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# Genome Editing in Agriculture: Methods, Applications, and Governance

## *A paper in the series on The Need for Agricultural Innovation to Sustainably Feed the World by 2050*

**The power of genome editing suggests that, if conducive social and regulatory conditions are in place, it can substantially increase the positive impacts of plant and animal breeding on human welfare and sustainability. (Shutterstock photos from Yaroslava [corn], vchal [gene manipulation], and Shyamalamuralinath [calf].)**

## Abstract

Genome editing is the process of making precise, targeted sequence changes in the deoxyribonucleic acid of living cells and organisms. Recent advances have made genome editing widely applicable, offering the opportunity to rapidly advance basic and applied biology. In the face of the mounting food, fiber, feed, and fuel needs and the decreasing availability of land and water caused by global population growth, as well as the challenges climate change poses to agriculture, genome editing for crop and livestock improvement is garnering increasing attention. This issue paper describes how genome editing is performed, the types of "edits" that can be made, how the process relates to traditional breeding and conventional genetic engineering, and the potential limitations of the approach. The paper also presents an overview of the current landscape of governance of genome editing, including existing regulations, international agreements, and standards and codes of conduct, as well as a discussion of factors that affect governance, including comparison with other approaches to genetic modification, environmental and animal welfare impacts of specific applications, values of producers and consumers, and economic impacts, among others. Recognizing both that genome editing for crop and livestock improvement has the potential to substantially contribute to human welfare and sustainability and that successful deployment of genome editing in agriculture will benefit from science-informed, value-attentive regulation that promotes both innovation and transparency (alongside strategies to improve food distribution, decrease socioeconomic disparities, mitigate barriers to trade, and moderate political and market dependencies), the paper aims to provide a conceptual and knowledge-based foundation for regulatory agencies, policy- and lawmakers, private and public research institutions, industry, and the general public.

## Introduction

Twentieth-century advances in plant and animal breeding and agricultural practices did much to help meet the increasing food, fiber, feed, and fuel needs of a burgeoning world population. As population growth continues through this century, those needs continue to increase while the amount of land and water available for production decreases. In addition, climate change is impacting land and water availability further and

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altering the incidence of droughts, floods, and other severe weather events, as well as the distribution and prevalence of diseases and pests. Meeting the increasing needs of the world population in the face of these challenges, sustainably, is a daunting yet essential task.

Continued successes in crop and livestock improvement will be critical. Resistance to pests and diseases, tolerance to adverse environmental conditions, and improved nutritional quality will be essential. In addition, adapting plants to increase their efficacy for environmental remediation and improving animals for use as models for human disease will be important. Meeting the needs of the increasing world population will also depend on social and engineering innovations, including changes to improve food distribution, decrease socioeconomic disparities, mitigate barriers to trade, and moderate political and market dependencies. The power of *genome editing*,[1] however, suggests that, if conducive social and regulatory conditions are in place, it can substantially increase the positive impacts of plant and animal breeding on human welfare and sustainability.

Genome editing is the process of making precisely targeted changes in the DNA (deoxyribonucleic acid) of living cells and organisms. Advances in recent years have made genome editing applicable in many contexts and for many purposes, including plant and animal improvement. This issue paper describes how genome editing is performed, the types of "edits" that can be made, how the process compares to traditional breeding and conventional genetic engineering, and the potential limitations of the approach. This paper also touches on ways in which genome editing can enhance related technologies such as the insertion of transgenes for genetic modification of plants and animals. Following these sections, the paper presents an overview of the current landscape of governance of genome editing in selected countries, including existing regulations, international agreements, and standards and codes of conduct. Gene drives, a new and widely discussed implementation of genome editing (Esvelt et al. 2014; Saey 2015; Wade 2015) that can modify the genetics of a wild population for purposes such as pest control, raise a unique and complex set of biosafety and regulatory issues beyond the scope of this paper and are not discussed.

The paper is intended to be a resource for U.S. and international regulatory agencies, policy- and lawmakers, private and public research institutions, industry, and the general public. It aims to provide a conceptual and knowledge-based foundation for informed regulatory and policy decision-making and for consumer choice.

## Genome Editing Methods

Genome editing, as it is most frequently practiced, uses reagents that specifically recognize and precisely cleave DNA targets within the genomes of living cells (Voytas 2013). These reagents are referred to as *site-directed nucleases* (SDNs; also called *sequence-specific nucleases* or SSNs). SDN-induced DNA damage is perceived by the cell and repaired; however, it is possible to direct the cell's DNA repair mechanisms to incorporate desired gene edits at or near the break site. In this section, the types of SDNs that have been developed to achieve targeted DNA cleavage, as well as the variety of targeted DNA modifications that can be realized through their use, are described briefly.

### Gene-targeting Reagents

Three types of SDNs—*meganucleases*, *zinc-finger nucleases* (ZFNs), and *transcription activator-like effector nucleases* (TALENs)—recognize their DNA targets through protein/DNA interactions. The DNA recognition domains of these reagents are engineered to achieve requisite target specificity.

[1] Italicized terms (except genus/species names and published material titles) are defined in the Glossary.

Meganucleases, produced by many prokaryotes and algae, typically recognize and cleave DNA sequence signatures ranging from 12 to 40 base pairs (bp) in length (Paques and Duchateau 2007; Smith et al. 2006). Whereas each meganuclease has evolved its own DNA sequence specificity, they can be engineered to recognize new DNA target sites. Engineering meganucleases for genome editing is often challenging because the amino acids that recognize DNA are in close proximity to the catalytic site that carries out DNA cleavage; changes introduced to alter DNA sequence specificity can compromise the ability of the engineered enzyme to cleave DNA. Nonetheless, a number of meganucleases have been successfully engineered and used to edit plant genomes (D'Halluin et al. 2013; Gao et al. 2010).

ZFNs bind DNA using a protein domain that is distinct and separate from the nuclease. DNA targeting is achieved using an engineered array of zinc-finger motifs (Bibikova et al. 2003; Carroll 2011). Each zinc finger typically recognizes 3 bp, and zinc fingers with different DNA recognition specificity can be strung together to recognize new DNA targets. As with meganucleases, protein engineering or genetic selections are often required to optimize DNA binding. Once optimized, the DNA binding domain is then fused to a catalytic domain of a type II restriction enzyme, typically *Fok*I. *Fok*I functions as a dimer, so two ZFNs are engineered to bind DNA targets in close proximity. This enables the *Fok*I monomers to dimerize at the target site and cleave the target DNA.

TALENs are similar to ZFNs in that their DNA binding and cleavage domains are distinct (Bogdanove and Voytas 2011; Christian et al. 2010). Sequence-specific DNA recognition is achieved using an engineered DNA binding domain based on transcription activator-like (TAL) effectors, which are proteins made by bacterial plant pathogens of the genus *Xanthomonas*. In nature, upon infection the bacteria deliver TAL effectors to plant cells where the proteins recognize specific DNA sequences upstream of target genes and activate the expression of those genes. Shortly after the mechanism of TAL effector DNA binding was elucidated (Boch et al. 2009; Moscou and Bogdanove 2009), the TAL effector DNA recognition domain is used to create targeted nucleases for gene editing (Christian et al. 2010). The TAL effector DNA recognition domain is structurally modular, with a pair of variable amino acids in each module specifying a single nucleotide in the target DNA sequence. Thus TALENs can be readily engineered for desired specificity by assembling the requisite modules into custom arrays. As in ZFNs, an engineered TAL effector DNA recognition domain is fused to the *Fok*I nuclease for use in genome editing. The use of meganucleases, ZFNs, and TALENs was instrumental in establishing successful approaches to plant and animal genome editing.

The most recent additions to the genome editing toolkit are reagents derived from an antiviral defense mechanism found in a wide range of bacteria and archaea (Wright, Nunez, and Doudna 2016). This adaptive immune system uses a short RNA (ribonucleic acid) derived from a CRISPR (clustered regularly interspaced short palindromic repeats) locus found in the bacterial genome. The RNA has a sequence complementary to DNA invaders such as viruses. The RNA base pairs with the viral DNA in association with a Cas (CRISPR-associated) protein, a nuclease, that precisely cleaves and thereby inactivates the viral DNA. The RNA sequence can be customized to target the nuclease to a DNA sequence of choice. In the most widely used system, from *Streptococcus pyogenes*, this protein is called Cas9 and the custom RNA is called a guide RNA (gRNA). The CRISPR/Cas9 mechanism for targeted cleavage has been used to make targeted DNA breaks in a variety of organisms.

Whereas all of the above reagents use proteins or protein/RNA complexes to recognize DNA targets, it is also possible to create targeted modifications using short pieces of single- or double-stranded DNA (*oligonucleotides*) (Lusser et al. 2011; Sauer, Mozoruk, et al. 2016). At some frequency, these oligonucleotides base pair with complementary sequences in the genome. If the oligonucleotide differs by one or a few bases from the genomic target sequence, it triggers a DNA mismatch repair mechanism; if the mismatch is repaired based on the oligonucleotide sequence, specific base modifications are made in the genome. Oligonucleotide-directed mutagenesis (ODM) is therefore an alternative to nuclease-based gene editing.

## Types of DNA Modifications Created with SDNs

Although there are a variety of SDN platforms from which to choose, the primary task of the reagent is to find the specific DNA sequence target within a complex genome and make a targeted DNA double strand break. The cell then recognizes the broken chromosome and activates one of two primary DNA repair mechanisms. The preferred mechanism for repair in most somatic (nonreproductive) cells appears to be *nonhomologous end-joining* (NHEJ). As the name implies, cellular machinery recognizes the break and simply rejoins the broken ends. An alternative repair pathway—*homology-directed repair* (HDR)—uses a DNA template and copies information from the template into the break site. The template must have regions with strong sequence similarity to the target to initiate HDR. In genome editing applications, these regions typically flank the DNA sequence changes to be introduced (ranging from single base changes to one or more genes). Crossing over between the flanking regions in the template and at the DNA break introduces the changes to the genome. To frame the discussion of the types of DNA repair outcomes that can be achieved, three classes—described in detail below and designated SDN-1, SDN-2, and SDN-3—are considered (EFSA 2012; Lusser et al. 2011; OECD 2014).

### SDN-1

One of the simplest targeted gene modifications to achieve involves allowing the broken chromosome to be repaired by NHEJ. Whereas NHEJ typically rejoins the broken chromosomes precisely, thereby restoring the DNA sequence and maintaining genome integrity, on occasion small deletions or, more rarely, insertions (collectively called *indels*) are introduced at the break site. The frequency at which such alterations occur

varies among SDNs and chromosomal targets, but indels are typically detected in the range of 5 to 75%. For example, after a TALEN was expressed in a transgenic *Arabidopsis* plant, 41 to 73% of surveyed chromosomes had mutations at the targeted site. Most of these mutations were deletions ranging from 1 to 55 bp in length (Christian et al. 2013).

Targeted mutagenesis by NHEJ can be used to inactivate a gene, i.e., to create a gene knockout. Indels within coding sequences frequently alter the reading frame, and such frameshift mutations prevent expression of a functional protein. Indels generated by NHEJ can also remove or insert codons without altering the reading frame, leading to a protein product with a few amino acids added or removed at the targeted site. Indel mutations in noncoding sequences can also have phenotypic consequences; for example, promoter mutations can disrupt key regulatory sequences and alter gene expression (see later).

### SDN-2

As mentioned earlier, if the break is repaired by HDR, information is copied from a DNA template during the repair process (Puchta 2005). The template can be either a homologous chromosome, a sister chromatid, or externally supplied DNA with sequence similarity to the DNA flanking the break site. SDN-2 typically refers to subtle modifications such as single nucleotide substitutions or small insertions or deletions that are made at the break site. Sequence modifications of one to a few bases can also be obtained by ODM, which does not depend on SDNs and which as currently understood does not involve integration of the repair template by HDR. Efficiency of ODM can be enhanced, however, by creating a DNA break at the site with an SDN (Sauer, Mozoruk, et al. 2016).

### SDN-3

Although not treated in detail in this paper, the third class of targeted modifications involves the site-specific integration of DNA. Integration into a specific locus is useful because it can circumvent the position effects on expression of an integrated gene or on genes at the site of integration that can occur with random integration. Also, targeted integration allows stacking of multiple genes into a single genomic locus, which simplifies the breeding process and avoids excessive linkage drag associated with trait introgression (Petolino and Kumar 2015). Targeted insertion can be achieved by either NHEJ or HDR. In the former case, the broken chromosome is joined to the ends of the DNA fragment to be inserted in the genome. In the latter, the repair template carries the DNA insert, flanked by DNA with sequence similarity to the broken ends for recombination. Contrary to SDN-1 and SDN-2, SDN-3 is characterized by the insertion of naturally occurring or synthetic, large sequences of DNA such as those used in *transgenesis*, *cisgenesis*, or *intragenesis*. Such sequences may range in content from multiple genes to a fragment of a gene. Where a new DNA is substituted for an existing DNA sequence, how different the new DNA must be before it is considered SDN-3 rather than SDN-2 has not been satisfactorily addressed.

## Base Editing without Double Strand Breaks

Recently, an approach to genome editing that allows specific mutations of individual DNA bases without requiring a double strand break has been developed. Called "base editing," this approach has attracted interest because double strand breaks are not repaired well in some organisms and cell types and because an alternative to HDR is needed for making specified changes in organisms and cell types in which HDR efficiencies are low. Base editing uses enzymes that convert one base in DNA without requiring or making a double strand break.

Cytidine deaminase was fused to derivatives of Cas9 that had been mutated to inactivate or partially inactivate the DNA cleavage domain. Such Cas9 derivatives, called dead Cas9 (dCas9) and nickase Cas9 (nCas9), respectively, have been used along with a gRNA to target various enzymes to specific sites in a genome. Fused to cytidine deaminase, the derivatives, particularly nCas9, achieved high efficiency and highly specific base editing in rice, maize, wheat, and tomato, as well as in human cells and animal models, including mouse and zebrafish (reviewed in Hess et al. 2017). Fusions of nickase Cas9 to an RNA adenine deaminase engineered to act on DNA achieved targeted edits of A to G in human cells (Gaudelli et al. 2017). TAL effectors and zinc finger arrays have been used to target a fused cytidine deaminase in *E. coli* and human cells, but off-target mutations occurred both proximal to the target and at sequences far away that were not similar to the target and were therefore unpredictable. These results indicated that the inherent DNA binding property of the cytidine deaminase allowed it to act independently of or to influence the targeting by the fused TAL effector or zinc finger array, and they revealed the need for further optimization of such fusions (Yang et al. 2016).

## Delivery of Genome Engineering Reagents to Plant Cells

A challenge in plant genome editing is the efficient delivery of editing reagents to cells. Most methods of DNA delivery were developed decades ago with the intention of creating transgenic plants that express foreign genes incorporated into their genomes. Such transgenic plants are identified by their expression of marker genes that confer selectable or screenable traits. Stable transformation, however, is not necessarily the objective of genome editing. Rather, the reagents only need to persist in the cell long enough to achieve the desired editing outcome. In fact, incorporation of foreign DNA is often undesirable, particularly from a regulatory point of view (see discussion later).

Plant cells are surrounded by a cell wall—a barrier that must be overcome when delivering genome editing reagents. Cell walls can be enzymatically removed to release membrane-bound cells called protoplasts. With protoplasts, DNA constructs encoding SDNs that can be delivered at high efficiency by *electroporation* or polyethylene glycol (PEG)-mediated transformation, often to >70% of treated cells. When normalized for transformation frequency, it is not uncommon for >25% of surveyed cells to have mutations created by imprecise NHEJ (SDN-1) using many of the common nuclease platforms (Shan et al. 2013; Zhang et al.

2013).

For plant species that can be regenerated from protoplasts, the phenotypic consequence of NHEJ-induced mutations can be assessed at the whole plant level. With high delivery efficiencies and effective nucleases, a substantial proportion of the plants regenerated from treated protoplast populations harbor mutations at the target locus (Clasen et al. 2016; Li et al. 2016; Sauer, Narvaez-Vasquez, et al. 2016). Among the regenerated plants, the majority lack foreign DNA; that is, the nuclease is expressed transiently, and the DNA construct is degraded before integration. Currently the number of crop plants that can be efficiently regenerated from protoplasts is limited (e.g., potato, tomato, canola, flax), but efforts in industry and academia are rapidly expanding that list, making it possible for genome editing through protoplast transformation to be more broadly applied.

Not only can DNA be delivered to protoplasts, but it is also possible to deliver SDN-encoding messenger RNA, or a purified SDN, or, in the case of CRISPR/Cas9, the Cas9-gRNA complex. Such approaches have been used successfully to make targeted mutations in plants (Luo et al. 2015; Woo et al. 2015). Unlike DNA, neither RNA nor protein become incorporated into the plant genome.

*Agrobacterium tumefaciens* causes crown gall disease (characterized by the formation of tumors) in a number of dicot plants. During infection, *Agrobacterium* transfers a segment of *plasmid* DNA into the plant cell, where that DNA integrates into the plant genome, giving rise to transgenic plants that express genes necessary for the formation of tumors. This naturally occurring DNA transfer process has been harnessed to deliver DNA from other sources into plant cells. *Agrobacterium*-mediated DNA transfer is one of the preferred methods to deliver SDN constructs and repair templates for genome editing. Often, the genome editing reagents are stably incorporated into the genome, and transgenic plants are recovered. As the transgenic plant grows and develops, editing takes place and can occur in cells that give rise to the germline and form seed. This makes it possible to recover heritable gene edits in the next generation. Because the DNA encoding the SDN integrates at random into the genome, nontransgenic "null-segregant" plants can be obtained in the next generation that carry the edit but have lost the SDN DNA through the random assortment of DNA that takes place during sexual reproduction.

Biolistics (also called particle bombardment) is an alternative to *Agrobacterium*-mediated DNA delivery, and it is widely used to transform cereal crops. In wheat, for example, biolistics achieves higher delivery efficiencies than *Agrobacterium* (Lazzeri and Jones 2009). One drawback is that particle bombardment promotes the integration of multiple transgenes (often dozens of copies), and it also causes physical damage to the tissues used for transformation, negatively affecting in vitro regeneration of the explants and therefore the transformation efficiency (Gil-Humanes et al. 2011).

Viruses are widely used to deliver gene editing reagents in animal systems; however, they have only recently been deployed for such purposes in plants. For example, autonomously replicating DNA "replicons" based on modified plant geminiviruses has been developed to deliver the coding sequences of SDNs and corresponding repair templates. Amplification of the reagents in this way significantly improves the frequency of HDR (up to ~100-fold compared to nonreplicating approaches) in tobacco (Baltes et al. 2014), tomato (Čermák et al. 2015), and wheat (Gil-Humanes et al. 2017). Because of the circular nature of these geminivirus-based DNA replicons, the frequency of DNA integration is significantly decreased, allowing the regeneration of modified plants without a transgenic intermediate. DNA replicons can be delivered via *Agrobacterium* or biolistic approaches into tissue explants or to protoplasts via PEG-mediated *transfection* or electroporation.

Plant RNA viruses can also be used to deliver gene editing reagents. RNA viruses have been widely used to deliver hairpin RNAs for virus-induced gene silencing (Lacomme 2015). RNA viruses engineered to express a ZFN were shown to induce mutations at a chromosomally integrated reporter gene in petunia and tobacco (Marton et al. 2010). Meganucleases have also been delivered by RNA viruses (Honig et al. 2015). The virus at some frequency infects meristematic tissue, which gives rise to floral organs, and it was thus possible to recover seeds with mutations from infected plants. One current limitation on the use of RNA viruses for mutagenesis is that they have limited cargo capacity, so it is difficult to deliver some SDN sequences because they are simply too large. For example, RNA viruses have been engineered to deliver gRNAs to Cas9-expressing transgenic plants; the Cas9 nuclease gene was integrated in the plant genome because it was too large to be carried by the virus (Ali et al. 2015). As RNA virus vectors improve and strategies are developed to more efficiently recover germinal mutations, the use of RNA virus vectors could emerge as an important means to achieve targeted mutagenesis in diverse plants.

## Delivery in Animals

Methods to deliver reagents for animal genome editing are reviewed in detail by Tan and colleagues (2016). Approaches include introducing DNA or RNA coding for the SDNs. Alternatively, SDNs can be delivered as purified protein or protein/RNA complexes. DNA delivery can result in integration into the animal genome, creating a transgenic animal. In contrast, RNA or proteins are not heritable and are degraded shortly after delivery. In many cases, reagents are delivered to fertilized embryos, which give rise to adult animals. Because expression of an SDN from RNA might not occur until an embryo has reached the two- or four-cell stage, a potential negative outcome of using RNA encoding the SDN is mosaicism, in which some cells in the developing embryo do not carry the genome edit or carry different edits. Injecting the SDN protein itself, or in the case of CRISPR/Cas9 the protein and gRNA complex, is being pursued as a strategy to bypass expression and achieve editing at the single-cell stage, so that as that cell divides and the embryo develops, all cells carry the edit.

Delivery of DNA may be achieved using vectors based on animal viruses (Luo et al. 2012; Yin et al. 2016) or by introducing plasmids (Choi et al. 2015). Protein and RNA can be delivered by direct injection (Whitworth et al. 2017).

The target cell type for reagent delivery varies depending on the editing approach or animal. For NHEJ-mediated edits (i.e., SDN-1) for which no donor or repair template DNA is needed, the use of cultured primary cells and subsequent fusion with an unfertilized egg cell from which the nucleus has been removed (i.e., animal cloning) has largely been replaced by treatment of single-cell embryos or embryonic stem cells (Hai et al. 2014). For allelic replacements or site-directed transgene insertions that rely on HDR, treatment of cultured cells en masse followed by screening and cloning is preferred. This is because the efficiency of HDR is usually too low to identify events in the small numbers of embryos that typically can be treated at a time. Donor or repair templates for HDR may be delivered on plasmids (Liu et al. 2013) or as single-stranded oligonucleotides (Park et al. 2017; Tan et al. 2013). In poultry, even for NHEJ-mediated mutagenesis, treatment of embryos or embryonic cells is infeasible because of the complexities of the avian reproductive tract, including, for example, presence of an egg shell. Reagents can be introduced into primordial germ cells (earliest form of germline stem cells) by stimulating uptake rather than by injection, either directly in ovo (Veron et al. 2015) or in culture. In the latter case, the modified germ cells are then reintroduced into the embryo in ovo (day 2–4) (Schusser et al. 2013).

## Genome-Editing Applications in Agriculture

### Applications in Plants

Genome editing has the potential to have a large, positive impact on plant agriculture (Belhaj et al. 2015; Montenegro 2016; Quétier 2016; Song et al. 2016). One reason is the efficiency of the technology. For example, gene knockouts are feasible for every crop into which reagents can be introduced and plants regenerated. This includes knocking out genes present in multiple copies in a genome or across the multiple genomes present in polyploid plants (Belhaj et al. 2015; Osakabe, Sugano, and Osakabe 2016). Genome editing by NHEJ contrasts with chemical and physical mutagenesis, in which populations of hundreds or thousands of individuals need to be generated to screen for rare mutations. For NHEJ-mediated mutagenesis, from 2 to 60% or more of the initial regenerated plants have been shown to have the desired edit (often in homozygous condition), so a much smaller population of plants can be used (Brooks et al. 2014; Feng et al. 2013; Hu et al. 2016; Ma et al. 2015; Zhang et al. 2014; Zhang et al. 2016; Zhou et al. 2014). Thus, at least for NHEJ, genome editing can be applied to crops for which regeneration and transformation frequencies are poor; it is not restricted to well-studied crops such as tobacco, or specific model genotypes within species, for which efficient tissue culture methods are established.

A second reason genome editing has potential for plant improvement is that in contrast to random mutagenesis, it causes relatively few or no mutations at unintended sites in the genome. In most cases, this obviates the need to perform additional crosses to remove unwanted mutations (Belhaj et al. 2015). Because the most likely sites of unintended mutation are those with sequence similarity to the intended target (so-called "off-target" sites), assessment of mutagenesis at those sites provides a gauge for overall off-target mutagenesis. Feng and colleagues (2014) found no mutations at four potential off-target sites across 60 edited lines of *Arabidopsis*. Similarly, Zhang and colleagues (2014) checked 13 potential off-target sites and found mutations at only one, a site that differed from the target by only 1 bp. Further, this particular off-target mutation occurred in only 7 of the 72 plants that were regenerated. Mutagenesis at DNA sequences unrelated to the intended target can be expected to be even less frequent.

Finally, genome editing allows knowledge-based alterations to a plant genome. This contrasts with conventional breeding, in which large populations with natural or artificially induced genetic variation are screened for a desired trait or traits. Those traits then have to be introduced into elite varieties through time- and labor-intensive breeding. Often, mobilization of such traits is confounded if the relevant genes are located at several different locations in a genome, making it considerably more challenging by virtue of the random segregation of genetic information that takes place. It is anticipated that by using genome editing, even multiple mutations might be made within a desired genetic background in a single step.

It is informative to consider the types of edits that can be made in relation to the types of genetic variation that have been used in traditional breeding and transgenic approaches. Loss-of-function mutations, either naturally occurring or induced by treatment with chemicals or radiation, have been a major source of genetic variation for crop domestication and crop improvement (Doebley, Gaut, and Smith 2006; Gepts 2002; Sang 2009). For example, loss-of-function mutations can eliminate plant toxins, decrease susceptibility to pests and pathogens, prevent seed dispersal and dormancy, slow ripening and/or senescence to extend shelf life and improve product quality, generate male or female sterility allowing the development of hybrids, and lessen plant stature to ease harvesting of fruits and prevent lodging in cereals. SDN-1 allows such loss of function alleles to be developed efficiently when the genes responsible for a trait are known. That knowledge may come from studies that associate specific gene sequences with specific traits in breeding populations or by directed studies of gene function. Often, knowledge in one plant species translates to others. Indeed, numerous potential targets can be found in the scientific literature.

Genes whose inactivation by SDN-1 can result in a valuable trait include those that drive the production of undesirable metabolites. Shukla and colleagues (2009) generated low phytic acid corn using ZFNs that mutated the inositol-1,3,4,5,6-pentakisphosphate 2-kinase 1 gene, whose enzyme product plays a major role in phytic acid biosynthesis. Phytic acid is the main form of phosphorus found in maize kernels, and decreasing its content in feed and food could decrease the amount of phosphorus runoff that leads to the deterioration of downstream aquatic habitats. SDN-1 could also be used to knock out repressors of pathways for desired metabolites,

leading to increases in those metabolites.

Other candidates for SDN-1 edits are genes exploited by pathogens. Bacterial blights are among the most important diseases of crops worldwide (Mansfield et al. 2012). Zhou and colleagues (2014) used TALENs to disrupt DNA sequences in rice that are used by the bacterial pathogen *Xanthomonas oryzae* to drive expression of sugar transport genes important for infection. The result of the targeted mutation was disease-resistant plants. Similarly, Chandrasekaran and colleagues (2016) generated cucumber immune to cucumber vein yellowing virus infection, and resistant to zucchini yellow mosaic virus and papaya ringspot mosaic virus-W, by using CRISPR/Cas9 to mutate the gene for eukaryotic translation initiation factor 4, which the viruses depend on for replication. Wang and colleagues (2014) generated wheat plants resistant to powdery mildew by using TALENs to knock out each of the three *homeoalleles* (copies of the same gene present in each of the three genomes of this hexaploid cereal) of mildew-resistance locus, which makes the plant susceptible to infection by the powdery mildew fungus. Powdery mildews cause large yield losses in wheat, grape, and other crops (Curtis, Rajaram, and MacPherson 2002; Lillemo et al. 2006).

Pharmaceutical production in plants can benefit from gene knockouts that eliminate plant-specific modifications of synthesized proteins. For example, plant modifications may affect function in humans. Li and colleagues (2016) used SDN-1 edits to decrease *glycosylation* (modification with sugars) of proteins in tobacco. The resulting plants showed glycosylation profiles expected to result in more efficacious pharmaceutical proteins. Also, although immunological problems from the plant modifications were not observed (Li et al. 2016), knockouts to prevent those modifications could guard against any increased immunogenicity.

Biofuel production can be significantly affected by the amount and quality of lignin in the cell walls of feedstock crops (Jorgensen, Kristensen, and Felby 2007; Zhao, Zhang, and Liu 2012). Decreasing the amount of lignin present in biofuel feedstock crops can increase biofuel yields (Chen and Dixon 2007; Fu et al. 2011; Jung et al. 2013; Saballos et al. 2008). Using TALENs, Jung and Altpeter (2016) induced SDN-1 mutations in the caffeic acid O-methyltransferase gene in sugarcane, decreasing the lignin content in the mutant lines by 29 to 32%. This approach could be used to improve the efficiency of biofuel production in crops that are difficult to breed with conventional methods as a result of their delayed onset of flowering and/or outcrossing mating system. Examples include alfalfa, poplar, and sorghum.

Genes that negatively impact nutrient content, taste, or safety of food are a major category of potential targets for knockout mutagenesis. In potato, *invertases* break down sucrose into glucose and fructose (reducing sugars). Invertases are activated by cold storage, and sucrose breakdown leads to softening, rendering potatoes unsuitable for processing. Further, when fried, the reducing sugars react with free amino acids to produce the potential carcinogen acrylamide, and they cause increased bitterness and browning. Thus, preventing the breakdown of sucrose is expected to prevent loss due to cold storage (currently up to 15% of the potato crop) and to improve safety and quality. A biotech company used transiently delivered TALENs directed against the vacuolar invertase gene to generate nontransgenic potatoes that have decreased levels of reducing sugars and acrylamide after frying (Li et al. 2016). Using the same approach, the same company also mutated the polyphenol oxidase gene to produce a potato that browns less when bruised. This potato variety was recently declared exempt under the regulation that governs transgenic plants by the U.S. Department of Agriculture (USDA) because it includes no plant pest sequences (Firko 2016). If sold commercially, this potato, along with a similarly produced, low-browning mushroom also found to be exempt under USDA transgenic regulation (Waltz 2016), would be among the first food products generated using SDNs.

Shelf life of vegetable oils is decreased by the presence of polyunsaturated fatty acids such as linoleic acid, which are more sensitive to oxidation. Partial hydrogenation to lessen the amount of such fatty acids, converting them to monounsaturated or saturated fatty acids, improves shelf life but creates trans fatty acids. Saturated and trans fatty acids can negatively impact human health. Haun and colleagues (2014) used the SDN-1 approach with TALENs to improve the fatty acid content of soybean seeds for oil production. They knocked out two fatty acid desaturase 2 genes (FAD2-1A and FAD2-1B). These genes convert oleic acid, a monounsaturated fatty acid, to linoleic acid, which is polyunsaturated. The resulting oil showed decreased levels of linoleic acid (Clemente and Cahoon 2009).

A final example of genes whose disruption can result in a desirable trait is the betaine aldehyde dehydrogenase 2 (BADH2) gene in rice. Disruption of this gene leads to accumulation of the fragrant compound 2-acetyl-1-pyrroline, one of the many volatile compounds that give basmati and jasmine rice their sought-out fragrance. Shan and colleagues (2015) used TALENs to knock out BADH2 in a nonfragrant variety and render it fragrant, bypassing the several generations of crossing and backcrossing otherwise needed to swap a natural BADH2 mutant gene into an elite rice variety.

SDN-1 has also been pursued to develop strategies for biocontainment of plants. Elorriaga and colleagues (2016) described the use of the CRISPR/Cas9 system to disrupt the function of floral genes in poplar; these mutations should give rise to trees, vegetatively propagated, that are both male and female sterile, which would prevent interbreeding and establishment from seeds in the field.

In SDN-2, defined by its use of a template to direct genome editing through HDR (Figure 1), it is feasible to make changes that modify the structure or function(s) of a protein, rather than simply knocking out gene expression. One example of an SDN-2 application in plants is generating herbicide tolerance. Herbicides often work by binding to specific domains of proteins to inhibit their activity. Thus, modifying those target sites by genome editing can give rise to herbicide-tolerant crops, facilitating weed control and decreasing costs of production. SDN-2 examples include edits by Sauer, Narvaez-Vasquez, and colleagues (2016) that modified 5'-enol-

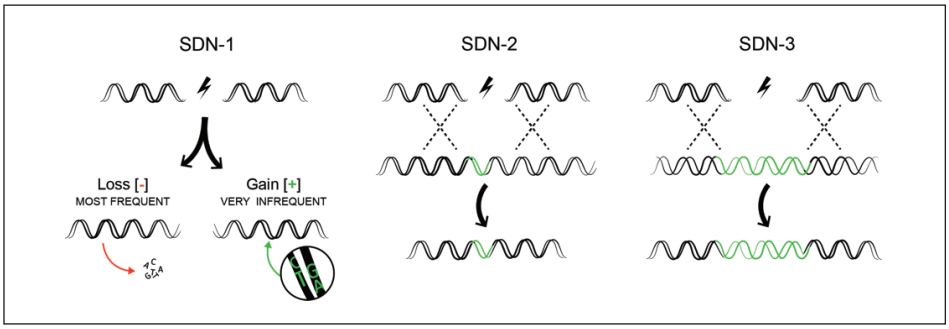


**Figure 1. In SDN-1, the broken chromosome is repaired by NHEJ. This results in indels at the break site. In SDN-2, a DNA repair template is provided that has subtle sequence differences to be incorporated at the break site through HDR. ODM (not shown) achieves the same outcome by using a single- or double-strand piece of DNA (oligonucleotide) with one to a few base differences from the target; the oligonucleotide base pairs with the target and acts as a template for mismatch repair to introduce the sequence change(s). In SDN-3, the repair template contains altogether new DNA sequences, such as one or more transgenes, that are incorporated at the break site through HDR.**

pyruvylshikimate-3-phosphate synthase to confer glyphosate tolerance in flax and an edit by Sun and colleagues (2016) that modified acetolactate synthase (ALS) in rice, resulting in rice plants tolerant to sulfonylurea and imidazoline herbicides.

In a proof-of-principle experiment, Townsend and colleagues (2009) edited ALS genes in tobacco cells using three different donor templates intended to produce different amino acid changes. Editing by SDN-2 might also be used to change or remove amino acids in proteins that contribute to allergenicity, including nut allergens and wheat gluten proteins. It should also be feasible to modify regulatory regions of genes to change how expression of a gene is regulated. Possible applications include the enhanced expression of genes for micronutrient biosynthesis in target tissues (e.g., vitamin A in grains). As detailed understanding of plant gene function continues to accrue, potential applications of precise modifications through SDN-2 will increase.

## Applications in Animals

There are numerous recent reviews on genome editing in livestock (Carlson et al. 2012; Laible, Wei, and Wagner 2015; Petersen and Niemann 2015a,b; Wang 2015), including one by Tan and colleagues (2016), that tally the multiple genome edits reported in pig, cattle, sheep, and goat. Gene edits have also been reported in fish, namely tilapia (Li et al. 2014) and carp (Zhong et al. 2016), as well as chicken (Park et al. 2014; Schusser et al. 2013; Veron et al. 2015) (see also review by Lee, Lee, and Han [2015]). A number of edits have been made or proposed with the aim of creating animal models for human disease (e.g., Ji et al. 2015) or improving pets (Reardon 2016). These will not be discussed; rather, applications directed at improving livestock for agriculture are reviewed.

Much effort has been devoted to improving production traits. For example, increased muscling for meat production has been achieved by knocking out the myostatin gene in pigs (Rao et al. 2016), goats (Ni et al. 2014; Wang et al. 2015), sheep (Crispo et al. 2015; Proudfoot et al. 2015), carp (Zhong et al. 2016), and cattle (Luo et al. 2014; Proudfoot et al. 2015) (SDN-1). Genome editing was also used to swap part of a gene between beef and dairy cattle (SDN-2). The version of the gene in beef cattle makes the breed hornless (polled), and the swap created polled dairy cattle (Carlson et al. 2016). Since horns are a source of injury both to the animals and to handlers, dairy cattle routinely have their horn buds cauterized or chemically removed as calves.

Eliminating the need for this painful and costly process, the edit is expected to improve animal welfare and decrease expense in dairy production. Other examples include targeted knockout (SDN-1) of the insulin-like growth factor 2 gene in an indigenous Chinese pig breed (the Lantang pig) to decrease backfat thickness (Jinqing et al. 2015) and knockout of the gene for lactoglobulin in cattle (Wei et al. 2015; Yu et al. 2011) and goats (Cui et al. 2015; Ni et al. 2014) to remove this major allergen from their milk.

A number of applications target improved livestock health. Insertion of a gene for either lysozyme (Liu et al. 2014) or lysostaphin (Liu et al. 2013) at the beta-casein locus (an SDN-3 edit) in cattle yielded cows resistant to mastitis (mammary gland infection). In cultured pig cells, knockout of the gene for elongation initiation factor 4E stimulated the interferon response and decreased replication of the vesicular stomatitis virus relative to unedited cells, showing promise for the eventual generation of pigs more resistant to infection by this virus (Ramirez-Carvajal et al. 2016). Genome editing (SDN-2) has produced domestic pigs with a gene sequence swapped in from their relative the warthog that decreases susceptibility to African swine fever, which is a viral disease that requires slaughter of entire herds when detected (Lillico et al. 2016).

Another viral disease, porcine reproductive and respiratory syndrome (PRRS) virus, is economically the most important disease in the U.S. pig industry. Pigs that are resistant to PRRS virus infection were achieved via genome-edited knockout of the gene for the CD163 receptor (SDN-1), which acts as a binding site for the virus to invade cells (Whitworth et al. 2014). Finally, resistance to bovine tuberculosis was also achieved by genome editing. Bovine tuberculosis causes loss of appetite and weight loss in cattle and can also infect people. Once detected, all infected animals are destroyed. Wu and colleagues (2015) generated resistant cattle by introducing the mouse SP110 nuclear body protein gene into the genome using the SDN-3 approach.

There is increasing effort to use genome editing technology to improve livestock as bioreactors. In eggs, ovalbumin (OV) is expressed in such high quantities that it is hard to purify any human proteins of interest expressed in the egg

without contamination by the egg protein, which can be allergenic. As a first step toward the use of eggs as bioreactors, chicks were generated from primordial germ cells in which the OV gene had been knocked out by genome editing (Park et al. 2014). For producing human antibodies in large animals, the first step was to generate a B cell-deficient animal. The IgM heavy chain gene, which is crucial for B cell development and differentiation, was knocked out in pigs (Chen et al. 2015). Also in pigs, DNA encoding human serum albumin was introduced into the native pig albumin locus by genome editing to produce this protein (Peng et al. 2015), which is a widely used human blood product in high demand.

Finally, as in plants, genome editing has been pursued to develop strategies for biocontainment of animals, with a focus on transgenic fish. For example, genome editing was used to knock out the Nanos 2 and Nanos 3 genes to develop germ cell-deficient tilapia (no sperm or eggs produced), providing both a model for studying fish reproduction and a proof of principle for containment (Li et al. 2014).

## Genome Editing Compared to Other Means of Genetic Modification

Although genome editing methods show great potential for crop and livestock improvement, the degree to which that potential may be realized is unclear. A comparison with other means of genetic modification is informative. For example, uncertainty about regulatory impacts on implementation of genome editing sets it apart from transgenic methods, for which regulatory frameworks are largely in place, and random mutagenesis and conventional breeding, which are generally regarded as safe and not subject to any premarket review or approval process. Regulation and other factors affecting implementation are discussed later under the section headed "Governance." In this section, distinctions among genome editing and conventional means of genome modification with regard to the respective biological processes and outcomes are presented (summarized in Table 1).

Like conventional genetic engineering, but unlike conventional breeding, genome editing requires delivery of reagents into cells, which for many species demands effective methods for in vitro culture and regeneration. Even in the major crop and livestock species, many varieties and breeds are recalcitrant to existing methods. Very little public research, however, has been provided to understand the physiological and molecular nature of recalcitrance and to design science-based means to overcome it.

It is generally desirable, from both a

**Table 1. Procedural and biological characteristics of genome editing relative to other methods of crop and livestock improvement.**

| | Precision | Time to Achieve | Changes from Original Parental Genome | Requires Genetic Transformation | Requires Genetic and Molecular Understanding of the Trait |
|---|---|---|---|---|---|
| Genome Editing | High | Months | Targeted edit(s); often no other changes, though edits at locations with sequence similarity to the target(s) may occur | Sometimes | Yes |
| Conventional Breeding (Crosses) | High for the trait determinant (governed by selection; typically introgresses at the same genomic location as in the donor); other donor DNA that introgresses is determined at random | Years | Introgressed gene and closely linked sequences from donor parent; after backcrossing, ~5% other donor DNA distributed at random through the genome | No | No |
| Random Mutagenesis | None | Months; with extensive backcrossing, years | Many and random; with extensive backcrossing, ~95% identical to parent | Sometimes | No |
| Conventional Genetic Engineering (Transgene Insertion) | None | Months to a few years | Presence of transgene; interruption of native DNA sequence with transgene | Yes | Yes |

technical and a regulatory standpoint, not to retain any reagent-encoding DNA in the edited genome. Although this is easily achieved in rapid cycling species by genetic segregation, or in those systems in which transient approaches such as delivery of RNA or protein can be used, it presents a much larger problem for plants that must be clonally propagated or for plants or animals with long generation times. Advances in methods such as transgene excision and transient expression from viral vectors, for a variety of crop and animal species, may be needed for the potential of genome editing to be broadly realized. These methods are not required for conventional breeding, and they are not applicable to conventional transgenic approaches.

Traits deriving from loss-of-function (knockout) mutations, such as those that can be generated by SDN-1, could also be obtained by conventional breeding. Introduction of a trait from one variety into another, however, typically requires extensive backcrossing to remove as much of the unwanted donor DNA (DNA not important for the trait) as possible, and this is time consuming. In some cases, undesirable genes closely linked to the gene conferring the desired trait cannot be removed even after extensive backcrossing. Sometimes, because it can be difficult to find the needed trait within the species, random mutagenesis is employed to generate a population of the target organism with new genetic variation, and that population is then screened for the desired trait. A typical chemical mutagenesis may result in random mutations every 150,000 bp in the genome of each individual (Sasaki et al. 2012). Multiple backcrosses to the parental type typically remove approximately 95% of these random mutations while retaining the one(s) responsible for the new trait, but, again, such backcrosses are time consuming. In contrast, genome editing may result in no or few unwanted changes to the target genome and can be achieved in a single generation.

For gain-of-function edits, such as those that might be achieved by SDN-2 or SDN-3, as noted earlier, genome editing differs from conventional genetic engineering in that it targets the change to a known locus, whereas conventional methods of genetic engineering result in one or more insertions of DNA into the genome typically at random. The latter can result in unintended disruption of genes and can impact the expression of the trait because of the effects of the surrounding DNA on expression of the introduced gene.

Finally, genome editing, like conventional genetic engineering, requires molecular genetic information about the trait. Conventional breeding does not strictly require such information, though it often contributes to establishing the link between traits and genes. Gene function is increasingly well understood in model organisms such as *Arabidopsis*, *Drosophila*, and mouse, and to a lesser extent in a few well-studied crops like rice and maize. Using insights from model organism research, the basis of important traits needs to be translated to many crop plants and animals. In the near term, genome editing for gene functional characterization will be a valuable approach toward achieving this goal.

# Governance

## What Is Governance?

For the discussion presented here, governance, oversight, and regulation are distinguished as follows. Governance involves a complex set of values, norms, processes, and formal or informal institutions through which society manages technological development and resolves conflict. It can include the act of governing at any point in the research and development chain from funding agencies making decisions about what projects to support, to researchers in laboratories making choices according to codes of conduct, to governments overseeing voluntary programs for safety-data submission, to consumers making choices in the marketplace. Oversight is defined more narrowly, usually involving activities such as standard setting, voluntary consultations or guidance, or mandatory government premarket review by an organization with some authority for the technology or product. Regulation is a subcategory of oversight and involves formal rules dealing with details or procedures issued by an executive authority or regulatory agency that have the force of law. Therefore, regulation can be an important element of governance, but it can also be excluded from a governance system.

Many scholars, practitioners, and organizations have published criteria for good governance. Sets of criteria vary and have included the balance of benefits and risks of technological development to promote innovation while minimizing environmental or human harms, transparency, opportunities for public and stakeholder input, and coordination among regulatory bodies, among others.

## Process or Method as a Focus for Governance

Some regulatory schema, such as that of the USDA for transgenic plants, center on the process used to develop a new product. Others (discussed later), such as that of the Canadian Novel Products Act, focus on characteristics of the product. In process-based systems, the SDN-1, SDN-2, and SDN-3 classes of edits, and base editing, may be treated differently because of the nature of the edit and whether or not it includes inserted DNA from a template as described earlier. Because of the insertion of entire genes (or new alleles of genes already present), SDN-3 might be used for transgenesis as well as cisgenesis and intragenesis. Whereas transgenesis is the introduction of a gene or a gene allele not found in the targeted organism or related, sexually compatible (crossable) species, cisgenesis involves the insertion of a gene originating from a crossable organism, including its introns and flanking native promoter and terminator in the original sense orientation. Intragenesis is the insertion of a gene comprising coding sequence from a crossable organism but promoter and terminator (i.e., expression controlling) sequences from another gene of the same species or crossable species. In the case of both SDN-2 and SDN-3, it is also possible to use templates derived from synthetic sequences that do not occur naturally or are partially inspired by nature.

A focal aspect for SDN-3 under process-based regulatory schema is whether the repair template comprises any sequence originating from a non-

crossable (sexually incompatible) organism (transgenesis) or is designed to enable a targeted form of intragenesis or cisgenesis instead. The former case involves "the formation of new combinations of genetic material and their incorporation into a host organism in which they do not naturally occur" (Council of the European Communities 1990; European Parliament and The Council of the European Union 2001). In the European Union (EU), introduction of any foreign nucleic acids is identified as "recombinant nucleic acid techniques" and, as such, is regulated. In oversight of transgenic plants in the United States, carried out by the USDA under the Plant Protection Act, the regulatory trigger is restricted to genetic changes that introduce any DNA sequence derived from a plant pest (Wolt, Wang, and Yang 2015). One point of uncertainty under any scheme is how long or complete SDN-2 sequences would need to be before being categorized as SDN-3. For example, whether a part of a gene introduced would be classified as SDN-2 or SDN-3 is unclear. These issues are being considered in various regulatory discussions across different countries (Wolt, Wang, and Yang 2015).

SDN-1 edits, and base edits, even though they introduce no foreign DNA at the target, may also be subject to restrictions under process-based regulatory schema. Specifically, the method of delivery of the nucleases may affect regulatory status. If the approach involves the stable genomic insertion of the (foreign) DNA sequences encoding the editing reagent, the earlier considerations concerning recombinant DNA techniques apply. Once the edit is made, however, null segregants (progeny that no longer harbor the nuclease-encoding DNA because of random assortment of parental genetic information during a cross) can be obtained. Such null segregants are exempted under some, but not all, process-based regulatory schema. ODM, or SDN, or base editor delivery methods that do not lead to integration of nuclease-encoding DNA, discussed earlier, circumvent the issue.

## Product Characteristics as a Focus for Governance

Under product-based regulatory schema, the effect(s) of the editing on product characteristics would be the basis for how the product is regulated. Factors that have been considered in regulation of conventionally genetically modified (GM) organisms are informative to consider. Guidelines and consensus documents established by the Organisation for Economic Co-operation and Development, the World Health Organization, and the Food and Agriculture Organization of the United Nations underlie an internationally accepted baseline for assessment of risks of GM products to human and environmental health.

In the case of food and feed products, GM organisms are compared with their non-GM counterparts for any undesirable change in toxicity, allergenicity, or nutritional quality. For environmental safety assessment, properties such as persistence or invasiveness, likelihood of gene transfer into sexually compatible relatives, interaction with target and nontarget organisms, and impact on biogeochemical processes are evaluated, relative to the non-GM counterpart. In both cases (food and feed assessment and environmental safety assessment), molecular characteristics of the particular GM event are also considered, including the potential impact on the function of any interrupted endogenous genes or the generation of new coding sequences at the site(s) of transgene insertion.

Whether these potential outcomes may be considered by regulatory bodies as applicable to the SDN-1, base editing, and SDN-2 classes of edit is yet unclear, but one might predict "no" because these are targeted edits that do not result in large insertions. Even for SDN-3 edits, it has been suggested that on a case-by-case basis "lesser amounts of data may be needed" (EFSA 2012) because of the targeted nature of the insertion, which can control for hazards associated with the disruption of genes or creation of new coding sequences at the insertion site.

Strauss (2003) and Bradford and colleagues (2005), among others, have suggested that in contrast to process-based regulatory systems, a more product-oriented approach would take into account genomic similarities and the long record of safe amplification of quantitative genetic variance and mutagenesis that are parts of traditional breeding. For example, a product-oriented system would require much less data or allow exemptions when unintended variation in transformed lines is similar to or smaller than what is seen in traditional breeding—a common observation (Strauss and Sax 2016)—and when using genetic engineering to modify the expression of native genes (e.g., by RNA interference). As discussed later, unintended effects of gene editing are generally expected to be lower than either conventional breeding or transgenesis. Another distinction between product- versus process-based regulatory systems is that the latter must be revisited with every innovation in process; product-based regulatory systems are therefore more likely to be stable, in contrast to process-based ones, which risk becoming outdated prior to or soon after implementation because of the rapid pace of innovation in genome editing and related technologies.

In Canada, the regulatory framework takes into account the potential for unintended effects to occur in any type of breeding program—the trigger for regulation is irrespective of the method used and is focused on the "novelty" or the characteristics of the resulting product. For example, the Canadian system is set up to review plants with novel traits (PNTs), whether derived from genetic engineering or conventional breeding. A PNT for environmental release is defined by whether "it is not present in stable, cultivated populations of the plant species in Canada, or the trait in the plant species is present at a level significantly outside the range of that trait in stable, cultivated populations of that plant species in Canada" (CFIA 2012). Despite the focus on product, the process of genetic modification nevertheless affects what traits fall into the PNT definition, because genetic engineering makes possible the over-expression of traits through the use of nonnative, constitutive (always on) gene promoters and also facilitates the transfer of new traits across species borders through transformation. Therefore, in reality, the vast majority of plant

traits considered novel and reviewed in Canada by the regulatory agencies has been derived from the process of genetic engineering (CFIA 2016).

## Off-target Effects as a Factor in Governance

Although there is international agreement on how to assess the intended modification for safety, based on molecular understanding of the change, it is less straightforward to determine how much effort must be taken and evidence obtained to assess unintended effects for their harmful potential (Devos et al. 2015; Ladics et al. 2015). Although SDN and base editing approaches are targeted, as alluded to earlier, the sequence-specificity of site-directed engineered nucleases is not absolute and unwanted cleavage or base editing can occur at locations that show sequence similarity to the target site. In the case of off-target cleavage, imprecise repair can result in unwanted mutations at those sites but also chromosomal perturbations such as deletions (Lee, Kim, and Kim 2010), inversions (Lee et al. 2012), and translocations (Brunet et al. 2009; Cho et al. 2014).

No method of genetic modification, including conventional plant or animal breeding, is without the possibility of unintended effects (Ladics et al. 2015). Unintended effects may occur as a result of recombination during crosses, as a result of random mutations induced by chemical or radiation treatment to generate variation, as a consequence of plant tissue culture, or, as noted, because of the location of a transgene insertion (Evans 1989; Larkin and Scowcroft 1981). Conventional agriculture widely uses mutagenized varieties (IAEA 2017), and many jurisdictions regard random mutagenesis with chemicals or irradiation, which can cause changes ranging from single bp substitutions to large deletions, as a conventional breeding technique because of its long history of safe use. With regard to unintended genetic changes posing risks to human or animal health or the environment, plants or animals obtained by SDN-1, base editing, or SDN-2 are not likely to differ from products obtained by conventional breeding, provided the genes for the machinery used for genome editing are absent from the final product (Podevin et al. 2013). Because of the targeted nature of mutagenesis by SDN-1, base editing, and SDN-2, such plants and animals may in fact have fewer, if any, such unintended changes.

It should also be noted that targeting specificity, i.e., predictability of the site(s) at which DNA cleavage or base editing will occur, is not equally important for every species or application in agriculture. For example, it may be less important in crop breeding strategies. For many crop improvement programs, following introduction of a new trait, extensive backcrossing to the original plant type is carried out, a large number of individuals are subject to selection, and then resulting varieties are tested over multiple years across multiple locations for performance in yield, resistance to biotic and abiotic stresses, and quality defined by growers, processors, and consumers. This process allows individual lines with undesirable compositional, agronomic, or other phenotypic features to be eliminated. On the other hand, in cases where such selection and phenotypic characterization is restricted by constraints of time, resources, or the number of individuals that can be generated, such as in animal breeding, nuclease specificity takes on greater importance. In the case of animal breeding, its importance extends to guarding against unintended mutations that may negatively affect animal welfare.

## Traceability of the Edit as a Factor in Governance

For foods derived from conventional GM technology, some countries have established regulations encompassing measures for traceability and labeling at all stages of production to allow consumers to make an informed choice about the types of products they purchase and consume—for example, the EU (European Parliament 2003). Under such regulations, effective detection and identification techniques are a prerequisite to regulatory approval. Detection is determining the existence of a change in the genetic material of an organism relative to an appropriate reference. It should be distinguished from the concept of identification, which also entails the determination of whether or not the genetic modification was made intentionally by a certain technique. With regard to modifications made by genome editing, in terms of detection, high-throughput sequencing technologies offer the possibility of identifying sequence variations genomewide, including small indels, with increasing precision. In terms of identification, however, it is not possible to distinguish the types of modifications made by SDN-1, ODM or base editing, or SDN-2 from variants that might derive from conventional breeding techniques or exist because of natural genetic variation (Lusser et al. 2011). This is one reason some member states of the EU and others have argued that plants obtained by SDN-1 and SDN-2 be exempted from GM regulation (e.g., ACRE 2013; BVL 2015; Haut Conseil des Biotechnologies 2016; NAS 2016; SBA 2015). Base editing is new enough that it has not yet been taken up in such recommendations.

## The Current Regulatory Landscape

For the assessment and regulation of products of biotechnology in the United States, in 1986 a Coordinated Framework for the Regulation of Biotechnology (CFRB)—which describes roles and responsibilities for the Environmental Protection Agency (EPA), the USDA's Animal and Plant Health Inspection Service (USDA–APHIS), and the Food and Drug Administration (FDA)—was put in place (OSTP 1986). The CFRB instructed three federal agencies to regulate the products of biotechnology and GM organisms (GMOs) under existing laws: the EPA to use the Toxic Substances Control Act and the Federal Insecticide, Fungicide, and Rodenticide Act for GM microorganisms and GM plants with engineered pesticidal proteins or molecules; the FDA to use the Federal Food, Drug, and Cosmetic Act (FFDCA) for GM food and feed as well as veterinary drugs; and the USDA to use the Federal Plant Pest Act (modified later in 2000 as the Plant Protection Act [PPA]) for GM plants.

The CFRB framework relied on principles that the "product, not process"

should be the focus of regulation, that regulation should be based on "sound science," and that the risks of GMOs are the "same in kind" as those of conventionally bred plants and animals. The framework was clarified by the White House Office of Science and Technology Policy and the federal agencies in an interagency process under the Obama administration. Public comments regarding genome editing were documented, but decisions about genome-editing were not made (Kuzma 2016a; White House 2017). The National Academy of Sciences issued a report reviewing the CFRB and whether or not the risk analyses performed for regulatory decision-making under it suitably address emerging technologies (NAS 2016).

In addition to recommending increased surveillance of the many new and more complex products and methods that biotechnology could deliver in upcoming years, the National Academies of Science report suggests reduced scrutiny of familiar types of products and emphasizes novel products and pathways, not methods: "Regulatory agencies should build and maintain the capacity to rapidly triage products entering the regulatory system that resemble existing products with a history of characterization and use, thus reducing the time and effort required for regulatory decision making, and they should be prepared to focus questions on identifying new pathways to risk-assessment endpoints associated with products that are unfamiliar and that require more complex risk assessments." The report does not make specific recommendations about regulation of genome editing, nor whether use of recombinant DNA methods in general (whether SDN-3 genome editing or conventional transgenesis) should be a regulatory trigger.

Under the PPA, the USDA regulates new crop varieties that contain any plant pest sequences. In the early years of genetic engineering, most GM crops were produced using sequences from the plant pest Agrobacterium in order to introduce genes into plants, and those sequences integrated along with the transgene, triggering regulatory review by the USDA. Triggers include the short T-DNA border sequences from Agrobacterium that do not encode any proteins or RNAs. Varieties engineered to contain no plant pest sequences following review are exempt under regulation (USDA–APHIS 2016a); if such varieties are not used for food or do not contain pesticide proteins, they are approved for release (Kuzma 2016b; Ledford 2013). If the variety is used for production of a vaccine, drug, or industrial compound, it may be submitted, voluntarily, for evaluation by the FDA. Letters from the USDA in response to inquiries from companies and public institutions concerning the regulatory status of new crop varieties they have produced are regularly published (Camacho et al. 2014; USDA–APHIS 2016a). Several genome-edited crops have been considered, many of them SDN gene null segregants, and found not to be subject to further regulation by the USDA because they lack plant pest sequences. The increase in the number of new varieties, both conventional GM and genome edited, found to be exempt under USDA regulation in recent years is shown in Figure 2.

A major update of the USDA rules for GM plants under the PPA had been proposed by the Obama administration that was expected to entail new specific regulatory considerations, including those for which the genetic modification was obtained by genome editing or other new approaches (USDA–APHIS 2016b). It was withdrawn, however, by the Trump administration in late 2017. Also in 2017, the USDA considered invoking its noxious weed authorities under the PPA. It published a notice of intent (NOI) to perform a programmatic environmental impact statement to capture not only GM plants posing plant pest risk, but also those that pose potential noxious weed risks. The definition of a noxious weed broadly covers potential harms such as "damage to the natural resources of the United States, the public health, or the environment." To date, the APHIS has interpreted these authorities in a limited way, restricting them to plants that are aggressively invasive, have significant negative impacts, and are extremely difficult to manage or control once established.

Under the NOI, different options were considered regarding whether or not and how to use both the plant pest and noxious weed risk authorities to regulate new crop varieties produced by genetic engineering. An extended public comment period followed during which several concerns were raised, and the NOI was eventually withdrawn, the USDA announcing it would engage with stakeholders to re-evaluate (USDA–APHIS 2017). In March 2018, U.S. Secretary of Agriculture Sonny Perdue issued a definitive statement addressing genome-edited plants, stating that the USDA "does not regulate or have plans to regulate plants that could otherwise have been developed through traditional breeding techniques as long as they are not plant pests or developed using plant pests," and that "[w]ith this approach, USDA seeks to allow innovation when there is no risk present" (USDA 2018).

In Canada, as noted earlier, the focus is on the properties of the PNT, not the process used for introducing the trait. Products derived through biotechnology are treated as any other novel product. For example, herbicide-resistant crops that have been developed from conventional breeding, mutagenesis, transgenesis, or genome editing each have been subject to evaluation and have been approved as a PNT.

Many countries, including Japan, South Africa, Australia, and Argentina, have introduced specific GMO legislation. Most Latin American and Caribbean countries are parties to the Cartagena Protocol on Biosafety (CPB), and many of them have developed national biosafety frameworks on this basis (Araya-Quesada, Craig, and Ripandelli 2012). Argentina (not a party to the CPB) has devoted substantial effort toward developing biosafety regulatory expertise in the field of GMO management and authorization processes. Its National Advisory Commission on Agricultural Biotechnology is in the process of developing a regulatory framework for new breeding techniques, whereby SDN-1 and SDN-2 mediated genetic modifications would not be considered as new combinations of genetic material in the plant genome. In cases in which a trait is developed by introducing an SDN transgene, however, evidence of removal of the SDN transgene from the final product must be provided to be exempt from consideration as a GMO (Whelan and Lema 2015). In

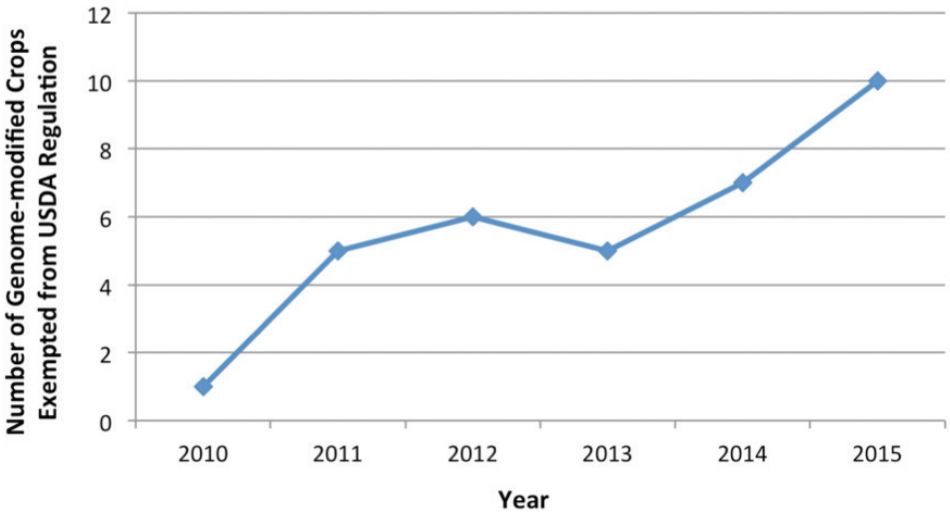


**Figure 2. Estimate of the number of new crop varieties reviewed by the USDA and found to be exempt under current USDA regulation (includes both genome-edited and conventional GM crops without plant pest sequences in final product) (data from Kuzma [2016a]).**

2016, a technical review of the Australian gene technology regulations was initiated to provide clarity on the legal status of organisms obtained by the use of a range of new technologies. The review proposed to exclude organisms obtained without the use of a repair template (i.e., SDN-1) from regulation as GMOs, but to regulate organisms obtained by SDN-2 and SDN-3 as GMOs.

Notably, decisions of countries to exclude SDN-1 and SDN-2 reflect the fact that SDN-1 and SDN-2 edits are generally indistinguishable from genetic variants that could be obtained with conventional chemical- or radiation-induced mutagenesis, which is exempted. Base edits are likely to be treated similarly. Also due to the molecular similarity of the outcomes, another consideration in the EU is that methods to unambiguously identify edited varieties do not exist. The recommendations of the National Academy of Sciences report and the practice of the Canadian regulatory authority may signal an overall trend toward product- rather than process-focused risk assessment going forward, at least in the United States and Canada. The EU and many countries, however, have highly process-focused regulation, which may not change absent major political shifts. Nonetheless, there was general agreement among an independent New Techniques in Agricultural Biotechnology Working Group mandated by the European Commission to assess a list of new plant breeding techniques that SDN-1 should be excluded from the European GMO Directive because of its similarity to conventional mutagenesis. For SDN-2 and ODM, views were not unanimous; though there was agreement that both are equivalent to mutagenesis, some could not conclude that oligonucleotide templates used in ODM are no different from repair templates integrated during SDN-2. Also, in cases in which a repair template is incorporated into the genome by homologous recombination, there have been debates about what size of change should be regulated (Lusser and Davies 2013). For organisms obtained by SDN-3, there was general agreement that, because of the formation of new combinations of genetic material, the technique should be covered by GMO directives. A report titled *New Techniques in Agricultural Biotechnology* (European Commission 2017) was prepared to provide scientific advice for policymaking. In it, SDN-1, -2, and -3 are compared and contrasted in depth to conventional breeding techniques, including transgenesis, with regard to detectability and identification, unintended effects, presence of exogenous DNA, end products, ease of use and efficiency, speed and cost, and maturity of the technology, laying a foundation for science-informed policymaking. More recently, an advocate general in the European Court of Justice issued an opinion that, though complex, recognizes the distinctions between genome editing and conventional GM technology and suggests that some genome-edited organisms need not be regulated in the same way as conventional GM organisms (Bobek 2018). In particular the opinion generally equates SDN-1 and SDN-2 with mutagenesis, such that products of those techniques either would not fall under the GMO Directive or, in the case of null segregants, which might fall under the GMO Directive as having resulted from GM technology, would be exempt from that directive under a "mutagenesis exclusion." A definitive legal interpretation from the European Court of Justice regarding whether SDN-1 and SDN-2 are to be excluded from the European GMO Directive is expected to follow shortly.

## Precedents and Uncertainties in Regulation of Genome-edited Animals versus Plants

As discussed earlier under Genome-editing Applications in Agriculture, the new technology has accelerated the development of improved crop varieties and livestock with commercial potential, making clarity in how they should be governed paramount. The question arises whether or not there should be any difference in the treatment of edited animals relative to edited plants. It is again informative to consider current treatment of conventional GM plants versus animals. In many countries, regulations regarding GMOs do not distinguish between plants and animals with respect to the genetic modification and the triggers for regulation. In the United States, however, engineered plants and animals are treated very differently. Current interpretations of the CFRB are shown in Figure 3; however, as previously stated, authorities will be clarified under the ongoing process led by the Office of Science and Technology Policy (White House 2015). Whereas plants are regulated by the USDA, and by the EPA when they may have pesticidal properties, and may be subject to FDA premarket approval also depending on the nature of the inserted DNA, GM animals are in large part regulated by the FDA. The FDA exerted its authority in 2009 for

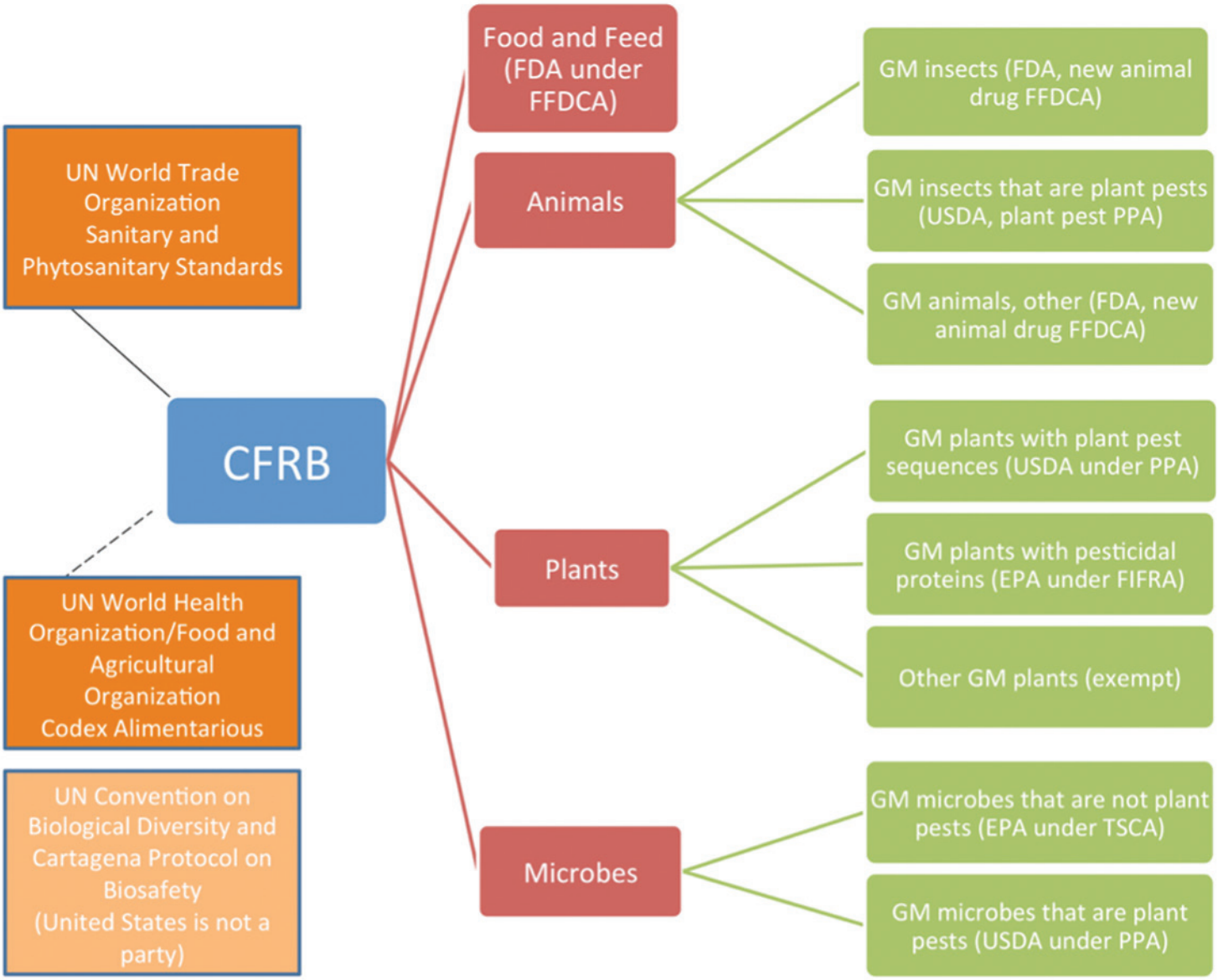


**Figure 3. United States Coordinated Framework for the Regulation of Biotechnology as it relates to living GM organisms. Solid lines show legal authorities; dotted lines show guidelines. The United States is not a party to the UN Convention on Biological Diversity Cartegena Protocol. (FIFRA—Federal Insecticide, Fungicide, and Rodenticide Act; TSCA—Toxic Substances Control Act)**

GM animals under the new animal drug provisions of the FFDCA. Genetically modified salmon was recently approved for food in the United States, and GM mosquitos for disease control are actively under consideration by the FDA.

In January 2017, the FDA made available draft rules stating that animals with intentionally altered genomes would be subject to safety testing similarly to new drugs (DHHS–FDA 2017). Whether the FDA draft rules will be implemented by the Trump administration has not been made clear at the time of this writing.

An additional aspect applicable to the assessment of GM animals is animal welfare—i.e., whether or not a modification might cause an unintended harmful effect on the animal (EFSA 2012; Jackson et al. 2010). SDN-3 modifications would likely follow principles established for transgenic animals in different countries. It is uncertain currently whether or not animals obtained by SDN-1, base editing, or SDN-2 will be exempted from animal welfare considerations. For any class of edit, there exists the possibility that an off-target mutation could impact animal welfare.

Containment as a consideration applies to both GM animals and GM plants, though routes and likelihood of release into the environment or gene transfer to nontarget organisms differ between plants and animals and among different types of animals (consider containment of seed versus containment of livestock animals and gene transfer by pollen relative to gene transfer from engineered fish or

insects, for example). Specific environmental or ecological risks associated with such events may apply to genome-edited organisms similarly to how they apply to conventional GM organisms derived through conventional genetic engineering.

## The Challenge of International Coordination

The scope and application of regulatory frameworks for products of modern biotechnology are determined by individual jurisdictions, which vary substantially but typically adhere to a case-by-case principle. These differences have resulted in asynchronicity in approvals of new products and have led to trade disruptions—e.g., when traces of GMOs are detected in countries where they have not been authorized. The consideration of products of new plant breeding techniques, including organisms developed by SDN approaches, has exemplified the lack of a harmonized regulatory framework. Consideration of such products raises a range of policy, legal, and trade issues, making decision-making by individual countries a lengthy process. Meanwhile, several nongovernmental organizations, governance or risk assessment experts, and academic or public sector scientific initiatives have issued their opinions (Camacho et al. 2014; Conko and Miller 2010; Harvey 2014; Kokotovich and Kuzma 2014; Kuzma and Kokotovich 2011; Podevin et al. 2013; Wolt, Wang, and Yang 2015).

Following a workshop and a questionnaire with the aim of gathering background information on the technologies and country experiences on new plant breeding techniques, including SDN approaches, the Working Group on Harmonization of Regulatory Oversight in Biotechnology of the Organization for Economic Cooperation and Development encouraged parties to share experiences and views; however, they did not propose harmonization.

The CPB is an international and environmental agreement on biosafety and establishes an advanced informed agreement procedure for GM products, based on a risk assessment, to enable informed decisions on transboundary movements of "living modified organisms." Though it may provide a forum to have a harmonized interpretation of the terms "living modified organism" and "novel combination of genetic material obtained through the use of modern biotechnology," it should be noted that important international players such as Australia, Russia, Argentina, Canada, and the United States are not signatories to the CPB.

## Economic Issues

There are few empirical studies on political and economic issues related to genome editing in agriculture, although some insight has been gained through interviews with stakeholders and experts (Kokotovich and Kuzma 2014; Kuzma, Kokotovich, and Kuzhabekova 2016; Lusser et al. 2011). Experts interviewed indicated that genome editing of plants and animals is generally easier and less costly than conventional genetic engineering techniques and that, as a result, genome editing may help smaller companies and public sector organizations innovate in the development of improved crops and livestock, particularly in specialty crops or livestock species for which there are not large commodity markets. Patent protection of genome editing tools, especially exclusive licensing, however, can present large barriers to entry (Lusser et al. 2011), particularly for academic institutions and small companies. A nonexclusive licensing arrangement for foundational CRISPR technology recently announced by the Broad Institute and DuPont Pioneer would appear to be a step toward addressing this problem.

## The Public

Public attitudes toward genome editing technologies applied to plants or animals have not been specifically studied yet to the authors' knowledge. There have been studies of public perception of cis- versus transgenesis. In the EU, a lower willingness to pay to avoid cisgenic versus GM crops was found (Delwaide et al. 2015); however, in this study, the question identified the transgenic crop as GM, but did not identify the cisgenic crop as GM—rather as "bred"—and therefore the results are not reliable given the confounding variables of GM/non-GM and cis/trans. In another study in Switzerland, two apple products were described as "genetic engineering, apple genes only" (cisgenic) versus "genetic engineering, genes from other species" (transgenic). The researchers found slightly more support for a cisgenic approach over transgenic, but still much less support than for traditional breeding (Haller 2009). This study is a closer approximation to what might be found in public perceptions of the mutational approaches of SDN-1, base editing, and SDN-2 versus cis- or transgene insertion by SDN-3.

Using focus groups with a total of 35 people, a study in Denmark found that some perceive cisgenesis more favorably than transgenesis, but this depended on different individual ideas about "naturalness" (Mielby, Sandøe, and Lassen 2013). Finally, a study that used experimental data and a representative survey (Eurobarometer) revealed that European public concerns were stronger when the boundaries of species were crossed; however, even with cisgenics, human intervention in the process amplified concerns and a majority of respondents across countries thought cisgenic products should be labeled (Kronberger, Wagner, and Nagata 2013). In summary, it is largely unknown whether or not trends and preferences revealed in these studies will hold as members of the general public consider the differences among SDN-1, SDN-2, and SDN-3, and between these and traditional genetic engineering approaches.

Genome editing, however, is likely to be subject to the same underlying factors of information processing and risk perception by individuals that have been found across multiple other emerging technologies. The psychometric paradigm explains that numerous factors influence how people perceive the degree of risk of a new product or technology. These include whether the risk is of something dreaded, something stigmatized, or something not experienced before; whether exposure to the risk is voluntary; and whether the risk is uncertain, among other factors. These elements ultimately influence attitudes or judgments and decisions about that risk (Fischhoff et al. 1978). Governance systems that mitigate these psychometric factors are likely to be more widely accepted than those that

do not. Indeed a survey of 1,600 people in the United States found that although two-thirds or more think genome editing for human therapeutic purposes (somatic or germline) is acceptable, there was substantial variation among respondents' perceptions, associated with amount of education and degree of religiosity, of whether or not the scientific community alone is able to provide enough oversight in the development and application of new technologies (Scheufele et al. 2017). The investigators concluded that their findings support a mandate for broad public engagement.

There are several social science frameworks that emphasize social and cultural factors in consumer attitudes toward products. Trust and confidence in social networks (e.g., social groups, communities, extended families, and friends) and societal systems (e.g., the market, the political system, the regulatory system, news media) play an important role in perceptions of risk for products, especially when those risks are new, uncertain, or ambiguous. They also influence people's reactions or behaviors in response to risk. For example, people who lack trust in the ability (or willingness) of companies to control risk have greater levels of political activism (Rohrmann and Renn 2000). The social amplification of risk framework focuses on the importance of intermediaries through which individuals receive risk information (e.g., media, government, industry, advertising, social groups). These sources can either amplify or attenuate risk information (Kasperson et al. 1988). From a practical standpoint, these considerations overall would suggest that governance systems that help to decrease some of the anxiety-provoking factors associated with consumer products—such as uncertainty, involuntary exposure, unfamiliarity, and catastrophic risk—might be more broadly accepted than those that do not.

Other theories focus on the values that people hold as predictors of perceptions and attitudes toward products, technologies, and risks. Core values are relatively stable over the course of an individual's life and provide a basis for attitudes and decisions, especially in the face of new information. Values can also play a significant role in whom or what institutions people trust. For example, the more closely aligned people's values are with those of institutions responsible for managing products and risk, the more trust they have in those institutions (Whitfield et al. 2009). In particular, this suggests that governance systems for genome editing that accommodate the values of a variety of consumers and stakeholders will be more trusted by a wider range of groups and be more effective.

Also related to values and their effects on perceptions and attitudes is the cultural cognition of risk. According to cultural theory, differences in risk perception arise from differences in individuals' views of the world and ways of living (Douglas and Wildavsky 1983). Worldviews can be classified according to two cross-cutting dimensions—egalitarian versus hierarchical and communal versus individualistic. People who hold more egalitarian-collectivist worldviews tend to advocate for social institutions that remedy inequalities, whereas people with individualistic-hierarchical worldviews tend to gravitate toward private control of activities and defend those with power and authority. Egalitarian-collectivists are generally more concerned with environmental risk associated with technologies or products, whereas individualistic-hierarchical people are more dismissive of these risks. Cultural cognition theory suggests that governance systems that take into account a variety of worldviews as lenses for the regulation of genome editing are more likely to be broadly trusted by multiple groups.

### Subject Matter Experts

Experts and stakeholders disagree about how genome editing should be regulated (Kokotovich and Kuzma 2014; Kuzma, Kokotovich, and Kuzhabekova 2016). Their viewpoints, however, can be grouped under three major approaches: (1) regulate genome editing like conventional genetic engineering using current systems, but improve upon these systems incrementally to better balance regulation, safety, and innovation; (2) loosen regulatory scrutiny significantly to oversee genome editing like conventional breeding; and (3) tighten regulatory scrutiny because current systems have too many gaps and genome editing is likely to increase the number and variety of GM products, which could overwhelm current oversight systems. In some cases these attitudes correlate with perceptions about the greater precision of genome editing and safety, whereas in other cases they derive from individual worldviews about technology and society (Kokotovich and Kuzma 2014; Kuzma, Kokotovich, and Kuzhabekova 2016).

### Nation States

National moods and priorities are also likely to affect the governance of agricultural products generated through genome editing. Some countries are more technologically optimistic than others; some are more precautious. Developing countries may accept higher levels of perceived risk for solutions to urgent problems such as food security. Cultural perceptions of food, the environment, and nature also may come into play. Economics and politics are certain to influence treatment of genome-edited products as they relate to trade.

## Perspectives

Genome editing is a powerful new method that enables unprecedented control over genetic material and offers the opportunity to make rapid advances in basic and applied biology. Issues that will affect governance of this powerful technology as it relates to plant and animal improvement include how genome editing compares to other methods of genetic manipulation, environmental and animal welfare impacts of specific applications, values of producers and consumers, and economic impacts, among others. Much remains to be learned regarding the variety of sociocultural factors that influence risk perception and technological acceptance at the national, group, and individual levels. As is true of other novel technologies, however, it is clear that, in democracies, successful deployment of genome editing for crop and livestock improvement will benefit from science-informed, value-attentive regulation that promotes both innovation and transparency.

## Abbreviations and Acronyms

| | |
|---|---|
| ALS | Acetolactate synthase |
| bp | Base pair |
| Cas | CRISPR-associated |
| CFRB | Coordinated Framework for the Regulation of Biotechnology |
| CPB | Cartagena Protocol on Biosafety |
| CRISPR | Clustered regularly interspaced short palindromic repeats |
| DNA | Deoxyribonucleic acid |
| FFDCA | Federal Food, Drug, and Cosmetic Act |
| *Fok*I | Restriction enzyme composed of a DNA recognition domain and a catalytic domain |
| GM | Genetically modified |
| GMO | Genetically modified organism |
| gRNA | Guide RNA |
| HDR | Homology-directed repair |
| NHEJ | Nonhomologous end joining |
| NOI | Notice of intent |
| ODM | Oligonucleotide-directed mutagenesis |
| OV | Ovalbumin |
| PEG | Polyethylene glycol |
| PNT | Plant with novel traits |
| PPA | Plant Protection Act |
| PRRS | Porcine reproductive and respiratory syndrome |
| RNA | Ribonucleic acid |
| SDN | Site-directed nuclease |
| SSN | Sequence-specific nuclease |
| TAL | Transcription activitator-like |
| TALEN | Transcription activator-like effector nuclease |
| ZFN | Zinc-finger nuclease |

## Glossary

**Cisgenesis.** The introduction into an organism's genome of a gene from a member of the same species or a closely related species that can be crossed with that species.

**Electroporation.** The process of introducing molecules, typically DNA or RNA, into living cells using an electric pulse.

**Genome editing.** The process of making precise, targeted changes in the DNA of living cells and organisms.

**Glycosylation.** Modification with sugars.

**Homeoalleles.** The versions of a gene found across the multiple sets of chromosomes present in a polyploid organism; a polyploid organism has more than two sets of chromosomes.

**Homology-directed repair.** Repair pathway that uses a DNA template with sequence similarity at its ends to the ends of the broken DNA and copies information from the template into the break site.

**Indel.** Insertion or deletion.

**Intragenesis.** The introduction into an organism's genome of a recombinant gene consisting of coding and regulatory sequences from two different genes, both from a member of the same species or a closely related species that can be crossed with that species.

**Invertase.** An enzyme that catalyzes the breakdown of sucrose into glucose and fructose.

**Meganuclease.** A type of site-directed nuclease based on homing endonucleases, which are enzymes that recognize specific sequences of between 12 and 40 bp in a DNA molecule and cut that DNA molecule there or nearby.

**Nonhomologous end-joining.** The preferred mechanism for repair in most somatic (nonreproductive) cells; it reattaches the broken ends of a DNA.

**Oligonucleotides.** Short pieces of DNA, typically single stranded.

**Plasmid.** A circular, self-replicating DNA molecule in a bacterial cell.

**Sequence-specific nuclease.** An enzyme that recognizes a specific sequence in a DNA molecule and cuts that DNA molecule there or nearby.

**Site-directed nuclease.** A sequence-specific nuclease, typically one that has been engineered for custom specificity.

**Transcription activator-like effector nuclease.** A type of site-directed nuclease that combines a customizable array of protein modules, found in bacterial proteins called transcription activator-like effectors, that each recognize a single DNA base and the catalytic domain of a DNA cutting enzyme called *Fok*I.

**Transfection.** A process for introducing molecules, typically DNA, into living eukaryotic cells.

**Transgenesis.** The introduction into an organism's genome of DNA from another, nonsexually compatible organism, or of synthetic DNA; if a gene, it is referred to as a transgene.

**Zinc-finger nuclease.** A type of site-directed nuclease that combines a customizable array of protein modules called zinc fingers that recognize specific triplets of bases in DNA and the catalytic domain of a DNA-cutting enzyme called *Fok*I.

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