



GENOME EDITING

in Food and Farming

RISKS AND UNEXPECTED
CONSEQUENCES

Acknowledgements

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Glossary

DNA: Deoxyribonucleic acid. In plants and animals, a molecule inside a cell's nucleus that carries genetic information.

Genetic engineering: Also commonly called genetic modification, leading to the creation of genetically modified organisms (GMOs). Genetic engineering makes changes directly to the genetic material of an organism, without mating, by introducing genetic material or using techniques that induce change to an organism's genome. The material introduced into the cell is produced, or at least handled, in the laboratory by humans.

Genetic errors: In the context of genome editing, genetic errors are either unintended changes to the DNA (such as rearrangements or deletions), or changes in RNA and protein composition (e.g. by misreading of DNA). Genetic errors resulting from genome editing can lead to unexpected and unpredictable effects in the resulting genetically modified organism (GMO).

Gene: A section (or sections) of DNA in a genome that codes for protein production via an intermediary, mRNA.

Genome: The entire set of genetic material in an organism, including DNA.

Genome editing or gene editing: A collection of genetic engineering techniques that alter the genetic material of organisms. The aim is to insert, delete or otherwise change a DNA sequence at a specific, targeted site or sites in the genome. Also called "genome engineering."

Host: The organism that is undergoing genome editing.

Nuclease: An enzyme that can cut DNA. Different nucleases can break either one or both strands of DNA.

Off-target effects: Unintended changes to other (non-target) genes.

Plasmid: Small, circular, double-stranded DNA molecules found in bacteria. They can be genetically engineered to contain genes of interest and introduced into the cell of a host organism, where the DNA may be taken up into the genome of the host, as in first-generation genetic engineering. In genome editing, the DNA coding for the genome editing components and the plasmid may or may not be assimilated into the host organism's genome, intentionally or unintentionally.

RNA: Ribonucleic acid. In a cell, there are different types of RNA, including messenger RNA (mRNA), which carries genetic information from DNA and directs the production of proteins.

Site-directed nuclease (SDN): The technology that guides DNA cutters to a target location to make a cut to DNA, as part of genome editing systems such as CRISPR. The SDN consists of a guide (generally constituted of RNA) and a DNA cutter (a nuclease). Often called "molecular scissors."

Unintended on-target effects: Errors that occur when the intended change is achieved at the target location but also triggers genetic errors.

Acronyms of genome editing techniques

(See [Box 1: Genome editing techniques](#) & [Box 2: Classification of genome editing](#))

CRISPR: Clustered regularly interspaced short palindromic repeat

TALENs: Transcription activator-like effector nucleases

ZFN: Zinc-finger nuclease

ODM: Oligonucleotide-directed mutagenesis

Executive Summary

Genome editing (also called gene editing) is a collection of new genetic engineering techniques that alter the genetic material of plants, animals and microbes, creating genetically modified organisms (GMOs). These techniques aim to change DNA in the cell of an organism. This allows a novel trait to be induced without necessarily inserting genes from another organism or producing a novel protein, as is the case with almost all of the currently commercialized products of genetic engineering.

Many studies have now shown that genome editing can create genetic errors, such as “off-target” and “on-target” effects, leading to unexpected and unpredictable outcomes in the resulting GMO.

Genome editing can be imprecise. For example, genome editing techniques can make unintended “edits” to genes that were not the target of the editing system, giving rise to off-target effects. The genome editing technique called CRISPR-Cas9 appears to be particularly prone to creating off-target effects.

Genome editing can also result in unintended on-target effects, when a technique is successfully used to make the intended change at the target location, but also triggers genetic errors. For example, unintended on-target effects can change the way that a gene is read and processed into proteins, with potential implications for food and environmental safety. Additionally, genome

editing can inadvertently cause extensive deletions and complex re-arrangements of the host’s DNA.

New scientific publications also indicate that the integration of unwanted DNA during the genome editing process is more common than previously thought. For example, foreign DNA was unexpectedly found in genome-edited hornless cows that were claimed to be free of foreign DNA, demonstrating the need for systematic risk assessment.

There are several types of possible genetic errors that need to be investigated in each GMO resulting from genome editing, including unintended DNA integration. However, as yet, there are no standard protocols for the detection of off-target or on-target effects of genome editing.

Some types of intended changes to genetic material induced by genome editing techniques are sometimes described as “mutations” because only very small parts of DNA are altered and no novel genes have been intentionally introduced. However, **even small changes in a DNA sequence can have big effects.** The orchestration of gene function in an organism is part of a complex regulatory network that is still poorly understood. This means it is not possible to predict the nature and consequences of all the interactions between altered genetic material and other genes within an organism. Making one genetic change could, for example, impact an organism’s ability to express or suppress other genes.

As the evidence in this report shows, even when a genome-edited organism does not contain foreign genes or express a novel protein, it cannot be considered safe for environmental release or human consumption on this basis alone. **Although widely promoted on the basis of specificity and precision, genome editing – like all other types of genetic engineering – can cause unexpected and unpredictable effects.**

In addition to opening up possibilities to genetically engineer a wider range of species, (more animal species, for example) for many different traits, genome editing techniques have facilitated experimentation with powerful “gene drives,” where genome-edited organisms are being designed to speed up the inheritance process and push new genes through entire populations of a species, with potentially serious and irreversible impacts.

In this report, we provide an overview of genome editing techniques being explored in agriculture, and the range of potential unexpected effects that can arise from them. The report draws on recent scientific publications, in a rapidly evolving field of research. The purpose of providing this information is to encourage broad public discussion about the potential implications of using genetic engineering, and specifically the new techniques of genome editing, in food and farming, and the ways in which decisions about the use of the technology should be made.

Introduction

“Gone are the days when life was shaped exclusively by the plodding forces of evolution. We are standing on the cusp of a new area, one in which we will have primary authority over life’s makeup and all its vibrant and varied outputs. Indeed, we are already supplanting the deaf, dumb, and blind system that has shaped genetic material on our planet for eons and replacing it with a conscious, intentional system of human-directed evolution.”

– Jennifer A. Doudna and Samuel H. Sternberg, 2017¹

Widespread excitement over new genetic engineering techniques called “genome editing” or “gene editing”, and the wide-ranging promises for their applications, mirrors those that accompanied the first-generation of genetic engineering. As with earlier genetic engineering, genome editing techniques are moving towards commercial application even while our knowledge of the mechanisms that underlie how genomes work remains incomplete.

Scientist Jennifer Doudna, discussing the genome editing technique CRISPR that she co-developed, called it “a transformative genome engineering technology”² and believes the technique gives us the ability to “rewrite the code of life” and control evolution.³ At the same time, she says that the main mechanism that is triggered to accomplish these changes (cell repair) is “a process we don’t fully understand...sort of magic happens, this is where the editing actually happens.”⁴

In the laboratory, genome editing is a new set of research tools that are being used to increase our understanding of the functions of genes and the regulation of the genome. At the same

time, these new techniques are being used to genetically engineer more crop plants and also farm animals.

Genome editing techniques are often described as being precise.⁵ In fact, some argue that genome editing is so precise that the resulting agricultural products can automatically be considered safe and can therefore be exempt from government regulation and safety assessment.⁶ However, as detailed in this report, research is showing that these techniques can cause genetic errors, and even precise edits do not necessarily result in precise outcomes. **Like all genetic engineering, genome editing can result in unexpected effects in the resulting organism.**

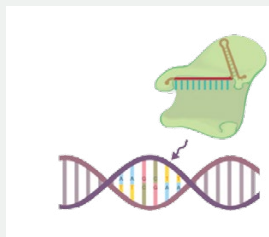
Genetic engineering in agriculture was first introduced to the public over twenty-five years ago with a description of precision, speed, and tremendous promise. However, the technology failed to deliver the products it promised,⁷ and, in animals for example, was ultimately described as “ham-fisted.”⁸ Now, as then, advances in genetic engineering such as genome editing are being widely celebrated as the future of agriculture. However, the technologies have limitations and risks with respect to food and environmental safety.

What is genome editing?

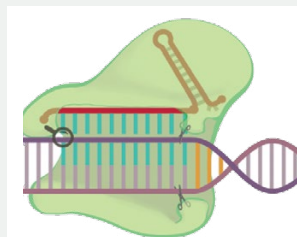
Genome editing, also called gene editing, is a term used to describe a collection of new techniques that alter the genetic material (usually DNA) of plants, animals and microbes. In general, these techniques consist of different types of DNA “editing” systems that aim to insert, delete or otherwise change a DNA sequence at specific, targeted sites in the genome. The organism’s genetic material is changed, not through the breeding process, but directly and artificially by humans, making these techniques a type of genetic engineering, resulting in the creation of genetically modified organisms (GMOs).

Genome editing techniques are a type of genetic engineering, resulting in the creation of genetically modified organisms (GMOs).

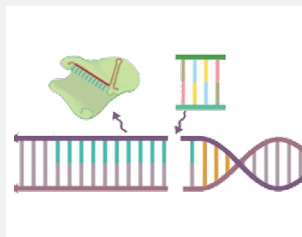
How Genome Editing Works



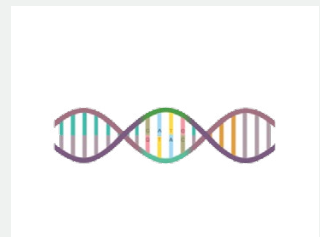
“DNA cutters” (nucleases) are guided to a location (the target site) on an organism’s DNA.



The DNA cutter docks onto the target site and cuts through the DNA.



The repair of DNA is then initiated and occurs either with (SDN-2) or without (SDN-1) a synthetic repair template. Alternatively, genes can be inserted (SDN-3).



The DNA is now “edited”. However, in reality, genome editing is prone to creating unintended changes and errors that can lead to unexpected effects in the genome-edited organism.

Genome editing is a set of new genetic engineering techniques that alter the genetic material of plants, animals and microbes, most often using DNA cutters that are guided to a location within an organism’s DNA and used to cut the DNA. This cut DNA is then repaired by the cell’s own repair mechanism, which creates “edits” or changes to the organism.

Genome editing systems are comprised of molecular components that are programmed to make changes (perform “edits”) at a target location in the genome. The most frequently used genome editing technique is CRISPR-Cas9 or CRISPR, but other techniques follow similar principles (see [Box 1: Genome editing techniques](#)).

The CRISPR editing system uses the technology of site-directed nucleases (SDNs), which are guided DNA cutters, often referred to as “molecular scissors.” The editing system consists primarily of a guide (RNA) and a DNA cutter (nuclease). In plants, commonly a package (“cassette”) of DNA encoding the CRISPR components is inserted into the organism’s genome at random, using first-generation genetic engineering techniques. **The CRISPR system then guides the DNA cutter to a location on the organism’s DNA, to cut specific, targeted DNA.** When a cut is made to DNA, the cell responds by repairing the damage, and it is through this repair mechanism that the system makes “edits,” which can be insertions, deletions, or other changes to DNA. Afterwards, the inserted CRISPR DNA is bred out of the genome-edited plant via conventional breeding, so that the resulting genetically engineered organism (GMO) may no longer be transgenic (i.e. no longer contain genes from another species). Some genome editing approaches, known as “DNA-free” genome editing,⁹ do not require a DNA cassette to be inserted. Instead, either only the guide and cutter are introduced. Alternatively, DNA in the form of a plasmid is introduced into the cell,¹⁰ but not expected to join with the host’s DNA. These two latter methods of introducing the CRISPR components into the genome are common for genome editing animals.

During genome editing, the cut made to DNA triggers the cell’s own repair mechanism, and the type of repair is key to how genome-edited organisms are classified. There are three types of genome editing, dependent on how the repair is achieved: one that does not use a synthetic repair template (SDN-1), one that uses a synthetic repair template (SDN-2) and one where a gene (or genes) is inserted (SDN-3) (see [Box 2: Classification of genome editing](#)).¹¹

Genome editing is a form of genetic engineering, resulting in the creation of genetically engineered organisms (commonly called genetically modified organisms or GMOs).¹ The principal difference between earlier genetic engineering and this new generation of techniques is that, while both change genetic material directly, with genome editing, genes do not necessarily have to be permanently incorporated into the organism to produce the new trait.

Currently, most commercial genetically engineered crop plants, which are predominantly herbicide tolerant and insect resistant,¹² were made using first-generation genetic engineering techniques. This earlier genetic engineering – devised in the 1970s and first commercialized in the mid-1990s – inserts genes at a random location in an organism’s genome.¹³ If those genes are from a different, unrelated species (often called “foreign” genes), then the resulting genetically engineered organism is transgenic. Almost all of the genetically engineered crops currently on the market are transgenic, with the inserted gene(s) producing a protein that, for example, makes the plant tolerant to a particular herbicide (e.g., Roundup Ready soy) or toxic to certain plant pests (e.g., Bt corn).

1 The terms genetic engineered (GE) and genetically modified (GM) are used interchangeably. The term GM is widely used in Europe, as well as in the North American marketplace. Many national regulatory regimes and international agreements use the term genetic modification. The U.S. and Canadian governments both refer to genetic engineering. Canadian regulation defines GM broadly to include conventional breeding as well as genetic engineering (including genome editing). In international convention, the use of the term GM refers to genetic engineering and excludes conventional breeding.

BOX 1:

GENOME EDITING TECHNIQUES

Genome editing techniques such as **CRISPR, TALENs, ZFN and meganucleases*** use the technology of site-directed nucleases (SDNs), which are guided DNA cutters, often referred to as “molecular scissors.” The SDN consists of a guide and a DNA cutter, and makes a cut at the location on the genome where the DNA change is intended to take place. The cutter cuts the DNA, which then undergoes repair carried out by the cell’s own repair mechanism.

CRISPR, the most frequently used technique, commonly uses a type of DNA cutter called “Cas9,” and it is therefore often referred to as the **CRISPR-Cas9** genome editing system. Other types of cutters, such as Cpf1 (also called Cas12a) and Cas 13a (which edits mRNA), can also be used.¹⁷ In addition, new CRISPR strategies are under development, including those that silence genes,¹⁸ as well as prime and base editing.¹⁹

The genome editing technique known as **oligonucleotide-directed mutagenesis (ODM)** does not use a guided DNA cutter, but instead introduces a short strand of DNA that attaches itself to the organism’s DNA at a particular location and causes a change to that DNA.²⁰

*See Glossary, page iv

There are different classes of genome editing, depending on how the repair to the cut DNA is achieved.

BOX 2:

CLASSIFICATION OF GENOME EDITING

There are different classes of genome editing, depending on how the repair to the cut DNA is achieved, although the distinction between categories can be blurred. Different components can accompany the **site-directed nuclease (SDN)**. For example, a synthetic DNA template is often used to direct the cell’s repair and achieve a particular change in the DNA.²¹ The use of these different components give rise to three different types of genome editing:

- 1) **SDN-1:** No repair template is used;
- 2) **SDN-2:** A repair template is used;
- 3) **SDN-3:** Genes are inserted during the genome editing process and are left in the organism in order to confer the novel trait. If the genes are from other species, this type of genome editing results in a transgenic organism.

In some countries, these different classes of genome editing are regulated differently (see [Regulation](#)).

Though they are often referred to as being among a range of “new breeding techniques”; genome editing techniques are wholly different from conventional breeding. Conventional breeding has been used by farmers and breeders for thousands of years¹⁴ to develop plant and animal varieties with desired traits, such as crops with resistance to pests and diseases. Conventional breeding relies on male and female mating to produce offspring with desired traits that are then selected for further breeding. In contrast, **genome editing, like other techniques of genetic engineering, directly alters the genetic material of an organism using laboratory techniques.**

There is controversy over the terms used to describe genome editing techniques. Genome editing is often called “gene editing,” but this terminology does not include the alteration of multiple genes or gene regulators, which is possible with these techniques. The term “editing” has also been criticized,¹⁵ because it likens genome editing to a precise and predictable text editor, when, in fact, the techniques of genome editing can give rise to several different types of genetic errors, as described in this report. The term “editing” also invites the perception of DNA as linear text, while genetic processes are complex and multidimensional. Genome editing has also been termed “genome engineering,” implying greater intervention than “editing.”¹⁶ In this report, we use the term genome editing.

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Genome editing applications

Genome editing techniques are enabling experiments with new traits, and with a wide range of plant and animal species. In particular, genome editing has facilitated the creation of genetically engineered animals, which was more technologically difficult with earlier genetic engineering techniques, resulting in numerous scientific publications on genome-edited farm animals.²²

Studies have demonstrated initial feasibility for a number of genome-edited plants and animals. However, the majority of these “proof of concept” studies, although they demonstrate that the desired DNA change can be achieved, do not examine (or do not examine thoroughly) the resulting GMO for possible genetic errors, such as off- and on-target effects (see [Genetic errors caused by genome editing](#)).²³

With farm animals, feasibility studies include the development of pigs with resistance to certain diseases,²⁴ “super-muscly” pigs,²⁵ and hornless cows.²⁶ There is also research on genome editing insects and rodents for use in gene drives (see [Gene Drives](#)). Genome-edited traits being researched in plants include drought tolerance in corn, virus resistance in cucumbers, altered flowering time in tomatoes, and altered composition in soybeans.²⁷

However, one of the most common plant traits in development is herbicide tolerance,²⁸ the trait that overwhelmingly dominates current genetically engineered crop acres globally.²⁹ In fact, **the first genome-edited organism commercialized in North America is a herbicide-tolerant canola**, developed by the company Cibus to be tolerant to the herbicide sulfonylurea.³⁰ It is on the market in Canada and the U.S. The only other genome-edited crop plant currently on the market is a soybean with higher oleic acid content, developed by the company Calyxt, and sold in the U.S. only.³¹

Genome-edited crops that could be commercialized soon include high-fiber wheat (from Calyxt, expected to be marketed “as early as 2022”)³² and a waxy corn by DuPont (now Corteva; expected to be launched in 2020).³³ However, although many other experiments are reported in the scientific literature and media, **there is no reliable way to anticipate or identify what GMOs are in the product pipeline.** The processes to develop, regulate and commercialize new products are not transparent to the public, and are affected by many variables that shift over time, including technical limitations, commercial viability and interest. Ultimately, it is not possible to determine which products are most likely to be approved or commercialized.

CIBUS' NON-TRANSGENIC CANOLA

The first genome-edited organism commercialized in North America is a herbicide tolerant canola, developed through the use of oligonucleotide mutagenesis (ODM) (see [Box 1: Genome editing techniques](#)). It was developed by the U.S. company Cibus, to be tolerant to the herbicide sulfonylurea, and is now sold under the seed brand Falco™. It was introduced in the U.S. in 2016, and in Canada in 2018.

Cibus initially advertised the canola as “non-GMO”³⁴ but is now more commonly advertising it as “non-transgenic,”³⁵ with some reference to it also being “non-GMO.”³⁶ However, as of the 2018 court decision in the European Union, the canola would be regulated as a GMO in Europe (see [Regulation](#)).

In 2019, the company claimed that “products derived using Cibus’ patented technology have been certified as non-GMO in countries including the United States, Canada, Argentina and Chile and the process for review is underway in the European Union and Japan.”³⁷ However, North America’s largest non-GMO product certifier, the Non-GMO Project, defines genome editing as GM and will not certify the Cibus canola as Non-GMO Project Verified.³⁸

Genetic errors caused by genome editing

Genome editing can cause genetic errors, including “off-target” effects in the genome, unintended “on-target” effects, interference with gene regulation, and intended and unintended insertion of DNA. These genetic errors are important because they can lead to unexpected and unpredictable effects in the resultant genome-edited organisms, that could be important for food and environmental safety (see [Unexpected and unpredictable effects in genome-edited organisms](#)).

OFF-TARGET EFFECTS

Genome editing can be imprecise, creating genetic errors such as “off-target” effects, which are changes to other genes that were not intended.

In genome editing, an editing system (such as CRISPR-Cas9) is introduced into the genome, but this editor can make mistakes. **The editor can make unintended edits to the host’s DNA at additional sites to the target location**, if the DNA sequences are similar. These unintended alterations cause genetic errors known as off-target effects.³⁹ Such unintended changes may be close to the target gene or can be at distant locations within the genome.

The frequency of off-target effects depends on the genome editing technique (i.e. ZFN, TALENs, etc.) and the protocol (e.g. dosage) used,⁴⁰ but **the CRISPR-Cas9 system appears to be particularly prone to creating off-target**

Genome editing can be imprecise, creating genetic errors such as off-target effects, which are changes to other genes that were not intended.

effects.⁴¹ In addition, many crops, such as corn, wheat and sugar beet, have multiple sets of genomes (i.e. are polyploid). This means there are similar and/or repeated gene sequences in these organisms, which increases the likelihood that they will be unintentionally changed during the genome editing process.⁴²

Off-target effects have been observed in studies on genome-edited plants, such as rice, soybean⁴³ and wheat.⁴⁴ Off-target effects are also a concern in genome-edited farm animals, such as pigs and cattle,⁴⁵ and have been detected in genome-edited pigs,⁴⁶ mice⁴⁷ and human cells.⁴⁸

Off-target effects can cause changes in biochemistry or protein production, both of which are important for food and environmental safety (see [Unexpected and unpredictable effects in genome-edited organisms](#)). Most studies on the potential uses of genome editing techniques in agriculture consider off-target effects to be both a major challenge and a major concern.⁴⁹ Despite this, **few studies search thoroughly for off-target effects.**⁵⁰

As yet, there are no standard protocols for detecting off-target effects. They can either be examined by predicting potential off-target sites in the genome and then examining the DNA in those sites to see if they have been unintentionally changed, or by sequencing the whole genome. Whole genome sequencing enables a search for off-target effects genome-wide instead of being limited to only computationally predicted sites, but it is costly.⁵¹ At the moment, most studies that examine for off-target effects use computation of predicted off-target sites.⁵²

Studies examining only those sites predicted by computation may miss off-target effects. Even with whole genome sequencing, some studies report a lack of detectable off-target effects in genome-edited organisms.⁵³ This could be either because off-target effects are not present, or they are present but it is difficult to distinguish between off-target effects and natural genetic variation.⁵⁴

UNINTENDED ON-TARGET EFFECTS

Genome editing can also cause unintended “on-target” effects, where the intended change occurs at the target location but also triggers genetic errors. Unintended on-target effects can occur even in the absence of off-target effects. On-target effects refer to an assortment of possible genetic errors, including errors in the way genes are processed or “read,” as well as rearrangements and deletions of parts of the host’s DNA. Even though they are called on-target effects, they may occur at some distance from the intended edit.

During normal cell function, parts of genes within DNA are “read” to produce an intermediary product, called messenger RNA (mRNA). This mRNA is then used as a template to produce proteins. **Unintended on-target effects can be caused by both large and small changes to DNA.** Even a small intended edit of the gene (e.g. an insertion or deletion of just

Genome editing can also cause unintended on-target effects, where the intended change occurs at the target location but also triggers genetic errors.

one DNA base pair), although on-target, can disrupt the way a gene is read and processed into proteins. The mRNA may form in a different way (i.e. the alternative splicing mechanism is disrupted) or important parts of the gene (those coding for protein production) may be missed or “skipped” completely.⁵⁵ In addition, **genome editing can inadvertently cause duplications, extensive deletions and complex rearrangements of DNA sections.**⁵⁶ These deletions and rearrangements can also cause genes to be misread.

The misreading of DNA has the potential to produce unintentionally altered (or malformed) proteins. For example, one study using a laboratory culture of CRISPR genome-edited human cells found a malformed protein produced in error when DNA was misread as a result of the genome editing process.⁵⁷ Another study found unexpected changes in protein expression or mRNA in approximately 50% of commercially available CRISPR-edited human cell lines.⁵⁸

Food allergens are mostly proteins, so altered proteins could have significant implications for food safety.⁵⁹ Concerns with the allergenicity of proteins have long been an important preoccupation with GMOs created by standard genetic engineering techniques. For example, “Starlink” corn was approved in the U.S. in 1998 for animal, but not human, consumption, due to concerns over the potential allergenicity of the protein (Cry9C) produced by the inserted Bt toxin that conferred insect resistance.⁶⁰ After it was discovered contaminating human food supplies in the U.S. and Canada in 2000,

products with Starlink corn were recalled from shelves at great cost to food companies, and the corn was withdrawn from the market.⁶¹

The misreading of DNA in a genome-edited plant or animal could, like first-generation GMOs, also impact biodiversity. For example, if the chemistry of a genome-edited plant or animal is changed by the misreading of DNA, it could potentially produce a compound that is toxic to the wildlife that feeds on it. Despite these possible impacts, **unintended on-target genetic errors created by the genome editing process may be missed because detailed, genome-wide examinations of DNA are not routinely performed, and there are no standard protocols for such examinations.**⁶² Similarly, there is rarely any examination of gene products, such as mRNA and proteins,⁶³ which could be important to food safety.

INTERFERENCE WITH GENE REGULATION

In addition to altering an organism's DNA, **genetic engineering may have unintended impacts on an organism's ability to express or suppress other genes.** Within an organism, genes are switched on (expressed) and off (silenced) in different parts of the organism at different times as the organism grows, reproduces, or responds to environmental factors such as light, heat or drought. In addition, genes interact with each other, either suppressing or reinforcing their expression.

The orchestration of gene function in an organism is part of a complex regulatory network. The precise way this regulatory network operates is still poorly understood, as exemplified by recent advances in our

Even precise edits do not necessarily result in precise outcomes.

knowledge of how gene expression is regulated.⁶⁴ For example, for several decades, a dominant theory in molecular biology, known as the central dogma, was that each gene had a single function (i.e. produces one protein), but it is now known that genes can have several functions, and interact with one another.⁶⁵ Also, DNA that does not produce proteins was previously thought to be "junk" DNA with no identified purpose, but recent scientific research now considers much of this DNA important for controlling gene expression in plants, animals⁶⁶ and in human genomes.⁶⁷

There have already been reports of an unexpected response from the cell regulatory network during genome editing. In experiments with human cells, the cuts in DNA created by CRISPR were unexpectedly found to kill cells or stop them from growing.⁶⁸ **The lack of understanding about how genomes are regulated means it is not possible to predict the nature and consequences of all the interactions between altered genetic material (whether intentionally or unintentionally altered) and other (unedited) genes within the organism.** This means that edits to DNA may inadvertently affect the operation of the organism's regulatory network. This could result in the organism's own (unedited) genes not responding as they should, by being produced incorrectly, in the wrong amount, or at the wrong time. **Any unexpected and unpredictable effects in the genome-edited organism could result in alterations to biochemical pathways or protein composition, which could have implications for food and environmental safety.**

INTENDED AND UNINTENDED INSERTION OF DNA

Several genome editing techniques are in development (see [Box 1: Different genome editing techniques](#)). Although, in the future, genes may not need to be inserted to perform genome editing, currently **genes encoding the editing components are commonly inserted into a plant's genome in order to perform the genome editing**. Most market-oriented genome-edited plants in development have genes encoding for gene-editing components inserted into them at random locations.⁶⁹ However, instead of these genes conferring a novel trait, they code for the CRISPR components that perform the genome editing process. This applies to plants developed by SDN-1 and SDN-2 types of genome editing (see [Box 2: Classification of genome editing](#)), which aim to have no transgenes in them.

In the case of CRISPR, a package of DNA (DNA “cassette”) containing the CRISPR components is commonly inserted into the plant's genome at a random location, in exactly the same way as earlier genetic engineering techniques. This DNA codes for the CRISPR components. This complex targets a specific location in the host genome to perform the genetic change. Afterwards, the inserted CRISPR DNA is bred out via conventional breeding so that the GMO may no longer be transgenic (i.e. no longer contains genes from another species). The genome-edited high-fiber wheat produced by Calyxt was developed in this way.⁷⁰ However, it is inevitable that, **in some cases, not all the inserted DNA will be removed and some of the DNA encoding for genome editing components will inadvertently remain in the genome-edited organism.**⁷¹

When DNA is inserted in this manner into a plant's genome, the insertion may not be precise. Just as earlier genetic engineering could not direct the location for the insertion of transgenes and control their copy number,

multiple copies and additional fragments of the DNA cassette can be unintentionally introduced into the plant's genome.⁷² The insertion of DNA coding for genome editing components can also cause sections of the host's organism's DNA to become rearranged, as has often happened with first-generation genetically engineered crops.⁷³ This is in addition to any deletions and rearrangements caused by the genome editing process itself (see [Unintended on-target effects](#)).

In some genome-edited plants and animals, DNA coding for CRISPR components is introduced as a plasmid into the organism's cell and performs the genome editing without becoming integrated with the organism's own genome, as is claimed with DuPont's genome-edited waxy corn.⁷⁴ However, **any introduced DNA could unintentionally become integrated, at random, into the organism's genome.**⁷⁵ Unintended integration of genome editing components (including any additional copies and fragments) has been found in both plants and animals, not only from CRISPR, but also from TALENs.⁷⁶ In a recent case, cows that had been genome-edited via TALENs to be hornless⁷⁷ were found to have DNA from the genome editing process, including antibiotic resistance genes, unintentionally incorporated into their genomes (see [Box 3: Case Study – Unintended Foreign DNA in Genome-Edited Hornless Cows](#)).⁷⁸ **Integration of unwanted DNA from the genome editing process appears to be more common than previously thought but a failure to identify integration events has led to a high rate of falsely claimed precision.**⁷⁹

Both unintended integration of DNA coding for CRISPR components, and additional fragments or rearrangements of intended DNA insertions, can give rise to unexpected effects in genome-edited organisms, in a similar manner to first-generation genetic engineering. It is, therefore, important that checks for unintended DNA and unintended effects from DNA insertion (intended or unintended) are carried out.

BOX 3: CASE STUDY

Unintended Foreign DNA in Genome-Edited Hornless Cows

“We can’t know if we don’t look.”

– Steven M. Solomon, Director Center for Veterinary Medicine, US Food and Drug Administration, 2020⁸⁰

“It was not something expected, and we didn’t look for it.”

– Tad Sonstegard, CEO, Acceligen, agricultural subsidiary of Recombinetics, 2019⁸¹

The development of genome-edited hornless cows⁸² (via TALENs, see [Box 1: Genome editing techniques](#)) was celebrated as an illustration of the power and ease of genome editing, and discussed as a demonstration of why genome-edited animals did not need to be regulated. As key university researchers involved in the project argued, “The effects of genome editing are largely identical to those of the natural processes that continually create variation in the genomes of food animals. From this point of view, it is hard to see why the process of genome editing to introduce defined genetic changes should be regulated.”⁸³ However, **the case of the hornless cows shows the potential for errors in the genome editing process and the need for independent safety assessment.**

The dairy cows were genome-edited to be hornless (polled), to eliminate the practice of manually dehorning cows. Two cows were developed by university researchers in collaboration with the U.S. company Recombinetics. The developers reported that they were created without foreign genes⁸⁴ and “our animals are free of off-target effects.”⁸⁵ However, in 2019, researchers at the U.S. Food and Drug Administration (FDA) found unexpected foreign DNA in the cows (on-target effects).⁸⁶

FDA researchers found two antibiotic resistance genes and various other gene sequences from bacteria in both cows. This genetic material was from the DNA carrier (a plasmid; see [What is genome editing](#)) that had been used to introduce the repair template into the cows’ cells. The FDA also found an extra copy of the DNA repair template, at the target site.

The genome-edited cows were checked for off-target effects but not for unintended on-target effects. In their 2016 study, the developers reported that the polled gene had been successfully changed and there were no unintended insertions or deletions from the cutting and repair process.⁸⁷ In 2017, the executive chair of Recombinetics said, “We know exactly where the gene should go, and we put it in its exact location,” and concluded, “we have all the scientific data that proves that there are no off-target effects.” He also said that the cows were “100% bovine [cow].”⁸⁸ However, upon discovery of the extra DNA, Recombinetics said, **“The company did not directly screen for the presence of the plasmid, it should have.”**⁸⁹

The extraneous DNA found in the cattle does not necessarily present a safety threat. This was stressed by the FDA in a related commentary that argued for government

regulation,⁹⁰ as well as in an editorial in the journal *Nature Biotechnology* that argued against mandatory government safety assessments.⁹¹ The editorial authors argued against regulation while acknowledging that the extra DNA was “unexpected and initially missed” and that **“gene editing is not the neat precise process that proponents tout.”** [emphasis added].

The FDA scientists suggested a number of reasons why the template plasmid may not have been detected in the cows. They said that their discovery “highlights a potential blind spot in standard genome editing screening methods” and that, across the scientific literature, **“we suspect that integration errors are either under-reported or overlooked.”**⁹² [emphasis added].

In this case, the discovery of unexpected DNA was not made by the developers, and it was only incidentally found by the FDA. The FDA researchers were not screening the genome-edited cows as part of any government regulatory process but because they were using the cows’ genome sequences to test a new bioinformatics method.⁹³ This is significant because, from the outset, the developers argued that regulating genome-edited animals was not necessary to ensure safety. In fact, the publication of the key scientific papers on the hornless cows was accompanied by arguments for and against regulation.⁹⁴

The director of the FDA’s Center for Veterinary Medicine wrote that the FDA’s finding emphasized the agency’s “critical role in risk-based evaluation of intentional genome alterations.”⁹⁵ However, this conclusion was disputed in a 2020 editorial in *Nature Biotechnology*, which argued that **the FDA’s proposal to regulate all GM animals “canonizes a precautionary stance to genome-edited animals” and, as such, “makes no economic sense.”**⁹⁶

Once the cows were found to be carrying extra DNA, the company stressed that their cows were only research animals⁹⁷ (“testing regulatory systems with our experimental gene-edited animal(s) is of interest”⁹⁸) and there “never was a commercial intent for these animals or their offspring.”⁹⁹ However, executives had, in 2017, mentioned that there were “multimillion-dollar deals in the final steps of negotiations.”¹⁰⁰ In 2019, the genome-edited dairy cows were being incorporated into a breeding program in Brazil that was then cancelled because the cows no longer qualified for Brazil’s regulatory exclusion of non-transgenic organisms, because they contained foreign DNA.¹⁰¹

“Our analysis demonstrated that genome editing in animals can have unintended consequences, and in this case, it caused foreign DNA to be integrated into the animals’ genomes. While the existence of an unintended alteration does not necessarily mean that the genome edit is unsafe to animals or consumers, it does show that both scientists and regulators need to be alert to the potential for such unintended alterations to take place.”

– Steven M. Solomon, director of the FDA’s Center for Veterinary Medicine, February 2020¹⁰²

Unexpected and unpredictable effects in genome-edited organisms

As with all genetically engineered organisms, genetic errors introduced by genome editing (e.g. off-target or unintended on-target effects) can result in unexpected effects in the genome-edited organism. Unexpected effects are unpredictable because they are caused by genetic errors and the lack of a full understanding of how genomes work means that effects cannot be reliably predicted. They can include changes in the chemistry, biochemical pathways or protein composition. Such effects could have implications for food and environmental safety if they alter toxicity (via chemical changes) or allergenicity (via changes in protein composition). Importantly, unexpectedly malformed proteins (i.e. with changed composition) have already been observed as an effect of genome editing (see [Unintended “on-target” effects](#)).

The intended changes to genetic material induced by some types of genome editing techniques (types SDN-1, SDN-2 and ODM) are sometimes described by developers as “mutations”¹⁰³ because only very small parts of DNA (e.g. one or a few base pairs) are altered, and no novel (or foreign) genes are intentionally introduced. While mutations do occur naturally, and indeed are an important source of genetic variability in breeding plants and animals, the process is very different from the direct changing of genetic material with genome editing. Importantly, **it does not**

As with all genetically engineered organisms, genetic errors introduced by genome editing can result in unexpected effects in the genome-edited organism.

follow that a genome-edited organism with only a small change to its DNA is always “safe.” To evaluate environmental and food safety, the changes to DNA (both intended and unintended) and other genetic material (e.g. mRNA) need to be carefully assessed.

Many genes are multifunctional, particularly in animals.¹⁰⁴ This means that a gene that is rendered dysfunctional, even by a single base change, could have an essential, unrelated function elsewhere in the organism. For example, in the case of genome-edited pigs reported to be resistant to porcine reproductive and respiratory syndrome virus (PRRS), the gene that has been knocked out (CD163) is known to be important in defending against other infections and to regulate blood composition.¹⁰⁵ Therefore, **intentionally disabling just one single gene (often referred to as a “genetic tweak”)¹⁰⁶ could have important consequences for other traits in the animal or plant.**

Genetic errors caused by genome editing can result in unexpected effects in the resulting GMO, irrespective of whether or not genes for a novel trait have been introduced. For example, super-muscly genome-edited pigs were found to have an extra vertebra compared to unaltered pigs, even though no genes had been inserted.¹⁰⁷ Although pigs can have slightly different numbers of vertebrae, the underlying mechanism of this change is not known, but is thought likely to be associated with the gene knocked out to make them super-muscly.¹⁰⁸

While there are many “proof of concept” publications about what genome editing might achieve, there are, as yet, no studies examining their potential environmental impacts. There are large gaps in the scientific literature on how new traits may impact the environment, particularly if they introduce novel compounds. For example, first-generation genetically engineered plants altered to produce omega-3 fatty acids unexpectedly induced toxic effects on caterpillar larvae, deforming wings in the adult butterfly and raising concerns about how this crop might affect the food web.¹⁰⁹ Ultimately, the full impacts of genetically engineered organisms on the environment cannot be predicted. Biological, ecological and social systems are interrelated and interdependent.

Gene Drives

Genome editing techniques, particularly CRISPR (type SDN-3) systems, have enabled the possibility of “genes drives.” Gene drives are a technology through which a few genetically engineered individuals are designed to intentionally push new genes through an entire population of a species.¹¹⁰ The gene drive mechanism ensures that the new genes will be inherited by all offspring (as opposed to an expected half of the offspring in normal inheritance) in subsequent generations.¹¹¹ Thus, **gene drives are designed to alter the genetic make-up of an entire wild population, or to eradicate a population or species.**

Gene drives are being proposed for pest and disease control in agriculture.¹¹² However, the most advanced research so far is in insects, specifically in gene drive systems that would alter genes to prevent mosquitoes from reproducing effectively, thus reducing the size of particular mosquito populations.¹¹³ The research group Target Malaria, funded by the Bill and Melinda Gates Foundation among others,¹¹⁴ aims to use gene drive mosquitoes to reduce the population of *Anopheles gambiae* mosquitoes, which transmit the parasite that causes malaria.

In agriculture, applications are being discussed to alter genes so that agricultural pests such as a type of fruit fly (spotted wing drosophila)¹¹⁵ and pigweed (*Palmer amaranth*)¹¹⁶ do not

reproduce effectively, in order to make them ultimately go extinct. Researchers are also devising a type of gene-drive mechanism for mammals,¹¹⁷ and have outlined a hypothetical gene drive system intended to drive a desired trait through a herd or population of a farm animal.¹¹⁸

Unlike GMOs that have thus far been engineered for confined use in agricultural production, gene drive organisms are expressly designed for intentional release into the wild. Releasing gene drive organisms can therefore be understood as a form of ecosystem engineering.¹¹⁹ **Once released, gene drive organisms cannot be recalled, and the changes they create in the genetic make-up of the population are most likely irreversible.** Acknowledgement of this challenge has led to theorizing various means to control or reverse gene drives, however these exist only as mathematical models and are accompanied by their own complex risks.¹²⁰

Scientists and others are already warning that the consequences of gene drives could be severe if there are any unexpected effects from the genome editing process¹²¹ (e.g. from off-target effects¹²²), or if other (e.g. ecological) unintended consequences arise.¹²³ The overarching concern is that genetic change to, or elimination of, an organism in the wild could disrupt ecosystems in unpredictable ways.

In 2018, governments at the United Nations Convention on Biological Diversity (CBD) agreed to apply the precautionary approach² in relation to the regulation of gene drives, including to conduct risk assessments and establish safety measures to prevent potential adverse effects.¹²⁴ Governments also agreed on the need to seek or obtain the approval of potentially affected indigenous peoples and local communities prior to considering any release. The CBD is further considering experiences, challenges and needs that may arise in relation to the risk assessment of gene drives.¹²⁵ Ultimately, however, the risks of releasing gene drives cannot be identified before they are deployed, suggesting that a precautionary approach would lead to a zero deployment of gene drives.

There is widespread agreement that research into gene drive systems requires some form of international regulation.¹²⁶ **However, some scientists and civil society organisations argue that gene drives cannot be regulated at all, and are calling for a global moratorium on gene drive research.**¹²⁷

Ultimately, the risks of releasing gene drives cannot be identified before they are deployed, suggesting that a precautionary approach would lead to a zero deployment of gene drives.

2 The precautionary approach prioritizes the protection of human health and the environment when faced with scientific uncertainty and gaps in our knowledge. The approach is based on Precautionary Principle, as defined in the UN 1992 Rio Declaration on Environment and Development, which states that, “Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.”

Regulation

“The advent of genome editing offers an opportunity to rethink the regulatory approach to the products of biotechnology.”

–Young et al., 2020¹²⁸

There is international debate about how genome-edited plants and animals for use in agriculture should be regulated, if at all.¹²⁹ The emergence of genome editing techniques has led to renewed arguments for reducing regulation.¹³⁰ However, as discussed in this report, the use of new genome editing techniques will challenge regulators with new traits and new processes, with increasing complexity and ongoing uncertainty.

There are divergent regulatory approaches to genome editing around the world. In some countries, certain genome-edited classes are excluded or included under existing GMO regulations (see [Box 2: Classification of Genome editing](#)). Other countries are changing regulation and definitions of genetic engineering in order to account for the new techniques. While some jurisdictions regulate based on the process used to genetically engineer organisms (e.g. the European Union), others regulate the end (“novel”) products on a case-by-case basis (e.g. Canada).

All regulatory authorities around the world regulate transgenic genome-edited plants (type SDN-3) as GMOs.¹³¹ However, some countries (Argentina, Brazil, Australia, Japan and the U.S.) have decided that non-transgenic genome-edited organisms should not be regulated in the same way as products of earlier genetic engineering:

- **Argentina** (2015) passed a resolution to not regulate products that do not have transgenic material (no “novel combination of genetic material”), unless their features lead to a significant risk hypothesis.¹³²
- **Brazil** (2018) has classified non-transgenic genome-edited organisms as non-GMO.¹³³
- **Australia** (2019) amended its regulations to exclude techniques that do not use a DNA repair template (SDN-1), or do not introduce other genetic material.¹³⁴
- **Japan** (2019) will also not regulate or require labelling of genome-edited foods unless they contain transgenes.¹³⁵
- In the **United States** most genome-edited crops would have already fallen outside existing environmental regulation which focused on GM crop plants that pose a plant pest risk.¹³⁶ However, new rules published in June 2020 also explicitly exclude plants produced through a range of genome editing classes, including those that do not use a repair template, make one small change (a single base pair substitution), and introduce a gene known to be in the plant’s gene pool.¹³⁷ These same crops may or may not undergo a food safety assessment because the Food and Drug Administration’s “premarket food safety consultation” is, technically, voluntary.¹³⁸

The European Union and Canada are relying on existing regulations rather than creating new rules for the new techniques:

- The **European** Court of Justice (2018) ruled that all genome-edited organisms, including those developed by ODM, SDN-1 and SDN-2, meet the definition of GMOs in current European Union regulations.¹³⁹
- In **Canada**, existing regulation means that all organisms with “novel traits” are regulated regardless of the technology used to produce them.¹⁴⁰ This means that most, or all, genome-edited products will be subject to government safety evaluation.

Many countries are thereby also excluding some products of genome editing from existing GMO labelling requirements. The new U.S. *National Bioengineered Food Disclosure Standard*, which will come into full effect in 2022, will mandate labelling for “bioengineered” foods but excludes those that do not contain detectable genetic material and “for which the modification could not otherwise be obtained through conventional breeding or found in nature.”¹⁴¹ Canada has no mandatory labelling for any genetically engineered foods. In addition to providing transparency in the marketplace, labelling and traceability can allow for post-market monitoring of safety and assist efficient food recalls if necessary.¹⁴²

As discussed in the sections above, all genome editing techniques can create genetic errors that can lead to unexpected and unpredictable effects in the resulting genome-edited organism. Such effects can go unnoticed if they are not adequately screened for. **CRISPR and other new genome editing techniques can lead to unintended consequences and therefore require robust regulation.**

One of the primary concerns for food safety is the question of unintended alteration to protein composition as a result of genetic engineering. This is because allergens are proteins, so any

inadvertent change in protein composition could cause the genome-edited plant or animal to trigger allergies in humans.¹⁴³ Importantly, altered proteins have already been identified as an unintended outcome of genome editing.¹⁴⁴ Protein composition needs to be examined carefully in genome-edited foods. Evaluations of GMOs need to identify **all potential hazards, no matter how theoretical they may initially appear.**

Such oversight is needed in order to avoid potentially damaging impacts that could otherwise be overlooked. For example, it is important that regulations include comprehensive examination for off-target effects, and that any found are fully evaluated to determine if they have caused any changes to chemistry or protein production. As discussed, however, there are, as yet, no standardized protocols to screen for off-target and on-target effects. The case of the genome-edited hornless cows illustrates that, even when developers state that they are certain their products show no unintended effects, third-party oversight is necessary (see [Box 3: Case Study – Unintended Foreign DNA in Genome-Edited Hornless Cows](#)).¹⁴⁵

Arguments to exclude some or all genome-edited products from government safety evaluation are being made. A 2020 editorial in the journal *Nature Biotechnology* argues that “mandatory oversight could be phased out” for genome-edited animals in the U.S. and replaced with a system whereby the U.S. FDA “exercises discretion over which genome-edited animals are regulated according to the hazard represented by the introduced trait.”¹⁴⁶ A similar argument is being made by the biotechnology industry in Canada, where the lobby group CropLife Canada recommends a tiered regulatory system for genome-edited products, one that predetermines the risk potential of products based on “complexity and familiarity” and could thereby exclude some products from a full scientific evaluation.¹⁴⁷

There are already long-standing critiques from many scientists and civil society organizations of GMO regulatory regimes around the world, largely focused on the need for increased independence and transparency.¹⁴⁸ For example, governments continue to largely rely on information and data generated and submitted by the same companies or institutions that are requesting product approval. In Canada, for example, all information submitted for safety evaluation is classified as confidential business information and is therefore not made available for review by independent scientists.¹⁴⁹ This also means that much of the science behind regulatory decisions is not peer-reviewed. This is significant because peer-review and independent corroboration are defining features of the scientific method.¹⁵⁰

This report focuses on describing the science of genome editing and the associated risks that could have implications for food and environmental safety. However, **the regulation of GMOs also affects our economies and societies, and there is increased discussion about the limitations of relying on solely “science-based” regulation for genome editing.**¹⁵¹ In particular, the use of genome editing to genetically engineer humans has prompted calls for mechanisms to enable debate over ethical questions.¹⁵² These include, for example, a proposal to establish an interdisciplinary network to function as a global observatory, “as a crucial step to determining how the potential of science can be better steered by the values and priorities of society.”¹⁵³

Excluding non-scientific considerations, such as possible economic and social impacts, can narrow the scope of scientific evaluation itself. For example, without consulting farmers, any regulatory process can miss or underestimate potential long-term environmental and agronomic impacts of the products being evaluated.¹⁵⁴ The Royal Society of Canada’s Expert Panel on the Future of Food Biotechnology argued that health and environmental safety issues “though largely scientific in nature, often cannot be addressed fully without reference to broader ethical, political and social issues and assumptions.”¹⁵⁵ This is in part because defining the scope of risk and levels of acceptable risk inherently involves value judgements.

A strictly scientific assessment could lead to regulatory agencies reviewing products for approval that may have little or no social worth. In Canada, for example, some farmer associations have requested an economic risk assessment of all GMOs before market release, in a process that would include consultations with farmers.¹⁵⁶ In the absence of such participatory processes, and in the context of a high level of corporate consolidation in the global seed and agrochemical markets,¹⁵⁷ companies are developing and commercializing products (e.g. GM glyphosate-tolerant alfalfa) that have little utility and, on the contrary, may pose serious risks to farming systems and the environment.¹⁵⁸

Conclusion

Genome editing techniques are leading to the commercialization of new genetically engineered plants and animals for food, and yet the orchestration of gene function in organisms is part of a complex regulatory network that is still poorly understood.

There is an increasing amount of evidence showing that genome editing techniques now being explored are not as precise as originally claimed. Describing these new genetic engineering techniques as enabling “edits” to the genome suggests a level of precision that has not yet been achieved, and may not be possible.

It is clear that genome editing can give rise to genetic errors. These include off-target effects, unintended on-target effects, interference with gene regulation, and effects from intended and unintended insertion of DNA. Genetic errors can result in unexpected and unpredictable effects in the resultant GMO. Unexpected effects such as altered protein composition could impact the food and environmental safety of genome-edited plants and animals. Ultimately, the precision of genome editing techniques and the food and environmental safety of genome-edited products cannot be assumed. Rather, robust regulation with thorough risk assessment is required to ensure food and environmental safety.

Genome editing, with such powerful applications and profound implications, requires precautionary regulation as well as mechanisms to consider societal values.

Genome editing could facilitate many new GMOs coming to market, enhancing the need to grapple with ongoing social and economic considerations. Additionally, genome editing is enabling the particularly powerful technology of gene drives, which pose profound environmental and social risks. Given the complexity of the ecosystems into which they would be introduced, assessing the full risks of gene drive organisms prior to their release is not possible. Any release of gene drive organisms is highly likely to be impossible to recall, with unpredictable and irreversible consequences.

Genome editing, with such powerful applications and profound implications, requires precautionary regulation as well as mechanisms to consider societal values.

Further Reading

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