Towards higher throughput GMO testing: matrix-based screening approaches and multi-target detection systems

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The need for screening in traceability of GMOs

The number of new GMOs is increasing constantly

Employing only event-specific methods for GMO testing thus becomes impractical

Time and cost effective strategies include the development of **screening approaches** using element- or construct-specific detection methods
Screening strategies for GMO testing: main concerns

1. Planning.
   How can I make sure the methods used in the screening strategy will not “miss” one of the GMO I want to screen for?

2. Drawing conclusions
   How can I gain the most information from the results of the screening results, i.e. narrowing down the pool of potential candidates.

A number of "GMO target matrices" have been developed to support the planning and evaluation of screening approaches.
Detection

Information
Information on GMO detection by screening methods

Event annotation

+ Many public sources of information (eg the BCH LMO registry)

- Elements given the same name in various GM events can differ at the level of the DNA sequence
Information on GMO detection by screening methods

- Event annotation

  Event sequence

  + Allows direct prediction of detection by bioinformatics tools

  - Event sequences difficult to obtain
Information on GMO detection by screening methods

+ Gives a "formal" answer for the detection of an event by a detection method (i.e. not a prediction)

- Very high confidence in the experiment is crucial (sample purity, quality of execution, ...). Significant work required.
Examples of available matrices

**GMOfinder**

Specific profile of 15 elements and constructs for >350 GMOs
Information taken from experimental data, literature, applications for GMO authorisation and other sources


**GMOseek**

Provides an overview of >270 genetic elements and their occurrence in >320 GMOs

The JRC GMO-Matrix application

Predicts (in silico) which GMO detection method(s) will detect which GMO event(s)

Links the information between

1. The JRC GMOMETHODS database

2. The internal Central Core Sequence Information System (CCSIS), a database that hold sequences of GMO inserts.
GMOMETHODS: The European Union Database of Reference Methods for GMO Analysis

Available at: http://gmo-crl.jrc.ec.europa.eu/gmomethods/

Mining of the public DNA sequence databases for GMO sequences

1 000 000 000s of sequences

Scientific literature

Patents

CCSIS

1000s of sequences
Available at: http://gmo-crl.jrc.ec.europa.eu/jrcgmomatrix/

From planning to analysis

From planning to analysis

Laboratory Sample

JRC GMO METHODS

Testing

JRC GMO Matrix

Interpretation and Report
EURL-GMFF support to harmonisation of GMO screening

Reference methods
(Validation, GMOMETHODS database)

Decision-support tools
(JRC GMO-Matrix, JRC GMO-Amplicons)
EURL-GMFF support to harmonisation of GMO screening

Reference methods
(Validation, GMOMETHODS database)

Decision-support tools
(JRC GMO-Matrix, JRC GMO-Amplicons)

Ready-to-use system
(Pre-spotted plates)
Pre-Spotted Plates (PSP) for GMO detection

qPCR plastic support whose wells are spotted with dried primers & probes (GMO detection assays)

Advantage

Perform up to 96 assays in a single experiment, with limited steps
Development of a GMO Screening System

I. Which assays?

- p35S
- iNOS
- Ctp2-CP4EPSPS
- PAT
- BAR
- Cry1Ab/Ac

16 assays

- CV127
- DAS-40278
- DP-305423

+ 7 crop reference genes

GMOMETHODS database

JRC GMO-Matrix

II. Assay standardization for use on PSP (and re-assessment of method performance)

III. Building a screening strategy: combine PSP with a Decision Support System

Best identification strategy!
PSPs perform well and are functional

Performance of the strategy (PSP + JRC GMO-Matrix)

95.9%
Proof of concept study

Integration of PSPs in the quality system of official testing laboratories working under ISO17025 accreditation

- L1 screening
- L2 Experiments
- L3 Documents (SOP, forms, validation reports)
- L4 Accreditation Request
- L5

NEW
19 GM Maize ev.

NEW
13 GM Soy ev.
Semi-quantitative use of ePSP

Applicability of the “Real-Time PCR-Based Ready-to-Use Multi-Target Analytical System for GMO Detection” in processed maize matrices

Linda Kluga · Silvia Folloni · Marc Van den Bulcke · Guy Van den Eede · Maddalena Querci

Table 5 Theoretic correlation between the ΔCt, the dilution factor and the GM%:

<table>
<thead>
<tr>
<th>ΔCt</th>
<th>Dilution</th>
<th>GM%</th>
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<td>16384</td>
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</tbody>
</table>

ΔCq = Cq_{GM} − Cq_{Txn} ≈ %GM
Estimation and assessment of ΔCq Thresholds

Above 0.9%

Below 0.9%

Assessment on simulated samples

<table>
<thead>
<tr>
<th>GM%</th>
<th>&lt;0.9%</th>
<th>Quant.</th>
<th>&gt;0.9%</th>
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<td>0.1</td>
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<td>0.9</td>
<td>0.0</td>
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<td>41.1</td>
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<tr>
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<tr>
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<td>0.7</td>
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Thank you for your attention!