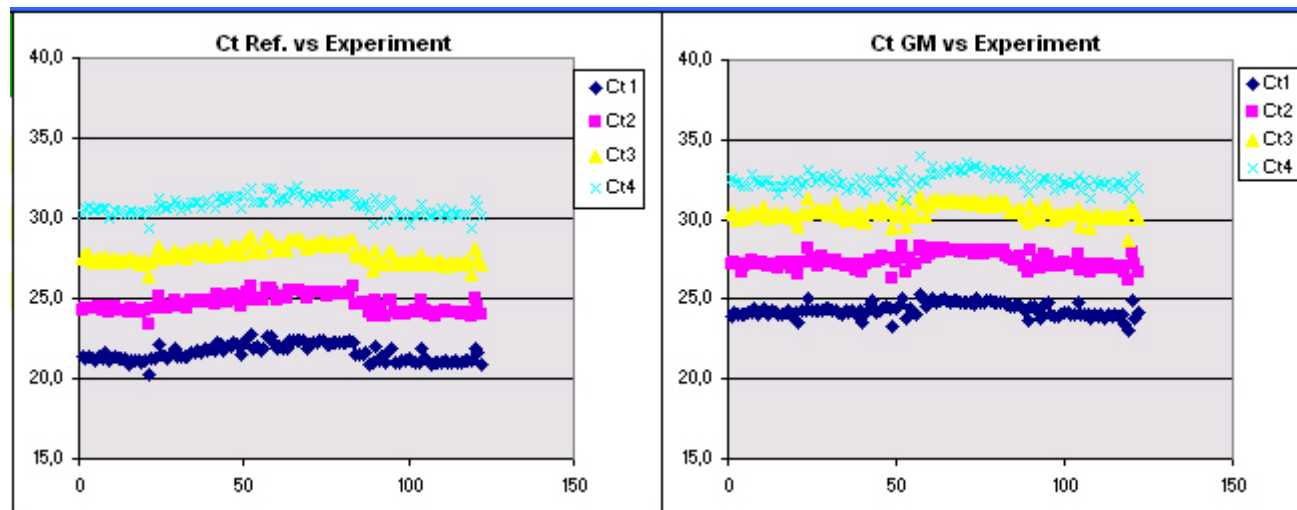
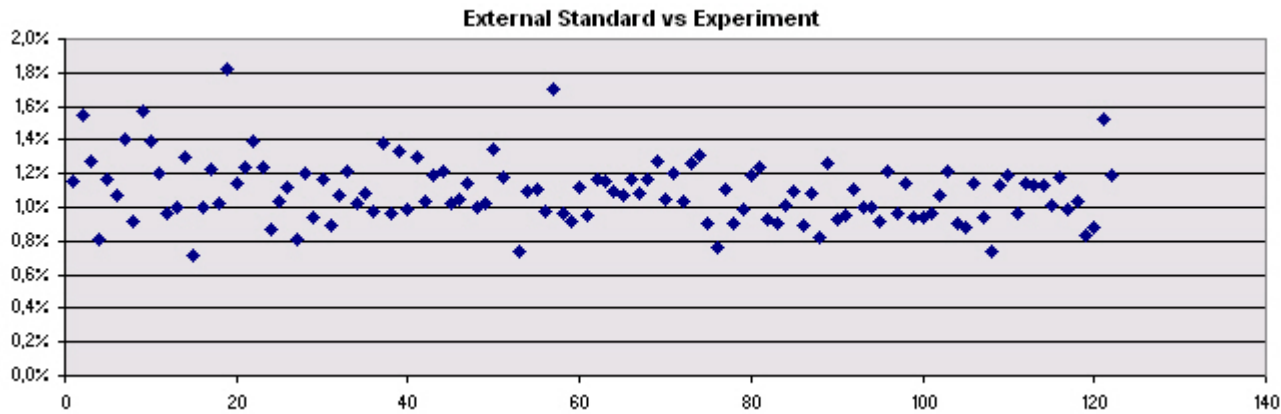
Shortening of DNA Fragments by Heat

Reagent QC and performance monitoring for equipment

- A new batch of reagent may be different in quality (define QC acceptance criteria)
- A system may not run robustly on a cycler or a cycler may be out of spec



QC Charts as indicators to alert failure

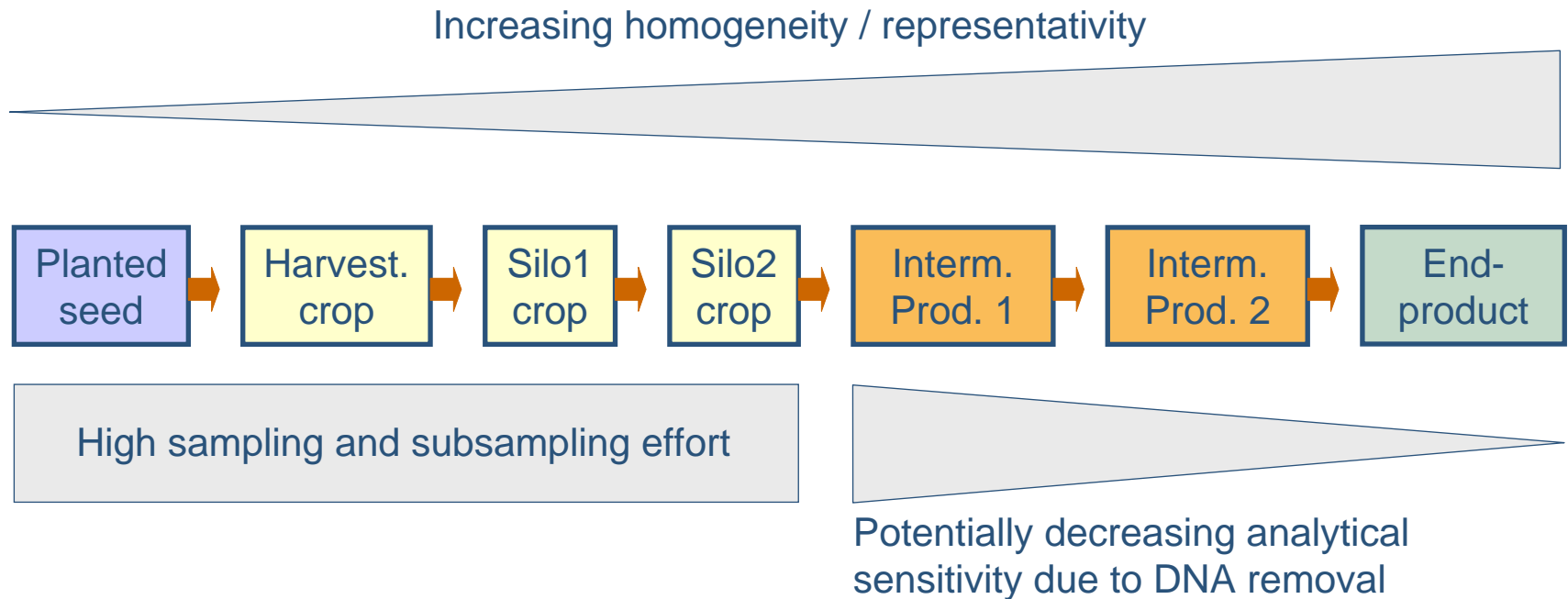




Experience and evaluation of results

- If DNA content is low: Is it method failure or matrix property?
- A positive result could be misleading (e.g. caMV in rape, soy contamination in corn) or it could be an „unknown“ GMO (Example LL601 rice or BT63 rice)
- Quantitative results can only be interpreted in a meaningful way if statistical knowhow is applied that spans the knowledge from the sampling point and situation, subsampling and homogenisation aspects to final DNA content and sensitivity considerations

Statistics and matrix experience



Ongoing development and innovation is needed:

- **New GMO call for adaption of screening strategies - Examples:**

Mavera™ corn (line LY038) or

TREUS™ soybean (line 305423)

... both lacking screening target sequences like 35S or nos

- **„Unforeseen“ GMO incidences like Bt10 corn, Bt63 rice, LLRice601**
- **Experimental stage or “unknown GMO”**

Analytical quality in a GMO analytical laboratory requires the right quality principles but is a question of management on the detail level.

When setting up a GMO analytical laboratory the specific conditions must be considered and a „General Blueprint“ - concept may only serve as a baseline that needs to be specifically adapted.

Thank you for your attention !



GeneScan



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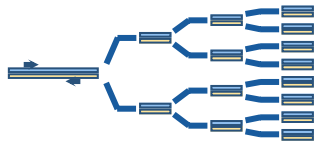
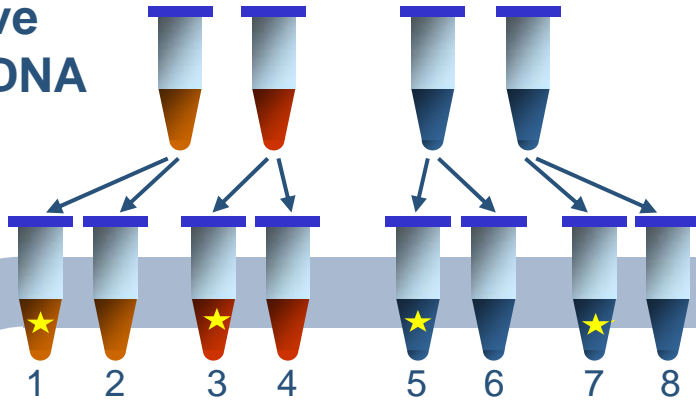
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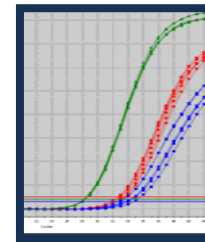
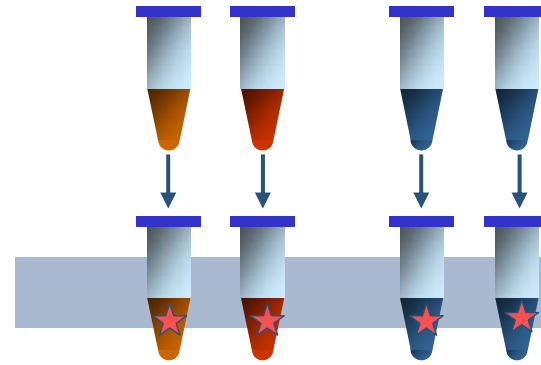
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Qualitative Analysis

positive control DNA



IPC: internal positive control



Control			IPC			IPC			IPC		
Run	Well	Result	Run	Well	Result	Run	Well	Result	Run	Well	Result
Control	1	OK	IPC	1	OK	IPC	1	OK	IPC	1	OK
IPC	2	OK	IPC	2	OK	IPC	2	OK	IPC	2	OK
IPC	3	OK	IPC	3	OK	IPC	3	OK	IPC	3	OK
IPC	4	OK	IPC	4	OK	IPC	4	OK	IPC	4	OK
IPC	5	OK	IPC	5	OK	IPC	5	OK	IPC	5	OK
IPC	6	OK	IPC	6	OK	IPC	6	OK	IPC	6	OK
IPC	7	OK	IPC	7	OK	IPC	7	OK	IPC	7	OK
IPC	8	OK	IPC	8	OK	IPC	8	OK	IPC	8	OK

Intelligent strategies for modern GMO testing

Step	Purpose	suitable methods
Screening	broad screening for the presence of GMOs	Element-specific (Modification-specific)
↓		
Identification	exclude non-approved, identify approved GMOs	Modification-specific (Event-specific)
↓		
Quantification	check for labelling requirements	Event-specific (validated CRL methods)

Increasingly complex approval situation

- **GMOs with new genetic modifications enter the market**
e.g. **Mavera™** corn (line LY038) or **TREUS™** soybean (line 305423)
both lacking known screening target sequences like 35S or nos
 - **New approvals lead to an asynchronous approvals situation**
(zero-tolerance for non-approved GMOs in the EU)
- ➔ **New GMO detection methods (screening, specific ...) are needed**

Detection of unknown (non-approved) GMOs

- **GMO incidences with unapproved GMOs are increasing**
e.g. Bt10 corn, Bt63 rice, LLRice601 ...
 - **Only GMOs with knowing screening elements are detectable**
- ➔ **New Screening targets for unknown GMOs have to be defined to guarantee GMO-analysis according EU requirements**