

Guidelines for Molecular Characterization of Genetically Modified Higher Plants to be Placed on the Market

According to Annex III B of Directive 2001/18/EC

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- any relevant scientific articles and reports referred to should accompany the dossier
- the quality of the experimental data should be sufficient to verify clearly any statements made by the applicant
- all sequences should be submitted in electronic format

1. Description of the genetic material used for the transformation

- 1.1. For each of the vectors used for transformation, provide a detailed **map** including the genetic elements listed in 1.2, indicating their location, order and orientation in the vector, and the position of relevant restriction sites.
- 1.2. For each of the vectors used for transformation, provide a **list** (and a table summarizing name, position and brief description) of all genetic elements, including coding and non-coding sequences (e.g. origins of replication, T-DNA borders of *Agrobacterium*, bacterial transposable elements, promoters). For each of these elements:
 - 1.2.1. Provide a **description** of the genetic element, or a **citation** where the genetic element was isolated and characterized (include an accession number in a publicly available database).
 - 1.2.2. Indicate the **portion** and **size** of the genetic element that was inserted in the vector, and its **location** in the vector.
 - 1.2.3. Provide information about its **source**. Give the scientific and the common or trade name of the donor organism. Describe the history of use of the donor organism (or that of relevant elements thereof) and its relevance to risk assessment: indicate whether the donor organism is responsible for any disease or injury to plants or other organisms (e.g. produces toxicants, allergens, pathogenicity factors or irritants).
 - 1.2.4. Indicate whether the genetic element itself is coding for or involved in the production of proteins responsible for **disease** or **injury** to plants or other organisms (e.g. a toxicant, allergen, pathogenicity factor or irritant).

- 1.2.5. Provide information about the molecular, biochemical and physiological **properties of its products**, as known in the donor organism and aimed at in the transgenic plant.
- 1.3. For direct transformation methods, provide data on how the part(s) of the vector(s) used for the transformation was purified and indicate how **purity** was assessed.

2. Description of the transformation method

- 2.1. **Describe** the transformation protocol and provide relevant **references** for the transformation method. In case of direct transformation pure DNA has to be used, implying the absence of carrier DNA.
- 2.2. For *Agrobacterium*-mediated transformation, provide the **strain** designation of the *Agrobacterium* used during the transformation process, and indicate if and describe how the Ti/Ri plasmid based vector was **disarmed**.
- 2.3. For transformation methods that involve the use of **helper plasmids**, describe these plasmids in detail.

3. Description of the transgene loci

- 3.1. Provide experimental data revealing the **number of sites** where (part of the) DNA used for the transformation is inserted and indicate whether it is located in the **nucleus, mitochondria or chloroplasts**. For allopolyploid plants indicate into **which parental genome** these insertions have occurred. Describe the methods that were used and assess their **sensitivity**.
- 3.2. For each of the insertion sites:
 - 3.2.1. Provide the **sequence** of the entire **insert** and of both **flanking regions** (about 500 basepairs, proven to correspond to plant DNA using appropriate methods). Delineate the different genetic elements and indicate any rearrangements on a schematical representation of the transgene locus. Provide a list of all the genetic elements and rearrangements and, for each of these, indicate its position, its origin and evaluate its integrity.
 - 3.2.2. Examine the **nature of the flanking sequences** using up-to-date bioinformatics tools (database searches, prediction models, etc.).
 - 3.2.3. Evaluate the presence and functionality of **novel chimaeric open reading frames** applying up-to-date bioinformatics tools (database searches and application of prediction models designed to reveal the presence of open reading frames, searches for immunologically and toxicologically relevant similarity, etc.) on the complete sequence that was obtained in 3.2.1. If a

chimaeric open reading frame is detected that extends beyond the region that was sequenced in 3.2.1, more of the flanking region should be sequenced until the putative end of this open reading frame is reached.

4. Transcript and protein characterization

- 4.1. Analyse the **expression** of all **open reading frames** identified in 3.2.1 and 3.2.2. Describe the methods that were used for the expression analysis and assess their sensitivity.
 - 4.1.1. For open reading frames intended to be expressed in the transgenic plant, provide data on the levels and the spatial and temporal specificity of expression at the protein level. In case the purpose of the transformation is to alter the expression of endogenous genes (e.g. by antisense constructs, ribozymes, or via the mechanism of RNA silencing), provide data on the expression of the target.
 - 4.1.2. For all other genes present on the DNA used for the transformation and (partly) inserted in the genome of the transgenic plant, provide data on the levels and tissue specificity of expression at the transcript and/or protein level, unless it can be demonstrated that the necessary regulatory sequences for expression are not linked to the open reading frame, or unless the open reading frame is linked to a non-plant promoter for which it can be demonstrated or for which reference can be provided that it is not functional in the plant, or unless it can be otherwise demonstrated that the open reading frame is not expressed. If transcription occurs, it should be determined whether the transcript is translated.
 - 4.1.3. For novel chimaeric open reading frames identified in 3.2.3, provide data on the levels and tissue specificity of expression at the transcript and/or protein level. If transcription occurs, it should be determined whether the transcript is translated.
- 4.2. Describe the **properties** of the expressed **proteins** or the target proteins referred to in 4.1.
- 4.3. If there has been a DNA modification that affects the amino acid sequence of the plant expressed protein, the **modified amino acid sequence** must be provided. Indicate whether the modifications are known or expected to result in changes in the properties of the protein.
- 4.4. On a case by case basis, data on protein stability in the cell and the environment may be required.

5. Inheritance and stability

- 5.1. Provide statistically significant data that demonstrate the inheritance pattern and the stability of the sequences inserted, and data that demonstrate the stability of expression of all proteins indicated in 4.2.
- 5.2. For plants which are either infertile or for which it is difficult to produce seed (e.g. vegetatively propagated male-sterile potatoes, plants with long sexual generation times such as trees), provide statistically significant data to demonstrate that the transgene trait is stably maintained and expressed during vegetative propagation.

6. Detection and identification

- 6.1. Provide the sequence of a **primer pair** which enables the unequivocal identification of the transformation event, as well as a detailed **protocol** for its use for identification, detection and quantification purposes.
- 6.2. **Reference** transgenic and control **material** should be provided at the time of deposition of the dossier.