



GENE EDITING AND CRISPR

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CRISPR is a breakthrough tool for genetic technology that opens new frontiers for science and new questions for society.

In 2003, scientists announced that the first human genome sequence had been mapped in full. The map took 13 years and more than US\$3 billion in public funds to complete, and was one of the largest global scientific collaborations ever attempted.

By 2016 – just 13 years later – a complete individual human genome

Terms to know

DNA (deoxyribonucleic acid) is nature's way of storing genetic information. It can be viewed as a program or recipe book containing instructions on how a particular organism will develop.

A **gene** is a section of DNA that controls a specific trait – for example, hair colour, eye colour, or blood type. In some cases, several genes interact to produce the final result.

Gene editing involves making precise changes to DNA in order to change a gene or the expression of a gene. This can be used to turn some genes 'on' or 'off' (a potential treatment for genetic disorders), or to enhance a particular trait (better crop yields).

could be sequenced in a day for US\$1,000. With falling costs has come growing availability and a wealth of genetic information in all manner of organisms. We are living in a golden age for biological research.

Against the background of this veritable tsunami of genetic sequence information, the rapid development of a technology known as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has opened a bold new chapter. CRISPR is transforming not just how we undertake genetic research, but how we live our lives – from the food we eat, to the industries we develop, to the years of healthy living we enjoy.

This paper explains what CRISPR is, why it is having such an impact on the scientific world, and what it could mean for our future.

GENE TECHNOLOGIES: A TIMELINE

Life has always been exposed to changes in genetic material through natural processes. Random changes (or 'mutations') can be caused by environmental factors or errors in organisms' biological processes. They can occur in all organisms, including humans, and are one of the driving forces of evolution.¹

Key messages

- ▶ CRISPR provides an unprecedented level of precision and control in gene editing work.
- ▶ It is a versatile tool with potential applications in healthcare, agriculture, industry, and environmental management.
- ▶ Effective regulation and community engagement are essential to responsibly develop this technology.

With the dawn of agriculture some 12,000 years ago, a new kind of modification began to take off – domestication and selective breeding (Figure 1).

Our ancestors selected and bred the plants and animals most useful to them. Advantageous traits were chosen in the offspring. Over many generations this selective breeding, together with influence from the environment, resulted in significant changes in the genetic information of these species. This process continues in agriculture and ecology today.

With growing understanding of the processes of genetic inheritance and the nature of DNA, scientists began to search for ways to directly target genes encoding specific traits, rather than laboriously breeding organisms to favour desired characteristics.





Figure 1: The teosinte plant (left) has been selectively bred over millenia to yield sweetcorn (right) – a much larger and sweeter vegetable. (source: Stock image)

From the 1970s, this research provided the first products classed as genetically modified organisms (GMOs), most of which were produced by introducing DNA sequences from different species into host organisms.²

Gene editing is the next step in precision and control.

Where previous methods of gene modification were laborious and expensive, editing technologies allow scientists to pinpoint the exact site in the DNA that they want to alter.

As an analogy, if the text of this paper were equivalent to an organism’s genetic information, older gene modification tools could insert new words – copied from another document – randomly anywhere in the sequence. New gene editing techniques can add, delete, or change one letter in the middle of a specific word.

ENTER CRISPR

In a fast-developing suite of gene editing technologies, CRISPR stands out for its simplicity, versatility, speed and precision.³ As a result, CRISPR is a rapidly-growing topic of research (Figure 2).

“This is not genetic modification as people have imagined it since the 1980s. This is something fundamentally different: a pair of scissors that we can wield with nuance, efficiency and control.”

- Dr Alan Finkel, Australia’s Chief Scientist

CRISPR gets its name from a DNA ‘library’ that acts as an immune system in bacteria. In nature, the library documents snippets of DNA from viruses and other organisms that have attacked the host bacterium in the past – like a wall of criminal mug shots in a police station.

The ‘police’ in this situation are CRISPR-associated endonucleases (Cas). These are enzymes that cut up DNA. The Cas enzyme is given a guide sequence or target ‘mug shot’ from the library. This allows the Cas enzyme to head out, recognise and arrest that specific invader.

By aiming the Cas enzyme at a new target – or providing it with a new mug shot – this system is able to accurately defend against a wide range of attackers.

While CRISPR naturally occurs in bacteria, it can work wherever a Cas enzyme and a guide sequence (mug shot) are introduced.

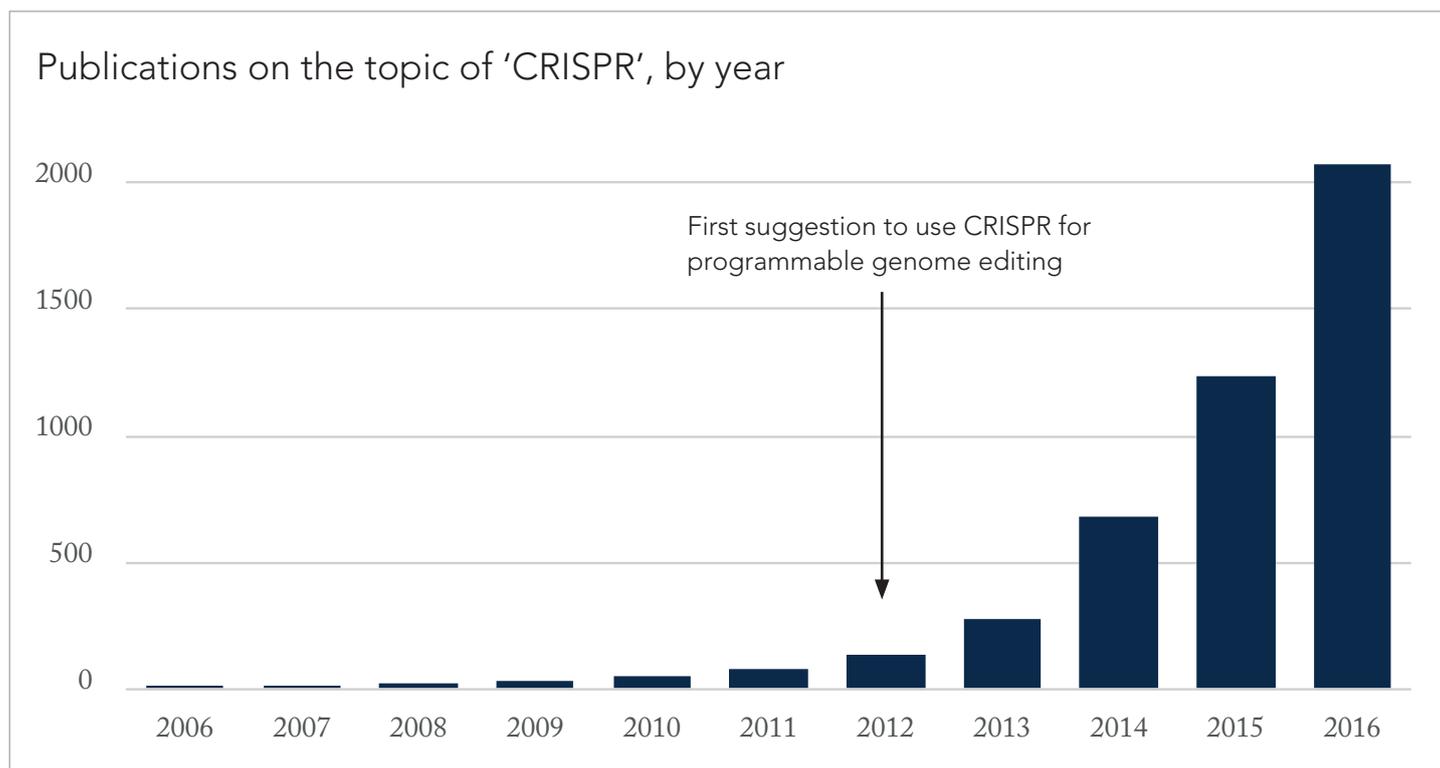


Figure 2: The number of CRISPR publications has vastly increased in recent years. (source: Web of Science)

In the laboratory, researchers can write their own guide sequences and direct the Cas enzyme to cut the cell's DNA at any chosen target location specified by that guide sequence. The editing occurs when the cell repairs the cut DNA. If we supply new DNA then the cell will stitch in the new code. In this way, we can write new words in an organism's genetic code.

Editing genes with CRISPR

A cut made by the Cas enzyme can be repaired in two ways. 'Non-homologous end-joining' is prone to introducing errors: for instance, it may delete a small DNA region during repair, effectively 'knocking out' that gene.⁴ Alternatively, DNA containing desired new edits can be added to alter the nature of a gene. Insertions made by this process can incorporate point mutations or deletions in the precise location of the cut, often used intentionally by scientists to turn a particular gene off.^{5,6,7}

APPLICATIONS OF CRISPR

Agriculture and the environment

CRISPR techniques could transform the agricultural sector: mushrooms that resist browning and disease-resistant wheat strains are just two examples of what has already been achieved.

Projects to improve yield, reduce waste or improve the health of crops and farm animals are widespread. In September 2016, a researcher from Umeå University in Sweden captured media attention by enjoying a meal including CRISPR-modified plants that he cultivated and grew himself.⁸

As well as increasing the efficiency of agriculture, gene editing could address a range of ecological issues associated with farming. One example is the potential use of CRISPR to knock out reproductive genes in farmed species to make them sterile unless provided with specific chemical signals while in captivity.⁹ Any potential escapees could not reproduce in the wild and disrupt ecosystems. This technology could be used to provide an additional safeguard for the farming of animals such as rabbits.

Health and medicine

Gene editing holds great potential to improve human health, particularly in the area of 'precision' medicine.¹⁰

For example, CRISPR has the capacity to redefine gene therapy for treating cancer, allowing reprogramming of a person's own immune cells to recognise and attack malignant tumours.¹¹

We can expect CRISPR to contribute to the development

of 'farmaceuticals' – medicines produced through existing animal farming practices.⁹ Prior to CRISPR, in 2006 the European Union and in 2009 the US Food and Drug Administration approved a breed of goat that produces an anti-clotting protein in its milk. In 2015, both agencies approved a genetically-modified chicken breed that expresses an anti-cholesterol drug in its eggs. Now, through CRISPR, CSIRO scientists are developing chickens that produce hypoallergenic eggs.⁹

The first clinical trial involving the injection of cells containing CRISPR-edited genes into a human patient is already underway in China. This trial is using CRISPR-edited cells to treat an aggressive form of lung cancer.¹²

New trials are also anticipated to begin in the US in 2017, with an advisory committee of the US National Institutes of Health approving a proposal to remove, edit and re-infuse T cells (human immune system cells that can destroy tumour cells) from patients with several types of cancer.¹³

CRISPR also opens the door to editing genes that cause serious illness in humans. These changes would be limited to particular individuals when performed on somatic (non-heritable) cells, such as immune or lung cells. Caution must be exercised, however, before conducting modification of germline (reproductive) cells that would allow changes inheritable by future generations.¹⁴ This potential application raises serious ethical concerns, with a recent report by the US National Academies of Science, Engineering and Medicine offering advice on the science, ethics and governance of human genome editing.¹⁵

NEW RESEARCH FRONTIERS

CRISPR could potentially prevent the spread of vector-borne diseases, such as dengue fever or malaria, by modifying mosquito populations through the use of 'gene drives' – an emerging system that allows rapid propagation of introduced genes through a large population.¹⁶ Based on studies conducted on mosquitoes, yeast, and fruit flies, these introduced genes have on average about a 95 percent probability of propagation through each generation¹⁷ – far greater than the 50 percent probability common in nature.

These gene drives could be used to either rapidly reduce the populations of specific types of mosquitoes known to carry target diseases, or to make populations of mosquitoes less likely to transmit diseases. However, the effects of gene drives altering or eliminating entire populations requires careful environmental and ethical consideration before implementation.^{18,19}

Researchers have also proposed using CRISPR to bring extinct species back to life. Potential targets of de-extinction include the woolly mammoth, passenger pigeons, dodo birds, and the thylacine (Tasmanian tiger).^{20,21,22} While the feasibility of such proposals is unknown, they prompt us to ponder the implications of such life-altering interventions.

CRISPR in Australia

Australia has emerged as an important player in the gene editing age, building on its traditional strengths in the life sciences and commercialisation of biotechnology research. It is currently the sixth most prolific producer of CRISPR publications worldwide per capita.

Combined with a strong record of responsible regulation, robust intellectual property framework, and a highly regarded clinical trials sector, the potential for future investment and discovery could be substantial.

Australia is developing applications of gene editing to solve issues for agricultural industries, such as sex determination for egg production. CRISPR will also have impacts in generating new biotechnology industries.

In the context of the Australian environment, gene editing holds particular promise in helping to deal with invasive species and biocontrol measures. Programs are underway using CRISPR editing to develop mitigation strategies for cane toad invasion, whilst daughterless technology is under development at CSIRO to safely control carp by removing females from populations.

Gene drives could also play a key role in eradication of mosquito-borne diseases, such as dengue fever, Ross River virus, and Murray Valley encephalitis.

PROCEEDING RESPONSIBLY

The rapid uptake and increasing sophistication of gene editing tools presents a series of highly complex economic, social, legal and ethical questions. Different countries have attempted to resolve these questions in different ways, with progress in science sometimes outpacing the capacity of governments to legislate for appropriate regulatory coverage of rapidly-developing technologies.¹⁹

The regulatory frameworks developed to regulate older gene modification techniques are constantly being updated for new technologies. Despite this, delays may deter investment and frustrate researchers seeking to develop the technology in line with community aspirations and values. On the other hand, caution demands that a high level of attention is paid to safety, efficacy and utility before the introduction of a new technology.

In Australia, the Gene Technology Regulator is the statutory office holder regulating dealings with GMOs, including organisms with genes inserted using CRISPR techniques (e.g. engineered gene drives). They assess all licence applications for work with GMOs, and contribute substantially to Australia's strong record of responsible and effective regulation of biotechnology research and commercialisation. Products produced from GMOs – such as food from a genetically modified (GM) crop – are regulated by other bodies. Food Standards Australia New Zealand (FSANZ), for example, is responsible for the safety assessment and approval of GM foods before they can be sold for human consumption in Australia or New Zealand.

In 2012 and 2013, FSANZ convened workshops that concluded that food derived from gene edited crops with small changes – such as those produced using CRISPR techniques – need not be considered GM because such mutations are indistinguishable from mutations that might occur naturally or through traditional mutagenic techniques. However, it was concluded that foods from edited crops with inserted genes should be categorised as GM. FSANZ is currently reviewing the definitions of GM food to consider whether food derived from new

techniques, including gene editing, should be subject to assessment and approval as GM foods.

Australia's strong regulatory environment will be key to applying, monitoring and commercialising these techniques, particularly as these techniques become more available outside the traditional academic laboratory.

At the time of writing, the Gene Technology Regulator is undertaking a technical review of the Gene Technology Regulations 2001 to provide clarity about whether organisms developed using a range of new technologies, including CRISPR techniques to make small changes, are subject to regulation as GMOs. The review also aims to ensure that new technologies are regulated in a manner commensurate with the risks they pose to human health and safety and to the environment.

CONCLUSIONS

While the potential of gene editing is vast, the use of these powerful tools has only just begun. It is up to governments, scientists and the community to work together to decide what outcomes we want, what risks we are prepared to manage, and how the benefits will ultimately be shared.

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References

1. J. A. de Visser, J. Hermisson, G. P. Wagner, L. Ancel Meyers, H. Bagheri-Chaichian, J. L. Blanchard, L. Chao, J. M. Cheverud, S. F. Elena, W. Fontana, G. Gibson, T. F. Hansen, D. Krakauer, R. C. Lewontin, C. Ofria, S. H. Rice, G. von Dassow, A. Wagner, M. C. Whitlock, Perspective: Evolution and detection of genetic robustness. *Evolution; international journal of organic evolution* **57**, 1959-1972 (2003).
2. M. Vaeck, A. Reynaerts, H. Hofte, S. Jansens, M. De Beuckeleer, C. Dean, M. Zabeau, M. V. Montagu, J. Leemans, Transgenic plants protected from insect attack. *Nature* **328**, 33-37 (1987).
3. J. K. Nunez, L. B. Harrington, J. A. Doudna, Chemical and Biophysical Modulation of Cas9 for Tunable Genome Engineering. *ACS chemical biology* **11**, 681-688 (2016).
4. J. K. Moore, J. E. Haber, Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. *Molecular and cellular biology* **16**, 2164-2173 (1996).
5. M. Babu, N. Beloglazova, R. Flick, C. Graham, T. Skarina, B. Nocek, A. Gagarinova, O. Pogoutse, G. Brown, A. Binkowski, S. Phanse, A. Joachimiak, E. V. Koonin, A. Savchenko, A. Emili, J. Greenblatt, A. M. Edwards, A. F. Yakunin, A dual function of the CRISPR-Cas system in bacterial antiviral immunity and DNA repair. *Molecular microbiology* **79**, 484-502 (2011).
6. L. Cong, F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini, F. Zhang, Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science (New York, N.Y.)* **339**, 819-823 (2013).
7. B. Wiedenheft, S. H. Sternberg, J. A. Doudna, RNA-guided genetic silencing systems in bacteria and archaea. *Nature* **482**, 331-338 (2012).
8. J. Cohen, Did a Swedish researcher eat the first CRISPR meal ever served? *Science*, (2016).
9. S. Reardon, Welcome to the CRISPR zoo. *Nature* **531**, 160-163 (2016).
10. T. B. Meissner, P. K. Mandal, L. M. Ferreira, D. J. Rossi, C. A. Cowan, Genome editing for human gene therapy. *Methods in enzymology* **546**, 273-295 (2014).
11. D. Cyranoski, Chinese scientists to pioneer first human CRISPR trial. *Nature* **535**, 476-477 (2016).
12. D. Cyranoski, CRISPR gene-editing tested in a person for the first time. *Nature* **539**, 479 (2016).
13. S. Reardon, First CRISPR clinical trial gets green light from US panel. *Nature News*, (2016).
14. K. S. Bosley, M. Botchan, A. L. Bredenoord, D. Carroll, R. A. Charo, E. Charpentier, R. Cohen, J. Corn, J. Doudna, G. Feng, H. T. Greely, R. Isasi, W. Ji, J. S. Kim, B. Knoppers, E. Lanphier, J. Li, R. Lovell-Badge, G. S. Martin, J. Moreno, L. Naldini, M. Pera, A. C. Perry, J. C. Venter, F. Zhang, Q. Zhou, CRISPR germline engineering--the community speaks. *Nature biotechnology* **33**, 478-486 (2015).
15. Human Genome Editing: Science, Ethics, and Governance. *The National Academies Press*, (2016).
16. J. Champer, A. Buchman, O. S. Akbari, Cheating evolution: engineering gene drives to manipulate the fate of wild populations. *Nature Reviews: Genetics* **17**, 146-159 (2016).
17. C. Noble, J. Olejarz, K. M. Esvelt, G. M. Church, M. A. Nowak, Evolutionary dynamics of CRISPR gene drives. *Science Advances* **3**, (2017).
18. B. L. Webber, S. Raghu, O. R. Edwards, Opinion: Is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat? *Proceedings of the National Academy of Sciences of the United States of America* **112**, 10565-10567 (2015).
19. R. A. Ary Anthony Hoffmann, Owain Edwards, Marianne Frommer, Keith Hayes, TJ Higgins, Oliver Mayo, Sue Meek, Charles Robin, Andy Sheppard, Ian Small, Synthetic Gene Drives In Australia: Implications of Emerging Technologies, *Australian Academy of Science*, (2017).
20. E. Callaway, Mammoth genomes hold recipe for Arctic elephants. *Nature* **521**, 18-19 (2015).
21. H. Nicholls, De-extinction: A behemoth revived. *Nature* **521**, 30-31 (2015).
22. K. Sanderson, Tasmanian tiger gene lives again. *Nature News*, (2008).

