Detection and identification of NGT organisms
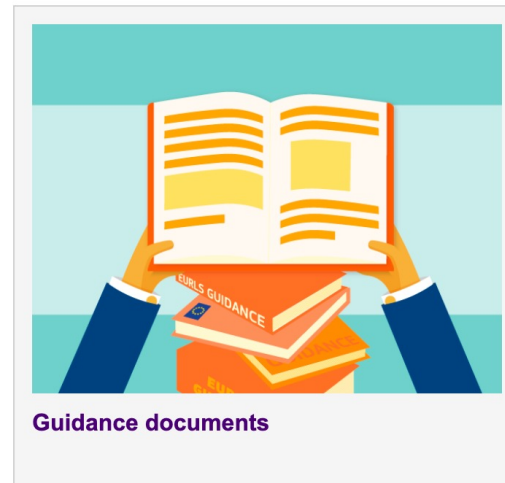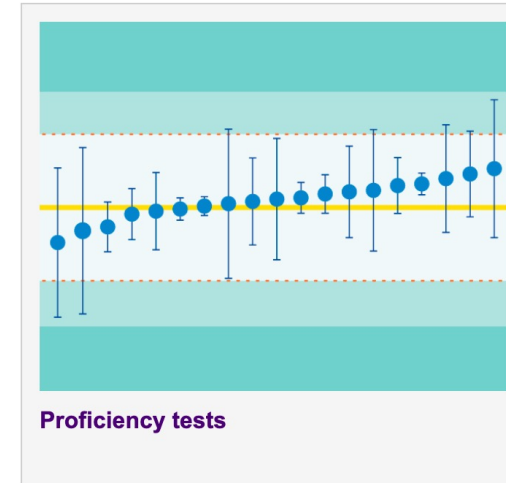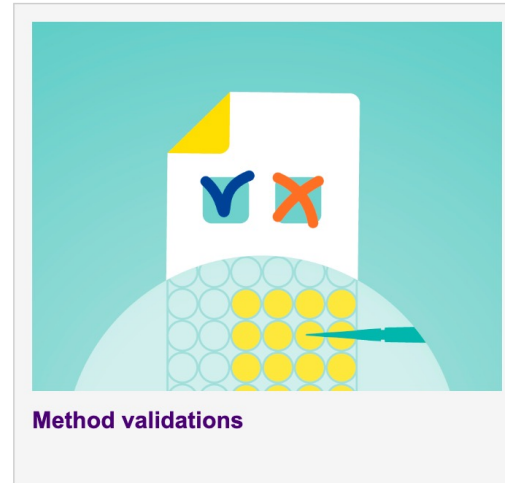
Sarah Agapito
Research Professor

14.06.2023

Detection and identification of GMOs

Legal basis

Method validations

Proficiency tests

Unauthorised GMOs

Guidance documents

Training & Workshops

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

Research on detection and identification in NORCE

FoodPrint Project

Traceability and labelling of gene-editing products in the food chain

Funded by The Research Council of Norway (2020-2024; 1M EUR)

Partners in Austria, France, Germany, Lebanon and Brazil

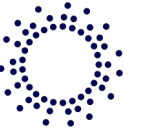Stakeholders include industry, regulators and academia

SeqApp Project

Sequencing strategies and their applicability for the molecular characterization of current and next generation GMOs

Funded by The German Federal Agency for Nature Conservation BfN (2021-2024; 500k EUR)
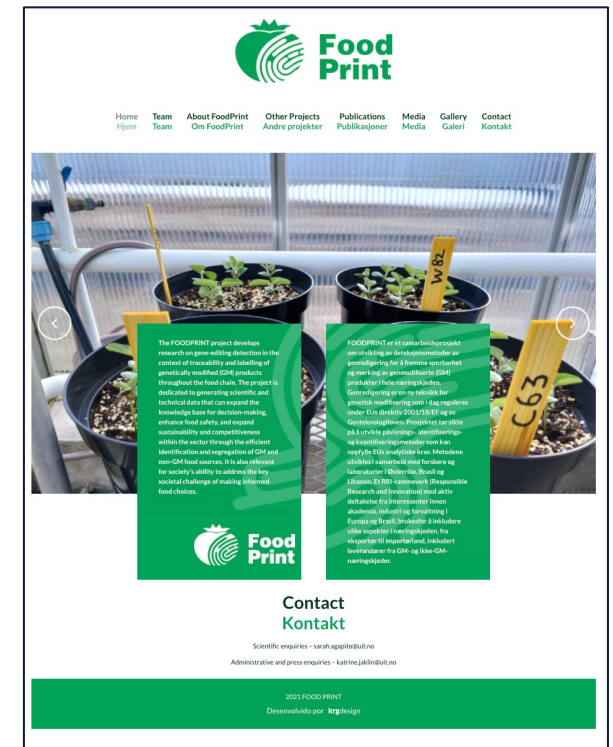
www.foodprintproject.org

Identified challenges for the detection and identification of NGT organisms

No common transgenic elements (promoter, transgene, terminator sequences)

No unique DNA sequences (no unique recombinant DNA insertions or junctions regions)

SNP genotypes or small InDels require sensitive PCR methods

Lack of biological reference material

Ribarits et al. *Agronomy* **2021**, *11*(1), 61

Typology of NGT organisms

Small nucleotide changes (InDels) that already occur in non-GMOs

Small nucleotide changes (InDels) that do not occur in non-GMOs

Insertion of complete genes by gene editing

Large deletions by gene editing

Insertion of DNA sequences by gene editing

We anticipate there will be a range of different gene-editing products and these might require different detection and identification methods.

It will be no longer possible to have a single method approach as before.

Our research questions

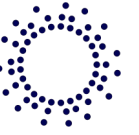How do different gene-editing outcomes impact their detection and identification?

And

How do new and alternative detection methods advance the current EU GM identification approach in the pursuit of sustainable food systems?
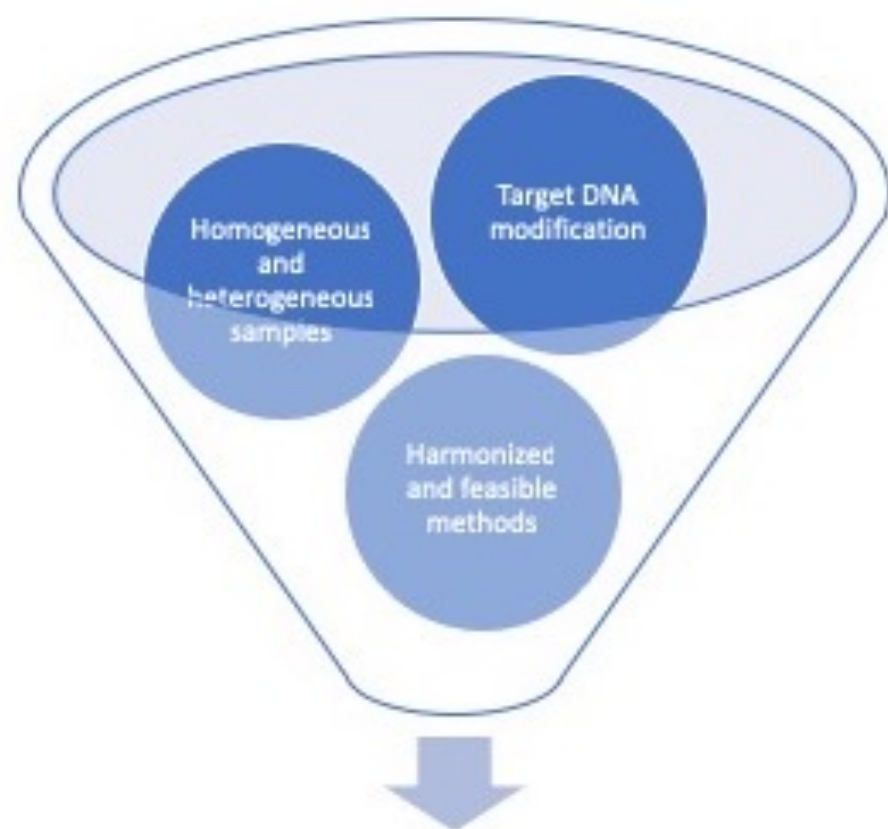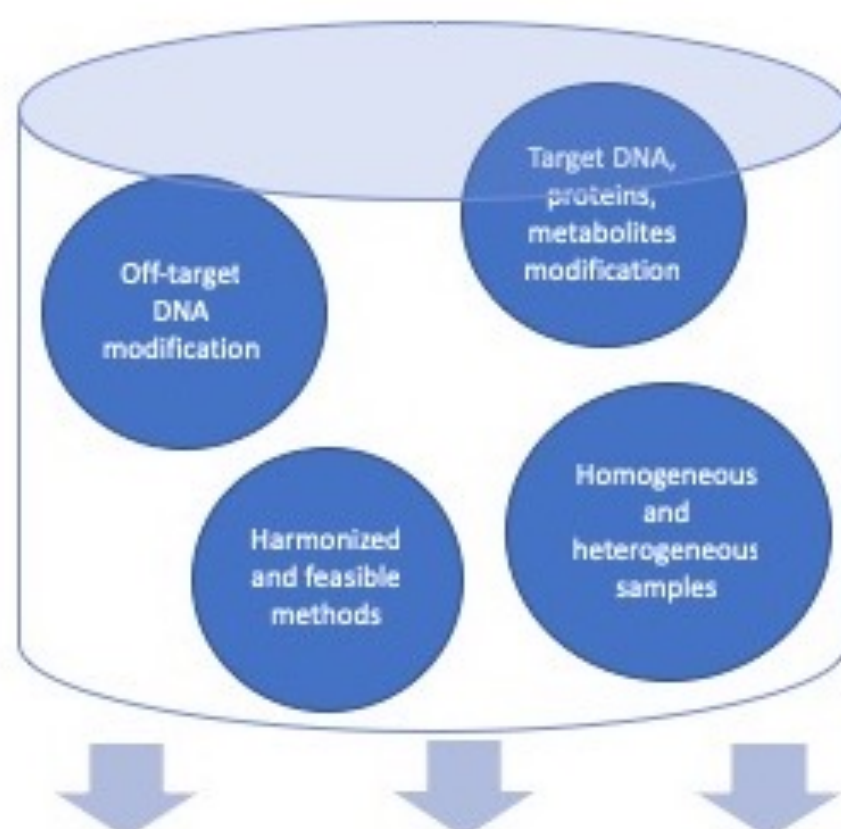
FOODPRINT goes beyond the idea that GMO detection has to be based on a single "transgenic tag"

Current EU GMOMETHODS approach

Homogeneous and heterogeneous samples

Target DNA modification

Harmonized and feasible methods

Target transgenic DNA detection via PCR amplification

FOODPRINT project detection approach

Off-target DNA modification

Target DNA, proteins, metabolites modification

Harmonized and feasible methods

Homogeneous and heterogeneous samples

Target transgenic DNA detection via PCR amplification

Multi-target DNA detection via PCR amplification

Molecular profiling (multi-target) based on *omics*

Flexible multicriteria decision analysis model (matrix) suitable for evaluation and comparison of analytical methods

How can research overcome analytical challenges for NGT detection?

Challenges for NGT market control and authorization	Research and development
No reliable detection/quantification of NGT mutations	Enhance chemistry of PCR based methods and evaluation for their compliance with minimum performance criteria (EU regulation)
Common transgene sequences for screening do not exist in NGT	Develop sequencing methods to screen key genetic elements
No validated/harmonized methods	ENGL is currently updating their validation guidelines (soon to be published) and continuous updating is planned
Only known NGT lines are detectable	Untargeted sequencing methods need to be developed and databases need to be improved
NGT mutations already exist in other organisms	Develop sequencing methods to provide a genetic fingerprint (AI)

Insights into implementation

Methods have to be validated (robustness, sensitivity, etc)

Methods need clear SOP for conclusive results and reporting

Cost and time effective

Law enforcement authorities will need further capacity – different methods, shift in PCR paradigm

HORIZON-CL6-2023-FARM2FORK-01-11: New detection methods on products derived from new genomic techniques for traceability, transparency and innovation in the food system

Scope: Proposals are expected to contribute to the development and validation of detection methods of products obtained through new genomic techniques, including all of the following activities:

- Examine innovative ways and/or specific markers that would allow for distinction between products resulting from new genomic techniques subject to the GMO legislation and products that are not subject to the GMO legislation. This should not only entail the detection of specific mutations, but also of other markers in the genome that are specific for the genotype containing the mutation/s. The methods should be able to distinguish between identical mutations obtained through different techniques;

- Development and validation of reliable detection methods including when possible quantification. Such methods could focus on products with known mutations (i.e. DNA sequence known) or on products with unknown mutations;

- The proposed detection methods should focus on a wide applicability of all or a subgroup of products, allowing for a screening approach. These methods should be assessed on pure products as well as on mixtures typical of food or feed products in the market. Proposals should always include plant-based products and may include also animal and/or microorganisms-based products.

- The proposal could also focus on the detection of unintended mutations or insertions (foreign DNA, CRISPR-Cas sequences, etc);

- The proposals could also include digital/virtual/AI modelling aspects along with the detection methods alternatives;

- The development and validation of standardized methodologies and the contribution to future standardisation processes is encouraged.

Thank you. Takk.
Merci. Gracias. Obrigado.

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NORCE