

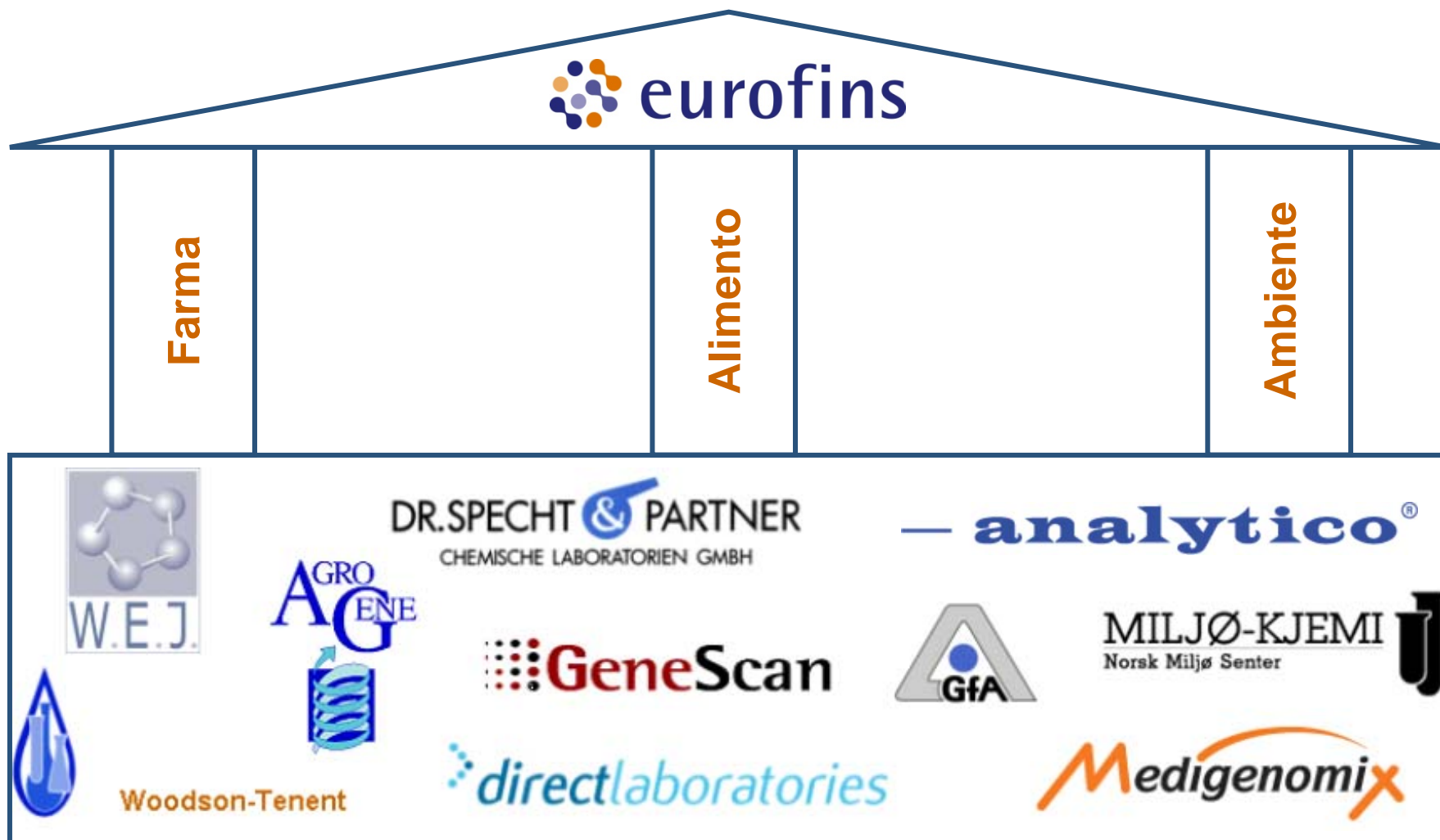


# **Setting up a GMO analysis laboratory in a GMO growing and commercialising country – aspects of the production chain quality**

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- Introduction
- Relevant dimensions (specific customer requirements and cost efficiency)
- Quality dimensions
- Challenges in practice



- 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				
Control	Ct	dRn	Out		Control	Ct	dRn	Out		Control	Ct	dRn	Out	
SD	31.7	0.78	0		SD	32.1	0.82	0		SD	32.5	0.81	0	
Delta	0.35	0.05			Delta	0.35	0.04			Delta	0.39	0.03		
CutOff	8	0.2			CutOff	8	0.2			CutOff	4	0.5		
	31.7	0.16				38.1	0.12				36.5	0.30		
NTC	Ct	neg	pos		NTC	Ct	neg	pos		Pos Control	Ct	dRn	Out	
Extr Control	45.0	0	0		Extr Control	45.0	0	0		Extr Control	32.9	0.88	valid	
Threshold	N/A	N/A	N/A		Threshold	N/A	N/A	N/A		Threshold	N/A	N/A		
	0.03					0.04					0.96			

Well	Task	35S	Ct	dRn	Result	NOS	Ct	dRn	Result	IPC	Ct	dRn	Total
A1	N	35S	45.0	0.90	neg	NOS	45.0	0.88	neg	IPC	32.6	0.88	valid
A2	N	35S	45.0	0.90	neg	NOS	45.0	0.88	neg	IPC	32.6	0.88	valid
A3	N	35S	45.0	0.90	neg	NOS	45.0	0.88	neg	IPC	32.9	0.88	valid
A4	N	35S	45.0	0.90	neg	NOS	45.0	0.88	neg	IPC	32.0	0.84	valid
A5	N	35S	45.0	0.90	neg	NOS	45.0	0.88	neg	IPC	32.6	0.88	valid
A6	N	35S	45.0	0.90	neg	NOS	45.0	0.88	neg	IPC	32.2	0.83	valid
A7	P	35S	32.0	0.74	pos	NOS	32.1	0.82	pos	IPC	32.0	0.88	valid
A8	P	35S	32.9	0.80	pos	NOS	32.1	0.82	pos	IPC	32.6	0.88	valid
A9	P	35S	32.0	0.73	pos	NOS	32.9	0.88	pos	IPC	32.0	0.88	valid
A10	P	35S	32.1	0.85	pos	NOS	32.5	0.82	pos	IPC	32.7	0.87	valid
A11	U	35S	45.0	0.90	neg	NOS	38.1	0.41	pos	IPC	34.4	0.40	valid
A12	U	35S	45.0	0.90	neg	NOS	38.9	0.37	pos	IPC	34.4	0.41	valid
B1	U	35S	45.0	0.90	neg	NOS	45.0	0.88	neg	IPC	34.2	0.43	valid
B2	U	35S	45.0	0.90	neg	NOS	45.0	0.88	neg	IPC	34.2	0.42	valid
B3	U	35S	45.0	0.90	neg	NOS	45.0	0.88	neg	IPC	34.8	0.81	valid
B4	U	35S	45.0	0.90	neg	NOS	45.0	0.88	neg	IPC	35.0	0.81	valid
B5	U	35S	34.4	0.86	pos	NOS	34.7	0.96	pos	IPC	32.5	0.73	valid
B6	U	35S	34.3	0.88	pos	NOS	33.9	0.98	pos	IPC	32.3	0.71	valid
B7	U	35S	45.0	0.90	inhibited	NOS	45.0	0.88	inhibited	IPC	45.0	0.82	inhibited
B8	U	35S	45.0	0.91	inhibited	NOS	45.0	0.81	inhibited	IPC	45.0	0.82	inhibited
B9	U	35S	36.6	0.40	pos	NOS	45.0	0.81	neg	IPC	34.8	0.58	valid
B10	U	35S	36.7	0.49	pos	NOS	45.0	0.82	neg	IPC	33.7	0.81	valid
B11	U	35S	45.0	0.91	neg	NOS	45.0	0.82	neg	IPC	32.5	0.84	valid
B12	U	35S	45.0	0.93	neg	NOS	45.0	0.88	neg	IPC	32.9	0.89	valid

## Examples:

- Short delivery times (express services)
- Complex analytical strategies, confirmations etc. dependent on screening results
- Electronic data exchange (EOL)
- Import and Export - Eurofins/GeneScan labs (Germany/USA/Brazil)
- High quality technical support

## ■ Quality

- ISO 17025 accreditation – Brazil - Inmetro
- MAPA accreditation – official analysis
- CQB – CTNBio (not mandatory) – routine analysis
- Proficiency testing schemes
- CRL list of methods and guidance documents for method validation
- ISO 21569:2005, ISO 21570:2005...  
(general and specific GMO testing prerequisites)
- GLP-OECD/Inmetro: Eurofins do Brasil is also in process of GLP recognition – for GMO analysis (before and post-monitoring approvals)

## UP-to date information

- Customer consultancy to apply the right systems and 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 ...)
- Internal “traffic” and air flows
- Number and location of work areas
- All primer, mastermix and standards are produced (ISO9001) and validated (R&D) by Eurofins/GeneScan GmbH



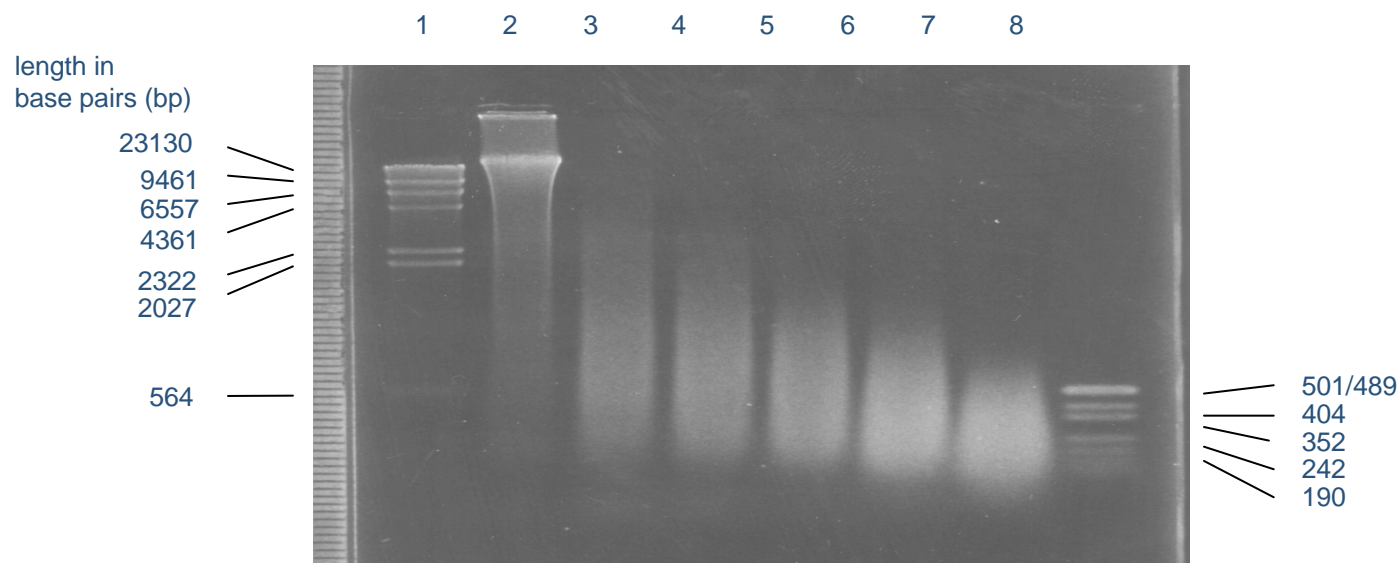


## Process architecture and IT (LIMS)

- To avoid human error
- To improve documentation
- To improve “best practices”

## Analytical controls

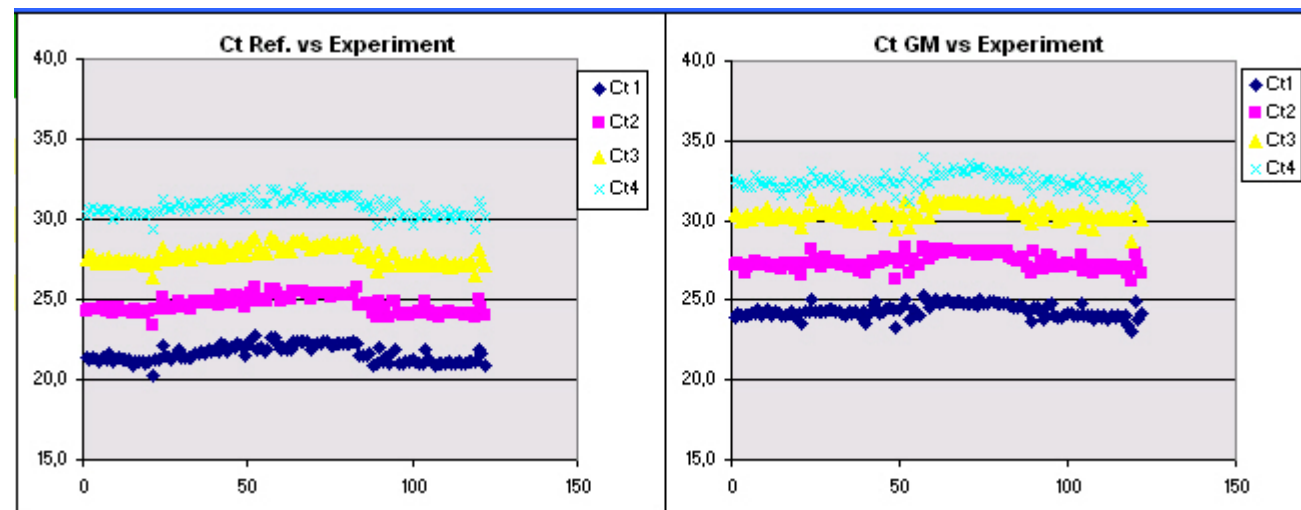
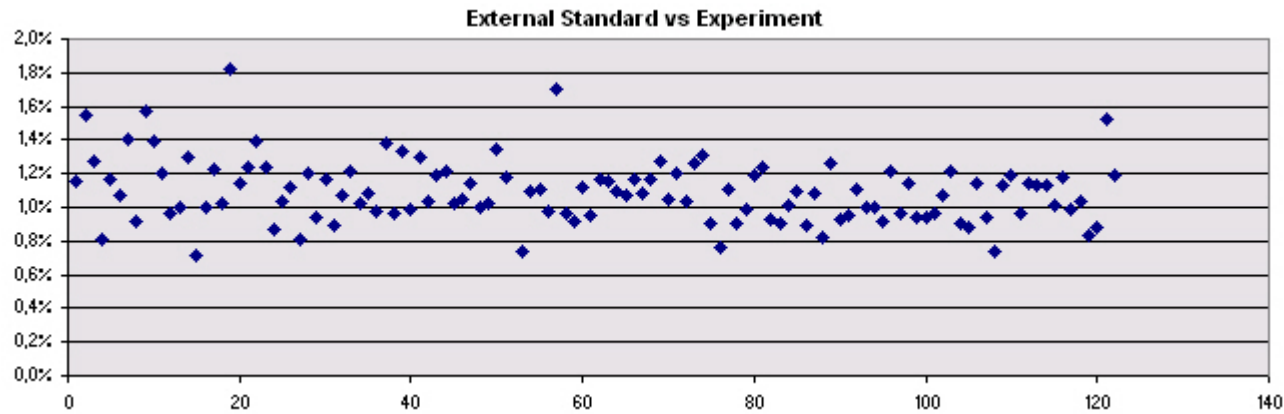
- DNA amount (misleading results of OD, fragmentation)
- Positive control (should include extraction process)
- Negative control (enough in number as compared to sample numbers)
- Inhibition control: Prefer low level spiked or quantitative IPC controls



**Shortening of DNA Fragments by Heat**



## QC Charts as indicators to alert failure

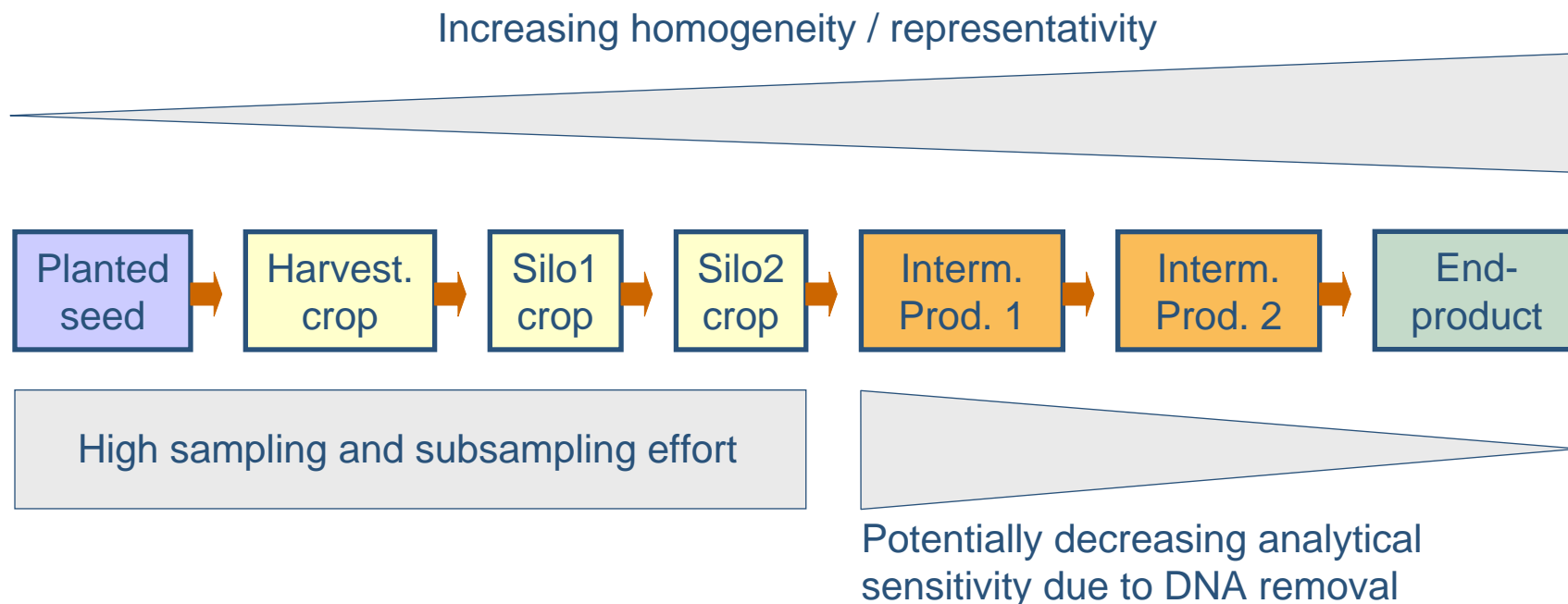




## Experience and evaluation of results

- If DNA content is low: Is it method failure or matrix property?
- A positive result could be misleading (e.g. CaMV, soy contamination in corn)
- Quantitative results can only be interpreted in a meaningful way if statistical knowhow is applied – evaluation sheet , controls and expertise

## Statistics and matrix experience



- Increase number of approved events in Brazil (new strategies)

Crop	# Approved events (CTNBio)
Soy	1
Corn	11 (8 last two year)
Cotton	6

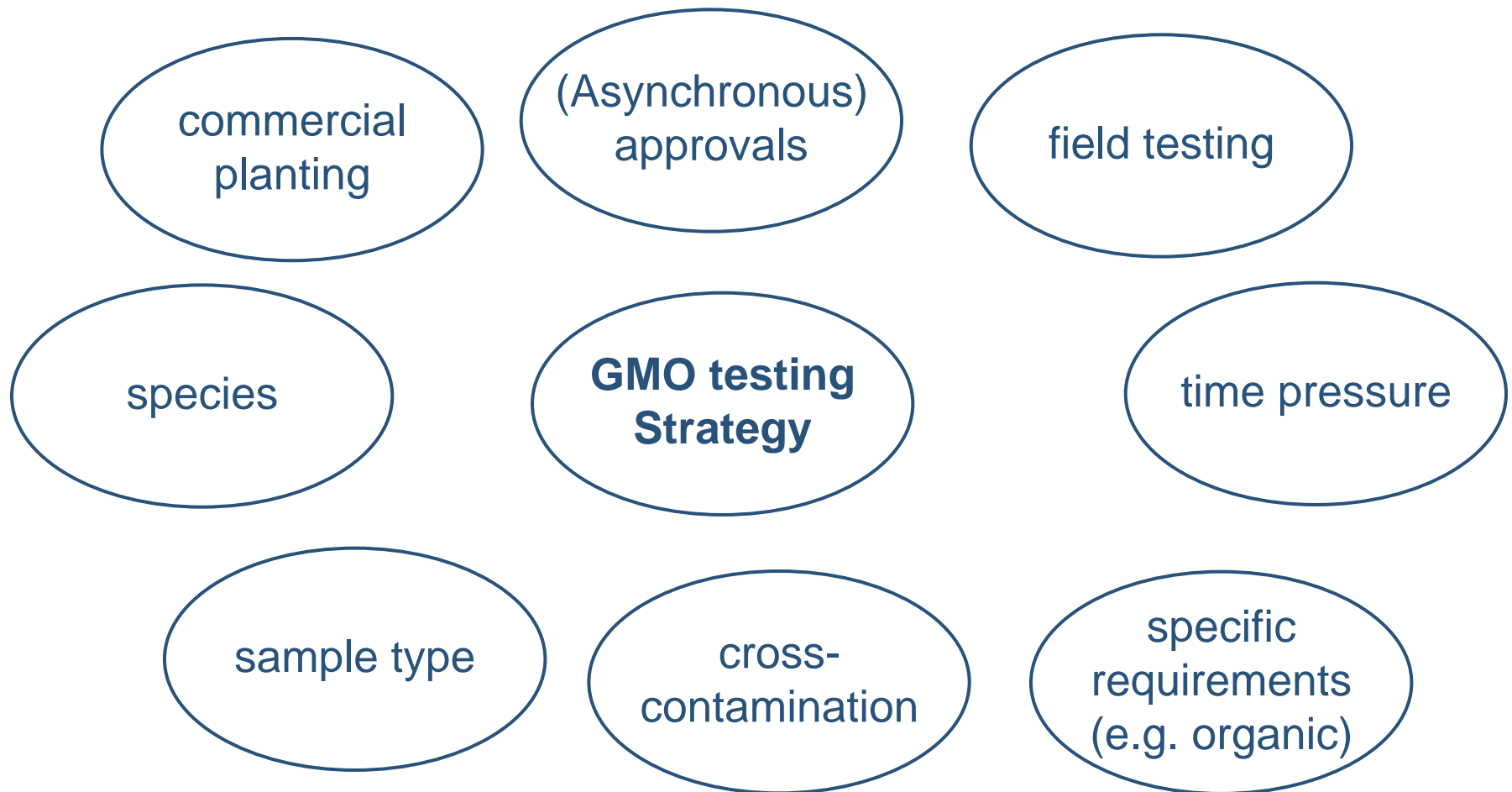
- New approvals at exporter countries
- Country infra-structure for segregation (GMO and Non-GMO)
- Presence of non-approved events (seeds contamination, country boundaries, GMO experimental fields) – eg: Flax Triffid (Canada)
- Stacked events (3 corn and 2 cotton varieties)
- Reagents and sample importation (Proficiency Tests)

## Intelligent strategies for modern GMO testing

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



→ Finding the right solution ....



**Thank you for your attention!**



GeneScan



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