

12 March 2026

Ms. Astrid Schomaker  
Executive Secretary  
Secretariat of the Convention on Biological Diversity  
413 Saint-Jacques Street, Suite 800  
Montreal, QC, H2Y 1N9  
Canada

**Subject: Submission of technical reference materials on detection and identification of living modified organisms (LMOs)**

Dear Executive Secretary,

On behalf of the Rio Institute, we are pleased to submit the attached technical contribution in response to the Secretariat notification (ntf-2026-010) inviting Parties and relevant organizations to provide technical reference documents and materials related to new detection and identification techniques for living modified organisms under the Cartagena Protocol on Biosafety.

This submission provides an overview of recent advances in molecular detection technologies, including developments in modified real-time PCR, digital PCR, next-generation sequencing tools. It also highlights recent scientific publications and ongoing international research initiatives that may contribute to strengthening analytical capabilities for detecting and identifying organisms developed through genetic engineering and genome editing.

We hope that the information provided will support the continued updating of the Training Manual on the Detection and Identification of Living Modified Organisms (Biosafety Technical Series No. 5) and contribute to ongoing capacity-building efforts under the Cartagena Protocol.

Please do not hesitate to contact us should additional information be required.

Yours sincerely,



**Nicolas Laarman**  
Director  
Rio Institute

## Recent Advances in Detection and Identification of Living Modified Organisms

### 1. Introduction

This technical contribution responds to the invitation of the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety to submit technical reference materials related to new techniques for the detection and identification of living modified organisms (LMOs).

Since the publication of Biosafety Technical Series No. 5, significant advances have occurred in molecular detection technologies and analytical strategies for identifying genetically modified organisms, including organisms developed using genome editing techniques. These developments expand the methodological toolbox available to laboratories responsible for biosafety monitoring and enforcement.

The following sections summarize key technological developments and recent scientific literature that may be relevant for consideration in future updates of the training manual.

### 2. Advances in PCR and Digital PCR

Polymerase chain reaction (PCR) techniques remain the cornerstone of molecular detection of genetically modified organisms. Since the publication of the *Training Manual on the Detection and Identification of Living Modified Organisms*, methodological developments have expanded the analytical capabilities of PCR-based detection systems, particularly through improvements in real-time PCR and the introduction of digital PCR technologies.

Digital PCR methods, including droplet digital PCR (ddPCR), enable **absolute quantification of target DNA molecules without the need for calibration curves** and offer improved sensitivity compared with conventional quantitative PCR methods. These characteristics make digital PCR particularly useful for detecting low-level presence of LMOs in complex matrices and for identifying subtle genetic modifications introduced through genome editing technologies.

For example, **Fraiture et al. (2022)** demonstrated the feasibility of using droplet digital PCR to detect plants carrying a **single nucleotide variation introduced through genome editing**, highlighting both the analytical sensitivity of the method and the interpretation challenges associated with distinguishing targeted edits from naturally occurring genetic variation.

More recently, **Zanatta et al. (2025)** evaluated the suitability of real-time PCR methods for detecting organisms developed using new genomic techniques within the context of European regulatory frameworks. Their study illustrates how established PCR-based detection systems can still play an important role in detecting certain genome-edited organisms when appropriate target sequences are known.

Together, these studies illustrate how improvements in PCR-based detection strategies continue to expand the analytical toolbox available for identifying LMOs. Future updates of the training manual could therefore benefit from expanded coverage of **digital PCR methodologies and advanced real-time PCR approaches**, including guidance on assay design, validation procedures, and interpretation

of results in the context of detecting genome-edited organisms carrying small sequence modifications.

### 3. Next-Generation Sequencing Approaches

Next-generation sequencing (NGS) technologies have significantly expanded the analytical capabilities available for detecting and characterizing genetically modified organisms. Unlike targeted PCR-based methods, sequencing-based approaches enable **genome-wide analysis**, allowing the identification of genetic modifications even when prior knowledge of inserted sequences is unavailable.

Several studies have demonstrated the potential of sequencing approaches for detecting genetically modified microorganisms in complex samples. For example, **D'Aes et al. (2021)** used a combination of short- and long-read whole-genome sequencing to characterize genetically modified microorganisms present in commercial enzyme preparations. Their analysis revealed contaminations originating from related genetically modified *Bacillus* strains used in industrial fermentation processes, demonstrating how sequencing approaches can help identify genetically modified organisms in situations where targeted detection methods are not available.

In a subsequent study, **D'Aes et al. (2022)** applied metagenomic sequencing approaches to detect multiple genetically modified *Bacillus* contaminations in microbial fermentation products, highlighting the value of metagenomic analysis for identifying genetically modified microorganisms within complex microbial communities.

Further developments in sequencing-based detection methods have focused on the use of targeted high-throughput sequencing for identifying genome-edited organisms. For example, **Fraiture et al. (2023)** demonstrated that targeted high-throughput sequencing approaches can enable the detection of **single nucleotide variations introduced through CRISPR/Cas9 genome editing**, providing a potential analytical strategy for detecting organisms carrying minimal sequence changes.

More recent studies have continued to refine sequencing-based detection approaches. **Fraiture et al. (2025)** showed that genetic fingerprints derived from genome database mining can allow accurate identification of genome-edited rice in the food chain through targeted high-throughput sequencing. Similarly, **Godfroid et al. (2025)** developed a whole-genome sequencing workflow capable of identifying genetically modified *Bacillus* isolates alongside foodborne pathogens.

In addition, broader methodological reviews highlight the growing importance of sequencing technologies for GMO detection. **Brod et al. (2024)** summarize recent developments in analytical technologies for detecting genetically modified organisms, emphasizing the increasing role of sequencing approaches in modern detection strategies. Likewise, **D'Aes et al. (2024)** demonstrated the use of metagenomics-based tracing approaches to identify genetically modified microorganism contaminations in commercial fermentation products.

Taken together, these studies demonstrate that sequencing-based approaches can provide powerful tools for the **detection and characterization of LMOs**, particularly in cases involving unknown or unauthorized organisms. Future updates of the training manual could therefore include expanded coverage of **whole-genome sequencing, metagenomic analysis, and targeted high-throughput sequencing workflows** for LMO detection.

### 4. Detection Challenges for Genome-Edited Organisms

Recent advances in genome editing technologies have enabled the development of organisms carrying highly precise genetic modifications, including single nucleotide substitutions and small

insertions or deletions. These types of modifications may be difficult to distinguish from naturally occurring genetic variation using conventional detection strategies based on transgene-specific markers.

Several studies have explored analytical approaches for detecting such modifications. For example, **Fraiture et al. (2023)** demonstrated that targeted high-throughput sequencing can detect **single nucleotide variations introduced through CRISPR/Cas9 genome editing**, highlighting the potential of sequencing-based methods to identify genome-edited organisms that do not contain foreign DNA sequences.

Similarly, **Fraiture et al. (2025)** showed that genomic fingerprinting approaches derived from large genomic databases can enable the identification of genome-edited rice in the food chain using targeted sequencing methods. Their work illustrates how bioinformatic analysis combined with high-throughput sequencing may support the detection of genome-edited organisms even when modifications consist of minimal sequence changes.

At the same time, PCR-based approaches may still play a role in detecting certain genome-edited organisms. As discussed by **Zanatta et al. (2025)**, real-time PCR assays can be suitable for detecting specific genome-edited events when sequence information about the modification is available.

These developments highlight both the opportunities and challenges associated with detecting genome-edited organisms. Future updates of the training manual could therefore include expanded discussion of the **analytical challenges associated with detecting genome-edited organisms**, as well as methodological approaches combining PCR-based assays and sequencing technologies to address these challenges.

## 5. Emerging Rapid Detection Technologies

The continued development of advanced analytical technologies has led to the emergence of integrated detection frameworks combining multiple molecular approaches. These strategies often integrate PCR-based detection methods, sequencing technologies, and bioinformatic analysis to improve the identification and characterization of genetically modified organisms.

Recent work has emphasized the importance of developing robust analytical workflows capable of detecting LMOs in increasingly complex contexts. For example, **Brod et al. (2024)** reviewed recent developments in analytical technologies for genetically modified organism detection, highlighting how emerging sequencing-based approaches and integrated molecular workflows are expanding the capabilities of GMO detection systems.

Similarly, **Godfroid et al. (2025)** demonstrated the development of a whole-genome sequencing workflow capable of simultaneously identifying genetically modified *Bacillus* isolates and foodborne pathogens, illustrating the potential of integrated sequencing approaches for monitoring microbial biotechnology products.

Metagenomic approaches also represent an important component of emerging analytical frameworks. As demonstrated by **D'Aes et al. (2022)** and **D'Aes et al. (2024)**, metagenomic sequencing can enable the detection and tracing of genetically modified microorganism contaminations within complex microbial communities, such as those present in fermentation products.

These developments suggest that future updates of the training manual could benefit from expanded discussion of **integrated analytical strategies for LMO detection**, including workflows

combining PCR-based detection, sequencing technologies, and bioinformatic analysis to improve the identification of genetically modified organisms across diverse applications.

## 6. International Research Initiatives

Two Horizon Europe research projects currently addressing analytical challenges related to new genomic techniques include:

DARWIN – Detection and Analysis of Genomic Modifications in Plants Developed with New Genomic Techniques - <https://darwin-ngt.eu/>

DETECTIVE – Detection and Traceability of Genomic Editing in Plants - <https://detective-ngt.eu/>

These initiatives aim to develop analytical strategies, sequencing approaches, and bioinformatic tools to support the detection and traceability of organisms developed using genome editing technologies.

## References

### *Sequencing strategies*

1. D'Aes, J., Fraiture, M.-A., Bogaerts, B., De Keersmaecker, S. C. J., Roosens, N. H. C., & Vanneste, K. (2021). Characterization of genetically modified microorganisms using short- and long-read whole-genome sequencing reveals contaminations of related origin in multiple commercial food enzyme products. *Foods*, 10(11), 2637. <https://doi.org/10.3390/foods10112637>
2. D'Aes, J., Bogaerts, B., Fraiture, M.-A., De Keersmaecker, S. C. J., Roosens, N. H. C., & Vanneste, K. (2022). Metagenomic characterization of multiple genetically modified *Bacillus* contaminations in commercial microbial fermentation products. *Life*, 12(12), 1971. <https://doi.org/10.3390/life12121971>
3. Fraiture, M.-A., D'aes, J., Guiderdoni, E., Meunier, A.-C., Delcourt, T., Hoffman, S., Vandermassen, E., De Keersmaecker, S. C. J., Vanneste, K., & Roosens, N. H. C. (2023). Targeted High-Throughput Sequencing Enables the Detection of Single Nucleotide Variations in CRISPR/Cas9 Gene-Edited Organisms. *Foods*, 12(3), 455. <https://doi.org/10.3390/foods12030455>
4. Brod, F. C. A., et al. (2024). Recent developments in analytical technologies for genetically modified organism detection. *Food Chemistry: Molecular Sciences*, 9, 100236. <https://doi.org/10.1016/j.fochms.2024.100236>
5. D'aes, J., Fraiture, M. A., Bogaerts, B., Van Laere, Y., De Keersmaecker, S. C. J., Roosens, N. H. C., & Vanneste, K. (2024). Metagenomics-based tracing of genetically modified microorganism contaminations in commercial fermentation products. *Food chemistry. Molecular sciences*, 10, 100236. <https://doi.org/10.1016/j.fochms.2024.100236>
6. Fraiture, M. A., D'aes, J., Gobbo, A., Delvoye, M., Meunier, A. C., Frouin, J., Guiderdoni, E., Deforce, D., De Vogelaere, C., De Keersmaecker, S. C. J., Vanneste, K., & Roosens, N. H. C. (2025). Genetic fingerprints derived from genome database mining allow accurate identification of genome-edited rice in the food chain via targeted high-throughput sequencing. *Food research international (Ottawa, Ont.)*, 221(Pt 1), 117218. <https://doi.org/10.1016/j.foodres.2025.117218>

7. Godfroid, M., Van Uffelen, A., Fraiture, M. A., De Keersmaecker, S. C. J., Vanneste, K., Roosens, N. H. C., & Bogaerts, B. (2025). A WGS workflow for identifying genetically modified and foodborne-pathogenic *Bacillus* isolates. *Food chemistry. Molecular sciences*, 12, 100338. <https://doi.org/10.1016/j.fochms.2025.100338>

*Enhanced Real-time PCR and Digital PCR strategies*

8. Fraiture, M.-A., Roosens, N. H. C., Taverniers, I., De Loose, M., & Deforce, D. (2022). ddPCR strategy to detect a gene-edited plant carrying a single variation point: Technical feasibility and interpretation issues. *Food Control*, 137, 108904. <https://doi.org/10.1016/j.foodcont.2022.108904>
9. Zanatta, C. B., Narendja, F., El Jawhary, H., Abou-Sleymane, G., Subburaj, S., Nodari, R. O., & Agapito-Tenfen, S. Z. (2025). Suitability of Real-Time PCR Methods for New Genomic Technique Detection in the Context of the European Regulations: A Case Study in Arabidopsis. *International Journal of Molecular Sciences*, 26(7), 3308. <https://doi.org/10.3390/ijms26073308>