



## Overview – status of organisms modified using gene editing and other new technologies

This document has been prepared to assist regulated organisations to understand which new technologies, including gene editing techniques, result in genetically modified organisms (GMOs) that are regulated under the *Gene Technology Act 2000* (the Act). This document is not intended to be a comprehensive summary nor does it provide legal advice. Refer to the Act and Gene Technology Regulations 2001 (the Regulations) for an authoritative statement of the law. If you are unsure about how to meet your legal obligations, OGTR suggests you seek your own legal advice.

### Organisms modified using SDN-1 are not GMOs

Schedule 1 of the Regulations lists organisms that are not GMOs for the purposes of the Act. Items on this list exclude organisms modified through unguided repair of site-directed nuclease (SDN) activity, also known as SDN-1 organisms, from regulation as GMOs. Unguided repair means that no nucleic acid template was added to cells to guide genome repair following SDN application. SDNs include, but are not limited to, CRISPR/Cas9, zinc finger nucleases, meganucleases and TALENs.

Site-directed nucleases can be applied in a variety of ways to produce SDN-1 organisms. Some of these methods generate GMOs in intermediate steps, and dealings with these GMOs will continue to require authorisation under the Act. Table 1 summarises the status of organisms with SDN-1 modifications, provided that the organisms have no other modifications from gene technology beyond those described in the table.

**Table 1:** Status of organisms with SDN-1 modifications, by method of SDN application

|   | SDN protein applied (with or without sgRNA) | SDN expressed from a transgene that is only transiently present in the organism  | SDN expressed from transgene integrated in the genome   |
|---|---|--|---|
| Status of the initial organism modified by SDN-1      | <b>Not a GMO</b><br>(Schedule 1 item 4)     | <b>GMO</b> while transgene or its expressed products are present<br><b>Not a GMO</b> when transgene and expressed products have degraded (Schedule 1 items 4+10) | <b>GMO</b>  |
| Status of offspring inheriting the SDN-1 modification | <b>Not a GMO</b><br>(Schedule 1 item 9(a))  | <b>Not a GMO</b><br>(Schedule 1 item 9(b))   | <b>GMO</b> if SDN transgene also inherited<br><b>Not a GMO</b> if no SDN transgene inherited (Schedule 1 item 9(b)) |

Some examples illustrating the status of organisms produced in the course of using SDN-1 are:

- An organism supplied with Cas9 protein and guide RNA/s in which an SDN-1 modification occurs is not a GMO.
- An organism expressing Cas9 and guide RNA/s from an expression cassette not integrated into the genome is a GMO while the expression cassette or its expressed products are present. If the expression cassette and its expressed products have degraded over time and only SDN-1 modifications remain, the organism is not a GMO.
- An organism with Cas9 and guide RNA transgenes integrated into its genome is a GMO, but those of its segregating offspring carrying an SDN-1 modification but lacking the Cas9 and gRNA transgenes are not GMOs.

In each example, this status depends upon:

- no nucleic acid template being supplied to guide genome repair through homology-directed recombination, and
- the organism having no other modifications as a result of gene technology.

It is the responsibility of proponents to comply with the law and ensure that these requirements have been met.

SDN-1 organisms may be subject to regulation by other agencies, depending upon their characteristics and intended uses.

The legislative provisions referred to above do not exclude organisms modified using base editing or prime editing methods from regulation as GMOs, because the provisions are specific to enzymes with nuclease activity. Base editing and prime editing use disabled CRISPR/Cas9 coupled with other enzymatic domains to modify genes or genetic material, e.g. cytidine deaminase or adenosine deaminase.

## **Organisms modified using template-guided SDN techniques and ODM are GMOs**

Schedule 1B, Organisms that are genetically modified organisms, provides that:

- organisms modified using oligonucleotide-directed mutagenesis are GMOs (**Schedule 1B item 1**)
- organisms modified using SDN techniques involving templates to guide repair of SDN action, also known as SDN-2 and SDN-3, are GMOs (**Schedule 1B item 2**).

In each case, the method used to modify the organism is central to determining whether or not the organism is a GMO. The number of resulting nucleotide changes, whether insertions or deletions, or whether the resulting nucleotide sequence may be found in sexually compatible species, is not a deciding factor.

## **Some RNA interference (RNAi) techniques are not gene technology**

RNAi techniques involving directly applying RNAs to temporarily induce RNAi are listed as a technique that is not gene technology in **item 11 of Schedule 1A**. As a result, organisms modified using these techniques are not classified as GMOs.

The RNAs could be introduced to the organism by any method including, but not limited to:

- the organism taking up an externally applied RNA (e.g. by spraying with or dipping in an RNA solution)
- injecting RNA into the organism

- electroporation, and
- methods leading to the organism consuming material to which the RNA has been applied (e.g. insects consuming RNA by feeding on plant material sprayed with RNA).

To ensure that only short-lived RNAi techniques are excluded, this exclusion only applies if:

- the organism's genomic DNA sequence cannot be changed by the technique (this requirement can be met even if changes to genomic DNA methylation can occur), and
- the introduced RNA cannot be translated into a protein or lead to the production of infectious agents.

Provided the above requirements are met, the applied RNAs could potentially include small interfering RNAs, artificial microRNAs, short or long double-stranded RNAs and hairpin RNAs, with sequence of any origin. It is the responsibility of proponents to comply with the law and ensure that the requirements above have been met.

Item 11 of Schedule 1A does not change the status of organisms to which other RNAi techniques have been applied, e.g. where an organism is stably or transiently transformed with a transgene able to express RNA that can induce gene silencing, this remains a GMO.

Product regulators such as the Australian Pesticides and Veterinary Medicines Authority or the Therapeutic Goods Administration may have requirements in relation to these techniques.

### **Organisms derived from GMOs but with no traits from gene technology**

Consistent with the definition of a GMO in the Act, and for the avoidance of doubt, the Regulations clarify the non-GMO status of organisms derived from GMOs but which do not possess traits as a result of gene technology. These organisms are:

- offspring of GMOs that have not inherited traits that occurred in a parent because of gene technology, commonly referred to as null segregants (**Schedule 1 item 8**)
- organisms temporarily modified using gene technology but which have lost all traits (e.g. transgenes, products expressed from transgenes) that occurred because of gene technology (**Schedule 1 item 10**).

Modifications produced using SDN techniques are traits that occurred because of gene technology, so item 8 does not exclude these organisms from being GMOs. However, other items described above do exclude SDN-1 organisms from regulation.