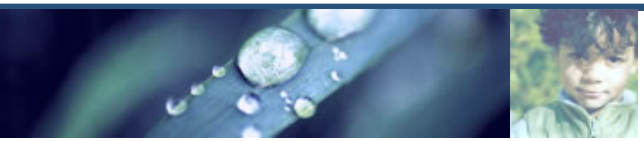


Setting-up a GMO detection laboratory

Success factors to reliable performance

Andreas Wurz, Eurofins GeneScan

April 2009

Agenda

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

The Basics

- 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

Criteria

Three main dimensions

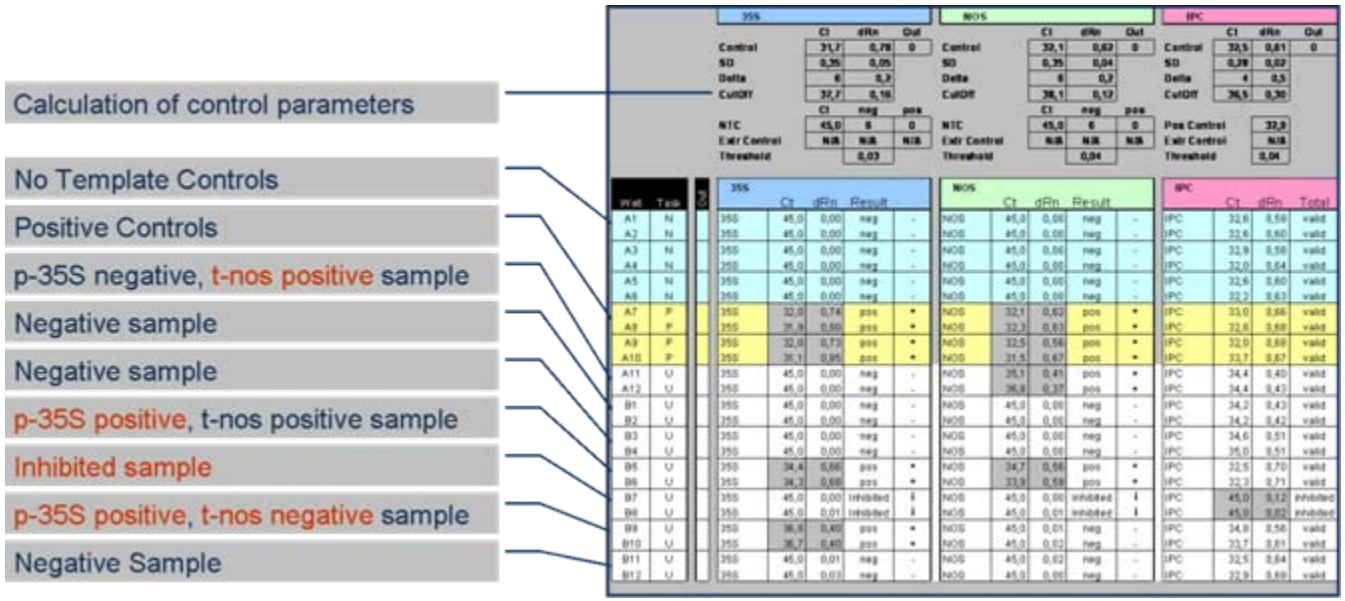
- Specific Customer requirements
- Cost efficiency
- **Quality**

Different Situations

- Inhouse-, private service- or public lab (what to confirm) ?
- Thoughput, 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 …?)

Cost Efficiency

- 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



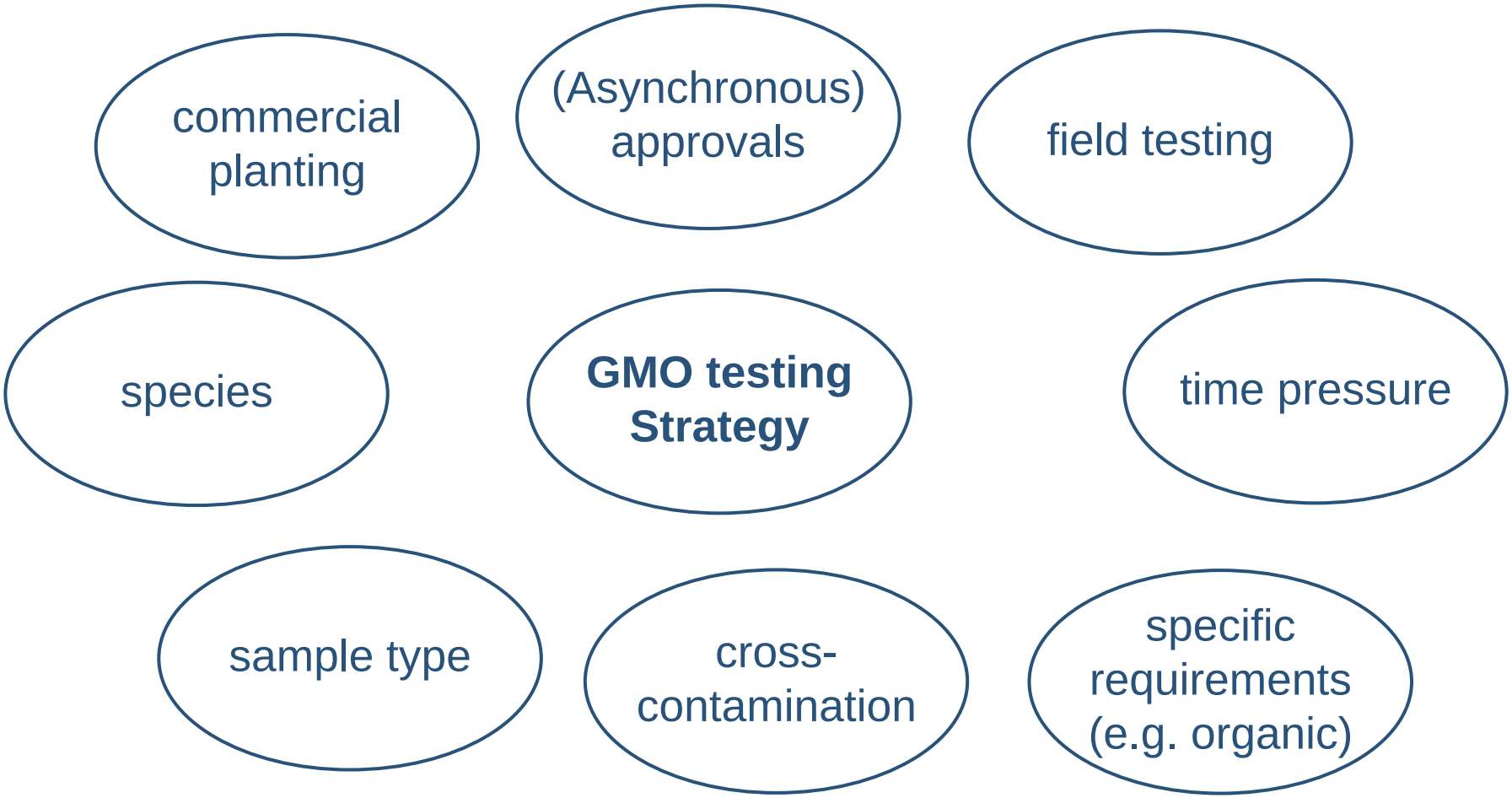
Customer Requirements

Examples:

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

„Real-life“ GMO Testing

→ **Finding the right solution ….**



Quality – Information/Knowledge

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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

Quality – Staff & Infrastructure

Contamination control

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

Quality - Process

Process architecture and IT (LIMS)

- To avoid human error
- To improve documentation

INHOUSE Method Validation

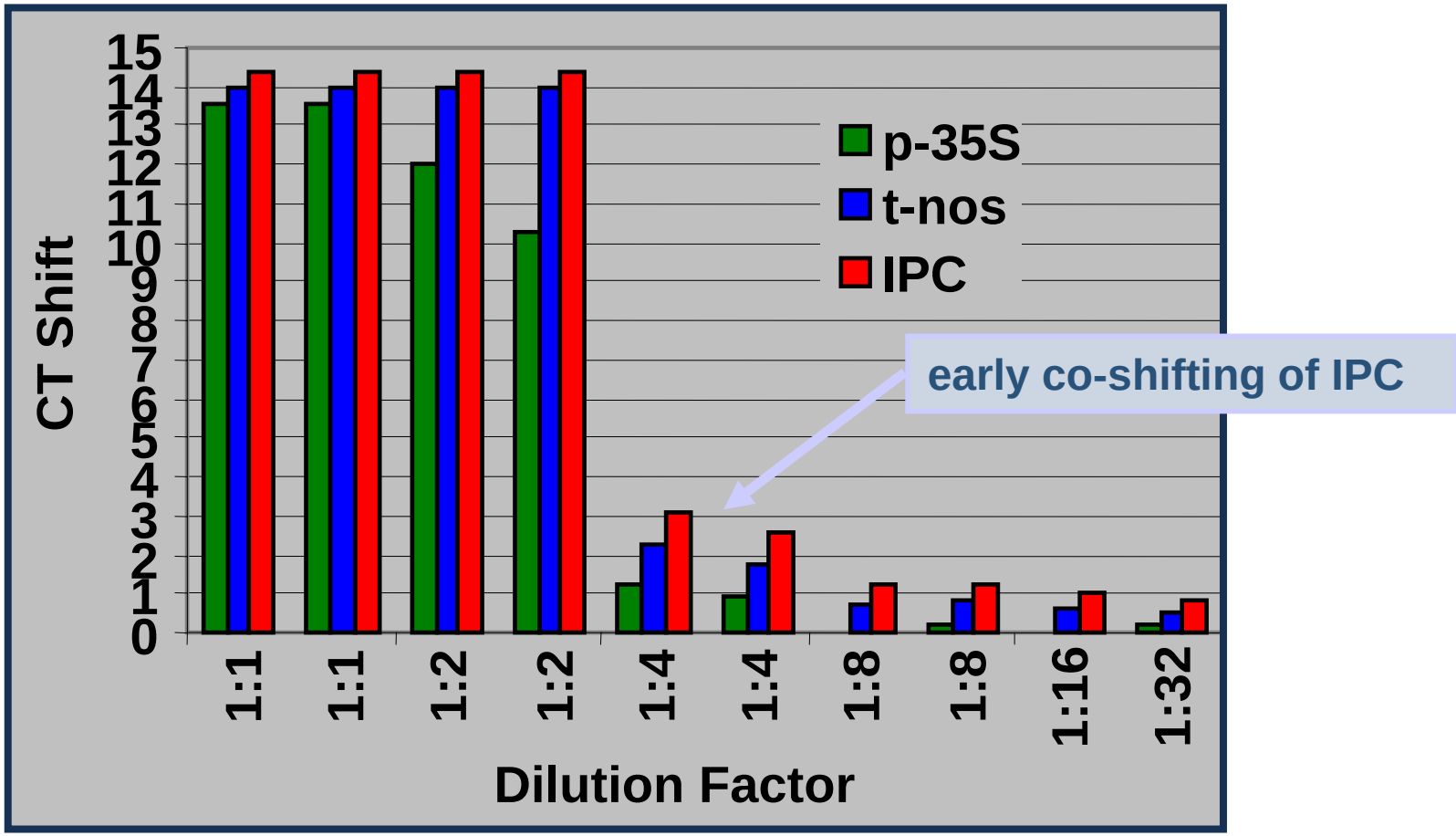
Why? Different equipment, supplies etc …

- 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.

Validation Aspects (Example)

Example Robustness: IPC reliability

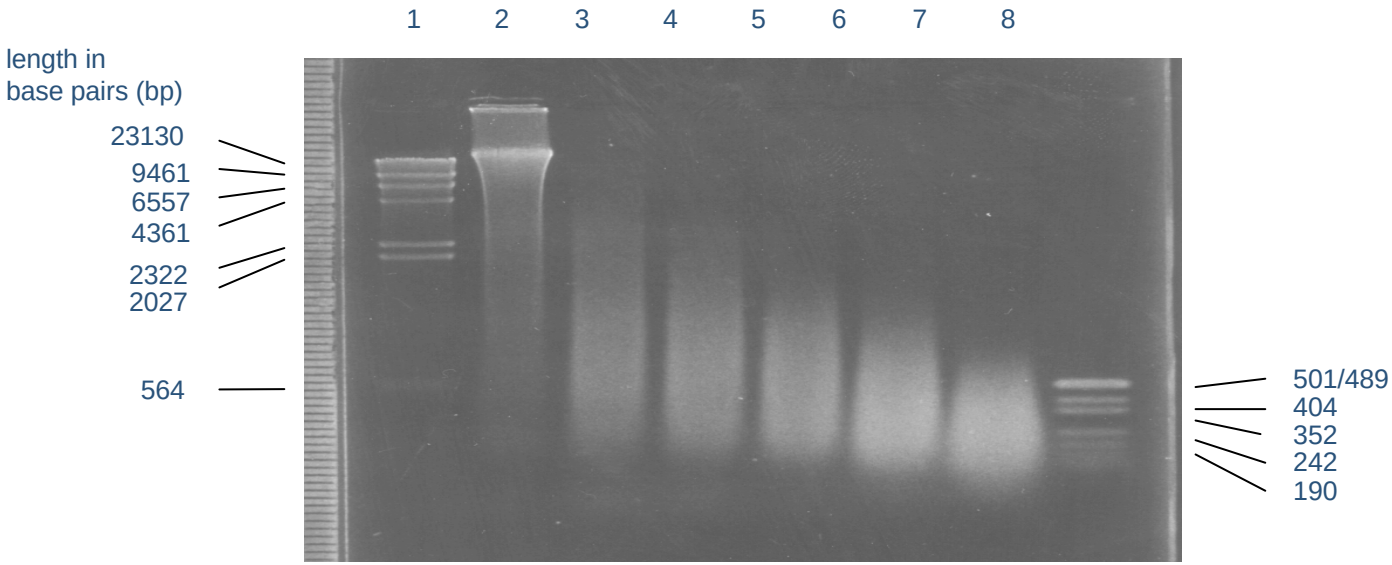
Ct shifts with dilution series of inhibitor-spiked DNA preparations



Quality – Analytical Controls

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

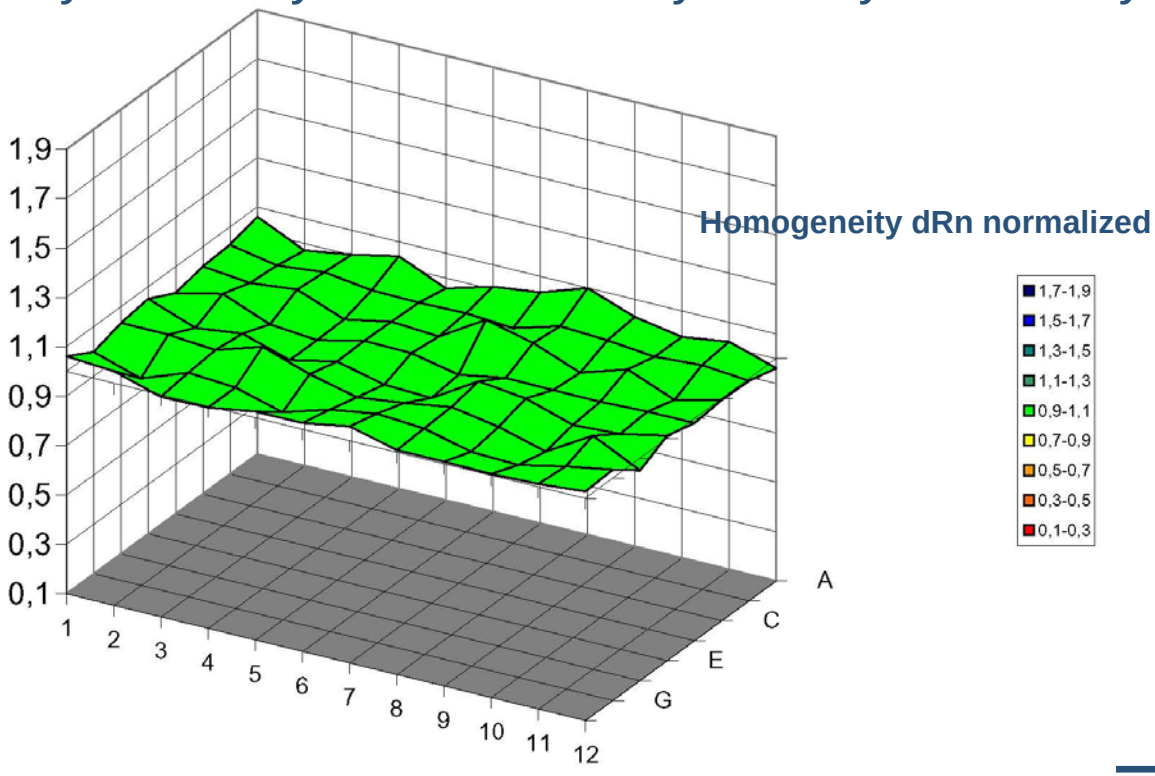


Shortening of DNA Fragments by Heat

Quality – Controls / Monitoring

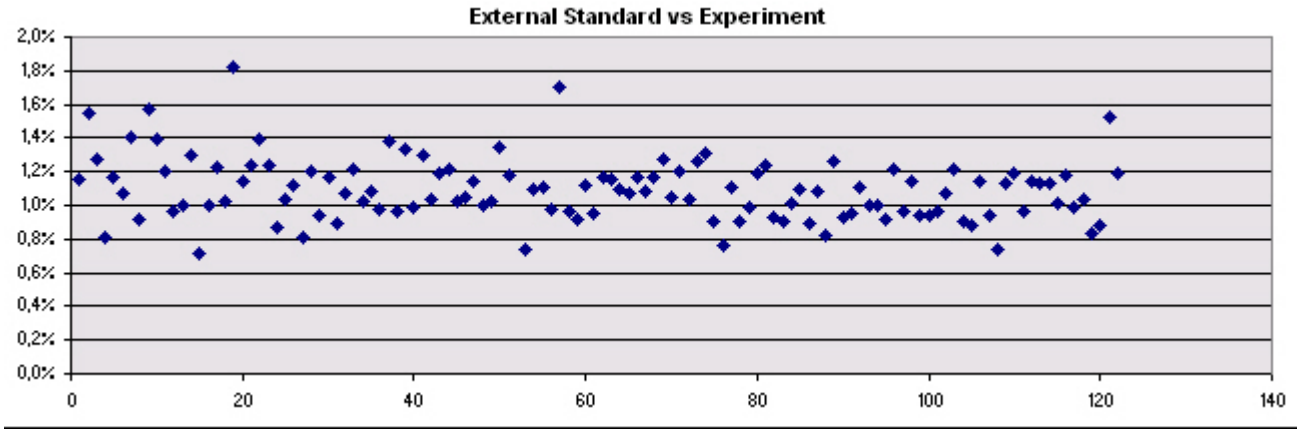
Reagent QC and performance monitoring for equipment

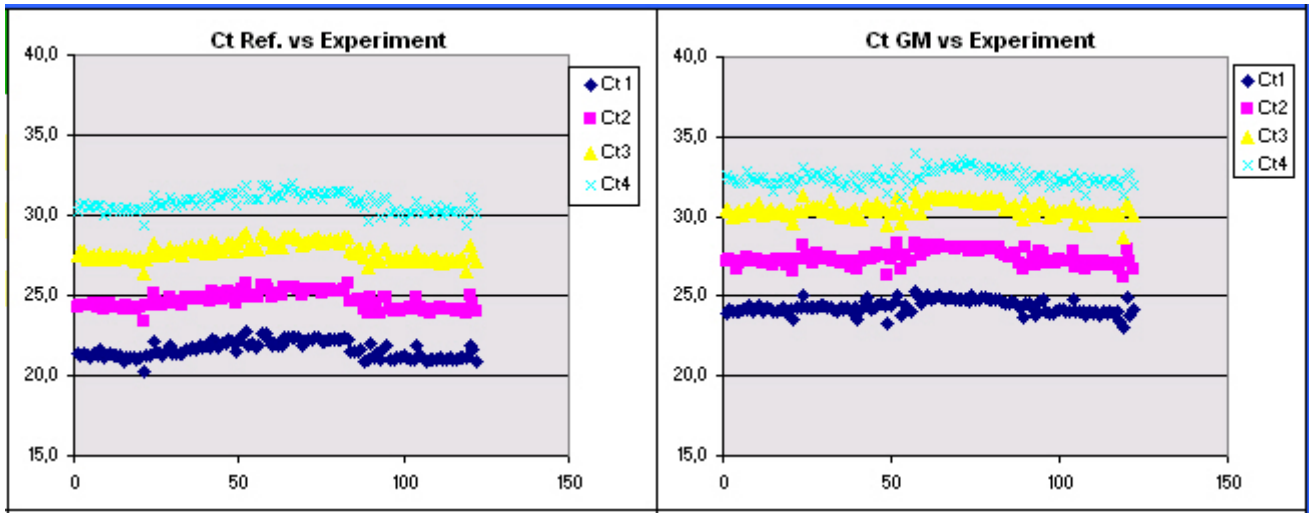
- A new batch of reagent may be different in quality (define QC acceptance criteria)
- A system may not run robustly on a cycler or a cycler may be out of spec



Quality – Controls / Monitoring

QC Charts as indicators to alert failure





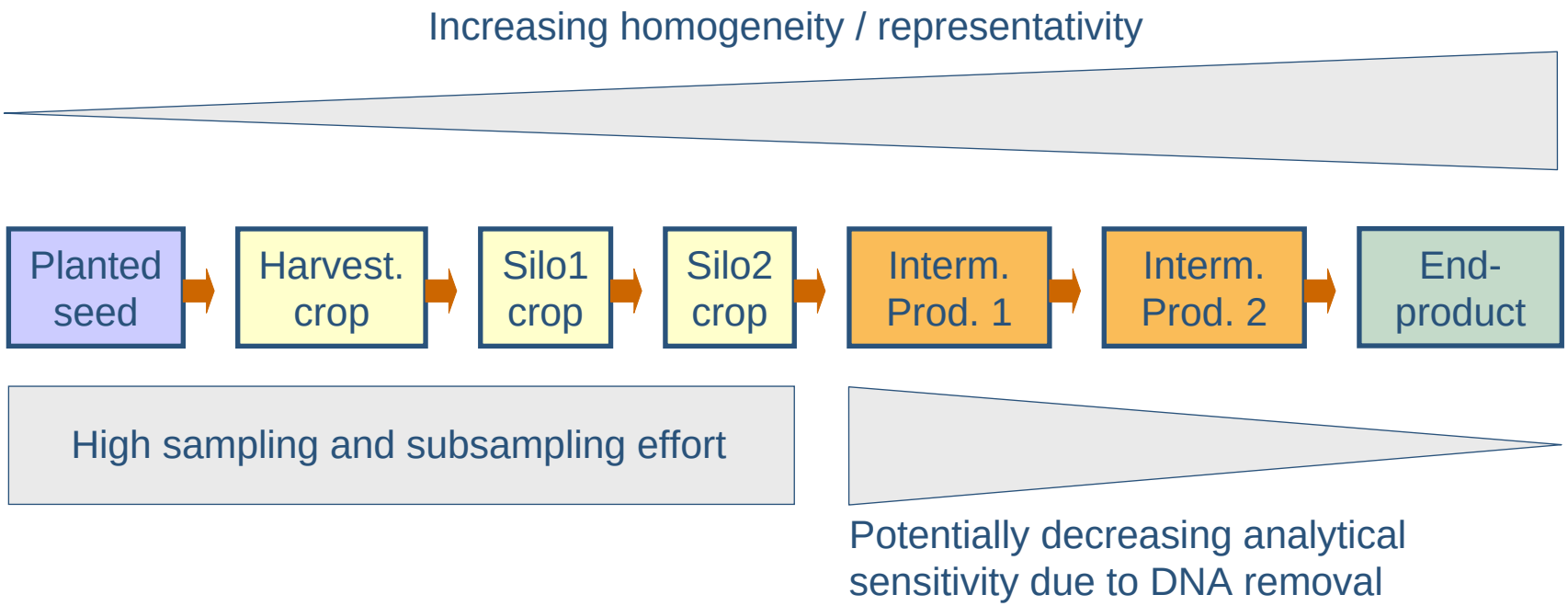
Quality – Interpretation of Results

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)
- Quantitaive 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

Experience along the Chain

Statistics and matrix experience



Challenges & Conclusions I

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”**

Summary & Conclusions II

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 !

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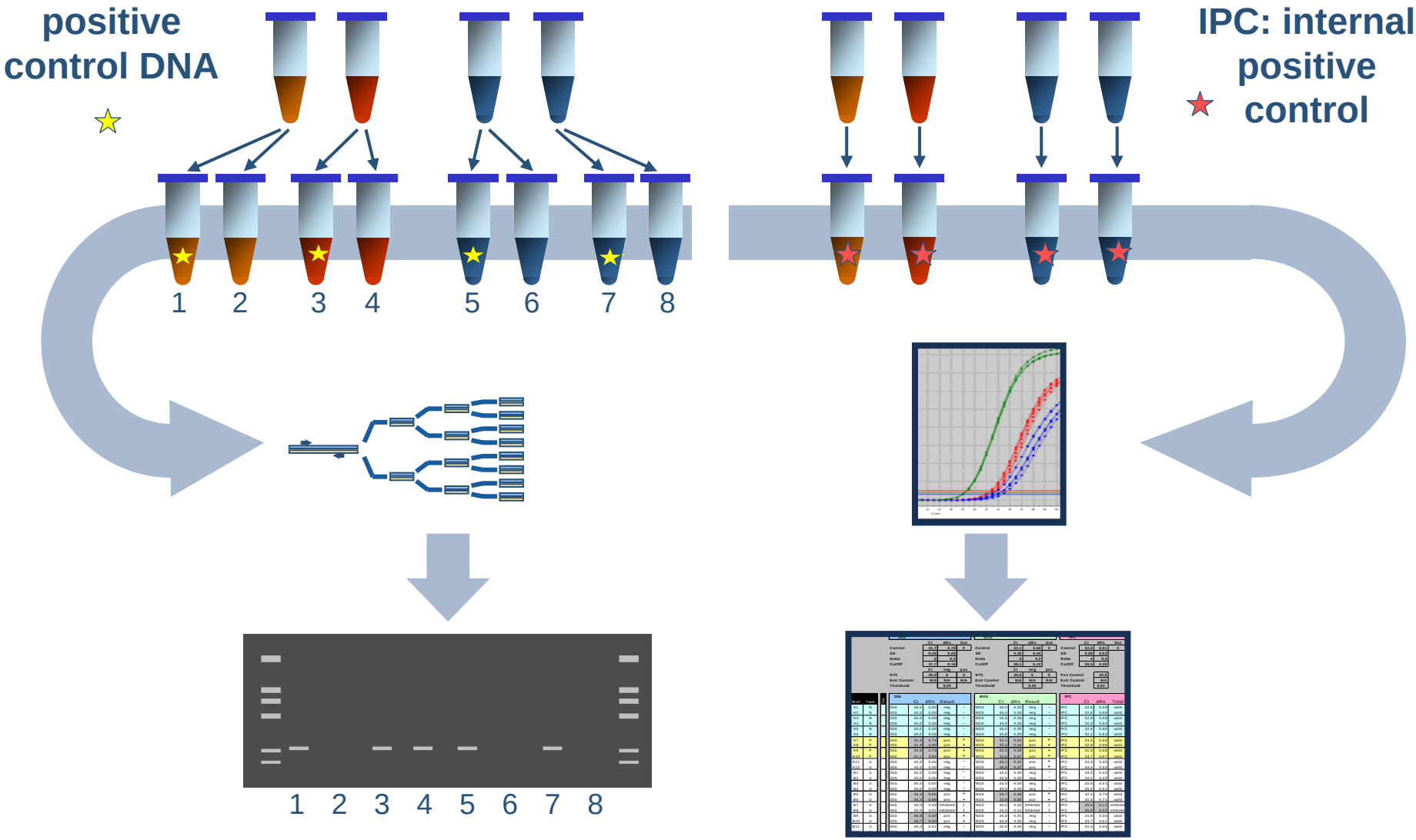
Add. slides

Qualitative Analysis



„Real-life“ GMO testing

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)

Future Challenges

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

Future Challenges

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