GMO detection method in the context of a Part B notification

Technical guidance

Version 2023

Biosafety and biotechnology (SBB) and Transversal activities in applied genomics
rue Juliette Wytsmanstraat 14
1050 Bruxelles – Belgium

Guideline for Part B PCR detection method

For any Part B notification (field trial or clinical trial) handed in under the Royal Decree of 21 February 2005 on the deliberate release into the environment and the placing on the market of GMOs or GMO containing products, the notifier has to deliver to Sciensano a control sample the latest 15 days after the start of the trial[1].

For the laboratory of the Sciensano that will receive and analyse these samples a detailed protocol regarding the methods for conservation and analysis of the control sample is needed.

This guideline describes the data to be presented by the notifier on the PCR detection method. Further, it provides information to the notifier on contact points for further information and reference material disposition.

The PCR detection method for any Part B notification should allow detection of the GMO at the level of its new trait(s). Univocal identification of the GMO is not required, but the method must be able to detect the inserted construct or modification unambiguously. However, univocal identification may be requested on a case-by-case basis by the competent authority.

1. Protocol

1.1 Primers

- Provide a primer pair, including sequence, which enables the detection of the GMO. In case the notification contains several GM lines, it should be clearly indicated which primer pair allows detection of which GM line. Specify the nature of the targeted sequence (e.g. (part of) the transgene, the selection marker, etc.) and clearly indicate which primer is the forward and which primer is the reverse primer
- The size of the expected amplicon needs to be specified.
- The position of the primers and its targets has to be illustrated by a figure.

1.2 DNA extraction method

The extraction procedure to obtain nucleic acid from a test sample must be given and referenced (e.g. in house protocol or name of kit).

1.3 PCR mix

The total volume and final reaction component concentrations (nucleic acid, buffer, primers, polymerase, dNTPs and water) must be described.

1.4 PCR program

The type of PCR (traditional or real-time) must be specified and the program (temperature, time, cycles) must be described as below.

Step (s)	Stage	T(°C)	Time (s)	Number cycles
1	Polymerase activation	-	-	-
2 (amplification)	Denaturation	-	-	-
	Annealing	-	-	-
	Extension	-	-	-
3	Elongation	-	-	-

[1] For clinical trials, it is recommended to send the requested material (and information related thereto) as soon as the trial gets approval and no later than 15 days after the first patient will be enrolled.

For digital PCR (dPCR), the device and the software (name, version) used must be mentioned, including whether the software is associated with the device or not.

1.5 Electrophoresis
In case PCR products are separated by electrophoresis, the parameters % agarose, time and voltage in case of gel electrophoresis, and the device used and critical consumables in case of capillary electrophoresis must be described.

1.6. Sanger sequencing
In case a PCR product is sequenced, the procedure to extract the DNA from the gel or DNA purification must be given or referenced and the procedure to sequence the PCR products, including the primer, should be given. The reference sequence to align the obtained DNA sequence should also be provided, as well as specific information on the analysis of the alignment.

1.7 Safety
The specific precautions to be taken (if any) have to be described

2. Description of parameters to measure

The relevant parameters to measure in the analysis have to be described. For a traditional PCR, the size of the amplicon; for a real-time PCR, the threshold cycle value (Ct); for dPCR, copy number /µl (preferably with respect to a positive control and negative control). In addition, the expected parameter values for the positive and negative control should be described by means of a table.

3. Reference material

Reference material should include a positive and a negative control. The positive control is the GMO or its genetic material that is used in view of detecting a positive signal. The negative control is the parental organism or its genetically material that is be used to detect a negative signal.

The amount of the positive and negative control to provide should enable the conduct of 5 to 10 runs, with each run including 4 samples, a positive control, a negative control and a no template control. Each control must be clearly labelled and accompanied by a file providing the following information:
• Number and name
• Nature of the reference material (genomic DNA, plasmid DNA,…)
• Origin of the material (supplier)
• Date of extraction
• Place and condition for storage
• Total amount in µg and concentration (ng/µl)

The reference material has to be properly packed (according to UN legislation[2] requirements if it concerns the GMO) and transported in appropriate conditions to avoid degradation and must be sent to the following address:

Sciensano
Transversal activities in applied genomics
GMO detection
Rue Juliette Wytsmanstraat 14
1050 Brussels

[2] UN, 2010. European agreement concerning the international carriage of dangerous goods by road.

4. Contact

Any additional information concerning this technical guidance may be obtained from Sciensano using the email “GMO-PartB@sciensano.be” and mentioning in the email as subject the reference of your application “B/BE/XX/XXXX – GMO DETECTION”.