

Legal Briefing Paper

The regulatory status of plants resulting from New Breeding Technologies

Produced by the NBT Platform¹
28 May 2013

¹ The NBT Platform is a coalition of SMEs, large industry representatives and members of prominent academic and research institutes which strives to bring clarity to the European debate on NBTs. Its aim is to provide policy makers and stakeholders with clear and precise information on NBTs and to generate awareness about their widespread benefits for the European economy and society as a whole. NBT Platform Secretariat, Rue Belliard 199, bte 22, 1040 Brussels, T: +32-2-5022008, info@nbtplatform.org.

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i. Executive summary

Recent scientific advances have led to the introduction of **New Breeding Techniques (NBTs)** that enable plant breeders to develop plants with new traits with greater speed, efficiency and precision; therefore offering **an improvement on traditional breeding techniques**. The NBT Platform has developed a Legal Briefing Paper² (LBP) for the purpose of elucidating the regulatory status of plants developed using these NBTs, since the practical use of these techniques will depend on the legal status according to Directive 2001/18/EC. This document summarises the main points raised in the LBP.

The LBP first explains the scope and purpose of Directive 2001/18/EC (from here on ‘the European Directive’), which provides a definition of ‘GMO’ (Genetically Modified Organism) and ‘non-GMO’. The analysis of the European Directive reveals that, contrary to what is often believed, the European legislator has chosen for a combined process and product approach. Consequently, a GMO is defined by a combination of the **process** (techniques of genetic modification) used to generate the plants and the characteristics of the resulting **product** (the plant itself).

Cumulative analysis

Based on the abovementioned definition, a cumulative analysis has been developed, for which **every question needs to be answered in the affirmative** in order to lead to a Genetically Modified Organism covered by the European Directive. If this is not the case, it leads to a plant not subject to the Directive. The analysis is composed of seven questions (see textbox below and the ‘Cumulative Multilevel Analysis’ on page (vii)).

1. Is it an organism?;
2. Is it non-human?;
3. Has the genetic material been altered (by 20bp or more) vis-à-vis the starting (parental plant) genetic material?;
4. The genetic alteration does not (and cannot) occur naturally (by mating and/or natural recombination)?;
5. Does the genetic modification occur at least through the use of the techniques listed in Annex I A part 1 of the Directive?;
6. Is the genetic modification not among the techniques listed in Annex I A Part 2?;
7. Is the genetic modification not among the techniques/methods listed in Annex I B?

The definitions in the European Directive form the basis for the analysis of each of the seven NBTs currently under evaluation **to confirm their legal and regulatory status** and thus to determine if, and for what reasons, any of these NBTs falls subject to the regulatory obligations imposed by the European Directive. The techniques covered are **1) Zinc Finger Nuclease (ZFN) technology³, 2) Oligonucleotide Directed Mutagenesis (ODM), 3) Cisgenesis, 4) RNA-dependent DNA methylation (RdDM), 5) Grafting (non-GMO scion on GMO**

² For access to this document, please contact the NBT Platform Secretariat using the contact information provided in this document.

³ Since it is a well documented group of techniques, ZFN technology is used as an example of a much larger group of related new genome editing techniques, commonly referred to as Site Directed Nucleases (which includes ZFNs, but also TALENs and Meganucleases).

rootstock), **6**) Reverse breeding, **7**) Agro-infiltration (Agro-infiltration '*sensu stricto*', Agro-inoculation). The analysis is carried out by applying a **cumulative multilevel test** to each technique, and is visualised in a **flowchart** (page iii of this summary) with supporting **checklist** (please refer to the main document).

This analysis shows that **none of the aforementioned NBTs necessarily lead to plants covered by the European Directive**, unless foreign DNA exceeding 20bp has been inserted in the resulting plant. See page (iv) for the specific conclusions per technique. For an in-depth analysis of the techniques, please refer to the Legal Briefing Paper.

Main Conclusions

The following main conclusions were drawn from the analysis:

- ZFN-1 & ZFN-2 are not regarded as leading to a product covered by the European Directive for the following reasons:
 - a) No foreign DNA (≥ 20 bp) is inserted into the genome (see LBP section 2.6).
 - b) The alteration is capable of occurring naturally by mating and/or natural recombination.
 - c) The oligonucleotides are not self-propagating entities and do not contain sequences necessary for replication.
 - d) ZFN1 and ZFN2 are a form of mutagenesis. Mutagenesis is subject to Annex 1 B, and the resulting plants are therefore excluded from GMO regulation.
 - e) Moreover, ZFN-1 falls within the 'natural processes' category in Annex I A Part 2 since in nature such deletions occur naturally (see point b). This technique replicates - in an accelerated and more efficient fashion - natural processes. Recombinant nucleic acid techniques (see LBP section 2.4) are absent because, among other considerations, ZFN-1 does not involve the *insertion of foreign DNA* produced *outside* the organism (see LBP section 2.6).
- ZFN-3 is regarded as leading to a product covered by the European Directive, except in the following situation:
 - a) There is no formation of new combinations of genetic material (see LBP section 2.5 and point 4 in the flow chart below), provided the inserted genetic material is naturally present in the plant genome.
 - b) The alteration is capable of occurring naturally by mating &/or natural recombination, provided the inserted genetic material is present in a naturally crossable plant. However, insertion of foreign DNA (≥ 20 basepairs) does lead to a plant covered by the European Directive.
- ODM is not regarded as leading to a product covered by the European Directive for the following reasons:
 - a) No genetic material is inserted into the genome (see LBP section 2.6).
 - b) The oligonucleotides are not self-propagating entities and do not contain sequences necessary for replication.
 - c) The alteration is capable of occurring naturally by mating and/or natural recombination.
 - d) ODM is considered a form of mutagenesis (subject to Annex I B) not involving recombinant nucleic acid molecules (see LBP section 2.7 on 'mutagenesis'). The fact that it is more precise does not necessarily exclude it from Annex I B or subject it to GMO regulation.
 - e) ODM falls within the 'natural processes' category in Annex I A Part 2 since in nature such mutations and deletions occur naturally (see point b). This

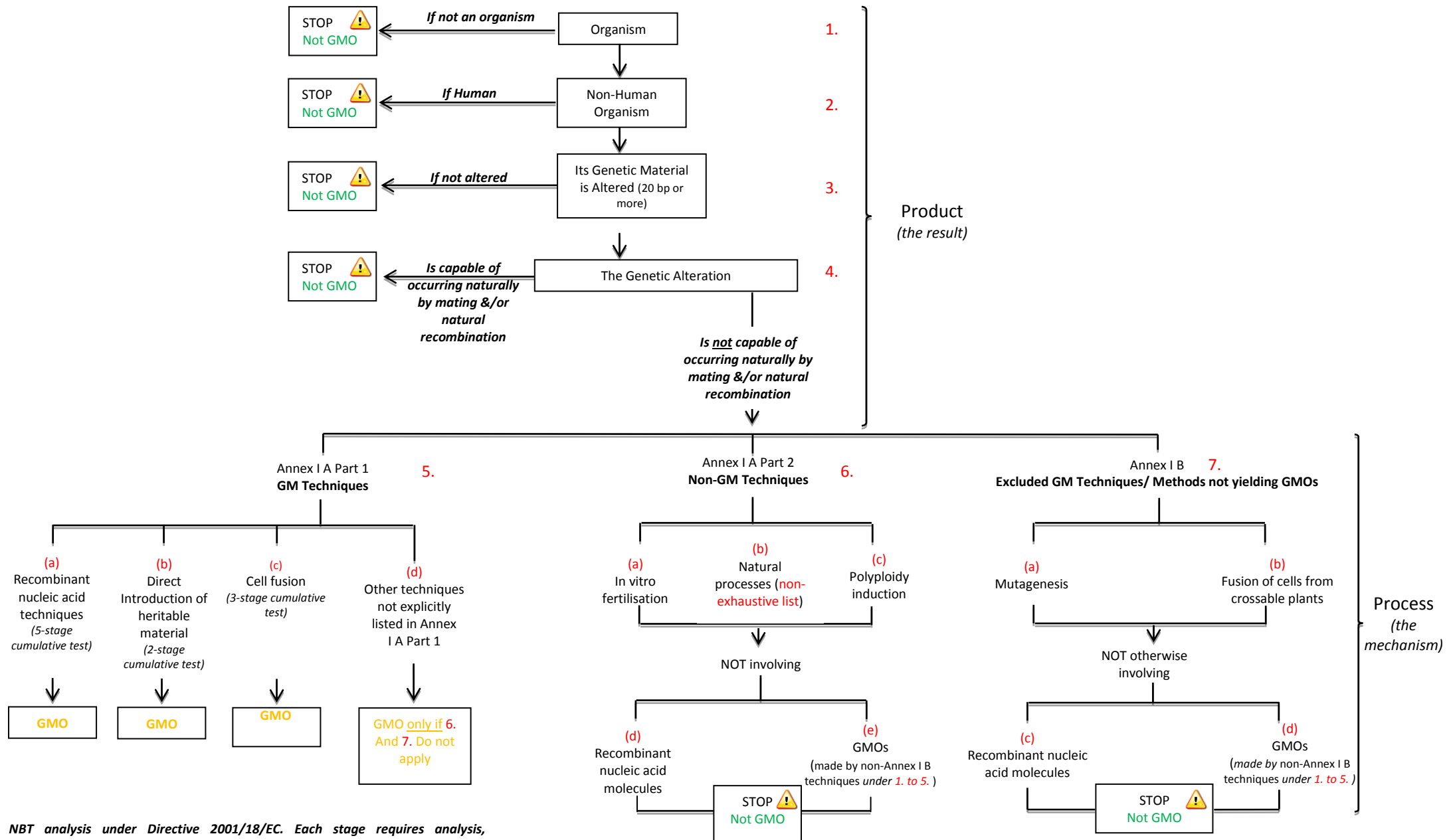
technique replicates - in an accelerated and more efficient fashion - natural processes. Recombinant nucleic acid techniques (see LBP section 2.4) are absent because, among other considerations, ODM does not involve the *insertion* of *foreign DNA* produced *outside* the organism.

- Cisgenesis is not regarded as leading to a product covered by the European Directive for the following reasons:
 - a) there is no formation of new combinations of genetic material, since the inserted genetic material is naturally present in the plant genome;
 - b) the alteration is capable of occurring naturally by mating &/or natural recombination; and
 - c) The nucleic acid molecules are capable of occurring naturally in the plant.
- RdDM is not regarded as leading to a product covered by the European Directive for the following reasons:
 - a) the offspring plants are substantially unaltered *vis-à-vis* the parental plant since the alteration is epigenetic and transient; and
- A harvested product from a non-GMO scion grafted on a GM rootstock⁴ is not covered by the European Directive for the following reasons:
 - a) the scion itself is not genetically altered, nor are the fruits, seeds or other materials from the scion; and
 - b) the process of grafting is not a recombinant DNA technique, nor a direct introduction of heritable material, nor a process of cell fusion.
- Reverse breeding is not regarded as leading to a plant covered by the European Directive for the following reason:
 - a) the genetic material of the hybrid plants (the product⁵) is altered in a way that is capable of occurring naturally by mating and/or natural recombination.
- Agro-infiltration is not regarded as leading to a plant/product covered by the European Directive for the following reason:
 - a) The progeny plant (the product) does not contain genetic material that is altered, since intermediate plants contain T-DNA and/or genetically modified *Agrobacterium* transiently and the foreign genetic material is not incorporated in the germline.

⁴ The GM rootstock itself is covered by the European Directive.

⁵ Intermediate plants that contain foreign DNA are covered by the European Directive.

Cumulative Multilevel Analysis



NBT analysis under Directive 2001/18/EC. Each stage requires analysis, ultimately allowing for classification of organisms developed with current and future breeding techniques.

1. Introduction and Background

Recent scientific advances have led to the introduction of new breeding technologies that enable plant breeders to develop new traits with greater speed, efficiency and precision; offering, therefore, an improvement on traditional breeding techniques. These technologies are grouped under the heading of ‘Novel Breeding Technologies’ or ‘New Breeding Techniques’ (NBTs).

The aim of the present legal briefing is to elucidate properly the regulatory status of organisms developed by means of NBTs. It does so in the following way:

- First, it revisits and explains the scope and purpose of Directive 2001/18/EC, focusing on the definition of a GMO. A close reading of the definition leads to the conclusion that GMOs are defined by a combination of the *process* (techniques) used to generate them *and* the properties of the resulting *product*. Chapter 2, below, explains this definition, drawing out the specific legal requirements that need to be fulfilled in order for a specific organism to be characterised and regulated as a GMO.
- Second, having articulated the legal requirements for what constitutes a GMO, these are applied to each of the seven principal NBTs available today to confirm their legal and regulatory status:
 1. Zinc finger nuclease (ZFN) technology (ZFN-1, ZFN-2 and ZFN-3)
 2. Oligonucleotide Directed Mutagenesis (ODM)
 3. Cisgenesis
 4. RNA-dependent DNA methylation⁶ (RdDM)
 5. Grafting (non-GM scion on GM rootstock)
 6. Reverse breeding
 7. Agro-infiltration (Agro-infiltration ‘*sensu stricto*’, Agro-inoculation)

Specifically, Chapters 3 to 9, below, address whether or not each of the abovementioned NBTs falls subject to the regulatory obligations imposed by Directive 2001/18/EC on GMOs and the reasons for this conclusion (applying the requirements elucidated in Chapter 2).

Finally, as NBTs continue to be developed, this analysis will enable regulators and operators to have a common understanding of the extent to which an individual organism developed by a new NBT is regulated by Directive 2001/18/EC or not. In that sense, the Legal Briefing Paper will provide a helpful resource to be used in the future.

⁶ The European Commission’s mandate to EFSA uses the term ‘RNA –*Dependent* DNA Methylation. Although the literature on these techniques uses different terminology, namely RNA-*Directed* DNA Methylation, this report will use the EFSA mandate’s terminology for clarity. This is in line with the Joint Research Commission (2011) and the New Techniques Working Group (2011) in their respective reports on NBTs.

2. Analysis of the scope of EU GMO regulation: Separating GMOs from other biological entities

2.1. The definition of a 'Genetically Modified Organism' (GMO) in EU law

Directive 2001/18/EC establishes the pivotal definition of what constitutes a GMO in EU law.⁷ It is defined by a combination of the *process* (techniques) used to generate a GMO *and* the properties of the resulting *product*⁸. The text below seeks to draw out the constituent elements of the legal definition, using underlining for emphasis, explanatory footnotes, colour coding and explanations [*in square brackets*].

Article 2(2) of Directive 2001/18/EC states that:

'genetically modified organism (GMO) means an organism, with the exception of human beings, in which the⁹ genetic material¹⁰ [*of the organism*] has been altered¹¹ in a way that¹² [*the resulting alteration*] does not occur naturally by mating and/or natural recombination

Within the terms of this definition:

- a) genetic modification occurs at least [*non-exhaustive*] through the use of the techniques listed in Annex I A, part 1 [*three illustrative techniques*]¹³;
- b) the techniques listed in Annex I A, part 2, [*exhaustive categories*]¹⁴ are not considered to result in genetic modification' [*and therefore there is no resulting GMO*].

It is apparent from the definition that a Cumulative Multilevel Analysis¹⁵ must be undertaken before a proper determination can be made as to whether an organism developed by an NBT is an organism

⁷ This is the reference definition used in subsequent EU regulation of modern biotechnology. See *inter alia* Directive 2009/41/EC on the contained use of GMMs (which differs significantly only as regards its additional express exclusion of self-cloning) and Regulation (EC) No. 1829/2003 on genetically modified food and feed.

⁸ 'Product' refers to the specific end product of a technology. It does not refer to any intermediate products that may arise during the process specific to a technology, nor does it refer specifically to materials obtained from the end product.

⁹ 'the' is referring to the genetic material of the organism. That means that the genetic material of the organism has been altered. It does not refer to other material being altered. This is important to keep in mind when reviewing the different elements of the NBTs.

¹⁰ 'Genetic material' is not defined explicitly in Directive 2001/18/EC, but is commonly understood as referring to heritable material containing information, where 'material' (i) refers to a double or single stranded DNA or RNA molecule, (ii) which is heritable, meaning that it contains an origin of replication, and (iii) the information is meaningful.

¹¹ The alteration (or 'change') needs to be considered by examining alterations *vis-à-vis* the starting plant material (parental plant) at the beginning of the process. See for example, the case of RdDM on page 24.

¹² 'that' is referring to the resulting alteration and focuses on the result (the alteration itself) rather than the process or mechanism of alteration. This is clear from the text itself but also from the legislative context. 'Processes' that occur in nature are considered at a separate stage of analysis under Annex I A Part 2; see stage 6(b) of the flow diagram visual aid on the Cumulative Multilevel Analysis on page 12). It is apparent that the stages 4 and 6(b) cannot be redundant duplication, so they must serve separate legislative and regulatory functions (one focused on product and the other on process). It is important to keep in mind that the resulting alteration should not be capable of occurring naturally. It does not refer to genetic material, and hence, the following situations do not fall under this definition:

(a) genetic material that has been altered through natural processes and the resulting genetic material does not occur in nature (e.g. triticale); (b) processes that do not occur naturally, but that do not lead to alteration of the genetic material (e.g. tissue culture techniques such as somatic embryogenesis). In other words, all of the criteria in the Cumulative Multilevel Analysis must be fulfilled before a GMO is determined to exist.

¹³ See the flow diagram visual aid on the Cumulative Multilevel Analysis on (page 12) at stage 5.

¹⁴ Whilst the three categories in Annex 1 A Part 2 (Non-GM techniques) are exhaustive, it is clear that the category of 'natural processes such as: conjugation, transduction, transformation' is open to all natural processes (some examples of which are listed).

subject to the requirements of Directive 2001/18/EC. A flow diagram visual aid on the Cumulative Multilevel Analysis (accompanying the text below) and a checklist for classifying products resulting from NBTs are both included at the end of this chapter to help follow the complex analysis required.

A GMO is:

1. an organism;
2. which is not a human being;
3. in which genetic material has been altered *vis-à-vis* the starting plant material;
4. where the genetic alteration does not (and cannot) occur naturally¹⁶ by mating and/or natural recombination;
5. where the genetic modification occurs at least through the use of the techniques listed in Annex I A part 1 (but is not limited to those techniques);
6. where the techniques in Annex I A Part 2 are considered not to result in genetic modification; and
7. where the use of the techniques/methods listed in Annex I B are considered not to result in an organism that is subject to the requirements of Directive 2001/18/EC (because of their longstanding conventional use and safety record¹⁷).

By focusing on both process (techniques and mechanisms) and product (the organism itself) the EU regulatory framework mirrors the approach taken at the international level. The Cartagena Protocol on Biosafety defines a 'Living Modified Organism' as:

'any living organism that possesses a novel combination of genetic material [the end product] obtained through the use of modern biotechnology' [the process].¹⁸

The Cartagena Protocol was ratified by the EC (now the EU) on 27 August 2002¹⁹ and as such forms part of the EU legal order and takes priority over secondary legislation (such as Directive 2001/18/EC) in the hierarchy of norms. Directive 2001/18/EC should accordingly be interpreted in so far as possible to reflect a consistent approach, to which the EU is bound in international law.²⁰

¹⁵ Each numbered stage is reflected in both the flow diagram visual aid on the Cumulative Multilevel Analysis and a checklist for classifying organisms developed through NBTs, on pages 12 and 13, respectively.

¹⁶ It is apparent that reference to how the genetic alteration 'occurs' means whether the resulting alteration which took place (as a result of technical intervention) *is capable of occurring naturally* (that it is a possibility) by mating and/or natural recombination, rather than whether *in this specific case* the alteration actually occurred naturally. See section 2.3 below.

¹⁷ Recital 17 of Directive 2001/18/EC provides that it 'should not apply to organisms obtained through certain techniques of genetic modification which have conventionally been used in a number of applications and have a long safety record'.

¹⁸ The Cartagena Protocol on Biosafety to the Convention on Biological Diversity, Article 3(g).

¹⁹ See <http://bch.cbd.int/protocol/parties>; Council Decision 2002/628/EC concerning the conclusion, on behalf of the European Community, of the Cartagena Protocol on Biosafety; and the Declaration by the European Community in accordance with article 34(3) of the convention on biological diversity.

²⁰ International agreements concluded by the EU form an integral part of the EU legal order once they enter into force. Article 216(2) TFEU provides that 'Agreements concluded by the Union are binding upon the institutions of the Union and on its Member States'. The Court of Justice has described them as ranking *between* primary law and secondary EU law (Regulations, Directives and Decisions); see Joined Cases C-402/05 P and C-415/05 P, *Kadi v Council & Commission*, paras. 306 to 308. It is clear that generally they prevail over provisions of secondary EU legislation; see Case C-61/94, *Commission v Germany*, para. 52: 'When the wording of secondary Community legislation is open to more than one interpretation, preference should be given as far as possible to the interpretation which renders the provision consistent with the Treaty. Likewise, an implementing regulation must, if possible, be given an interpretation consistent with the basic regulation (see Case C-90/92, *Dr Tretter v Hauptzollamt Stuttgart-Ost*, [1993] I-3569, paragraph 11)'.

Directive 2001/18/EC is also a measure subject to the WTO Sanitary and Phytosanitary Measures (SPS) Agreement.²¹ The WTO has traditionally shown concern about the regulation of Process and Production Methods (PPMs) being used as a disguised restriction on trade. It is therefore important to demonstrate that the EU regulatory framework for the assessment of GMOs has a sound scientific basis – restricting its application to considerations which are capable of raising potential risks to health and the environment. This is a further reason underlying the proper interpretation of the definition of a GMO in the EU.

CONCLUSION 1

We conclude that both the characteristics of the resulting organism and the processes that have generated it (the technology employed) are necessary considerations under Directive 2001/18/EC to determine whether or not that organism is properly considered a GMO. By applying the Cumulative Multilevel Analysis set out above (and represented in diagram on p. 12), this assessment can be made properly.

In order to be able to apply the Cumulative Multilevel Analysis, some of the terms used need to be defined (see sections 2.2.to 2.7).

2.2. The definition of an ‘Organism’

An *organism* is defined in Directive 2001/18/EC as:

‘any biological entity capable of replication or of transferring genetic material’.

The effect of this definition is that, even where a biological entity satisfies stages (3) to (5) of the Cumulative Multilevel Analysis, if it is nonetheless unable to replicate or transfer genetic material, it cannot constitute a GMO under Directive 2001/18/EC. This has been clearly affirmed by the Court of Justice of the EU in *Bablok and Others v Freistaat Bayern*:

‘The definitions of organism and GMO given by Directive 2001/18 necessarily imply that the genetic information included is capable of being transferred specifically to a suitable recipient for the purposes of recombination. Recital 4 in the preamble to Directive 2001/18 supports such an analysis. That directive thus seems to endorse conclusively two criteria which go together, namely viability and fertility, and not merely a transfer of DNA which is no longer capable of playing a role in reproduction.’²²

CONCLUSION 2

We conclude that biological entities in which the genetic material has been altered in a way that is NOT capable of occurring naturally by mating and/or natural recombination, but are NOT capable of replication or of transferring genetic material are NOT GMOs.

2.3. The definition of the notion ‘altered in a way that does not occur naturally by mating and/or natural recombination’

In order to evaluate whether the alteration does not occur naturally by mating and/or natural recombination - stage (4) in the Cumulative Multilevel Analysis - it is essential to define the term ‘naturally’. It is apparent that the reference to how the genetic alteration ‘occurs’ and whether this is by natural means, refers to whether the alteration which took place (as a result of a technical

²¹ See WT/DS291/R, WT/DS292/R, WT/DS293/R, Report of the Panel of 29 September 2006 in ‘European Communities — Measures Affecting the Approval and Marketing of Biotech Products’.

²² Case C-442/09, *Bablok and Others v Freistaat Bayern*, para. 55. See also paras. 59, 60 and 62.

intervention) *is capable* of occurring naturally by mating and/or natural recombination rather than whether *in this specific case* the alteration actually occurred naturally.²³ Indeed, the logic of Directive 2001/18/EC is to exclude both alterations which *are observed as* occurring in nature and alterations which *can possibly* occur in nature. This is apparent elsewhere in Directive 2001/18/EC in Annex I A Part 2 which excludes an open category of ‘natural processes such as: conjugation, transduction, transformation’ (stage 6(b) in the Cumulative Multilevel Analysis), and the fact that GM techniques and their respective products which have been conventionally used in a number of applications and have a long safety record²⁴ (listed in Annex I B) are also excluded from Directive 2001/18/EC (stage 7 in the Cumulative Multilevel Analysis).

CONCLUSION 3

We conclude that alterations which can possibly occur in nature do NOT give rise to GMOs.

2.4. The definition of techniques listed in Annex I A, Part 1

As in Chapter 2.1, the text below seeks to draw out the constituent elements of the legal definition, of those techniques which fall within the non-exhaustive list of techniques of genetic modification subject to the regulatory regime of Directive 2001/18/EC. Again, we use underlining for emphasis and colour coding to draw out core elements of the definition in Article 2(2)(a):

‘PART 1

Techniques of genetic modification referred to in Article 2(2)(a) are *inter alia*:

- (1) **recombinant nucleic acid techniques** involving the formation of **new combinations of genetic material** by the **insertion** of nucleic acid molecules **produced by whatever means outside an organism**, into any virus, bacterial plasmid or other vector system and their **incorporation into a host organism in which they do not naturally occur** but in which they are **capable of continued propagation**;
- (2) techniques involving the **direct introduction** into an organism of **heritable material prepared outside the organism** including micro-injection, macro-injection and micro-encapsulation;
- (3) **cell fusion** (including protoplast fusion) or hybridisation techniques where live cells with **new combinations of heritable genetic material** are formed through the fusion of two or more cells **by means of methods that do not occur naturally**.

Each of these techniques has common and distinct elements (as indicated by the use of common colour coding for thematic elements which are shared). Each is considered below because, although they are not exhaustive, they provide an insight into the characteristics which GMO producing techniques may share.

²³ A good example of genetic material altered by natural processes (cross breeding) is Triticale, where the entire genome of wheat (*Triticum aestivum*) is altered by the fusion with the rye (*Secale cereal*) genome.

²⁴ Recital 17 of Directive 2001/18/EC provides this rationale for its Annex I B.

2.4.1. recombinant nucleic acid techniques

Recombinant nucleic acid²⁵ techniques bear the following cumulative distinguishable characteristics (five-stage cumulative test):

- a) They lead to the formation of new combinations of genetic material.
- b) They do so by the insertion of nucleic acids produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system.
- c) They lead to incorporation of nucleic acids into a host organism.
- d) Such nucleic acids do not naturally occur²⁶ in the host organism.
- e) The host organism is capable of continued propagation [not merely a transient presence or effect²⁷] of the incorporated nucleic acid molecules (which links to the definition of 'organism', see above under section 2.2.).²⁸

It follows that this list of requirements for what constitutes 'recombinant nucleic acid techniques' must be fulfilled in *all* respects in order for an organism resulting from the use of these techniques to be considered a GMO. In absence of even one of these five elements there is no GMO formed by means of recombinant nucleic acid techniques.²⁹ This does not necessarily preclude (i) techniques which satisfy the five-stage cumulative test and *also* bear additional characteristics being classified as recombinant nucleic acid techniques or (ii) techniques which do not satisfy the five-stage cumulative test yielding GMOs. In fact, the definitions of all three illustrative techniques in Annex I A Part 1 provide a minimum harmonised set of core requirements for each of the three illustrative techniques listed therein (see section 2.4.2 and 2.4.3 below, for explanation of the two other illustrative techniques).

Whilst there is no express definition of 'recombinant nucleic acid molecules' *per se* - in either Annex I A Part 2 or Annex I B - it is apparent that recombinant nucleic acid molecules are those obtained by the five-stage cumulative test, outlined immediately above (see also the flow chart and the checklist for classifying products resulting from NBTs, at the end of this section).

²⁵ Recombinant nucleic acid molecules are nucleic acid molecules obtained by recombinant nucleic acid techniques and are characterised by the 5-stage cumulative test (see last paragraph of section 2.4.1). It is apparent from the test that recombinant nucleic acid molecules are molecules that constitute (i) a new combination of genetic material (see section 2.6) and (ii) which does not naturally occur (see section 2.5).

²⁶ The notion of incorporating nucleic acids into a host organism in which the nucleic acids 'do not naturally occur' refers to nucleic acids from exogenous ('foreign') sources (see section 2.5 below).

²⁷ Whilst there is no explicit reference to the absence of transient effect or presence, the notion of 'continued' presence does lead to the strong inference that this is a requirement. Indeed, this is also the conclusion reached by the majority of the New Techniques Working Group (see chapter 4.5 of their 2011 report).

²⁸ This notion of recombination is consistent with the generally accepted meaning of that term in the scientific literature, which focuses on the *recombining* of DNA molecules from two or more *different species* that are inserted into a host organism to *produce new genetic combinations*.

²⁹ Nucleic acid molecules are also not defined, but are generally understood in the scientific literature to be a polymer consisting of purine and pyrimidine nucleotides. The New Techniques Working Group defined this term in chapter 4.2 of their 2011 report: 'In line with the aforementioned Directives, a recombinant nucleic acid molecule is created outside the cells through the formation of a new combination of genetic material/nucleic acid molecules.' The requirements for recombinant nucleic acid techniques that result in the formation of GMOs relate both to the *product* ('*new combinations of genetic material*') and the *process* ('*by the insertion of nucleic acid molecules*'), again underlining the definition of what is a GMO, outlined in section 2.1.

2.4.2. direct introduction of nucleic acids

Similarly, whilst **direct introduction** of nucleic acids by micro-injection, macro-injection or macro-encapsulation into an organism could be a technique of genetic modification where they are incorporated into the organism and are capable of continued propagation, this would not be the case where the nucleic acid is either not incorporated or when once incorporated is unable to continue propagation. Therefore, techniques which constitute **direct introduction of nucleic acids bear the following cumulative distinguishable characteristics (2-stage cumulative test):**

- a) Direct introduction of heritable material into an organism.
- b) The heritable material has been prepared outside the organism.

2.4.3. cell fusion

Cell fusion (including protoplast fusion), or hybridisation techniques, **bears the following cumulative distinguishable characteristics (3-stage cumulative test):**

- a) They produce live cells with new combinations of heritable genetic material.
- b) They are formed by the fusion of two or more cells.
- c) They are formed by means of methods that are not capable of forming naturally.

This reflects the approach in Article 2(2) of Directive 2001/18/EC, that GMOs are defined by a combination of the *process* (techniques) used to generate them *and* the properties of the resulting *product*. This is underlined by the fact that cell fusion is listed in Annex I B as a GM technique which does not result in a GMO where the respective product can occur through traditional breeding methods or can occur naturally (see section 2.7 and Annex I B).

CONCLUSION 4

We conclude that:

a) Not all recombinant nucleic acid techniques lead to the formation of a GMO.

b) Not all nucleic acid techniques are recombinant and if they do not lead to the formation of new combinations of genetic material, and/or if the nucleic acids naturally occur in the host organism, they do not lead to the formation of a GMO.

c) Not all direct introduction of heritable material leads to the formation of a GMO.

d) Not all cell fusion leads to the formation of a GMO.

2.5. Definition of (not) 'naturally' occurring nucleic acids

Naturally occurring nucleic acid is nucleic acid of the same or from a related and crossable (sexually compatible) organism. For example, a resistance gene from a wild relative of a cultivated crop transferred to the cultivated crop should be regarded as a 'naturally' occurring nucleic acid under the condition that both plants are crossable. This common understanding of naturally occurring nucleic acids is also used and generally accepted for the definition of cisgenesis (see section 5, below). On the other hand, a bacterial resistance gene transferred into a cultivated crop should be regarded as a 'not naturally' occurring nucleic acid because bacteria and plants are not sexually compatible. Not-naturally occurring nucleic acid may also be described as exogenous or 'foreign' DNA.

2.6. The insertion of small nucleotide sequences

Relevant in the same context is the question how many newly inserted nucleotides could constitute a new combination of genetic material. The New Techniques Working Group, in section 4.2 of its 2011 report, noted:

‘A majority of experts concluded that in order to form a new combination, a nucleotide sequence of at least 20 bp is required’.

The number of 20 bp is not arbitrary. In large eukaryotic genomes³⁰, there is a high probability that by chance alone any short string of nucleotides is already present in the genomic material as a naturally occurring sequence. As a consequence, a sequence of that length cannot be regarded as a new combination of genetic material. The probability that a given nucleotide sequence is already present in the genome of an organism decreases at increasing nucleotide sequence length, and increases with the genome size.

Based on statistical calculations, the Joint Research Centre (2011) has arrived at a threshold for new combinations of genetic material of 20 nucleotides for plant genomes. On page 165 of their report, they state: ‘It can therefore be assumed that in the case of a plant genome, information on DNA sequence of at least 20 nucleotides is needed to be in a position to consider a certain DNA sequence as unique and to identify it as the result of a deliberate genetic modification technique’. For this reason, only foreign sequences of 20 nucleotides or more (‘20 bp or more’) are being regarded, from a biological and statistical point of view, as a potentially new combination of genetic material in plants.

CONCLUSION 5

We conclude that the use of techniques that result in the introduction of small sequences of foreign DNA (less than 20 bp) do NOT result in the formation of a GMO.

2.7. The techniques listed in Annex I B

Annex I B of Directive 2001/18/EC describes techniques of genetic modification yielding organisms that do NOT fall within the scope of the GMO legislation.

‘Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, **on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below** are:

- (1) mutagenesis,
- (2) cell fusion³¹ (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.’

Directive 2001/18/EC does not provide a definition of ‘mutagenesis’, probably because the legislator assumed the term sufficiently well known in the field. A review of various scientific and general dictionaries indicates that mutagenesis is understood to connote ‘the occurrence or induction of a

³⁰ Eukaryotic: consisting of cell(s) containing a membrane-bound nucleus in which most genetic material is carried. Most multi-cellular organisms –among which all plants and animals– are eukaryotic (Lawrence, 2011).

³¹ See section 2.4.3.

mutation', whereby a mutation is defined as 'a permanent, heritable change in the nucleotide sequence in a gene or a chromosome'³².

CONCLUSION 6

We conclude that techniques that do not involve recombinant nucleic acid molecules and are mutagenesis do NOT yield GMOs.

2.8. Basic Principles of Interpretation

Finally, it is worth noting that the legal text in Directive 2001/18/EC must be interpreted in line with a number of fundamental principles of EU law:

- (1) **Legal Certainty:** An EU legal text must be interpreted in line with the requirements of legal certainty so that those subject to its requirements are able to plan their actions accordingly. Moreover, ambiguities arising from the language of the law should be resolved in favour of the individual.³³
- (2) **Purposive (Teleological) Interpretation:** An EU legal text must be interpreted not merely by reading the back letter law or comparing linguistic versions. Rather, one must ensure that the underlying purpose of the legislation is realised, so that unity and coherence is ensured in the EU legal order and specific areas of regulation therein (such as in the field of biotechnology).³⁴ For example, one of the principles established by the EU legislator in Directive 2001/18/EC is that an alteration capable of occurring *naturally* does not present a risk requiring EU regulation (see section 2.5, above).
- (3) **Proportionality:** This requires action by the Union institutions to be proportionate to its aim: 'The content and form of Union action shall not exceed what is necessary to achieve the objective of the Treaties.'³⁵ A Protocol to the Treaty puts the point more strongly: '[...] any burden, whether financial or administrative, falling upon the Union, national government, regional or local authorities, economic operators and citizens, [is] to be minimised and commensurate with the objective to be achieved'.³⁶ The General Court has held, 'where there is a choice between several appropriate measures, recourse must be had to the least onerous, and the disadvantages caused must not be disproportionate to the aims pursued'.³⁷ Accordingly, a reading of Directive 2001/18/EC which is consistent with its purpose, yet imposes the least burden, will always be the correct means of interpretation.

2.9. Cumulative Multilevel Analysis under Directive 2001/18/EC

The following two pages contain a:

- (1) diagram setting out the Cumulative Multilevel Analysis described in section 2.1, above; and
- (2) checklist for classifying products resulting from NBTs, covering all of the considerations relevant to assessing whether an organism is a GMO.

³² See, for example, Lawrence (2011).

³³ Case C-169/80 *Administration des Douanes v Gondrand Frères*, paras. 17-18.

³⁴ This is manifest in case C-442/09, *Bablok*, noted in section 2.2, above.

³⁵ Article 5 TFEU.

³⁶ Protocol (No. 2) on the application of the principles of subsidiarity and proportionality, [2001] OJ C 83/207.

³⁷ Case T-13/99, *Pfizer*, para. 411; Case T-70/99, *Alpharma*, para. 324.

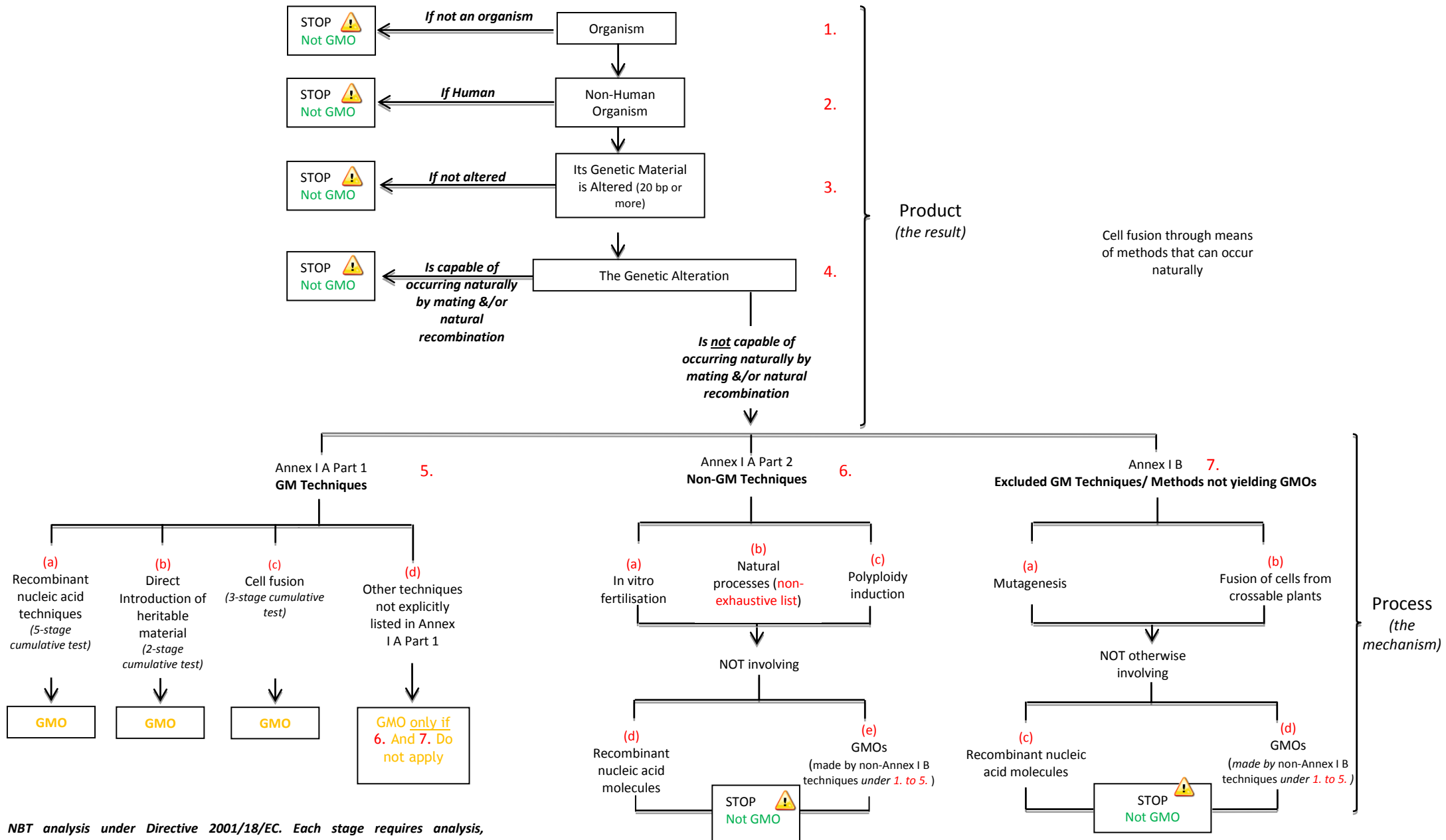
In chapters 3 to 9, the diagram setting out the Cumulative Multilevel Analysis has been completed for each separate NBT. The analysis (flowchart and checklist) has been completed with regard to the final organism, and separately also for intermediary stages where relevant³⁸.

In this way a clear visual representation is provided of the longer textual analysis provided under each NBT. The diagram has been completed in each instance to show the first (earliest) stage at which it can be determined that an NBT does or does not yield a GMO.³⁹ In the event that the reader takes a different view, and for the sake of completeness, the checklists in Appendix 2 include answers to all of the considerations under the legislative regime, going beyond the first stage at which it can be determined that an NBT does or does not yield a GMO. Nonetheless, the completed diagrams represent the NBT Platform's best analysis in each case.

³⁸ Any transient GMO-steps during the development and breeding process might be covered by Directive 2001/18/EC and related permits, any GMM-steps during the process might be covered by Directive 2009/41/EC and related permits.

³⁹ Thus, if it can be determined that there is no GMO at stage 4 of the diagram, it is not necessary to continue it and show how (if stage 4 is not considered to be exclusionary) the same conclusion can be arrived at by continuing to stages 6 or 7.

Cumulative Multilevel Analysis



NBT analysis under Directive 2001/18/EC. Each stage requires analysis, ultimately allowing for classification of organisms developed with current and future breeding techniques.

Check List for Classifying products resulting from NBTs

Questions		No	Yes	
1	Is it an organism?			If No - not a GMO
2	Is it a human organism?			If Yes not a GMO
3	Is genetic material altered? (20 bp or more)			If No - not a GMO
4	Is the result of the genetic alteration <i>capable</i> of occurring naturally by mating &/or natural recombination?			If Yes - not a GMO
5	Is one of the three <i>examples</i> of GMO techniques listed in Annex I A Part 1 employed?			If Yes = GMO
	(a) Recombinant nucleic acid techniques (<u>all 5 tests</u> below must be satisfied)?			If Yes = GMO
	(i) They lead to the formation of new combinations of genetic material?			If No - not a GMO
	(ii) They do so by the insertion of nucleic acids produced outside an organism into any vector system (including virus or bacterial plasmid)?			If No - not a GMO
	(iii) They lead to incorporation of nucleic acid into a host organism?			If No - not a GMO
	(iv) Such nucleic acids naturally occur in the host organism?			If Yes - not a GMO
	(v) The nucleic acids are capable of continued propagation in the host organism?			If No - not a GMO
	(b) Direct introduction (tests (i) and (ii) are <u>both</u> satisfied)?			If Yes = GMO
	(i) Direct introduction into an organism of heritable material?			If No - not a GMO
	(ii) Heritable material prepared outside the organism?			If No - not a GMO
	(c) Cell fusion by <u>not</u> naturally occurring means including protoplast fusion or hybridisation techniques (must satisfy <u>all</u> 3 tests below)?			If Yes = GMO
	(i) live cells with new combinations of heritable genetic material are formed?			If No - not a GMO
	(ii) formed through the fusion of two or more cells?			If No - not a GMO
	(iii) formed by means of methods that are not capable of forming naturally?			If No - not a GMO
	(d) Is another technique, not explicitly listed in Annex I A Part 1 employed (and 6 & 7 do not apply)?			If Yes = GMO
6	Is one of the three Non-GM techniques listed in Annex I A Part 2 involved?			If Yes - not a GMO
	(a) In vitro fertilisation?			If Yes - not a GMO
	(b) Natural processes (<i>non-exhaustive list</i>)?			If Yes - not a GMO
	(c) Polyploidy induction?			If Yes - not a GMO
	(d) Does the technique involve recombinant nucleic acid molecules?			If No - not a GMO
	(e) Does the technique involve GMOs (made by non-Annex I B techniques; see 7, below)?			If No - not a GMO
7	Is one of the two excluded modification techniques/methods not yielding GMOs listed in Annex I B involved?			If Yes - not a GMO
	(a) Mutagenesis?			If Yes - not a GMO
	(b) Fusion of cells from crossable plants?			If Yes - not a GMO
	(c) Does the technique involve recombinant nucleic acid molecules?			If No - not a GMO
	(d) Does the technique involve a GMO (made by other modification techniques covered by Directive 2001/18/EC under 1 to 5, above)?			If No - not a GMO

3. Legal reasons why most products resulting from Zinc Finger Nuclease techniques do not give rise to a GMO

3.1. Overview of Zinc finger nuclease techniques⁴⁰

ZFN-1 and ZFN-2 are forms of precision *mutagenesis* that result in far fewer alterations to the genetic material of an organism than classical mutation technologies (which use chemical agents or radiation). ZFN-1 and ZFN-2 induce alterations to the sequence of the genetic material by means of an oligopeptide which performs exactly the same function as classical mutagenesis but is limited to one mutation at one well-defined predetermined position in the DNA of an organism. This is in contrast with classical mutagenesis, which induces thousands of random alterations to the genetic material.

ZFN-3 (like ZFN-1 and ZFN-2) results in a double strand break at a predetermined location in the DNA of an organism. In contrast to ZFN-1, in ZFN-3 a nucleic acid molecule is added which is incorporated into the DNA of the host organism by the endogenous DNA repair machinery.

3.2. ZFN-1 Legal Analysis

ZFN-1 results in a double strand break at a predetermined location in the DNA of an organism. The *natural* DNA-repair machinery then repairs the break using the enzymes and nucleic acids that are *naturally available* in the cell. The double strand break and subsequent repair thereof can lead to site-specific deletion, of one or a few base pairs. ZFN-1 does not give rise to products subject to the requirements of Directive 2001/18/EC because of the following regulatory reasons:

- a) No genetic material is inserted into the genome (see section 2.6 above).
- b) The alteration is capable of occurring naturally by mating and/or natural recombination.
- c) The oligonucleotides are not self-propagating entities and do not contain sequences necessary for replication.
- d) When focussing on the process only, ZFN1 is considered a form of mutagenesis⁴¹ (subject to Annex I B) not involving recombinant nucleic acid molecules (see section 2.7, above, on 'mutagenesis'). The fact that it is more precise does not necessarily exclude it from Annex I B or subject it to GMO regulation.⁴²
- e) Even if it is not considered subject to Annex I B (because it is a refinement of classical mutagenesis), ZFN-1 falls within the 'natural processes' category in Annex I A Part 2⁴³ since in nature such deletions occur naturally (see point b). This technique replicates - in an accelerated and more efficient fashion - natural processes. Recombinant nucleic acid techniques (see section 2.4, above) are absent because, among other considerations, ZFN-1 does not involve the *insertion of foreign DNA* produced *outside* the organism (see section 2.6, above).

See the Flow Chart for classifying NBTs, completed for products arising from this technique on page 16, and the Checklist for the complete analysis of ZFN-1 (Appendix 2).

⁴⁰ Since it is a well documented group of techniques, ZFN technology is used as an example of a much larger group of related new genetic-alteration techniques, commonly referred to as Site Directed Nucleases (which includes ZFNs, but also TALENs and Meganucleases).

⁴¹ See checklist analysis, Appendix 2, 'ZFN-1'.

⁴² This is also the conclusion reached by the majority of the New Techniques Working Group for ZFN-1 and ZFN-2 (para. 5.2.1.5, A, 4th bullet, in their 2011 report) and the German Central Commission for Biological Safety (ZKBS) June 2012 'Opinion on New Techniques for Plant Breeding' (2012).

⁴³ See note 36.

3.3.ZFN-2 Legal Analysis

ZFN-2 also results in a double strand break at a predetermined location in the DNA of an organism. However, ZFN-2 technology makes use of a repair template which leads to the *sequence alteration* (which can also be a short deletion or addition) of one or a few base pairs (at any rate certainly less than the 20 bp required to be classified as a new combination of genetic material, discussed in section 2.6). No foreign DNA is inserted (see section 2.4, above). Accordingly, ZFN-2 does not give rise to products subject to the requirements of Directive 2001/18/EC for at least the same considerations as already noted above in section 3.2.

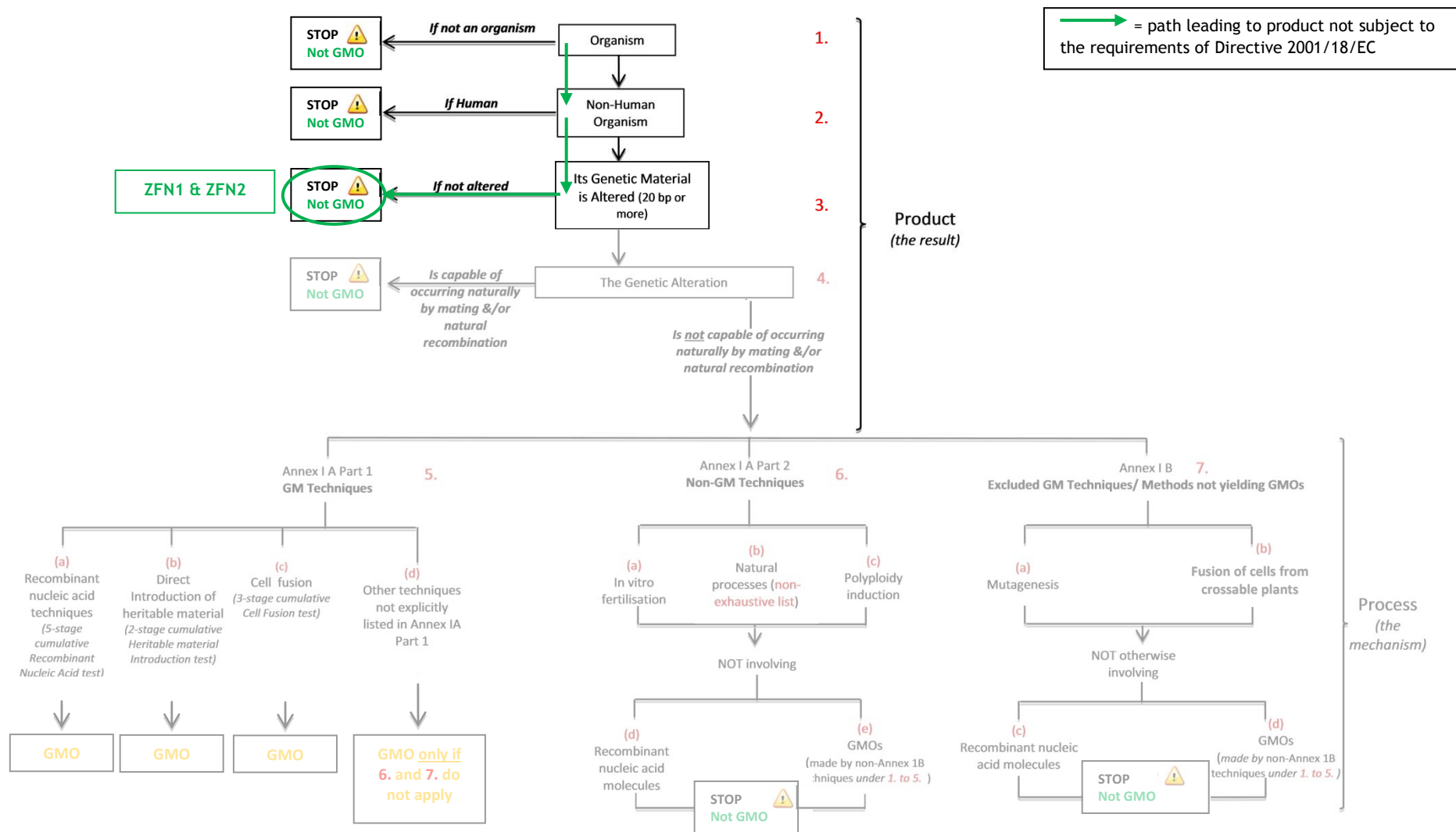
See the Flow Chart for classifying NBTs, completed for products arising from this technique on page 16, and the Checklist for the complete analysis of ZFN-2 (Appendix 2).

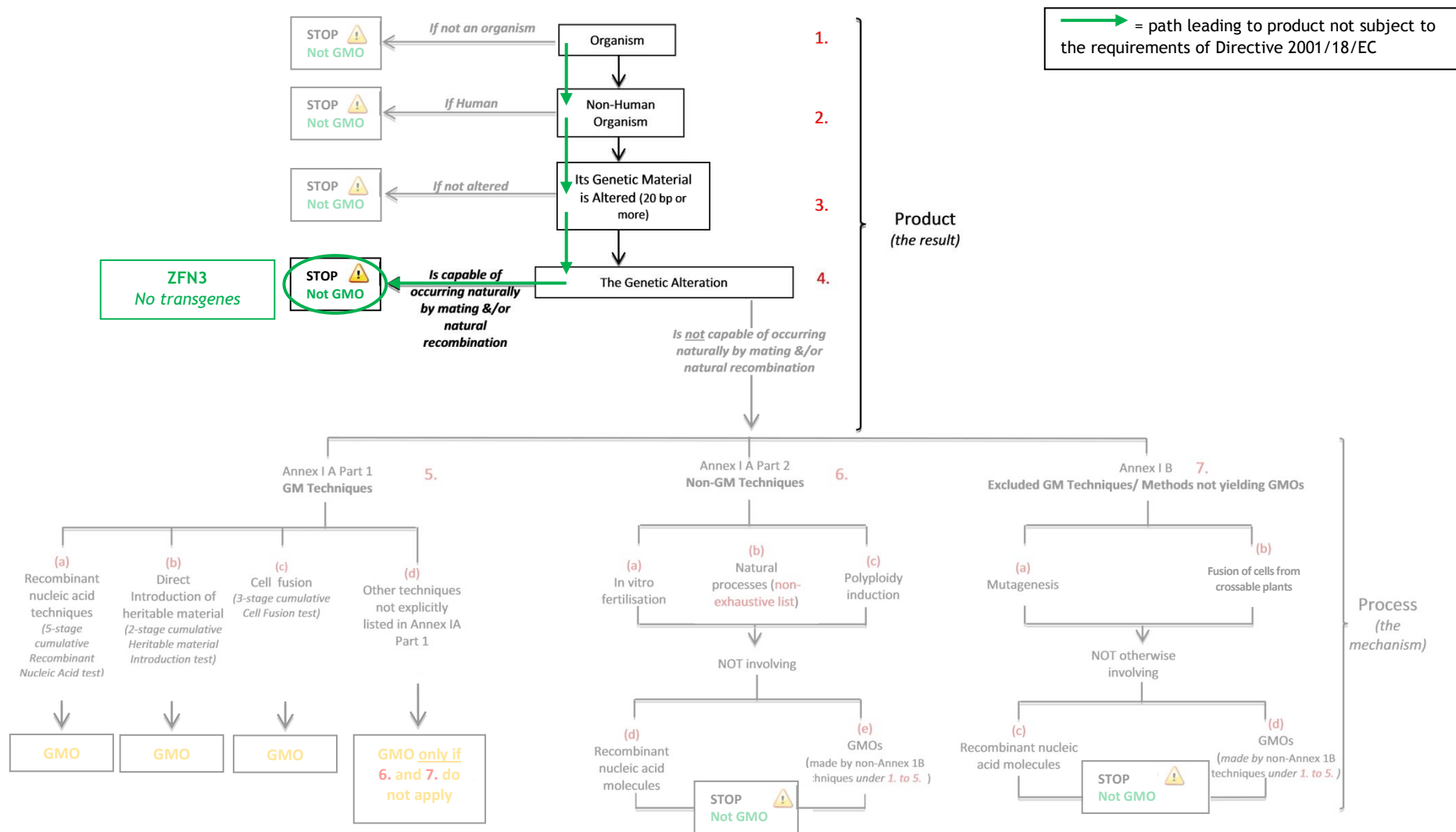
3.4.ZFN-3 Legal Analysis

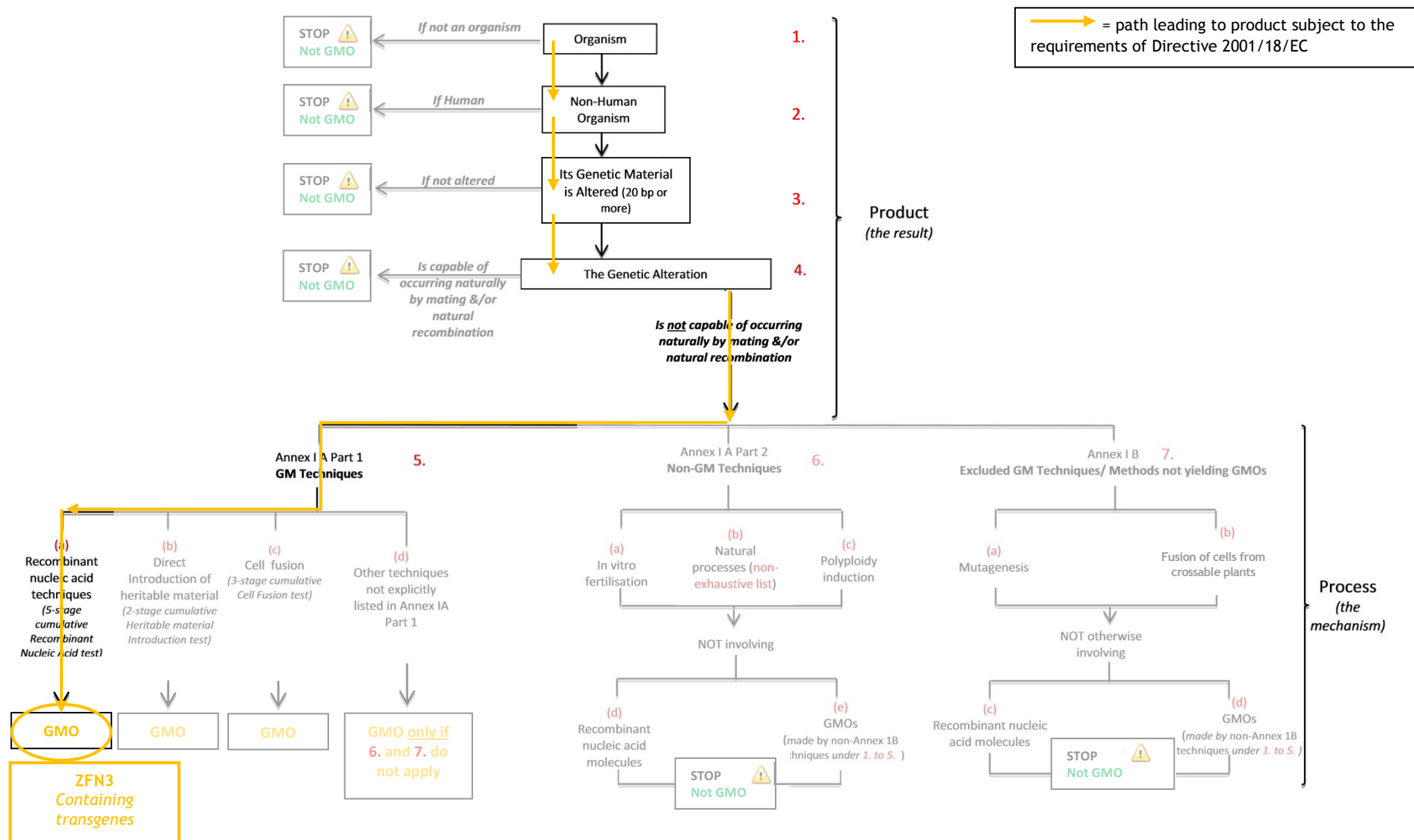
As already explained in section 2.1, above, a GMO is defined by a combination of the *process* (techniques) used to generate it *and* the properties of the resulting *product*. Whilst ZFN-3 targets delivery of genes (insertion), there will be occasions where ZFN-3 will not give rise to products subject to the requirements of Directive 2001/18/EC, since:

- a) There is no formation of *new combinations* of genetic material (see section 2.5 and point 4 in the flow chart), provided the inserted genetic material is *naturally present* in the plant genome.
- b) The alteration is capable of occurring naturally by mating &/or natural recombination, provided the inserted genetic material is present in a *naturally crossable* species.

It is therefore only possible to establish on a case-by-case basis whether the particular use of ZFN-3 will give rise to a GMO or not. See the Flow Chart for classifying NBTs, completed for products arising from this technique on page 18, and the Checklist for the complete analysis of ZFN-3 (Appendix 2).







4. Legal reasons why products resulting from Oligonucleotide-Directed Mutagenesis (ODM) do not give rise to a GMO

4.1. Overview of Oligonucleotide-Directed Mutagenesis

Oligonucleotide-Directed Mutagenesis (ODM) is a form of precision *mutagenesis* that results in far fewer alterations to the genetic material of an organism than classical mutation technologies (which use chemical agents or radiation)⁴⁴. ODM makes use of chemically modified oligonucleotides to generate a specific mutation at a predetermined location in the genetic material of an organism. The sequence of the oligonucleotide used is identical to the sequence of the target DNA, except for the intended mismatch. The oligonucleotide then pairs with one strand of the target DNA. The mismatch is recognised by the natural DNA repair machinery of the cell leading to the alteration of one or a few nucleotides at the predetermined location in the genetic material of the host organism. The oligonucleotide does not integrate into the genetic material of the host organism, since it is not chemically compatible with natural DNA propagation. The oligonucleotide is only present for a short period of time and is degraded by the cell.

4.2. Legal Analysis

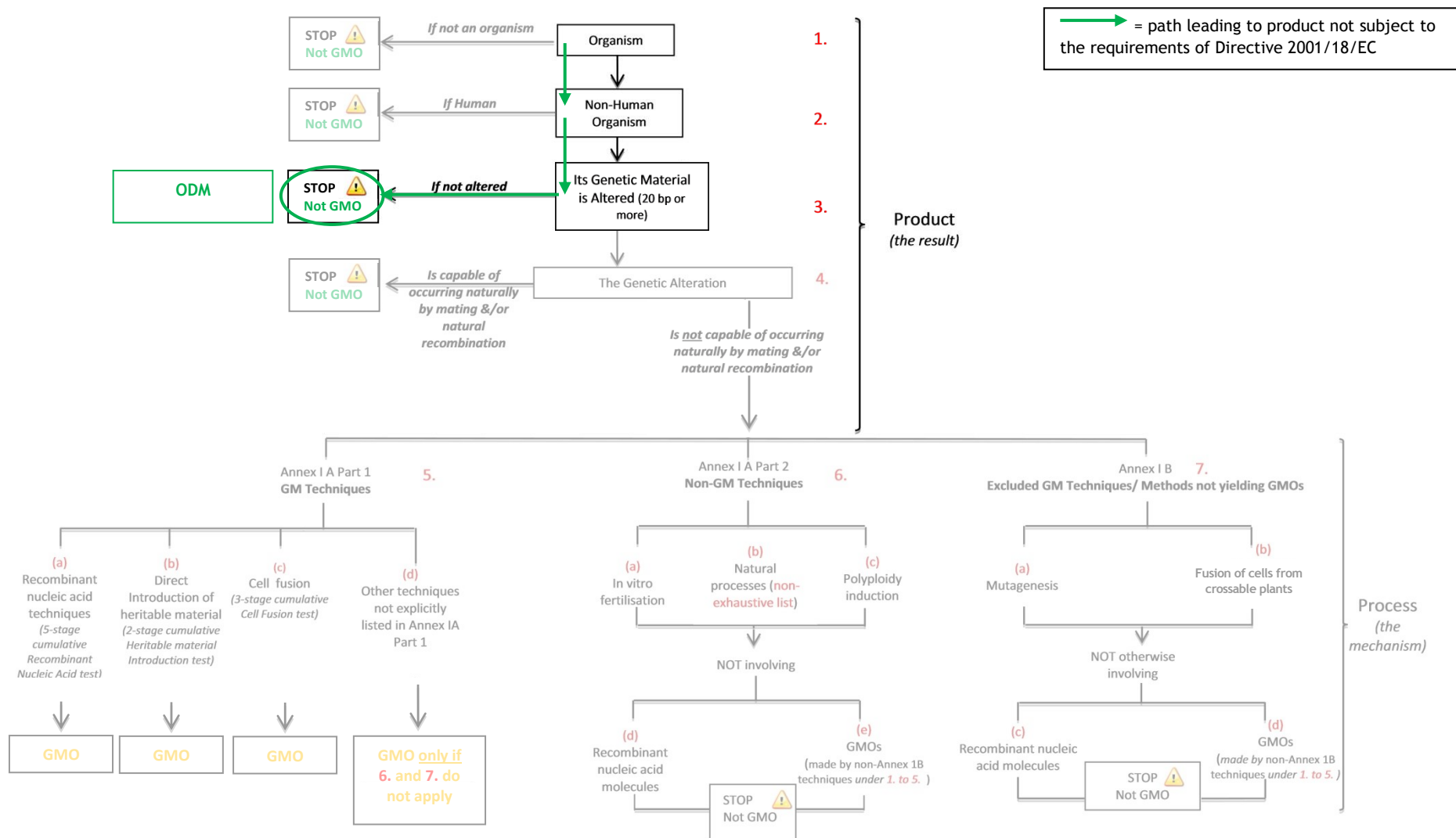
ODM generates a permanent structural alteration and a heritable change in an organism's genetic material by means of an oligonucleotide. ODM does not involve recombinant nucleic acid techniques or recombinant nucleic acid molecules (see section 2.4.1, above). ODM does not give rise to GMOs for a number of reasons⁴⁵:

- a) No genetic material is inserted into the genome (see section 2.6 above).
- b) The oligonucleotides are not self-propagating entities and do not contain sequences necessary for replication.
- c) The alteration is capable of occurring naturally by mating and/or natural recombination.
- d) When focussing on the process only, ODM is considered a form of mutagenesis (subject to Annex I B) not involving recombinant nucleic acid molecules (see section 2.7, above, on 'mutagenesis'). The fact that it is more precise does not necessarily exclude it from Annex I B or subject it to GMO regulation.
- e) Even if it is not considered subject to Annex I B (because it is a refinement of classical mutagenesis), ODM falls within the 'natural processes' category in Annex I A Part 2 since in nature such mutations and deletions occur naturally (see point b). This technique replicates - in an accelerated and more efficient fashion - natural processes. Recombinant nucleic acid techniques (see section 2.4, above) are absent because, among other considerations, ODM does not involve the *insertion of foreign DNA* produced *outside* the organism (see section 2.6, above).

See the Flow Chart for classifying NBTs, completed for products resulting from this technique on page 20, and the Checklist for the complete analysis of ODM (Appendix 2).

⁴⁴ ODM generates single and well defined alterations to the genetic material of an organism, whereas classical mutagenesis induces thousands of random alterations to the genetic material.

⁴⁵ It is noteworthy that overall conclusions in section 4.2 have been supported by the independent assessments made by the Netherlands Commission on Genetic Modification (COGEM, 2010)) and the UK Advisory Committee on Releases to the Environment (ACRE, 2011).



5. Legal reasons why products resulting from Cisgenesis do not give rise to a GMO

5.1. Overview of Cisgenesis

Cisgenesis is the genetic modification of a recipient plant with a natural gene from a crossable (sexually compatible) plant. Accordingly, cisgenic plants do not contain foreign DNA, so no new combinations of genetic material are formed when compared with plants resulting from conventional breeding by mating and/or natural recombination (see section 2.5, above). The genes that are introduced via cisgenesis may therefore also be present in an organism as the result of natural mating. Cisgenesis can be achieved through the use of different technologies which alter the genetic material of an organism, e.g. *Agrobacterium tumefaciens*-mediated transformation, micro-injection or ZFN-3 technology. The term cisgenesis therefore points more to the characteristics of the end product than to the actual technique that is used to achieve the genetic alteration.

The natural gene introduced includes its introns and is flanked by its native promoter and terminator in the normal sense orientation. Cisgenic plants can harbour one or more cisgenes, but they do not contain any transgenes.

5.2. Legal Analysis

Cisgenesis does lead to the insertion of nucleic acid molecules but (as set out in section 2.4.1) while this is a *necessary* condition of a process giving rise to a GMO this is not in itself sufficient⁴⁶. In fact, cisgenesis fails to satisfy a number of the core regulatory requirements necessary to give rise to GMOs:

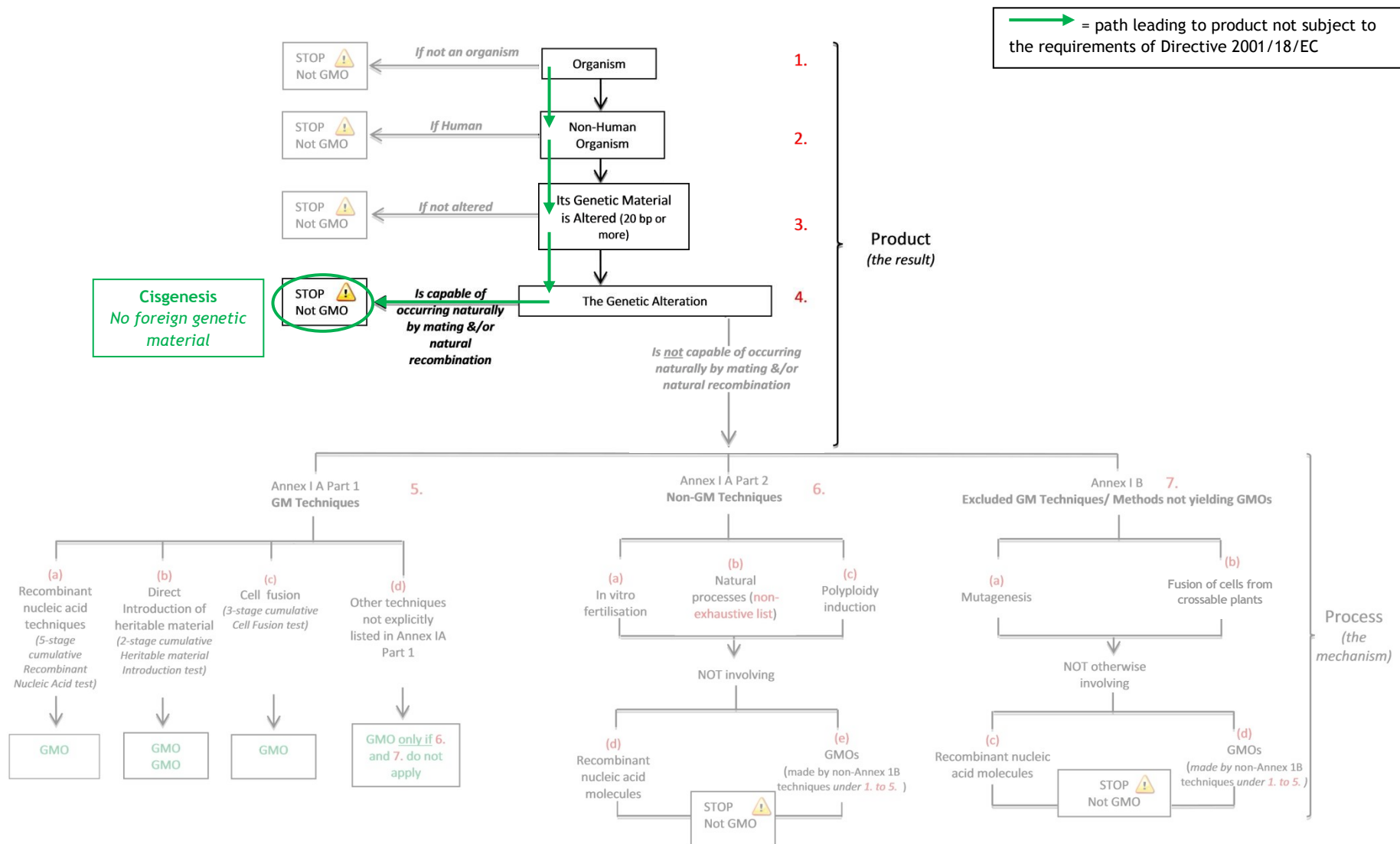
- a) New combinations of genetic material are not formed (because there is no foreign DNA)⁴⁷.
- b) The alteration is capable of occurring naturally by mating and/or natural recombination.
- c) The nucleic acid molecules are capable of occurring naturally in the host organism.

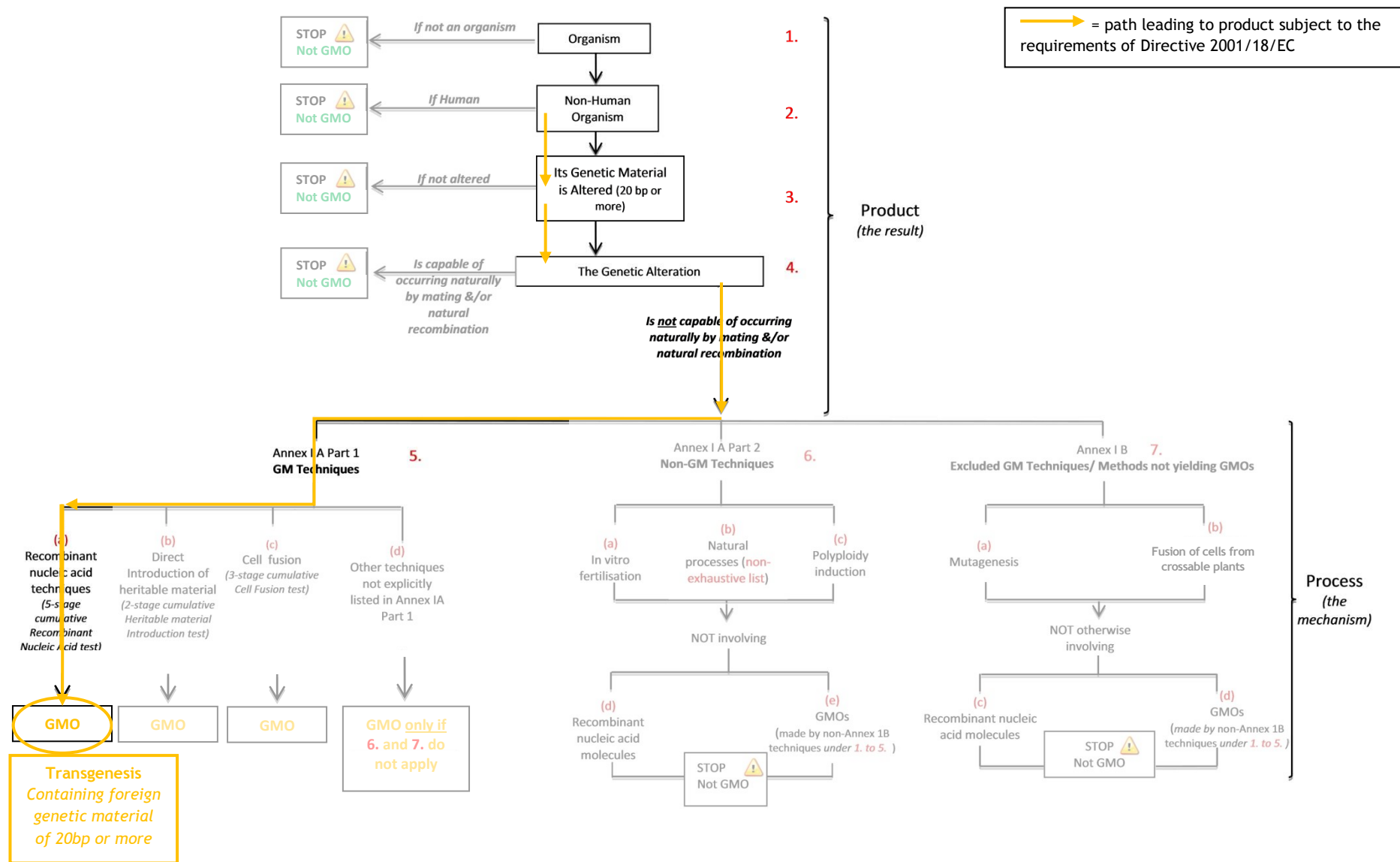
Further, when transformation by means of *Agrobacterium tumefaciens* is used to achieve cisgenesis, this may result in co-insertion of remainders of border sequences of the T-DNA, flanking the insert. In order to satisfy point 5 in the Cumulative Multilevel Analysis NBT analysis (see section 2.8 and below), the T-DNA border cannot exceed 20bp in length (see section 2.6) or must be shown to be naturally present in the plant genome.

See the Flow Chart for classifying NBTs, completed for products arising from this technique on page 22 (cisgenesis without the introduction of ‘foreign genetic material’ according to the 20bp rule discussed above) and the Checklist for the complete analysis of cisgenesis (Appendix 2). If foreign genes or foreign DNA sequences of 20 bp or more are introduced, this is regarded as transgenesis (see flowchart on page 23).

⁴⁶ The insertion of foreign marker genes into the plant genome (as a product) creates a transgenic species, classified as GMO at point 5 in the Cumulative Multilevel Analysis under Directive 2001/18/EC. See flowchart below.

⁴⁷ Some argue that insertion of cisgenes leads to ‘genomic disruption’ and that this is an unnatural process. However, this is not correct since the phenomenon of genomic disruption is a natural process that occurs on a very regular basis in plants resulting from transposon activity, natural mutagenesis, natural recombination and natural genomic rearrangements. Genomic disruptions do ‘occur naturally by mating and/or natural recombination’, and therefore do not give rise to GMOs. Gene duplication is a frequently occurring natural phenomenon in plant genomes; an example of naturally occurring cisgenesis in plants is described in the Science article “A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit” (2008, Vol. 319, pp. 1527-1530). This paper describes the natural duplication of a gene in tomato, and insertion of this gene elsewhere in the tomato genome, leading to elongated tomatoes that are sold around in numerous shops around the world.





6. Legal reasons why products resulting from RNA-dependent DNA methylation (RdDM) do not give rise to a GMO

6.1. Overview of RNA-dependent DNA methylation (RdDM)

RdDM is a technique which makes use of a small RNA molecule that induces methylation at a targeted DNA sequence. A construct coding for the small RNA molecule (inducer construct) is introduced and incorporated in a parental plant. The resulting small RNA molecule leads to methylation of the target DNA sequence, and hence the silencing of the targeted gene *without altering the DNA sequence*. Subsequently, the mother plant is crossed and the inducer construct is segregated away in the progeny. The gene remains silenced during one or a few generations, but eventually the trait will gradually disappear due to the natural removal of the methyl groups from the nucleotides. The technique is based on the naturally occurring mechanisms of the plant cell whereby small RNA molecules dynamically affect gene expression through methylation during plant growth and development.

6.2. Legal Analysis

When looking at the parental plant it is clear that recombinant nucleic acid techniques are used (step 5 in the decision Flow Chart; the inducer construct is inserted into the genome of the mother plant) and this is not an alteration which is capable of occurring naturally.

Intermediate plants containing transgenes (part of the *process*) are classified as GMO and can be subject to e.g. Directive 2001/18/EC.

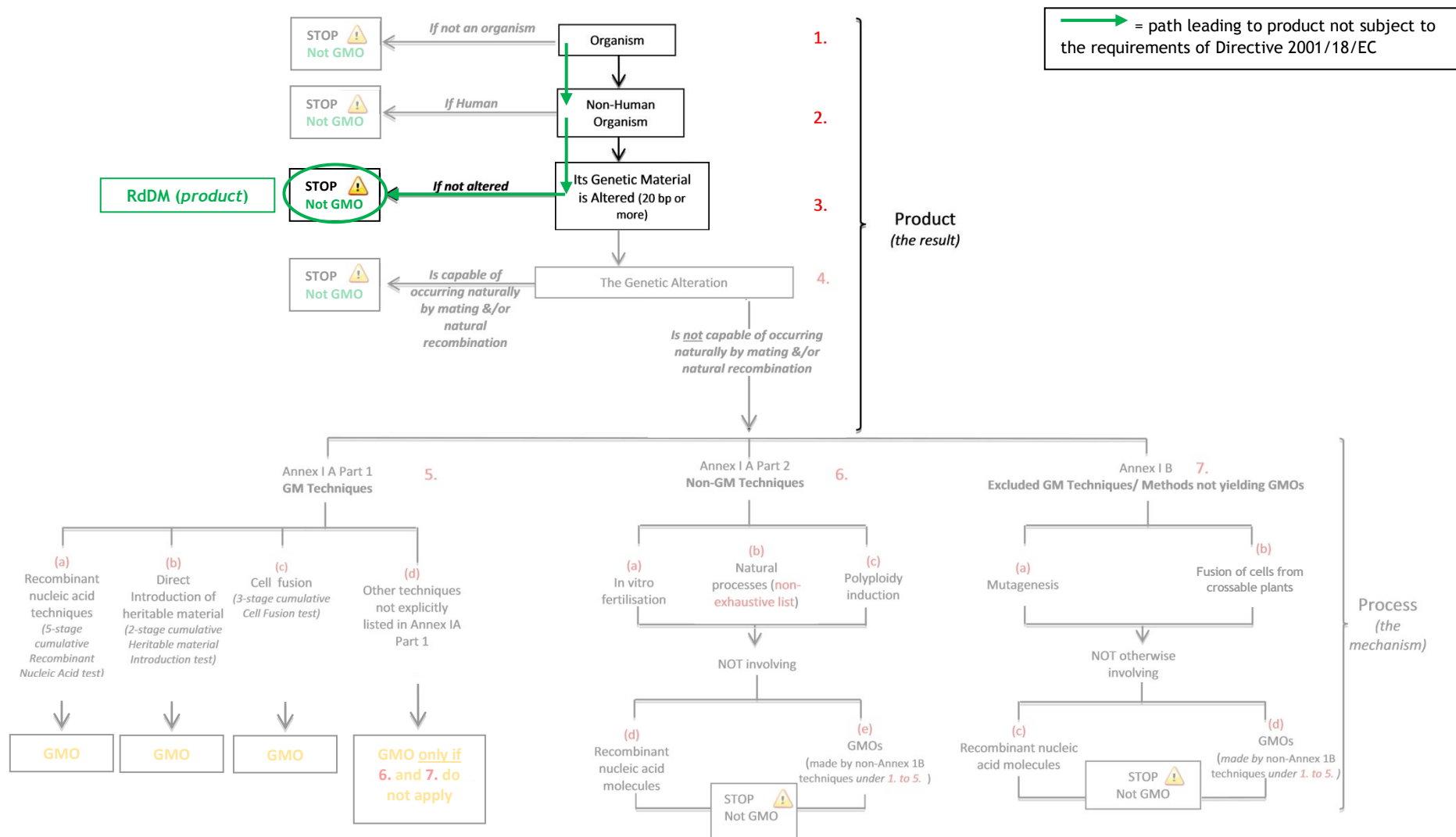
However, for the progeny (the *product*) that does not contain the inducer construct, RdDM fails to satisfy a number of the core regulatory requirements necessary to give rise to products subject to the requirements of Directive 2001/18/EC:

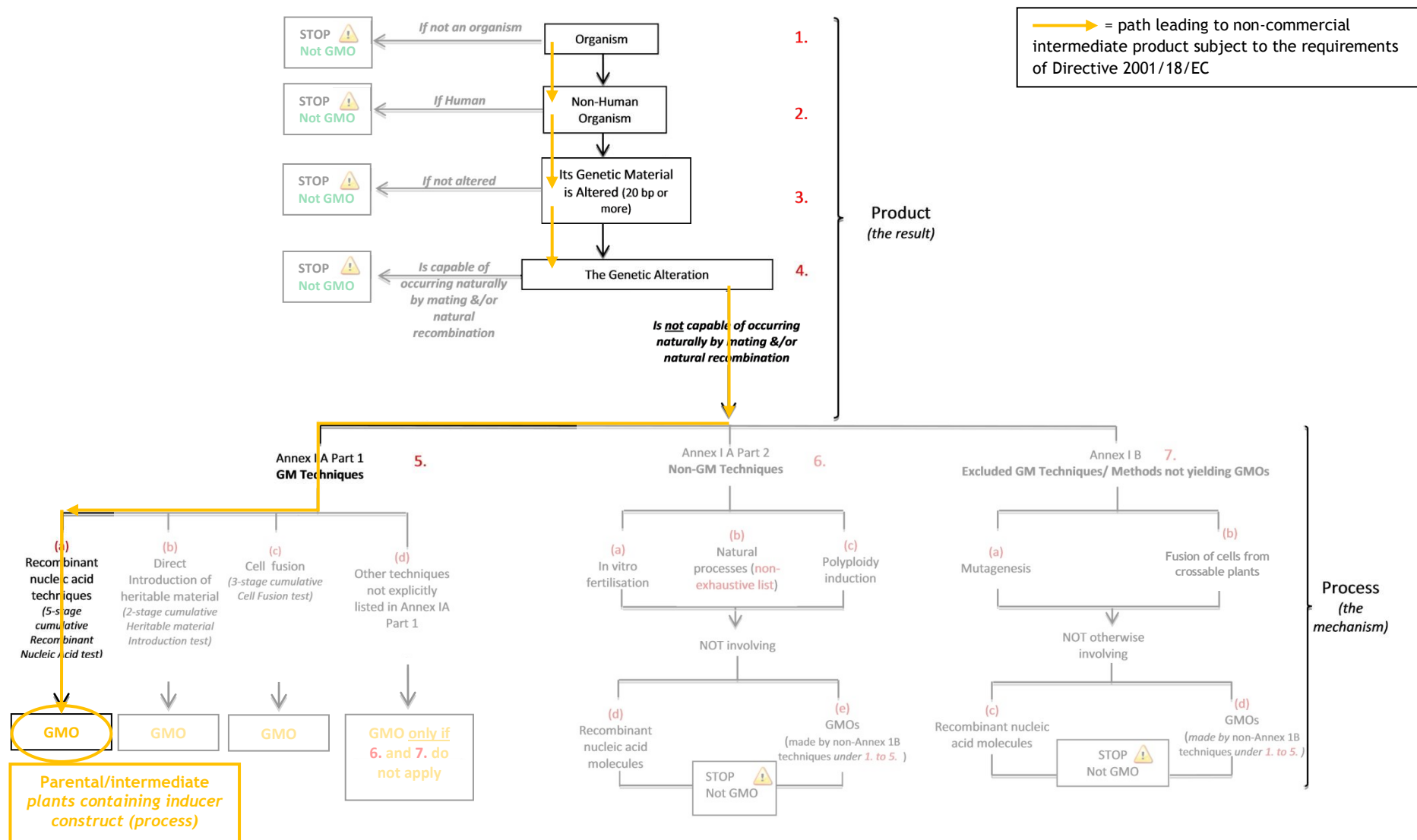
- a) Offspring plants still have the methylated silenced gene sequence, but do not contain the inducer construct. The offspring plants can therefore properly be classified as substantially *unaltered vis-à-vis* the parental plant since the alteration is epigenetic and transient⁴⁸.
- b) The resulting organisms and their offspring can be obtained by traditional breeding methods.⁴⁹

See the Flow Chart for classifying NBTs, completed for products arising from this technique on page 25, and intermediate plants on page 26. Also see the Checklist for the complete analysis of RdDM (Appendix 2)

⁴⁸ See section 2.4.1

⁴⁹ Needless to say (as noted by the New Techniques Working Group), this interpretation is only valid on the condition that only non-GM homozygous plants (doubled haploids) are chosen after the genetic modification step, in order to perform crosses to obtain the desired hybrids.





7. Legal reasons why products resulting from grafting do not give rise to a GMO

7.1 Overview of grafting (non-GM scion on GM-rootstock)

The technique of grafting has been used by breeders for thousands of years. Grafting refers to a process whereby two parts of two distinct plants are fused. For agricultural use, a vegetative upper part, or 'scion', is grafted onto a rooted bottom part of a second plant, the so-called 'rootstock'. This is a simple mechanical process of cutting and subsequent joining (bandaging) of the plant parts, which grow together and fuse. Grafting itself is thus not a method of genetic modification.

7.2 Legal Analysis

Assuming the rootstock is a GMO (and therefore can be subject to Directive 2001/18/EC) and the scion is not a GMO (not subject to Directive 2001/18/EC), the scion (the *product*) does not satisfy a number of the core regulatory requirements necessary to produce materials⁵⁰ subject to the requirements of Directive 2001/18/EC:

- a) The scion (the *product*) is not genetically altered, nor are the fruits, seeds or other materials from the scion genetically altered in any way;
- b) The *process* of grafting is not a recombinant DNA technique, nor a direct introduction of heritable material, nor a process of cell fusion.

The products resulting from a non-GM scion on a GM rootstock, such as fruits and seeds, are therefore not subject to the requirements of Directive 2001/18/EC, nor do they fall within the scope of the GM food & feed regulation 1829/2003/EC.

See the Flow Chart for classifying NBTs, completed for products arising from this technique on page 28, and for the rootstock on page 29. Also see the Checklist for the complete analysis of Grafting (Appendix 2).

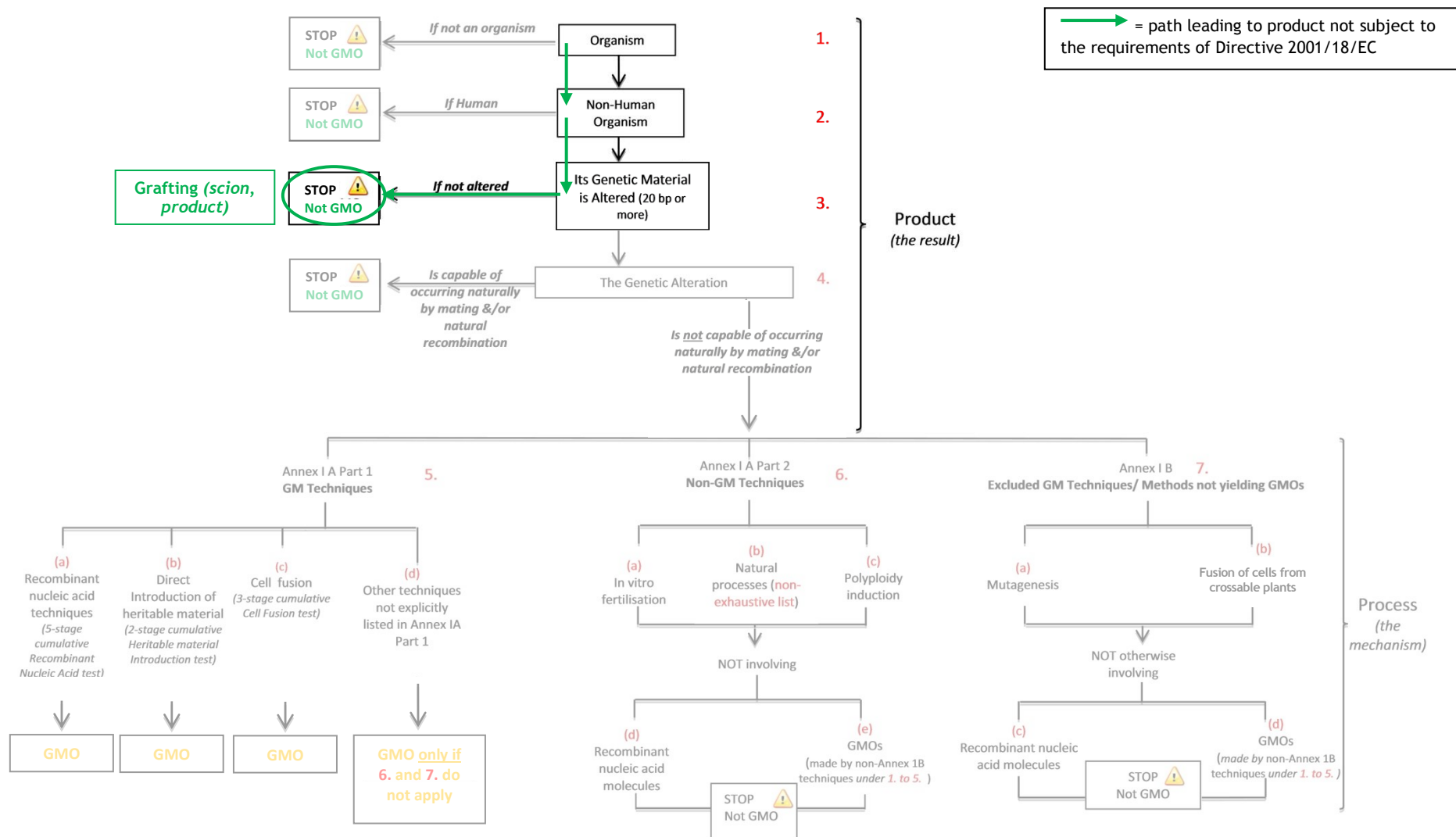
7.3 Note on the use of GM rootstocks

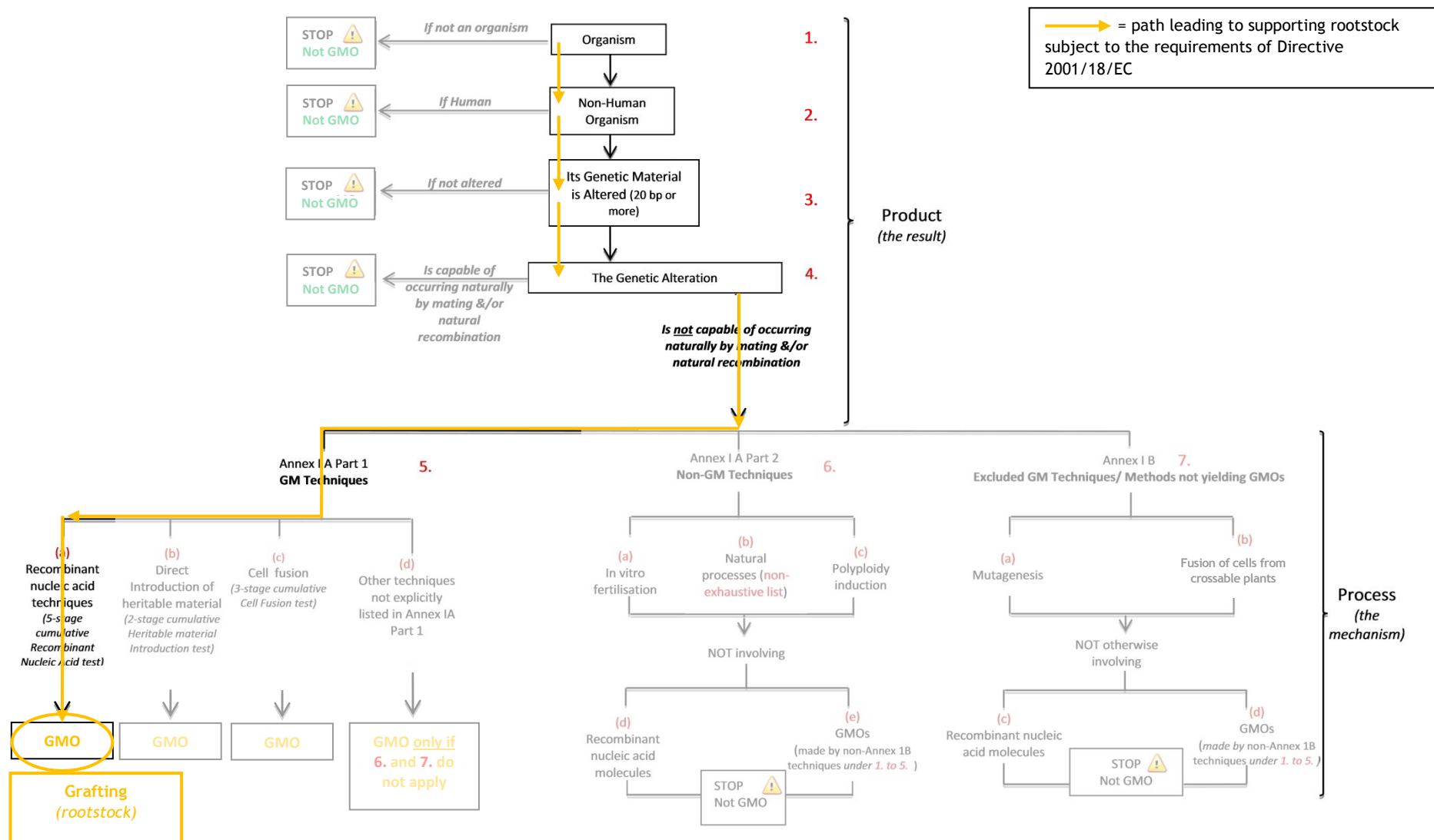
A genetically modified rootstock is subject to the requirements of Directive 2001/18/EC when it is capable of replication or of transferring genetic material (see 2.2.). In specific cases and depending on the crop, a GM rootstock may also be able to replicate vegetatively or by forming generative plant parts, with or without the presence of a non-GM scion. A GM rootstock will therefore be subject to the requirements of Directive 2001/18/EC.

However, in accordance with the principle of proportionality⁵¹ one GM rootstock should be able to be combined with all possible different non-GM scions, without requiring a new dossier for each new combination. Accordingly, the market authorization of a GM rootstock should include all possible combinations with non-GM scions.

⁵⁰ In this case, referring to the seeds and fruits harvested from the scion.

⁵¹ See section 2.8, point (3).





8. Legal reasons why products resulting from Reverse Breeding do not give rise to a GMO

8.1. Overview of Reverse Breeding

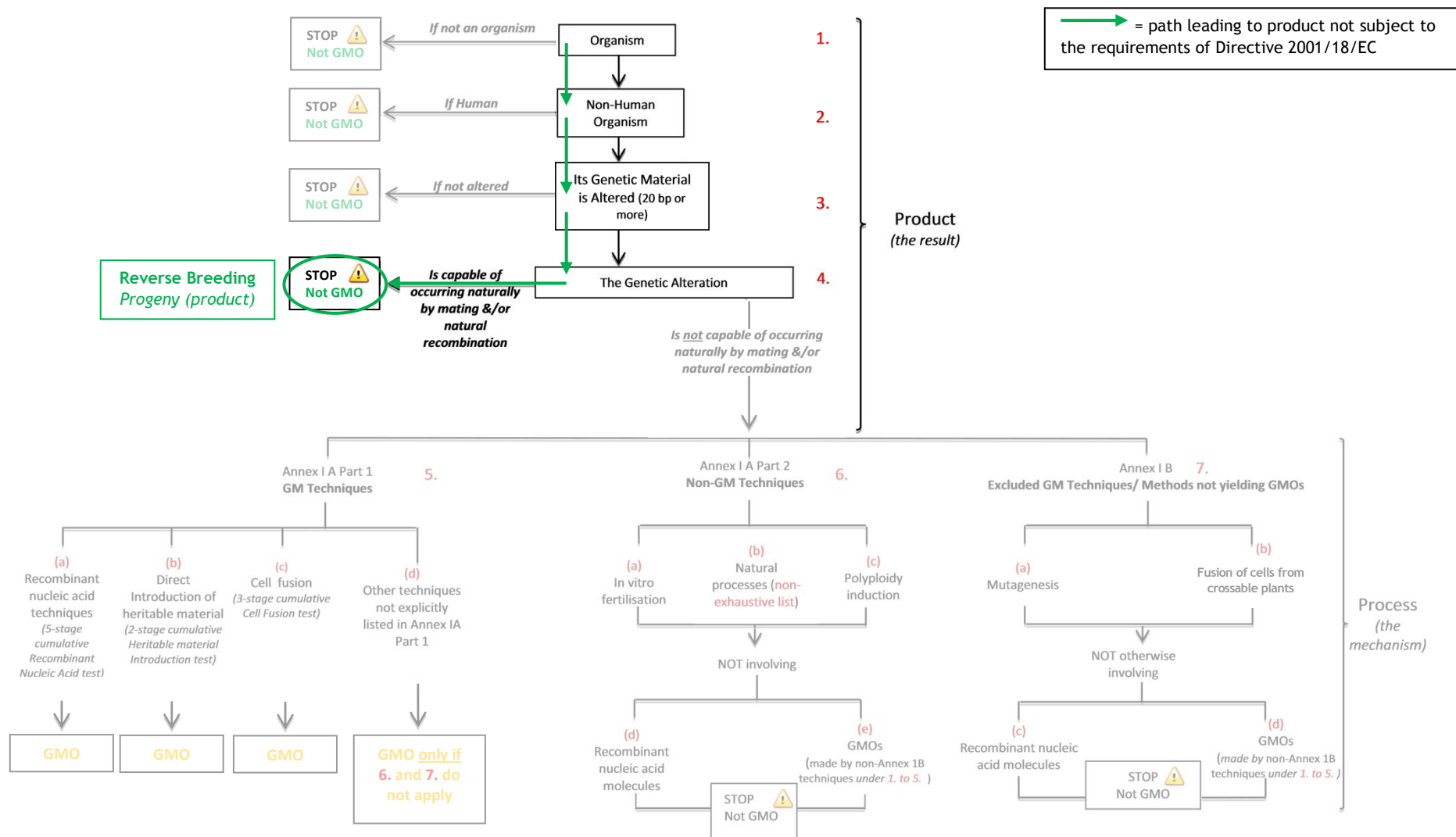
Traditional plant breeding makes use of heterosis by producing hybrid varieties which are superior to their known homozygous parents. Reverse Breeding is a new plant breeding technique that produces homozygous parental lines to be used for the reconstruction of any hybrid plant with (un)known parents.

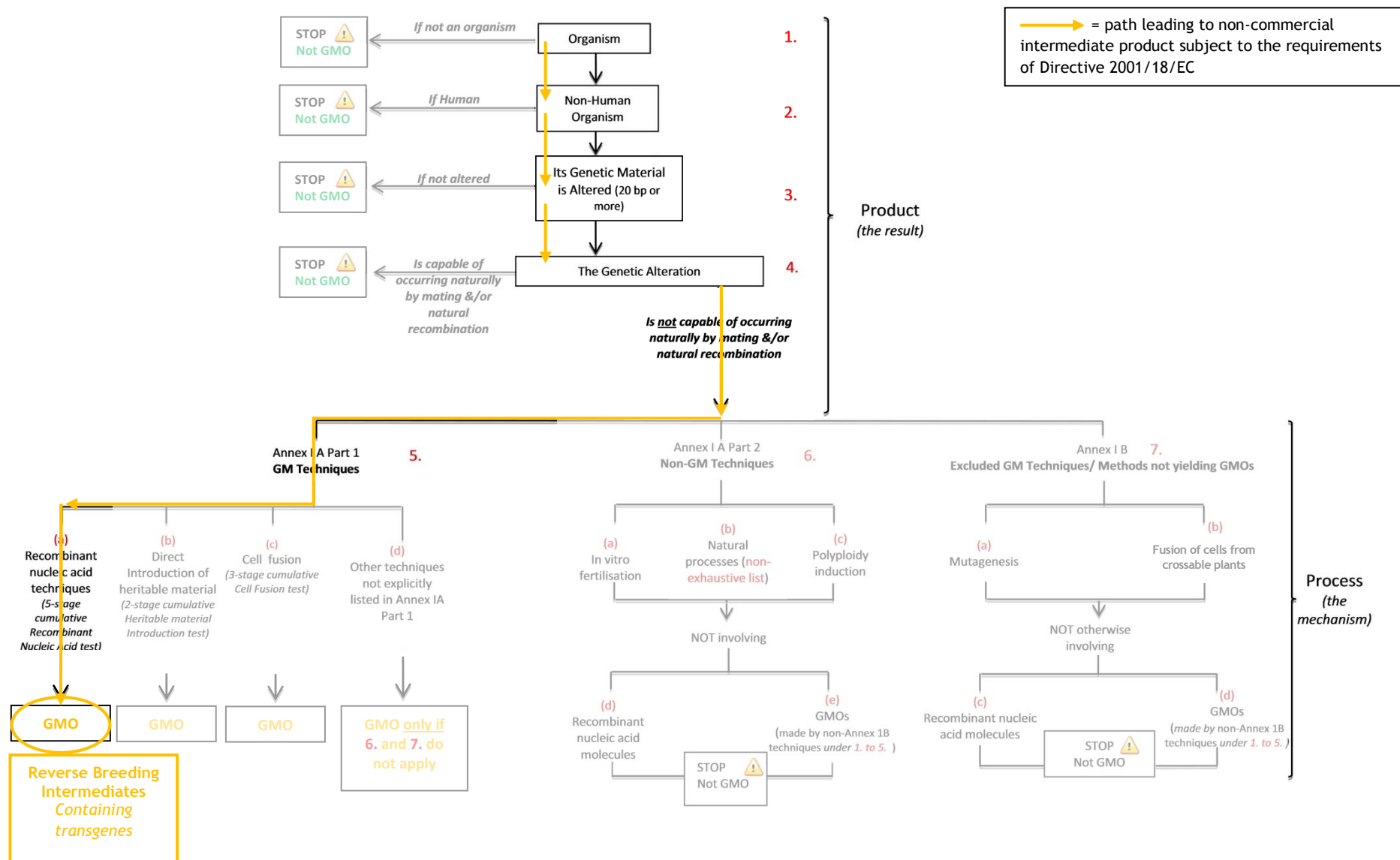
Reverse Breeding is based on the *suppression* of the genetic recombination, such that homozygous parental lines can be generated from a selected hybrid plant. The suppression of genetic recombination is achieved by introducing a construct through genetic modification. The genetic modification is only present in intermediate plants, and these intermediate plants containing transgenes (part of the *process*) are therefore classified as GMO and can be subject to Directive 2001/18/EC. During selection, complementing parental transgene-free plant pairs are selected, such that the desired transgene-free hybrid (the *product*) can be created by natural crossing.

8.2. Legal Analysis

Both the final transgene-free parental plant lines and the resulting hybrid plants (also transgene-free) do not satisfy the core regulatory requirement necessary to give rise to GMO since the genetic material of the hybrid plants (the *product*) is altered in a way that is capable of occurring naturally by mating and/or natural recombination.

See the Flow Chart for classifying NBTs, completed for products arising from this technique on page 31, and for the intermediate plants on page 32. Also see the Checklist for the complete analysis of Reverse Breeding (Appendix 2).





9. Legal reasons why products resulting from Agro-infiltration (Agro-infiltration '*sensu stricto*', Agro-inoculation) do not give rise to a GMO

9.1. Overview of Agro-infiltration⁵²

Agro-infiltration is an established technology in the field of plant molecular biology and plant breeding. Plant parts are brought into contact with cells of the bacterium *Agrobacterium tumefaciens* which has the innate ability to transfer and integrate a part of its own DNA (so-called T-DNA) into the genome of the plant. This capability has been exploited to (i) enable local introduction of genetic material in plants, and (ii) efficiently identify plants carrying a viral resistance gene by inducing a genetic modification of a non-generative plant part (this latter application is often termed 'Agro-inoculation'). Resistant plants identified through Agro-inoculation can be used to produce progeny which is then used to develop commercial varieties. As Agro-inoculation is applied very locally on a plant, the T-DNA is, as a rule, not stably incorporated in the germline and therefore not transmitted to progeny⁵³. Intermediate plants containing transgenes (part of the *process*) are classified as GMO and can be subject to Directive 2001/18/EC. Any intermediate GMMs are covered by Directive 2009/41/EC.

9.2. Legal Analysis

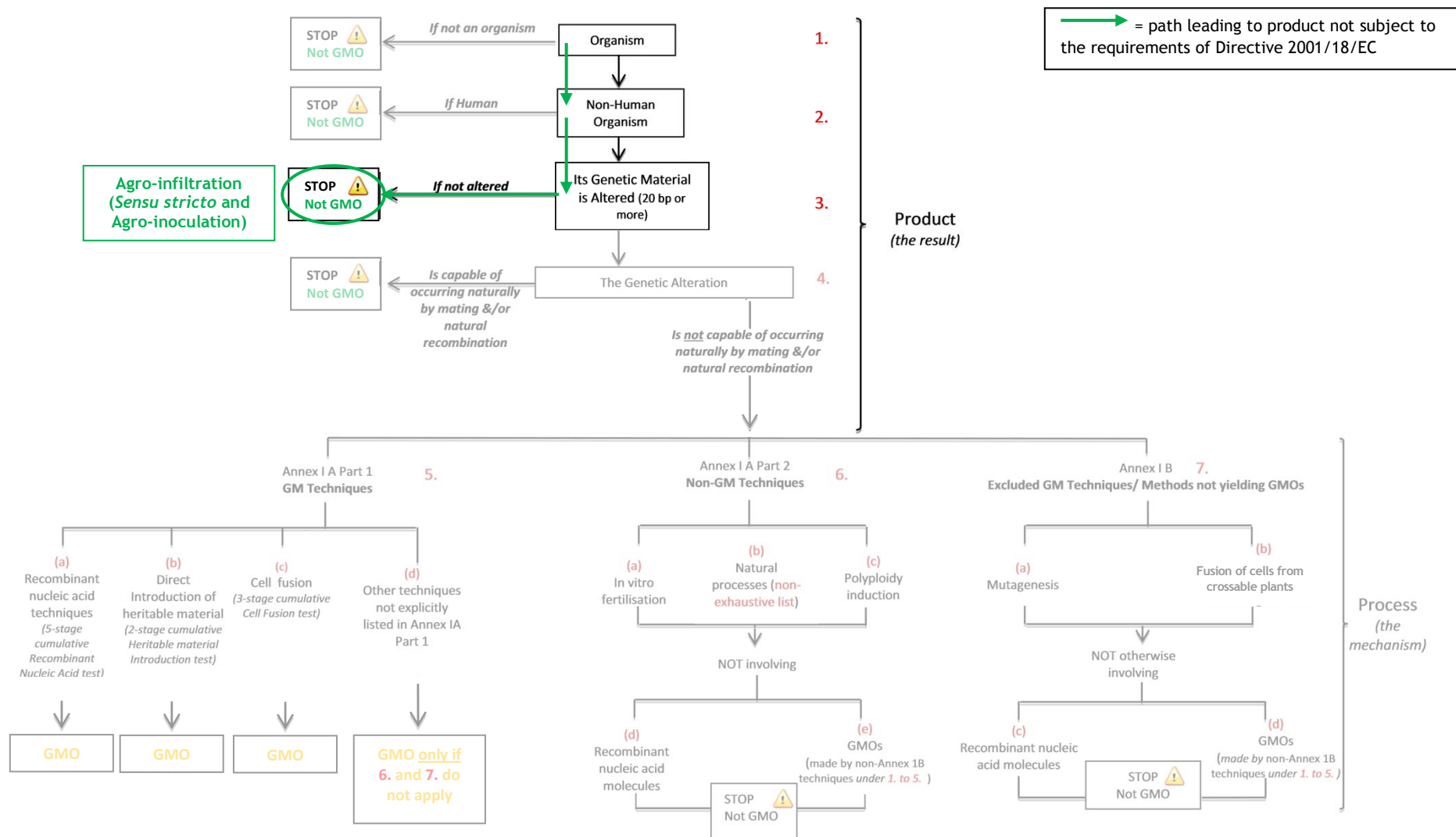
Agro-infiltration does not satisfy the core regulatory requirement necessary to give rise to GMOs:

- a) Intermediate plants contain T-DNA and/or genetically modified *Agrobacterium* transiently and do not carry any alterations in the germline. The progeny (the *product*) therefore does not contain genetic material that is altered.

See the Flow Chart for classifying NBTs, completed for products arising from this technique on page 35, and the Checklist for the complete analysis of Agro-infiltration *sensu stricto* (Appendix 2).

⁵² The derived technique 'floral dip' is excluded since it invariably leads to GMOs and is not relevant as a technique for cultivated crops.

⁵³ Quality checks ensure the absence of *Agrobacterium* and genetic modifications in seeds and progeny.



10. Additional references

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Appendix 1: Key Legislative Provisions from Directive 2001/18/EC

ANNEX I A

TECHNIQUES REFERRED TO IN ARTICLE 2(2)

PART 1

Techniques of genetic modification referred to in Article 2(2)(a) are *inter alia*:

- (1) recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation;
- (2) techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;
- (3) cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

PART 2

Techniques referred to in Article 2(2)(b) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms made by techniques/ methods other than those excluded by Annex I B:

- (1) in vitro fertilisation,
- (2) natural processes such as: conjugation, transduction, transformation, [i.e. all natural processes]
- (3) polyploidy induction.

ANNEX I B

TECHNIQUES REFERRED TO IN ARTICLE 3

Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below are:

- (1) mutagenesis,
- (2) cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.

Appendix 2: Checklists in support of classifying products of described NBTs

The following Appendix contains the checklists in support of classifying the products of NBTs described in this document. The most right hand column describes the result of each question.

All questions must be answered to come to a full analysis of the product of an NBT.

N.B. Only if the remark following a question is 'If Yes = GMO', and the answer is 'Yes' (e.g. Question 5 in the '*Checklist for classifying products resulting from NBTs*'), is the product considered subject to Directive 2001/18/EC.

ZFN-1: Checklist for classifying products resulting from NBTs

Questions		No	Yes	
1	Is it an organism?		X	If No - not a GMO
2	Is it a human organism?	X		If Yes not a GMO
3	Is genetic material altered? (20 bp or more)	X		If No - not a GMO
4	Is the result of the genetic alteration <i>capable</i> of occurring naturally by mating &/or natural recombination?		X	If Yes - not a GMO
5	Is one of the three <i>examples</i> of GMO techniques listed in Annex I A Part 1 employed?	X		If Yes = GMO
	(a) Recombinant nucleic acid techniques (<u>all 5 tests</u> below must be satisfied)?	X		If Yes = GMO
	(i) They lead to the formation of new combinations of genetic material?	X		If No - not a GMO
	(ii) They do so by the insertion of nucleic acids produced outside an organism into any vector system (including virus or bacterial plasmid)?	X		If No - not a GMO
	(iii) They lead to incorporation of nucleic acid into a host organism?	X		If No - not a GMO
	(iv) Such nucleic acids naturally occur in the host organism?		X	If Yes - not a GMO
	(v) The nucleic acids are capable of continued propagation in the host organism?	X		If No - not a GMO
	(b) Direct introduction (tests (i) and (ii) are <u>both</u> satisfied)?	X		If Yes = GMO
	(i) Direct introduction into an organism of heritable material?	X		If No - not a GMO
	(ii) Heritable material prepared outside the organism?	X		If No - not a GMO
	(c) Cell fusion by <u>not</u> naturally occurring means including protoplast fusion or hybridisation techniques (must satisfy <u>all</u> 3 tests below)?	X		If Yes = GMO
	(i) live cells with new combinations of heritable genetic material are formed?	X		If No - not a GMO
	(ii) formed through the fusion of two or more cells?	X		If No - not a GMO
	(iii) formed by means of methods that are not capable of forming naturally?	X		If No - not a GMO
	(d) Is another technique, not explicitly listed in Annex I A Part 1 employed (and 6 & 7 do not apply)?	X		If Yes = GMO
6	Is one of the three Non-GM techniques listed in Annex I A Part 2 involved?	X		If Yes - not a GMO
	(a) In vitro fertilisation?	X		If Yes - not a GMO
	(b) Natural processes (<i>non-exhaustive list</i>)?	X		If Yes - not a GMO
	(c) Polyploidy induction?	X		If Yes - not a GMO
	(d) Does the technique involve recombinant nucleic acid molecules?	X		If No - not a GMO
	(e) Does the technique involve GMOs (made by non-Annex I B techniques; see 7, below)?	X		If No - not a GMO
7	Is one of the two excluded modification techniques/methods not yielding GMOs listed in Annex I B involved?		X	If Yes - not a GMO
	(a) Mutagenesis?		X	If Yes - not a GMO
	(b) Fusion of cells from crossable plants?	X		If Yes - not a GMO
	(c) Does the technique involve recombinant nucleic acid molecules?	X		If No - not a GMO
	(d) Does the technique involve a GMO (made by other modification techniques covered by Directive 2001/18/EC under 1 to 5, above)?	X		If No - not a GMO

ZFN-2: Checklist for classifying products resulting from NBTs

Questions		No	Yes	
1	Is it an organism?		X	If No - not a GMO
2	Is it a human organism?	X		If Yes not a GMO
3	Is genetic material altered? (20 bp or more)	X		If No - not a GMO
4	Is the result of the genetic alteration <i>capable</i> of occurring naturally by mating &/or natural recombination?		X	If Yes - not a GMO
5	Is one of the three <i>examples</i> of GMO techniques listed in Annex I A Part 1 employed?	X		If Yes = GMO
(a) Recombinant nucleic acid techniques (<u>all 5 tests</u> below must be satisfied)?		X		If Yes = GMO
	(i) They lead to the formation of new combinations of genetic material?	X		If No - not a GMO
	(ii) They do so by the insertion of nucleic acids produced outside an organism into any vector system (including virus or bacterial plasmid)?	X		If No - not a GMO
	(iii) They lead to incorporation of nucleic acid into a host organism?	X		If No - not a GMO
	(iv) Such nucleic acids naturally occur in the host organism?		X	If Yes - not a GMO
	(v) The nucleic acids are capable of continued propagation in the host organism?	X		If No - not a GMO
(b) Direct introduction (tests (i) and (ii) are <u>both</u> satisfied)?		X		If Yes = GMO
	(i) Direct introduction into an organism of heritable material?	X		If No - not a GMO
	(ii) Heritable material prepared outside the organism?	X		If No - not a GMO
(c) Cell fusion by <u>not</u> naturally occurring means including protoplast fusion or hybridisation techniques (must satisfy <u>all 3</u> tests below)?		X		If Yes = GMO
	(i) live cells with new combinations of heritable genetic material are formed?	X		If No - not a GMO
	(ii) formed through the fusion of two or more cells?	X		If No - not a GMO
	(iii) formed by means of methods that are not capable of forming naturally?	X		If No - not a GMO
(d) Is another technique, not explicitly listed in Annex I A Part 1 employed (and 6 & 7 do not apply)?		X		If Yes = GMO
6	Is one of the three Non-GM techniques listed in Annex I A Part 2 involved?	X		If Yes - not a GMO
(a) In vitro fertilisation?		X		If Yes - not a GMO
(b) Natural processes (<i>non-exhaustive list</i>)?		X		If Yes - not a GMO
(c) Polyploidy induction?		X		If Yes - not a GMO
(d) Does the technique involve recombinant nucleic acid molecules?		X		If No - not a GMO
(e) Does the technique involve GMOs (made by non-Annex I B techniques; see 7, below)?		X		If No - not a GMO
7	Is one of the two excluded modification techniques/methods not yielding GMOs listed in Annex I B involved?		X	If Yes - not a GMO
(a) Mutagenesis?			X	If Yes - not a GMO
(b) Fusion of cells from crossable plants?		X		If Yes - not a GMO
(c) Does the technique involve recombinant nucleic acid molecules?		X		If No - not a GMO
(d) Does the technique involve a GMO (made by other modification techniques covered by Directive 2001/18/EC under 1 to 5, above)?		X		If No - not a GMO

ZFN-3: Checklist for classifying products resulting from NBTs

Questions		No	Yes	
1	Is it an organism?		X	If No - not a GMO
2	Is it a human organism?	X		If Yes not a GMO
3	Is genetic material altered? (20 bp or more)		X	If No - not a GMO
4	Is the result of the genetic alteration <i>capable</i> of occurring naturally by mating &/or natural recombination?	Case-by-case		If Yes - not a GMO
5	Is one of the three <i>examples</i> of GMO techniques listed in Annex I A Part 1 employed?	Case-by-case		If Yes = GMO
(a) Recombinant nucleic acid techniques (<u>all 5 tests</u> below must be satisfied)?		Case-by-case		If Yes = GMO
	(i) They lead to the formation of new combinations of genetic material?	Case-by-case		If No - not a GMO
	(ii) They do so by the insertion of nucleic acids produced outside an organism into any vector system (<u>including virus or bacterial plasmid</u>)?	Case-by-case		If No - not a GMO
	(iii) They lead to incorporation of nucleic acid into a host organism?	Case-by-case		If No - not a GMO
	(iv) Such nucleic acids naturally occur in the host organism?	Case-by-case		If Yes - not a GMO
	(v) The nucleic acids are capable of continued propagation in the host organism?	Case-by-case		If No - not a GMO
(b) Direct introduction (tests (i) and (ii) are <u>both</u> satisfied)?		X		If Yes = GMO
	(i) Direct introduction into an organism of heritable material?	X		If No - not a GMO
	(ii) Heritable material prepared outside the organism?	X		If No - not a GMO
(c) Cell fusion by <u>not</u> naturally occurring means including protoplast fusion or hybridisation techniques (must satisfy <u>all</u> 3 tests below)?		X		If Yes = GMO
	(i) live cells with new combinations of heritable genetic material are formed?	X		If No - not a GMO
	(ii) formed through the fusion of two or more cells?	X		If No - not a GMO
	(iii) formed by means of methods that are not capable of forming naturally?	X		If No - not a GMO
(d) Is another technique, not explicitly listed in Annex I A Part 1 employed (and 6 & 7 do not apply)?		X		If Yes = GMO
6	Is one of the three Non-GM techniques listed in Annex I A Part 2 involved?	X		If Yes - not a GMO
(a) In vitro fertilisation?		X		If Yes - not a GMO
(b) Natural processes (<i>non-exhaustive list</i>)?		X		If Yes - not a GMO
(c) Polyploidy induction?		X		If Yes - not a GMO
(d) Does the technique involve recombinant nucleic acid molecules?		Case-by-case		If No - not a GMO
(e) Does the technique involve GMOs (made by non-Annex I B techniques; see 7, below)?		X		If No - not a GMO
7	Is one of the two excluded modification techniques/methods not yielding GMOs listed in Annex I B involved?	X		If Yes - not a GMO
(a) Mutagenesis?		X		If Yes - not a GMO
(b) Fusion of cells from crossable plants?		X		If Yes - not a GMO
(c) Does the technique involve recombinant nucleic acid molecules?		Case-by-case		If No - not a GMO
(d) Does the technique involve a GMO (made by other modification techniques covered by Directive 2001/18/EC under 1 to 5, above)?		X		If No - not a GMO

ODM: Checklist for classifying products resulting from NBTs

Questions		No	Yes	
1	Is it an organism?		X	If No - not a GMO
2	Is it a human organism?	X		If Yes not a GMO
3	Is genetic material altered? (20 bp or more)	X		If No - not a GMO
4	Is the result of the genetic alteration <i>capable</i> of occurring naturally by mating &/or natural recombination?		X	If Yes - not a GMO
5	Is one of the three <i>examples</i> of GMO techniques listed in Annex I A Part 1 employed?	X		If Yes = GMO
(a) Recombinant nucleic acid techniques (<u>all 5 tests</u> below must be satisfied)?		X		If Yes = GMO
(i) They lead to the formation of new combinations of genetic material?		X		If No - not a GMO
(ii) They do so by the insertion of nucleic acids produced outside an organism into any vector system (<i>including virus or bacterial plasmid</i>)?		X		If No - not a GMO
(iii) They lead to incorporation of nucleic acid into a host organism?		X		If No - not a GMO
(iv) Such nucleic acids naturally occur in the host organism?		X		If Yes - not a GMO
(v) The nucleic acids are capable of continued propagation in the host organism?		X		If No - not a GMO
(b) Direct introduction (tests (i) and (ii) are <u>both</u> satisfied)?		X		If Yes = GMO
(i) Direct introduction into an organism of heritable material?		X		If No - not a GMO
(ii) Heritable material prepared outside the organism?		X		If No - not a GMO
(c) Cell fusion by <u>not</u> naturally occurring means including protoplast fusion or hybridisation techniques (must satisfy <u>all</u> 3 tests below)?		X		If Yes = GMO
(i) live cells with new combinations of heritable genetic material are formed?		X		If No - not a GMO
(ii) formed through the fusion of two or more cells?		X		If No - not a GMO
(iii) formed by means of methods that are not capable of forming naturally?		X		If No - not a GMO
(d) Is another technique, not explicitly listed in Annex I A Part 1 employed (and 6 & 7 do not apply)?		X		If Yes = GMO
6	Is one of the three Non-GM techniques listed in Annex I A Part 2 involved?	X		If Yes - not a GMO
(a) In vitro fertilisation?		X		If Yes - not a GMO
(b) Natural processes (<i>non-exhaustive list</i>)?		X		If Yes - not a GMO
(c) Polyploidy induction?		X		If Yes - not a GMO
(d) Does the technique involve recombinant nucleic acid molecules?		X		If No - not a GMO
(e) Does the technique involve GMOs (made by non-Annex I B techniques; see 7, below)?		X		If No - not a GMO
7	Is one of the two excluded modification techniques/methods not yielding GMOs listed in Annex I B involved?		X	If Yes - not a GMO
(a) Mutagenesis?			X	If Yes - not a GMO
(b) Fusion of cells from crossable plants?		X		If Yes - not a GMO
(c) Does the technique involve recombinant nucleic acid molecules?		X		If No - not a GMO
(d) Does the technique involve a GMO (made by other modification techniques covered by Directive 2001/18/EC under 1 to 5, above)?		X		If No - not a GMO

Cisgenesis: Checklist for classifying products resulting from NBTs

Questions		No	Yes	
1	Is it an organism?		X	If No - not a GMO
2	Is it a human organism?	X		If Yes not a GMO
3	Is genetic material altered? (20 bp or more)		X	If No - not a GMO
4	Is the result of the genetic alteration <i>capable</i> of occurring naturally by mating &/or natural recombination?		X	If Yes - not a GMO
5	Is one of the three <i>examples</i> of GMO techniques listed in Annex I A Part 1 employed?	X		If Yes = GMO
(a) Recombinant nucleic acid techniques (<u>all 5 tests</u> below must be satisfied)?		X		If Yes = GMO
(i) They lead to the formation of new combinations of genetic material?		X		If No - not a GMO
(ii) They do so by the insertion of nucleic acids produced outside an organism into any vector system (including virus or bacterial plasmid)?			X	If No - not a GMO
(iii) They lead to incorporation of nucleic acid into a host organism?			X	If No - not a GMO
(iv) Such nucleic acids naturally occur in the host organism?			X	If Yes - not a GMO
(v) The nucleic acids are capable of continued propagation in the host organism?			X	If No - not a GMO
(b) Direct introduction (tests (i) and (ii) are <u>both</u> satisfied)?		X		If Yes = GMO
(i) Direct introduction into an organism of heritable material?		X		If No - not a GMO
(ii) Heritable material prepared outside the organism?			X	If No - not a GMO
(c) Cell fusion by <u>not</u> naturally occurring means including protoplast fusion or hybridisation techniques (must satisfy <u>all</u> 3 tests below)?		X		If Yes = GMO
(i) live cells with new combinations of heritable genetic material are formed?		X		If No - not a GMO
(ii) formed through the fusion of two or more cells?		X		If No - not a GMO
(iii) formed by means of methods that are not capable of forming naturally?		X		If No - not a GMO
(d) Is another technique, not explicitly listed in Annex I A Part 1 employed (and 6 & 7 do not apply)?		X		If Yes = GMO
6	Is one of the three Non-GM techniques listed in Annex I A Part 2 involved?	X		If Yes - not a GMO
(a) In vitro fertilisation?		X		If Yes - not a GMO
(b) Natural processes (non-exhaustive list)?		Case-by-case		If Yes - not a GMO
(c) Polyploidy induction?		X		If Yes - not a GMO
(d) Does the technique involve recombinant nucleic acid molecules?		Case-by-case		If No - not a GMO
(e) Does the technique involve GMOs (made by non-Annex I B techniques; see 7, below)?		X		If No - not a GMO
7	Is one of the two excluded modification techniques/methods not yielding GMOs listed in Annex I B involved?	X		If Yes - not a GMO
(a) Mutagenesis?		X		If Yes - not a GMO
(b) Fusion of cells from crossable plants?		X		If Yes - not a GMO
(c) Does the technique involve recombinant nucleic acid molecules?		X		If No - not a GMO
(d) Does the technique involve a GMO (made by other modification techniques covered by Directive 2001/18/EC under 1 to 5, above)?		X		If No - not a GMO

RdDM: Checklist for classifying products resulting from NBTs

Questions		No	Yes	
1	Is it an organism?		X	If No - not a GMO
2	Is it a human organism?	X		If Yes not a GMO
3	Is genetic material altered? (20 bp or more)	X		If No - not a GMO
4	Is the result of the genetic alteration <i>capable</i> of occurring naturally by mating &/or natural recombination?	X		If Yes - not a GMO
5	Is one of the three <i>examples</i> of GMO techniques listed in Annex I A Part 1 employed?	X		If Yes = GMO
	(a) Recombinant nucleic acid techniques (all 5 tests below must be satisfied)?	X		If Yes = GMO
	(i) They lead to the formation of new combinations of genetic material?	X		If No - not a GMO
	(ii) They do so by the insertion of nucleic acids produced outside an organism into any vector system (including virus or bacterial plasmid)?	X		If No - not a GMO
	(iii) They lead to incorporation of nucleic acid into a host organism?	X		If No - not a GMO
	(iv) Such nucleic acids naturally occur in the host organism?	N/A		If Yes - not a GMO
	(v) The nucleic acids are capable of continued propagation in the host organism?	X		If No - not a GMO
	(b) Direct introduction (tests (i) and (ii) are <u>both</u> satisfied)?	X		If Yes = GMO
	(i) Direct introduction into an organism of heritable material?	X		If No - not a GMO
	(ii) Heritable material prepared outside the organism?	X		If No - not a GMO
	(c) Cell fusion by <u>not</u> naturally occurring means including protoplast fusion or hybridisation techniques (must satisfy <u>all</u> 3 tests below)?	X		If Yes = GMO
	(i) live cells with new combinations of heritable genetic material are formed?	X		If No - not a GMO
	(ii) formed through the fusion of two or more cells?	X		If No - not a GMO
	(iii) formed by means of methods that are not capable of forming naturally?	X		If No - not a GMO
	(d) Is another technique, not explicitly listed in Annex I A Part 1 employed (and 6 & 7 do not apply)?	X		If Yes = GMO
6	Is one of the three Non-GM techniques listed in Annex I A Part 2 involved?	X		If Yes - not a GMO
	(a) In vitro fertilisation?	X		If Yes - not a GMO
	(b) Natural processes (non-exhaustive list)?	X		If Yes - not a GMO
	(c) Polyploidy induction?	X		If Yes - not a GMO
	(d) Does the technique involve recombinant nucleic acid molecules?	X		If No - not a GMO
	(e) Does the technique involve GMOs (made by non-Annex I B techniques; see 7, below)?	X		If No - not a GMO
7	Is one of the two excluded modification techniques/methods not yielding GMOs listed in Annex I B involved?	X		If Yes - not a GMO
	(a) Mutagenesis?	X		If Yes - not a GMO
	(b) Fusion of cells from crossable plants?	X		If Yes - not a GMO
	(c) Does the technique involve recombinant nucleic acid molecules?	X		If No - not a GMO
	(d) Does the technique involve a GMO (made by other modification techniques covered by Directive 2001/18/EC under 1 to 5, above)?	X		If No - not a GMO

Grafting (non-GMO scion and products): Checklist for classifying products resulting from NBTs

Questions		No	Yes	
1	Is it an organism?		X	If No - not a GMO
2	Is it a human organism?	X		If Yes not a GMO
3	Is genetic material altered? (20 bp or more)	X		If No - not a GMO
4	Is the result of the genetic alteration <i>capable</i> of occurring naturally by mating &/or natural recombination?	X		If Yes - not a GMO
5	Is one of the three <i>examples</i> of GMO techniques listed in Annex I A Part 1 employed?	X		If Yes = GMO
(a) Recombinant nucleic acid techniques (<u>all 5 tests</u> below must be satisfied)?		X		If Yes = GMO
(i) They lead to the formation of new combinations of genetic material?		X		If No - not a GMO
(ii) They do so by the insertion of nucleic acids produced outside an organism into any vector system (including virus or bacterial plasmid)?		X		If No - not a GMO
(iii) They lead to incorporation of nucleic acid into a host organism?		X		If No - not a GMO
(iv) Such nucleic acids naturally occur in the host organism?		X		If Yes - not a GMO
(v) The nucleic acids are capable of continued propagation in the host organism?		X		If No - not a GMO
(b) Direct introduction (tests (i) and (ii) are <u>both</u> satisfied)?		X		If Yes = GMO
(i) Direct introduction into an organism of heritable material?		X		If No - not a GMO
(ii) Heritable material prepared outside the organism?		X		If No - not a GMO
(c) Cell fusion by <u>not</u> naturally occurring means including protoplast fusion or hybridisation techniques (must satisfy <u>all</u> 3 tests below)?		X		If Yes = GMO
(i) live cells with new combinations of heritable genetic material are formed?		X		If No - not a GMO
(ii) formed through the fusion of two or more cells?		X		If No - not a GMO
(iii) formed by means of methods that are not capable of forming naturally?		X		If No - not a GMO
(d) Is another technique, not explicitly listed in Annex I A Part 1 employed (and 6 & 7 do not apply)?		X		If Yes = GMO
6	Is one of the three Non-GM techniques listed in Annex I A Part 2 involved?	X		If Yes - not a GMO
(a) In vitro fertilisation?		X		If Yes - not a GMO
(b) Natural processes (non-exhaustive list)?		X		If Yes - not a GMO
(c) Polyploidy induction?		X		If Yes - not a GMO
(d) Does the technique involve recombinant nucleic acid molecules?		X		If No - not a GMO
(e) Does the technique involve GMOs (made by non-Annex I B techniques; see 7, below)?		X		If No - not a GMO
7	Is one of the two excluded modification techniques/methods not yielding GMOs listed in Annex I B involved?	X		If Yes - not a GMO
(a) Mutagenesis?		X		If Yes - not a GMO
(b) Fusion of cells from crossable plants?		X		If Yes - not a GMO
(c) Does the technique involve recombinant nucleic acid molecules?		X		If No - not a GMO
(d) Does the technique involve a GMO (made by other modification techniques covered by Directive 2001/18/EC under 1 to 5, above)?			X	If No - not a GMO

Reverse Breeding: Checklist for classifying products resulting from NBTs

Questions		No	Yes	
1	Is it an organism?		X	If No - not a GMO
2	Is it a human organism?	X		If Yes not a GMO
3	Is genetic material altered? (20 bp or more)		X	If No - not a GMO
4	Is the result of the genetic alteration <i>capable</i> of occurring naturally by mating &/or natural recombination?		X	If Yes - not a GMO
5	Is one of the three <i>examples</i> of GMO techniques listed in Annex I A Part 1 employed?	X		If Yes = GMO
(a) Recombinant nucleic acid techniques (all 5 tests below must be satisfied)?		X		If Yes = GMO
(i) They lead to the formation of new combinations of genetic material?		X		If No - not a GMO
(ii) They do so by the insertion of nucleic acids produced outside an organism into any vector system (including virus or bacterial plasmid)?		X		If No - not a GMO
(iii) They lead to incorporation of nucleic acid into a host organism?		X		If No - not a GMO
(iv) Such nucleic acids naturally occur in the host organism?		X		If Yes - not a GMO
(v) The nucleic acids are capable of continued propagation in the host organism?		X		If No - not a GMO
(b) Direct introduction (tests (i) and (ii) are <u>both</u> satisfied)?		X		If Yes = GMO
(i) Direct introduction into an organism of heritable material?		X		If No - not a GMO
(ii) Heritable material prepared outside the organism?		X		If No - not a GMO
(c) Cell fusion by <u>not</u> naturally occurring means including protoplast fusion or hybridisation techniques (must satisfy all 3 tests below)?		X		If Yes = GMO
(i) live cells with new combinations of heritable genetic material are formed?		X		If No - not a GMO
(ii) formed through the fusion of two or more cells?		X		If No - not a GMO
(iii) formed by means of methods that are not capable of forming naturally?		X		If No - not a GMO
(d) Is another technique, not explicitly listed in Annex I A Part 1 employed (and 6 & 7 do not apply)?		X		If Yes = GMO
6	Is one of the three Non-GM techniques listed in Annex I A Part 2 involved?	X		If Yes - not a GMO
(a) In vitro fertilisation?		X		If Yes - not a GMO
(b) Natural processes (<i>non-exhaustive list</i>)?		X		If Yes - not a GMO
(c) Polyploidy induction?		X		If Yes - not a GMO
(d) Does the technique involve recombinant nucleic acid molecules?		X		If No - not a GMO
(e) Does the technique involve GMOs (made by non-Annex I B techniques; see 7, below)?		X		If No - not a GMO
7	Is one of the two excluded modification techniques/methods not yielding GMOs listed in Annex I B involved?	X		If Yes - not a GMO
(a) Mutagenesis?		X		If Yes - not a GMO
(b) Fusion of cells from crossable plants?		X		If Yes - not a GMO
(c) Does the technique involve recombinant nucleic acid molecules?		X		If No - not a GMO
(d) Does the technique involve a GMO (made by other modification techniques covered by Directive 2001/18/EC under 1 to 5, above)?		X		If No - not a GMO

Agro-infiltration (sensu stricto and agro-inoculation): Checklist for classifying products resulting from NBTs

Questions		No	Yes	
1	Is it an organism?		X	If No - not a GMO
2	Is it a human organism?	X		If Yes not a GMO
3	Is genetic material altered? (20 bp or more)	X		If No - not a GMO
4	Is the result of the genetic alteration <i>capable</i> of occurring naturally by mating &/or natural recombination?	N/A		If Yes - not a GMO
5	Is one of the three <i>examples</i> of GMO techniques listed in Annex I A Part 1 employed?	X		If Yes = GMO
(a) Recombinant nucleic acid techniques (<u>all 5 tests</u> below must be satisfied)?		X		If Yes = GMO
(i) They lead to the formation of new combinations of genetic material?		X		If No - not a GMO
(ii) They do so by the insertion of nucleic acids produced outside an organism into any vector system (including virus or bacterial plasmid)?		X		If No - not a GMO
(iii) They lead to incorporation of nucleic acid into a host organism?		X		If No - not a GMO
(iv) Such nucleic acids naturally occur in the host organism?		X		If Yes - not a GMO
(v) The nucleic acids are capable of continued propagation in the host organism?		X		If No - not a GMO
(b) Direct introduction (tests (i) and (ii) are <u>both</u> satisfied)?		X		If Yes = GMO
(i) Direct introduction into an organism of heritable material?		X		If No - not a GMO
(ii) Heritable material prepared outside the organism?		X		If No - not a GMO
(c) Cell fusion by <u>not</u> naturally occurring means including protoplast fusion or hybridisation techniques (must satisfy <u>all</u> 3 tests below)?		X		If Yes = GMO
(i) live cells with new combinations of heritable genetic material are formed?		X		If No - not a GMO
(ii) formed through the fusion of two or more cells?		X		If No - not a GMO
(iii) formed by means of methods that are not capable of forming naturally?		X		If No - not a GMO
(d) Is another technique, not explicitly listed in Annex I A Part 1 employed (and 6 & 7 do not apply)?		X		If Yes = GMO
6	Is one of the three Non-GM techniques listed in Annex I A Part 2 involved?	X		If Yes - not a GMO
(a) In vitro fertilisation?		X		If Yes - not a GMO
(b) Natural processes (<i>non-exhaustive list</i>)?		X		If Yes - not a GMO
(c) Polyploidy induction?		X		If Yes - not a GMO
(d) Does the technique involve recombinant nucleic acid molecules?		X		If No - not a GMO
(e) Does the technique involve GMOs (made by non-Annex I B techniques; see 7, below)?		X		If No - not a GMO
7	Is one of the two excluded modification techniques/methods not yielding GMOs listed in Annex I B involved?	X		If Yes - not a GMO
(a) Mutagenesis?		X		If Yes - not a GMO
(b) Fusion of cells from crossable plants?		X		If Yes - not a GMO
(c) Does the technique involve recombinant nucleic acid molecules?		X		If No - not a GMO
(d) Does the technique involve a GMO (made by other modification techniques covered by Directive 2001/18/EC under 1 to 5, above)?		X		If No - not a GMO