European Commission

Detection of (plant) products developed by targeted mutagenesis techniques

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Joint Research Centre

European Commission - JRC

EURL
European Union Reference Laboratory
for GM Food & Feed

Providing scientific evidence throughout the whole policy cycle

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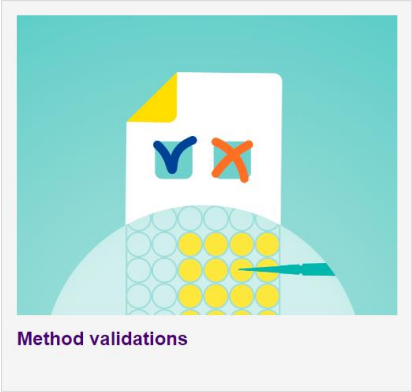
GMO analysis

1. Validated detection methods



2. Certified Reference Material for the GM event

3. Official controls

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GMO analysis – Validated detection method

- An applicant submits a detection method to the EURL GMFF
- The EURL GMFF validates the method in a step-wise workflow

Step 1: Documentary check

Step 2: Scientific assessment of the method according to ENGL MPR criteria

Step 3: Experimental analysis in-house and *in silico* specificity assessment

Step 4: Ring trial involving 12 National Reference Laboratories

Step 5: Assessment of methods performance, reporting and publication

Step 6: Inclusion of the EU reference method in the GMOMETHODS database

Supported by the European Network of GMO Laboratories, e.g. "*Guidance document on the Minimum Performance Requirements (MPR) for analytical methods of GMO testing (2015)*"

GMO METHODS database

GMOMETHODS

Last update: 15/02/2022

GMOMETHODS provides information on EU reference methods for GMO Analysis.

The tool assists control laboratories in selecting the appropriate methods, supplies core data on the experimental protocol and information on methods performance, ring-trial design, plasmid standards, reference materials and links to published articles or validation reports.

The assays are DNA-based detection methods that have been validated according to the principles and requirements of international standards and can assure therefore consistent and reproducible results in the analysis. Data is retrieved from peer-reviewed journals and final reports of collaborative studies. Few assays have been verified by the EURL GMFF for EU legal purposes.

Perform your search by keyword, select a GMO unique identifier or click a link in the section below.

keyword | Search | or by GMO unique identifier:

Quantitative methods

- GMO specific
  - Event specific
    - Cotton
    - Maize
    - Oilseed rape
    - Papaya
    - Potato
    - Rice

Qualitative methods

- GMO specific
  - Event specific
    - Carnation
    - E. coli
    - Maize
    - Oilseed rape
    - Papaya
    - Rice

Database of validated GMO detection methods

https://gmo-crl.jrc.ec.europa.eu/gmomethods/

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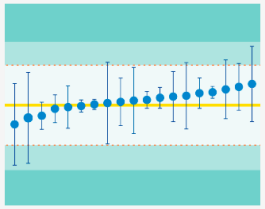
What we also offer

What we do

Legal basis

Method validations



Proficiency tests

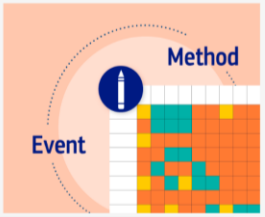
Unauthorised GMOs

Guidance documents

Training & Workshops

Tools

GMOMETHODS



GMO-Matrix

GMO-Amplicons

Other information

Our publications

European Network of GMO Laboratories

National Reference Laboratories

https://gmo-crl.jrc.ec.europa.eu/

GMO analysis – Certified Reference Material

- Analytical measurements require **CRMs** for calibration or as control materials to ensure **reliability** and **comparability** of the measurement results

- For **each GMO application**, the applicant needs a CRM (ISO 17034) produced by:
  - Joint Research Centre (JRC), BE (35 GMOs)
  - American Oil Chemists' Society, US (47 GMOs)

GMO analysis – EU food and feed control system

REGULATION (EU) 2017/625 applies among others to:

(a) food and food safety, integrity and wholesomeness at any stage of production, processing and distribution of food;

(b) deliberate release into the environment of Genetically Modified Organisms (GMOs) for the purpose of food and feed production;

(c) feed and feed safety at any stage of production, processing and distribution of feed and the use of feed;

…

(h) requirements for the placing on the market and use of plant protection products and the sustainable use of pesticides;

(i) organic production and labelling of organic products;

(j) use and labelling of protected designations of origin, etc.

The dual purpose of GMO analysis in the EU regulatory context

1. To **inform** and give the consumer a choice in buying (safe) GMO-containing products or not

2. To **identify** the presence of unauthorised GMOs which may potentially pose a safety risk for the consumer or the environment

GMO analytical workflow

First: preparation of the laboratory sample, homogenisation to fine powder, DNA extraction and assessment of the quality and quantity of DNA

Crop?

- Qualitative detection of plant species present in the sample
  - Maize, soybean, oilseed rape, cotton, sugarbeet (crops with authorised GMOs in EU)
  - Rice, potato, wheat, linseed, flax, papaya,… (crops with unauthorised GMOs in EU)

GMO?

- Qualitative detection of the presence of GMOs, using screening markers
  - Plus a few GM events that do not contain any of those common **screening markers**

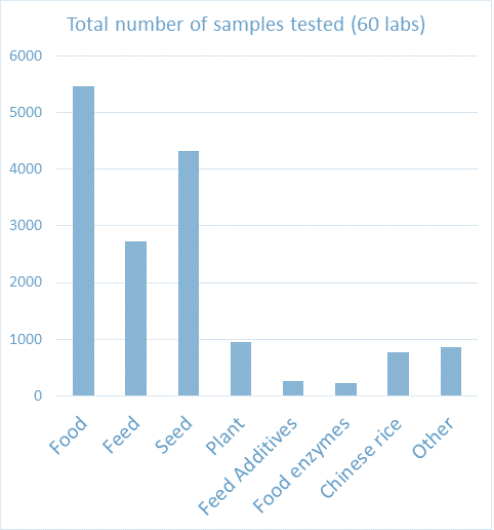
GM event(s) identification

- Qualitative identification of authorised GMOs or EU-pending authorisations
  - Using event-specific detection methods validated by the EURL GMFF
  - If unknown GMO: unauthorised GMO >> Rapid Alert System RASFF notification + further investigation

GM event(s) quantity

- Quantification of the GMO content
  - Using event-specific detection methods for each GMO identified
  - For authorised GMOs and EU-pending authorisations

GMO control analysis in the EU



37 National Reference Laboratories and >100 official laboratories perform GMO analysis for law enforcement

Every year > **30.000** samples are analysed for GMOs, including food, feed, seed, plants, etc.

Twice as much **food** samples are analysed compared to *feed* samples

While the large majority of **food** samples tested are negative for GMOs, many of the *feed* samples contain GMOs and are correctly labelled as such, and occasionally unauthorised GMOs are detected.

Conclusion: **the EU GMO control system works well**

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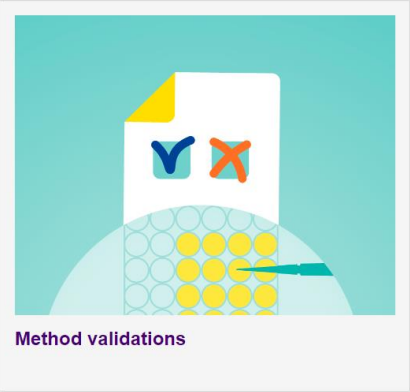
GMO analysis: fit for NGT products?



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Technology and market landscape

- Scientific Literature Review – State-of-the-art scientific and technological developments in NGTs
  - Dynamic and evolving field, many techniques and variants, different types of increasingly precise sequence alterations
- Market applications review - Products derived from NGTs that are, or in the near future are expected to be, on the market

April, 2021

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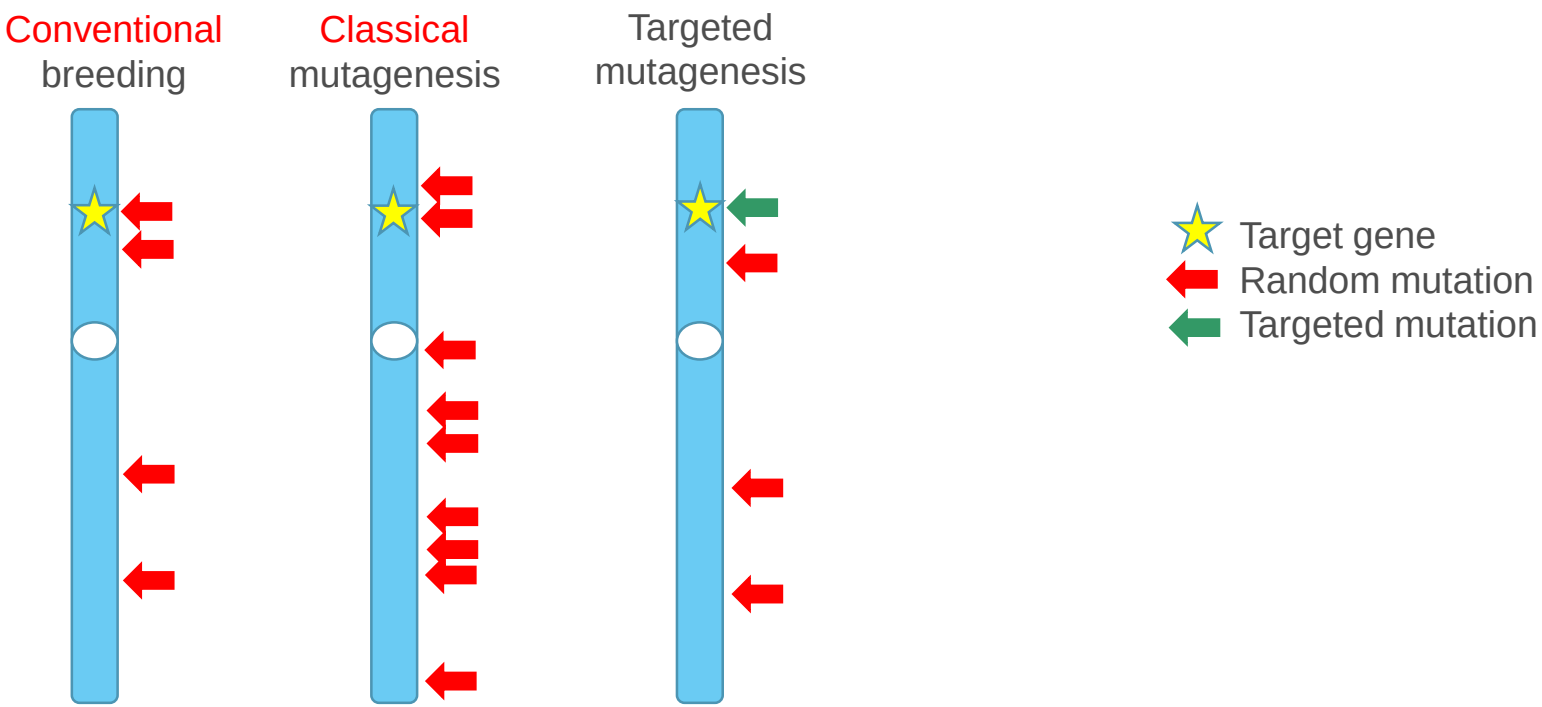
Specificity of methods for NGT products

- NGTs create mutations in the genetic material, *i.e.* in the DNA
- Spontaneous alterations in nature and conventional breeding may create similar mutations in the genetic material



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Validation of NGT products?

- An addendum to **Minimum Performance Requirements** guidance (MPR2) includes recommendations for the data requirements and validation of methods for the detection of NGT products (mainly if containing mutations of a few nucleotides)
  - Specificity tests: specifications on how to perform, on which materials, etc.
  - If more than one mutation: method for each mutation required
  - Technique update: MPR for digital PCR methods (before only for qPCR)

Remaining question:

What to conclude if same mutations already occur in nature?

Event-specific identification

- Detection methods rely on DNA sequence recognition
  - **If sequences are identical, no method can differentiate** two products made by different means (conventional or new techniques)
  - The **shorter** the sequence **difference** between two sequences, the **more difficult to discriminate** them by any method
  - These methods need not only to detect but also to **quantify** the GMO/NGT products
  - Methods for the detection of single nucleotide variations (SNVs) have been described in the literature, but their specificity also **depends on the sequence context** around the mutation and may not always be sufficient (e.g. repetitive or high G/C rich sequences)
  - Robustness and transferability of such methods to other laboratories **need to be demonstrated** before their use in enforcement
  - To remain specific when applied to complex and compound food and feed samples
- Detection without event-specific identification creates uncertainty
  - Is the mutated sequence derived from NGT or from conventional breeding?
  - Is there court-proof evidence for this?

The analysis of NGT products

- **Screening** not possible as there are no common markers
- Hence, only **event-specific** detection methods to be applied
  - For pure products, e.g. broccoli, to apply the methods for known broccoli NGT products
  - For compound products (e.g. feed), to apply all methods for all known NGT products in all species present, i.e. potentially hundreds of methods and continuously increasing
- Testing will only detect the NGT products with **known**, **unique** sequences and a validated detection method
- Moreover, one NGT product may contain **more than one mutation**, hence needs to be analysed with more than one detection method
- This will have a significant impact on the enforcement analysis
- There are no alternative high-throughput methods that can be applied in routine analysis

Certified Reference Material (CRM)

- In principle, CRM production is also possible for NGT products
  - Uncertainty: are CRM producers ready to face increasing demand?

Conclusions

- Analytical methods for the detection of NGT-plant products can be developed, but perhaps **not in all cases**

- Demonstrating the **specificity** of such methods remains the major problem and is opt for court disputes when applied for enforcement

- GMO analysis in official control laboratories may be *compromised* by the significantly increased number of (event-specific) tests per sample for NGT products

- **Unknown NGT products**, e.g. entering the market through imports, **cannot be detected** in routine analysis

Acknowledgements

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Thank you for your attention

Questions
Comments
Suggestions

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