Gene Editing

Evidence Update

the ROYAL SOCIETY of NEW ZEALAND
TE APĀRANGI

Summary

- **Gene editing involves the insertion, deletion or replacement of genetic material called DNA.**
- **New gene-editing technologies have been developed which have increased the speed, ease and accuracy of making changes to DNA in cells, and their use is increasing rapidly.**
- **These technologies are beginning to be used for new approaches in a variety of areas including research, medicine, agriculture, biotechnology and have the potential to be used in pest control.**
- **The three most widely used new gene-editing tools use bacterial proteins to find, cut, edit, add or replace genes, and are known as Zinc Fingers (ZFNs), TALENs, and CRISPR.**
- **Gene-editing technologies open up new opportunities and potential risks from new uses which may challenge people's views on what is acceptable.**
- **These new technologies pose challenges for regulators who will find it harder to distinguish between genetic changes in organisms generated by conventional breeding, gene editing, or natural mutation.**

What is a genome?

The characteristics of all living organisms are determined by their genetic material and their interaction with the environment. An organism's complete set of genetic material is called its genome which, in all plants, animals and microbes, is made of long molecules of DNA (deoxyribonucleic acid). The genome contains all the genetic information needed to build that organism and allow it to grow and develop.

Within the genome are regions of DNA called 'genes'. These 'genes' can carry instructions for making proteins, which in turn give the organism its characteristics or 'traits' [1]. For example, the red colour of a pōhutukawa flower is determined by the plant's genes, which carry the instructions for colour production within the flower. While every cell in an organism will have essentially the same genome, the differences between cells are determined by how and when different sets of genes are turned on or off. For example, genes in specialised cells in the eye are turned on to make proteins that detect light, while genes in red blood cells are turned on to make proteins for carrying oxygen.

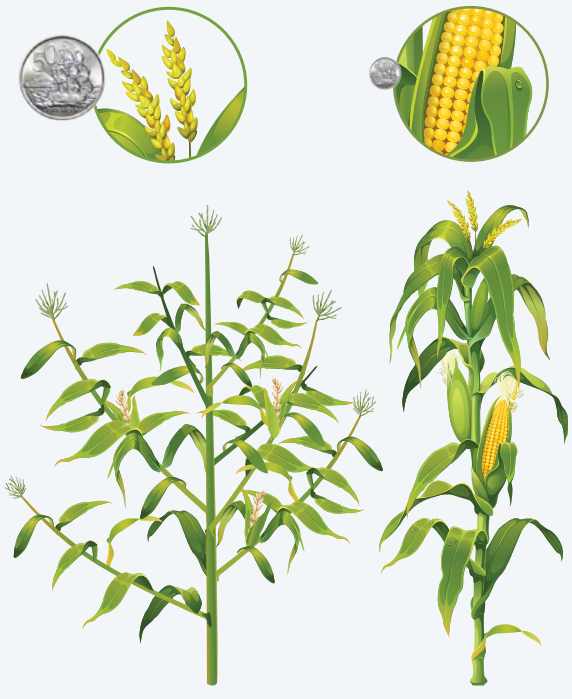
Occasionally, changes to DNA in cells can occur that create a new and different version of a gene which can then be carried by that organism's offspring. These changes are known as mutations and mean different individuals can carry different versions of that particular gene, which can result in differences in the trait within populations, for example for individual eye colour.

Identifying and using these different versions of genes, and the traits they create, has been an important part of agriculture for thousands of years. By cross-breeding plants with different versions of genes, and repeatedly selecting preferred plants from their offspring to serve as new parent lines [2], agricultural plants have been created over time with more desirable traits, such as higher yields, reduced toxicity, and improved flavour (see BOX 1). Much the same is true of livestock animals [6].

BOX 1

HISTORIC SELECTION IN AGRICULTURAL CROPS

Some 6,000 – 10,000 years ago, Meso-American farmers began the drastic changes to a grass species called teosinte to become what is now known as maize. Through selecting and growing plants based on very rare, desirable attributes caused by naturally occurring mutations, a plant was created with a single stalk and a cob with dozens or even hundreds of large seeds that were encased in husks, resulting in the maize that is grown today [3 – 5].



History of genetic engineering

Since the 1930s, chemical methods or ionizing radiation [7] have been used to change (or mutate) genomes, and to introduce new traits. This is a random process and breeders do not know what changes had actually occurred in the DNA. These methods are considered established tools of conventional plant breeding, along with 'marker-assisted selection' in the last 15 years. This latter process involves the genetic screening of agricultural plants and animals to see which individuals have useful versions of specific genes, and then selectively breeding from them. This selective breeding, however, would still introduce thousands of 'unwanted' genetic variations alongside any desired genes identified.

The introduction of genetic engineering in the 1970s and 80s enabled the possibility of moving beyond the conventional sources of random genetic variation, described above, by allowing researchers to introduce a specific single new or altered gene, or to disrupt or enhance an existing gene. While more targeted systems were available for some organisms, in many cases (such as in plants) the first set of tools provided little control over where new engineered DNA could be integrated into the organisms' genome. Two of these techniques used bacteria or viruses to transfer the DNA, and a third method involved coating small metal particles with the DNA, and then 'shooting' the particles into cells [2].

The impact on the biological sciences since these first tools were developed has been profound. However, in the past 10 years, researchers have developed tools to enable the manipulation of specific genes within a genome with greater and greater precision in the modification process, and fewer and fewer unintended changes elsewhere in the genome [8]. With their wide availability and simplicity, these gene-editing technologies are now being used to significantly accelerate research, and offer new treatments for a range of genetic diseases, while new agricultural products are beginning to be commercialised.

Alongside the development of the technology, the concept of genetic engineering, or genetic modification, has raised ethical and values-based questions in many societies [9, 10]. New Zealand has adopted a regulatory framework under the Hazardous Substances and New Organisms Act 1996 (HSNO Act[a]) to manage adverse effects on the environment and health and safety of people associated with the technology, which takes into account both benefits and risks. This act is based on the assumption that genetically modified organisms are different from unmodified organisms and can be distinguished from them. Food which has been derived or developed from an organism that has been modified by gene technology must also meet the Australia New Zealand Food Standards Code[b]. With the arrival of new gene-editing technologies, there are now increasing challenges to New Zealand's national regulatory system's ability to distinguish between genetically modified and conventionally produced products and organisms (see BOX 2).

BOX 2

WHAT IS CLASSIFIED AS A GENETICALLY MODIFIED ORGANISM?

In New Zealand, the HSNO Act defines genetically modified organisms as:
'any organism in which any of the genes or other genetic material have been modified by in vitro[c] techniques; or are inherited or otherwise derived, through any number of replications, from any genes or other genetic material which has been modified by in vitro techniques', and the Australia New Zealand Food Standards Code defines food produced using gene technology as **'a food which has been derived or developed from an organism which has been modified by gene technology'.**

However, the organisms, resulting from modern gene-editing techniques may show no direct trace of a genetic modification, and therefore it will be harder to distinguish from a fully conventionally produced organism [11]. For example, accelerated plant breeding. This process involves an intermediate generation of GM plants where a new gene is inserted to shorten the time to flowering of a plant, speeding up the breeding process [12]. The inserted gene is then removed later by conventional crossing with other non-GM plants, so that no foreign genetic material remains in the resulting crop [13, 14].

In New Zealand, this example would still be considered a genetically modified organism, however in other countries there may be no legislative requirement to record the genetic modification step as part of the process [15]. For example, in the US, the Department of Agriculture has ruled that commercial production of CRISPR gene-edited mushrooms [16] and waxy corn[d] do not need regulation, while in Europe, the Swedish Board of Agriculture have ruled that plants mutated by CRISPR, which do not contain any foreign DNA sequences, are exempted from GM legislation[e].

What are the new gene-editing technologies?

Gene-editing technologies use proteins, called enzymes, to cut targeted areas of DNA within a genome. Cells repair these cuts but if no instructions are provided for the repair, the repair process can make mistakes, resulting in altered DNA sequences. If specific DNA repair information is provided, however, the cell will use this to repair the cut in the way it is instructed. The use of this process provides an opportunity for researchers to modify the genome, by providing slightly different repair information from what was there before. In this way, it is possible to use gene editing to change a version of a gene from one that causes disease to one that does not (for example gene variants that contribute to Parkinson's disease [17] or genetic metabolic disorders [18]), or choose the version of a gene that confers better resistance to disease in agricultural plants and animals (for example resistance to powdery mildew in wheat [19]).

It is also possible to use the technique to modify genes without introducing foreign DNA sequences. For example, gene editing can be used to switch off genes [20] in laboratory-grown cells to identify their function [21], or to switch off genes that are causing disease, such as in animal models of Huntington's disease [106]. Alternatively, a DNA template could be provided for a whole new gene based on a gene found within the same species, or from a different species, providing a new set of traits such as new disease resistance or hornlessness in dairy cows [23 – 25].

While technologies to make cuts in DNA have been known since the 1970s, using them in a controlled and accurate way, and in organisms whose genome is poorly understood, has been a major hurdle. However, in the last 10 years, researchers have identified, or created, proteins that permit gene-editing technologies to make gene edits in specific areas of DNA, rather than introducing these changes randomly into the genome. Also, advances in very rapid genome sequencing now mean that genome DNA sequence information for any species can be quickly assembled [26], opening up the way for widespread use of gene editing approaches.

The three main new gene-editing technologies [27] which have been developed to do this (see FIG. 1) are:

- **Zinc-finger nucleases** [28, 29]
- **TALENs** [30]
- **CRISPR** [31]

Zinc-finger nucleases (ZFNs)

ZFNs use a bacterial DNA cutting enzyme [32] that has been combined with proteins called 'zinc fingers', which can be customized to recognise a specific section of DNA [27]. In 2005, this technology was first used to edit DNA in human cells [33]. ZFNs are small (one-third of the size of TALENs and much smaller than CRISPR) so they are easier to package inside delivery vehicles, such as viruses, to enable them to reach their targets in cells for genome-editing-based therapies [34].

TALENs

TALENs (transcription activator-like effector nucleases) again use a DNA-cutting enzyme combined with proteins from bacteria [35] that target areas of DNA, in a similar way to the zinc finger proteins. TALENs can be designed with long DNA recognition sections, and therefore tend to have lower unintended off-target cut sites, which can occur when parts of a genome have an identical or near-identical sequence to the target site [36, 37].

CRISPR

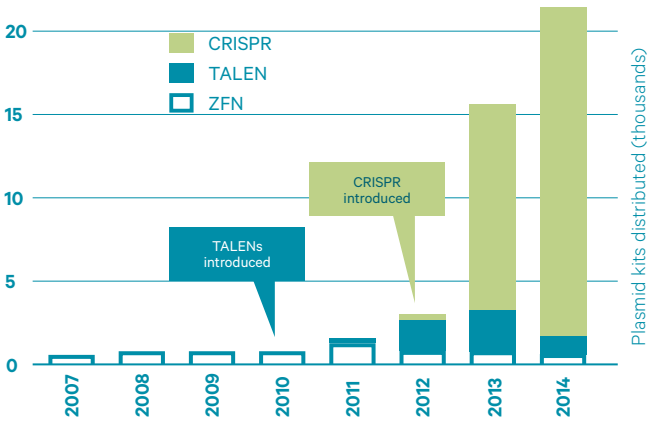
Bacteria possess an immune system which recognises invading viral DNA and cuts it up, making the invading virus DNA inactive. This type of immune system is known as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) [38]. In 2012, it was discovered that by modifying this mechanism, it was possible to target and cut any DNA sequence and edit genomes [39]. In 2013, this technology was modified further so that the target DNA is bound and blocked, rather than cut, allowing a gene to be turned off without altering the DNA sequence [40 – 42]. In 2014, a further advance allowed the blocking enzyme to be reactivated, enabling a way to turn genes on and off using chemical triggers, or blue light [43]. In 2016, researchers further improved on the performance of CRISPR by allowing for editing of single DNA letters [44].

CRISPR, unlike ZFNs and TALENs allows for many DNA sites to be edited simultaneously and easily [45]. It is also the most affordable and programmable genome editing technology. While much more accurate than earlier genetic modification technologies, there can still be unintended off-target effects, although these are detectable and new research is rapidly improving the technology's accuracy [46 – 50].

FIG. 1

POPULARITY OF GENOME-EDITING KITS

Popularity of genome-editing kits. The ease of use of CRISPR has seen a rise in the number of orders for genome-editing kits from Addgene, in the US.



How are these new technologies being used and applied?

New gene-editing technologies are enabling a broad range of applications from basic biological research to biotechnology and medicine [51] (see FIG. 2).

Medical applications in treatments and research

Of the approximately 25,000 identified genes in the human genome so far, mutations in over 3,000 have been linked to disease [52]. Gene-editing tools are now being used to understand how gene variants are linked to disease in mammalian cells and whole animal models, indicating the potential for this technology to be used to understand and treat human disease [20, 53–57] (see FIG. 3 [20]).

For example, CRISPR has been used in research mouse models to correct a mutation in genes responsible for Hepatitis B [58], haemophilia [53], severe combined immunodeficiency [59], cataracts [60], cystic fibrosis [61], hereditary tyrosinemia [62] and inherited Duchenne muscular dystrophy [22].

Clinical trials with patients are underway in the US using ZFN to modify the genes of immune-system cells to treat HIV [63]. HIV infects and destroys immune system cells and key genes within these cells have been modified using ZFN to make them resistant to HIV, and the cells then transplanted back into patients.

The Great Ormond Street Hospital in the UK has used TALENs for gene editing in donated blood cells to disable the gene which the immune system uses to recognise 'foreign' cells. This allowed a patient to receive donated blood cells, without the donor cells attacking the patients' healthy cells [64, 65]. In June 2016, a federal biosafety and ethics panel in the US approved a clinical study in patients using CRISPR-based genome-editing to create genetically altered immune cells to attack three kinds of cancer[f].

Gene editing is also being used by researchers to try to overcome allergic reactions to chicken eggs, which prevents about 2% of children worldwide from receiving many routine vaccinations. Researchers at Deakin University in Australia are working with gene modifications using CRISPR to produce hypoallergenic eggs [66].

The use of gene-editing technologies in the early stage embryo allows modifications which can be passed on to future generations. In the UK, the Human Fertilisation and Embryology Authority (HFEA) has approved an application for the use of CRISPR in healthy human embryos to help researchers to investigate the genes involved in early embryo development. This could lead to improvements in assisted reproductive technologies used to treat infertility, although the CRISPR technology itself will not form the basis of a therapy [67]. In China, researchers have used CRISPR in non-viable human embryos to genetically modify genes responsible for ß-thalassemia, a potentially fatal blood disorder [68], and to modify genes in immune cells to develop increased HIV resistance [69].

FIG. 2

APPLICATION OF GENOME EDITING
(Modified from Hsu et al. 2014)

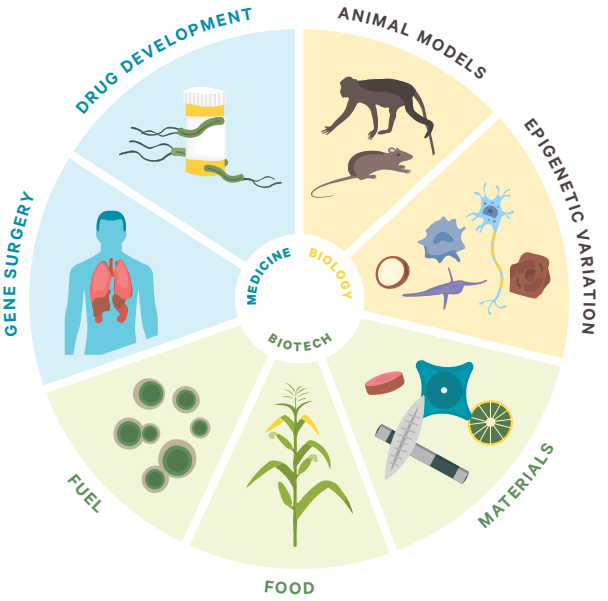
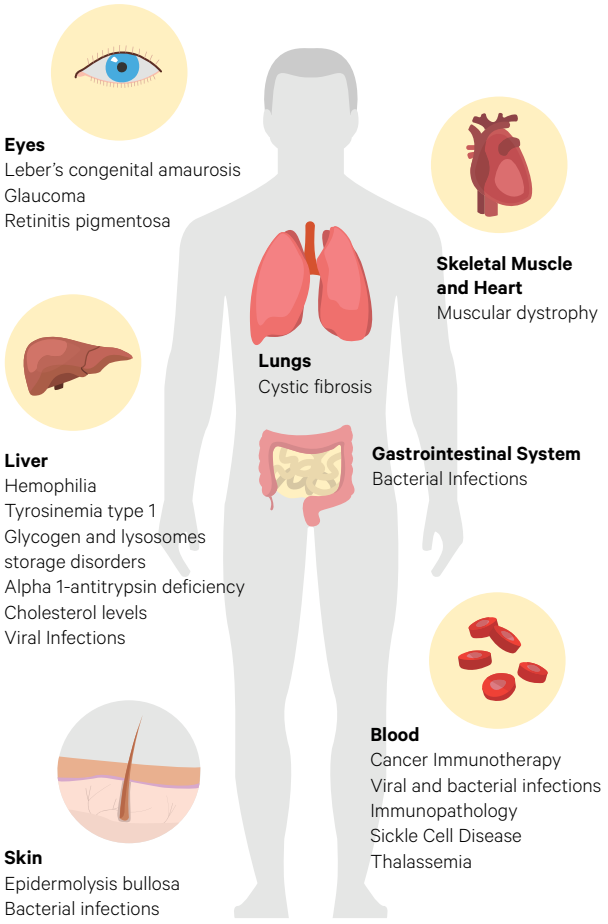


FIG. 3

DIVERSITY OF TARGETS FOR THERAPEUTIC GENOME EDITING (Maeder & Gersbach 2016)



Agricultural applications

In agriculture, the new gene-editing technologies make it possible to modify a range of agriculturally-important organisms easily, cheaply, and if desired, without introducing foreign DNA sequences [13, 70].

Food production

In the US, researchers have used gene-editing technologies on agricultural crops such as maize [29], soybean [71], sorghum [72], and developed a rice resistant to bacterial blight [73]. In commercial development, the common white button mushroom has been modified by CRISPR at Penn State University to prevent them from becoming off-colour by targeting a gene that produces an enzyme that causes browning [16]. Further, DuPont Pioneer have used CRISPR to produce a higher-yielding waxy corn variety [74] and Calyxt Plant Sciences Inc. have produced soybean lines that are low in polyunsaturated fats, using TALENs [75].

Chinese researchers have similarly used TALENs and CRISPR to modify a range of agriculturally important plants and animals, including maize [76], rice [77, 78], and wheat [79]. They have also used the techniques to develop goats with longer coats (for Angora) and more muscle (for increased meat yield) [80]. Elsewhere in the world, researchers have used the techniques to modify barley (Denmark) [81]; wheat (India) [82]; and to study allergenic milk protein production in cow embryos cultured in the laboratory (New Zealand) [83].

Animal health and welfare

In the US, hornless dairy cattle have been produced using gene editing to avoid the need for painful de-horning and to prevent animals injuring each other during transport. Using TALENs, the genetic code that makes dairy cattle have horns has been substituted for the one that makes Angus beef cattle have none [24]. The University of Missouri has also bred the first pigs resistant to Porcine Reproductive and Respiratory Syndrome by suppressing the production of a protein within the pigs that the virus uses to help it spread [84].

African swine fever is a highly contagious disease that kills up to two-thirds of infected animals. In Scotland, ZFN has been used by the University of Edinburgh to modify a gene in pigs to the version of the gene found in warthogs, to produce pigs that are potentially resilient to the disease [25]. The university has also used gene editing to modify chicken genes so they don't spread bird flu by introducing a gene that produces a 'decoy' molecule that interrupts the replication cycle of the bird flu virus, thereby restricting its transmission [85].

In China, TALENs have been used to add a gene that is found in mice into cattle to improve tuberculosis (TB)-resistance. The modified cattle have immune cells that are better at slowing the growth of the disease and are less susceptible to developing the internal symptoms of TB [86].

Pets

In China, CRISPR has been used to create micro-pigs which are approximately half the size of their non-modified counterparts, which can be sold as pets [87].

Biocompound production

By using gene-editing technology to manipulate biological pathways, new materials are being developed, such as algae-derived porous silica-based particles for drug delivery [88], CRISPR modified silkworms to produce spider silk, algae-derived lipids for biofuels [89], and microbial production of pharmaceuticals and commodity chemicals such as ß-carotene [90], L-lysine [91], and mevalonate [92].

Environmental applications

Gene-editing tools have not been used to date in the conservation of wildlife [93], but their use in the control of non-native invasive organisms is being explored with the use of gene drives.

Gene drives

In 2015, researchers demonstrated the use of CRISPR to develop 'gene drives', a genetic system named for the ability to 'drive' themselves and nearby genes through populations of organisms over many generations [94].

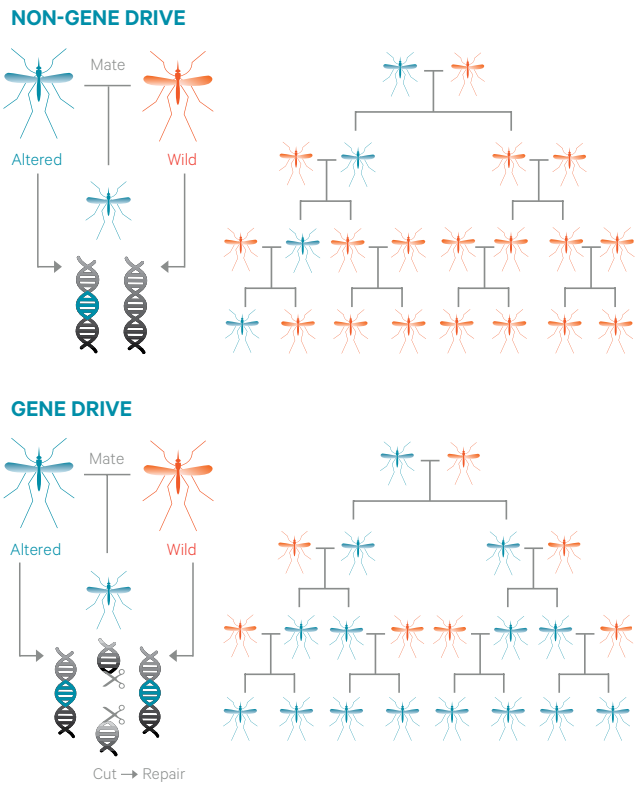
In sexual reproduction, offspring inherit two versions of every gene, one from each parent. Each parent carries two versions of the gene as well, so chance (50:50) normally governs which particular variant of the gene that will be passed on.

But 'gene drives' ensure that the genetic modification will almost always be passed on, allowing that variant to spread rapidly through a population (see FIG. 4). So far, 'gene drives' have been developed in yeast [95], the fruit fly [96], and two mosquito species.

One of the mosquito gene drives, developed in the US by researchers at the University of California, causes a malaria-resistance gene to be passed on to the mosquitoes' offspring, meaning they are unable to transmit malaria in mice [97]. The other mosquito gene drive strategy, developed by Imperial College in the UK, propagates a gene that sterilizes all female mosquitoes (which could suppress specific mosquito populations to levels that will not support malaria transmission) [98].

FIG. 4

CRISPR gene editing can be used to propagate a genetic modification rapidly through generations, using a gene drive which cuts the partner chromosome and copies the modification to this chromosome through the repair process.



Changing research approaches

In biological research, gene editing can increase the speed and ease of creating new animal-based or cellular models for disease, and it is proving to be an important tool in the study of cell development.

Rapid generation of cellular and animal models

Many human illnesses, including heart disease, diabetes, cancer and various neurological conditions, are affected by numerous variants in genes. Working out the impact of these variants on the illness with the help of animal models has been a slow process. To create these animal models, mutations need to be introduced into multiple genes. But using conventional tools to create a mouse with a single mutation can take up to a year and cost US$20,000 to produce [99]. If a scientist wants an animal with multiple mutations, the genetic changes must be made sequentially, and the timeline for one experiment can extend into years. In contrast, CRISPR has allowed the creation of a strain of mice with multiple mutations in a few weeks [100], with the CRISPR tools costing as little as US$30 [101].

Functional genomic screens

Cultures of cells developed from a single cell, which has a uniform genetic make-up, are used to examine the contribution of genes to biological processes. CRISPR can now be used to rapidly generate thousands of these different cell lines, with each cell line having a different gene switched off, to speed up the search for DNA sequences linked to specific biological processes [102].

Exploring gene expression

Almost every cell in the human body has roughly the same DNA sequence but cells use their DNA code in different ways, depending on where they are located in the body. One way that genes are controlled is by DNA-packaging proteins called histones [103]. In 2015 it was reported how CRISPR could be used to attach to and switch on these proteins, to determine whether they cause changes to the growth and development of the organism [104].

Tracing cells during development

In 2016 it was discovered that CRISPR can be used to mark cells whenever they divide based on a specific pattern (or barcode) of deletions and insertions. This technology now allows researchers to re-construct a 'family tree' of the cells that compose an animal's body, revealing which cells spawned others [105]. The use of this technique is now also being considered to record a cell's history in response to environmental signals, or to trace the evolution of tumours.

Implications of these technologies for New Zealand

With the falling cost and increasing simplicity and availability of these techniques, their application is increasing around the world, offering new opportunities and risks with legal and ethical implications. For example, gene editing poses challenges for regulators who will find it harder to distinguish between genetic changes generated by conventional breeding, gene editing, or natural mutation. The use of these techniques in human genome editing in particular has led to a global summit being held in December 2015 to consider human gene editing and the implications of these emerging technologies[g].

To explore these issues for New Zealand, the Royal Society of New Zealand has established an expert panel in 2016 to consider the implications of gene-editing technologies for New Zealand society, including the ethical, social, legal, environmental and economic considerations that reflect current and future trends in New Zealand's population and community diversity. The intention of the Panel will be to raise public awareness of the technologies and their uses, and provide insight and advice on the future implications associated with the application of these new technologies for New Zealand.

For further information

This paper was authored by the Royal Society of New Zealand, under the guidance of the following expert reference group: Professor Barry Scott FRSNZ, Professor Peter Dearden, Associate Professor Peter Fineran, Professor Neil Gemmell, Professor Emily Parker, and Professor Andrew Allan.

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For further information, please contact info@royalsociety.org.nz or go to the Royal Society of New Zealand web page: **www.royalsociety.org.nz/gene-editing**

Endnotes

[a] http://www.legislation.govt.nz/act/public/1996/0030/latest/DLM381222.html. The Act also implements New Zealand's obligations under the Convention on Biological Diversity and its Cartagena Protocol on Biosafety, which regulates living modified organisms resulting from modern biotechnology.

[b] http://www.foodstandards.govt.nz/code/Pages/default.aspx To date foods derived from 88 lines of genetically modified canola, corn, Lucerne (alfalfa), potato, rice, soybean and sugar beet are approved for use in foods in Australia and New Zealand. None of these have been derived from gene editing, [c] https://www.pioneer.com/CMRoot/Pioneer/About_Global/Non_Searchable/15-352-01_air_response_signed.pdf

[c] *In vitro* techniques are those that occur in a laboratory vessel or other controlled experimental environment rather than within a living organism.

[d] https://www.pioneer.com/CMRoot/Pioneer/About_Global/Non_Searchable/15-352-01_air_response_signed.pdf

[e] http://www.upsc.se/documents/Information_on_interpretation_on_CRISPR_Cas9_mutated_plants_Final.pdf

[f] https://www.statnews.com/2016/06/21/crispr-human-trials/

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