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Guideline for Part B
PCR detection method

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D. Breyer, A. De Schrijver, K. Pauwels, N. Roosens
Biosafety and Biotechnology Unit (SBB) and Platform Biotechnology and Molecular Biology
rue Juliette Wytsmanstraat 14
1050 Bruxelles – Belgium
www.iph.fgov.be

Guideline for Part B PCR detection method

For any Part B notification 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 the SBB a control sample the latest 15 days after the approval and the start of the trial.

For the laboratory of the Scientific Institute of Public Health 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 in his application dossier 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 construct (i.e. an intended trait) 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.
- 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 (in house protocol or references).

1.3 PCR mix

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

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	-	-	-

1.5 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). 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 is respectively 50 µg and 150 µg. 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[1] requirements if it concerns the GMO) and transported in appropriate conditions to avoid degradation and must be sent to the following address:

Scientific Institute of Public Health
Platform Biotechnologie en Moleculaire Biologie / Plateforme Biotechnologie et Biologie Moléculaire
Rue Juliette Wytsmanstraat 14
1050 Brussels

4. Contact

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

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