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Gene-Editing Technology to Disrupt Food & Agri Systems

Technology Will Affect Plants, Animals, and Microorganisms

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Summary

The impact of gene-editing technology on our world is tremendous. We are already applying this technology in four areas: humans, plants, animals, and microorganisms. Because genes are the code for physical traits such as yield, disease resistance, and appearance, the power of gene editing unlocks possibilities for designing a biological system to suit one's needs. Gene editing greatly reduces the time and cost of plant and animal breeding, which is crucial for a resilient supply chain, especially in this era of climate change. Also, it offers added value through innovative products and a longer shelf life. Microorganisms can become easier to engineer, which is beneficial for livestock farming, crop farming, and other uses such as industrial enzyme production and medical production.

To evaluate the application potential of gene editing in plants, animals, and microorganisms, we take into consideration research costs, system complexity, and social reputation. Based on this, microorganisms have the greatest potential for deploying gene technology since it costs less than in plant and animal systems. Microorganisms are also the simplest system and have a neutral social reputation. According to these criteria, the second potential system in which gene editing could play a role is that of plants, followed by that of animals.

Gene-Editing Technology Unlocks Possibilities for Designing Biological Systems

The impact of gene-editing technology on our world is vast and tremendous. We can apply this technology in four biological systems: humans, plants, animals, and microorganisms. That means that, once the genetic code of a trait is decoded, technically it's possible to edit that trait artificially. Since 2014, several gene-edited products have been developed and reviewed for a commercial launch: a wheat variety resistant to powdery mildew, a waxy corn variety with a higher waxy starch content, and a mushroom that doesn't turn brown and thus has a longer shelf life, to mention a few.

The regulation of genes is highly complex. As one gene can play several roles in multiple signaling pathways, a disruption may lead to other undesired changes. Thus, in breeding, the foremost concern is to change/edit the genome as little as possible. The advantage of gene editing in breeding lies in its precision, as most of the genetic background remains the same and only the edited parts are modified. This shortens the time for recovering the high-yield background after introducing a desired trait and brings down the costs, while minimizing disruptions to the genome. Take the plant breeding process for example: conventional processes of genetic modification cost an average of 13 years and USD 130m, but gene-editing technology reduces the required time to only three to six years. In some countries, such as the US, gene-edited products are not considered genetically modified organisms (GMO), saving regulatory time and costs.

A cell is the basic unit of a biological system. It can be found in all living things, from the simplest systems that have only one cell, such as bacteria, to more complex systems that have 30 trillion cells, such as a human body. These cells all share a common principle, despite their differences in appearance and nature.

Despite the huge difference in numbers, almost all cells follow the same rule to perform and maintain the function of the living creature. This rule is called 'the central dogma' in biology, which states that genetic information is stored in DNA, and that during the transcription process, DNA is transcribed onto RNA. After that, RNA is translated into an amino acid sequence to form protein, which is used to build up enzymes or structures such as hair, muscles, and bones.

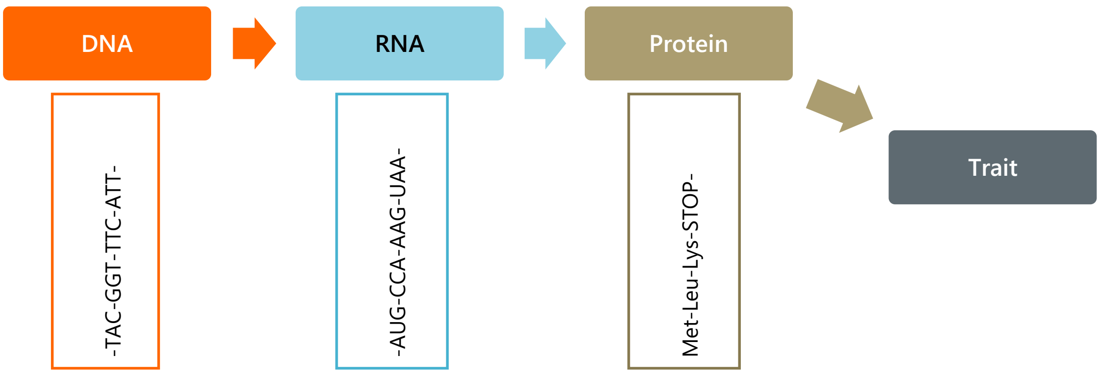
Imagine a cell as a restaurant, DNA would be the original version of the recipes (written in Latin), RNA the translated version (written in English), and protein the prepared dish. DNA contains genetic information and is stored in every cell in the nucleus. RNA is not stable by nature and is only copied during the production of a protein after which it soon degrades.

The sequence of DNA records all the information needed for regulating biological processes. The coding of DNA consists of four bases: adenine, thymine, cytosine, and guanine (A, T, C, G). Usually, protein synthesis starts with 'ATG,' while it ends with 'TAA.' Upon transcription, a piece of RNA synthesizes using DNA as the template. The bases of RNA are similar to those of DNA; the only difference is that T is replaced by uracil (U). The coding of RNA provides the information for protein synthesis; three bases encode for one amino acid. For example, 'CCA' encodes leucine (Leu) and 'AAG' encodes lysine (Lys). The sequence of amino acid forms the structure and property of a protein.

Gene-editing techniques can edit the bases of DNA or RNA, resulting in a changed final protein product. For example, editing 'AUG' into 'AAG' results in an amino acid change from methionine (Met) to lysine. Editing activities include insertion (of single or several bases), deletion (of single or several bases), and single-base editing.

Traditionally, gene editing that targets the DNA level leads to irreversible impacts, which have no buffer for errors. A more recent version of gene-editing techniques can target the RNA level to reduce the synthesis of abnormal protein.[1]

Figure 1: Gene-editing technology can edit DNA or RNA so that a cell can generate protein as desired, and ultimately exhibit the traits desired



Source: Lehninger Principles of biochemistry 2004[2]

[1] Ali, Zahir, Ahmed Mahas, and Magdy Mahfouz. "CRISPR/Cas13 as a tool for RNA interference." Trends in plant science 23.5 (2018): 374-378.

[2] Butterworth, P. J. "Lehninger: principles of biochemistry (4th edn)" DL Nelson and MC Cox, WH Freeman & Co., New York, 2004.

Most applications of gene editing lay in microorganisms, followed by plants and animals. To assess the possibilities of gene-editing applications in these three systems, several aspects were considered including: 1) the complexity of each system, 2) the costs of conducting the necessary experiments, and 3) the reputation of the final product among the general public.

Genome size and the number of genes determine the complexity of the application of gene-editing technology, for the larger the genome size, the greater the chance of off-target effects. And, the higher the gene number, the greater the total amount of testing. Usually, the lower the gene number, the easier it is to identify the gene function and the location to be edited. These two characteristics vary depending on the system. According to the National Center for Biotechnology Information (NCBI), microorganisms such as E. coli have a genome size of 5m base pairs (bps) and 4,736 genes, and yeast has 11m bps and 4,878 genes. Animals, such as a cow, have about 2,715m bps and 42,000 genes, while humans have 2,859m bps and 122,000 genes. In plants, the variation is more significant as some plants are polyploids. Diploids like corn have 2,198m bps and about 57,000 genes, and polyploids like bread wheat (hexaploid) have 14,439m bps and 131,000 genes.

After editing, the system needs to be tested to evaluate the impact, so the life cycle of each system determines the time and costs for screening the successful candidates. In microorganisms, the life cycle is one to three days, for plants it is around three to five months, and for animals around eight to twelve months. For each system, the number of genes and the life cycle are defined to determine how accessible the application of gene-editing techniques is.

Finally, the risk of reputational damage when conducting experiments in different systems is assessed. As far as plants and microorganisms are concerned, the general public pays almost no attention to such experiments. By comparison, the responses to animal experiments are much stronger and can give rise to protests that could potentially damage the reputation of companies. All in all, microorganisms have the simplest genetics and pose almost no threat to companies' reputations. Thus, they have the most potential to be engineered.

Table 1: Microorganisms have the most potential to be engineered

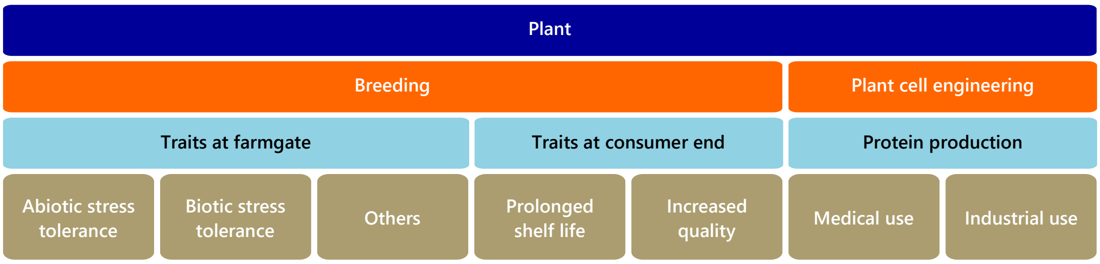
	Plants	*Microorganisms*	*Animals*
Life cycle	Medium (3-5 months)	Short (1-3 days)	Long (8-12 months)
Gene number	~3,0000 (corn)	~4,000 (E.coli)	~20,000 (cow)
Reputation	Neutral	Neutral	Negative
Overall ranking for applications	2	1	3

Source: NCBI, Rabobank 2021

Plant Breeding and Customized Protein Production Systems Will Benefit From Gene Editing in Plants

Plants are the core of agricultural production systems, and they serve various purposes, such as feed grain, food grain, vegetables, and fruits. Due to their different planting acreage and seed demand, they have different levels of development.

Figure 2: Plant breeding and customized protein production systems will benefit from gene editing in plants



Source: Crop, Environment and Bioinformatics 2018[3]

The breeding target varies according to the purpose of the crop. For example, feed grains such as corn and soybean are grown at a large scale and in open fields. Their ability to adapt to local conditions, such as drought, and good resistance to disease and pests are therefore important. On the other hand, fresh produce, such as vegetables, has a shorter shelf life and is mostly produced locally. Gene editing can also meet all breeding needs and can generate results faster than GMO.

Take corn, for example. Corn has a convenient structure for artificially moving its pollen (the male flower is on the top and can be easily removed with a machine), which is the basis of conventional hybridization breeding. Also, corn is used for a variety of purposes, including feed, food, and biofuel, and thus has a large acreage. Based on USDA records from 1989 to 2020, drought is the leading cause of loss of corn production in the US, followed by excessive precipitation. Therefore, resilient corn varieties with drought tolerance traits will lead to stable yields and increase farmer income.

Another use of gene editing is to engineer plant cells in such a way that they produce protein as designed – from high-value proteins for medical use to low-value industrial enzymes. Because cell culture can avoid the risks of contamination, it has a major advantage for the production of proteins for medical use.

Better Livestock and Fishery Genetics and Associated Techniques Lead to Higher Productivity While Enhancing Animal Welfare

For animal applications, gene-editing technology benefits the breeding process in a similar way to plants. Besides the common goals of disease resistance and increasing yield and quality, gene editing can also address animal welfare, which has recently drawn public attention. For example, Carlson, D.F. et al. published in 2016 in Nature Biotechnology,[4] reporting the production of hornless dairy cattle from genome-edited cell lines. This cattle line helps to maintain milk yield while avoiding the damage of horn removal.

For aquaculture, the main species for farming include salmonoid, tilapia, carp, and catfish. In 2016, a research group led by Dr. Han Wang established a line of common carp using a gene-editing technique to remove genes encoding myostatin, which resulted in increased body weight and muscle ratio.

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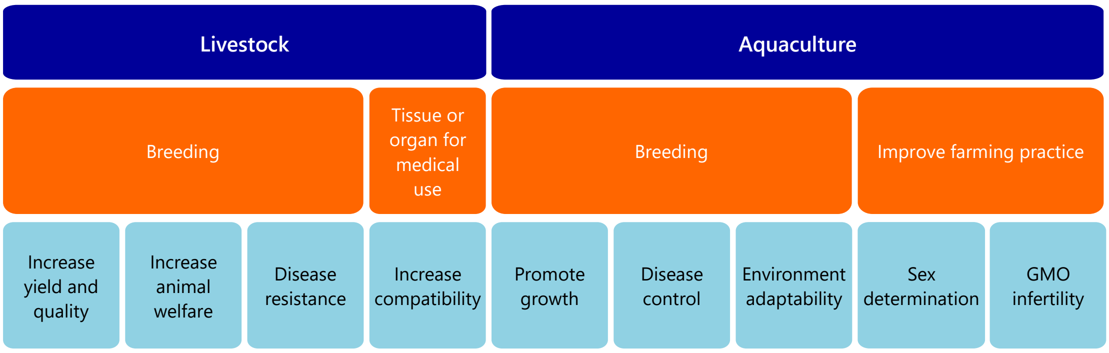
[3] Hong, Chwan-Yang. "Development and prospect of crop genome editing." Crop, Environment and Bioinformatics 15.2 (2018): 101-115.

[4] Carlson, Daniel F. et al. "Production of hornless dairy cattle from genome-edited cell lines." Nature Biotechnology 34.5 (2016): 479-481.

In tilapia, the male fish has a higher growth rate and thus a higher commercial value, which incentivizes research into mechanisms for sex control. In 2015 and 2016, at least three genes controlling sex in Nile tilapia were edited using gene-editing techniques.

Figure 3: Better livestock and aquaculture genetics and associated techniques lead to higher productivity while enhancing animal welfare



Source: CTCI 2018[5,6]

Gene-Editing Applications on Microorganisms Boost Animal Health and Crop Health, Resulting in Changes in Farming Practices

With the improvement of sequencing techniques for microorganisms, it becomes easier to identify beneficial specimens, resulting in more applications. Despite broad applications of gene editing on microorganisms, the concepts can be simplified as follows: 1) amplifying the symbiotic effects with hosts – for example, probiotics for animals, growth-promoting microorganisms for plants; 2) engineering its simple and inexpensive system for the manufacturing of high-value materials; and 3) use of its metabolic process for the production of desired products (fermentation).

Following these concepts, the applications in livestock farming include an oral vaccine for disease control, feed additives for animal health, and improved digestion leading to a higher feed-meat conversion rate. For example, one company produces probiotics for the digestive tract of pigs to support growth and health during dietary changes or disease.

In crop farming, a company produces microorganisms for corn, which colonize in the root and can fix nitrogen (N). Thus, the plant needs less synthetic N fertilizer. Another company uses microorganisms for pest control, including insecticide, fungicide, and herbicide. Using gene-editing technology, the efficacy of these microorganism-based farm inputs can be increased, leading to higher acceptance rates. This is expected to change farming practices in the long run.

The potential of the fermentation platform is enormous. Nowadays, the most commonly used microorganisms are E. coli and yeast. Research has focused on bioenergy and biorefinery. In the case of bioenergy, the fermentation processes usually generate alcohol, by-products, and heat. But these substances become toxic to the microorganisms when the concentration increases, which is a barrier to large-scale production. Gene-edited microorganisms have higher yields and

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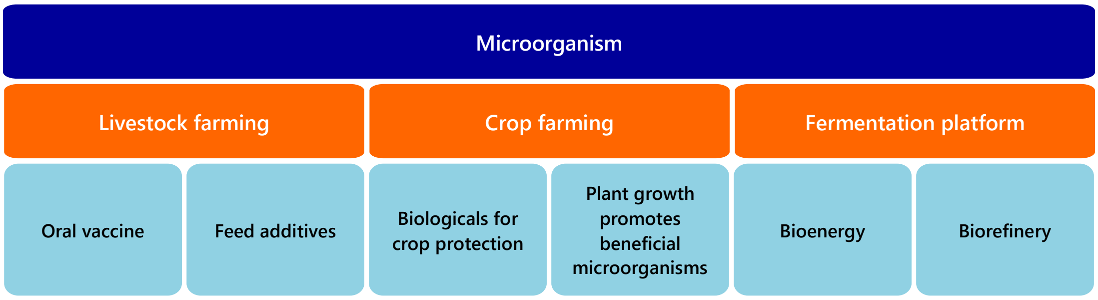
[5] Tu, Ching-Fu. "Application and development of gene-editing technology in livestock animals." Development of genome editing technology on agriculture and energy, edited by Wang, Jin-Guu and Lur, Hur-Seng, CTCI (2018): 107-117

[6] Li, Ya-Wen, Huang, Hsin-Chieh, Wu, Jen-Leih. "Application and development of gene-editing technology in aquaculture." Development of genome editing technology on agriculture and energy, edited by Wang, Jin-Guu and Lur, Hur-Seng, CTCI (2018): 155-164

are also more resistant to those toxic substances and high temperatures, making large-scale production (and ultimately, the replacement of fossil fuel) possible.

In the case of biorefinery, it is about manipulating the metabolic pathway in microorganisms to produce high-value compounds. The advantages of biorefinery lie in its simple production process (fermentation) compared to naturally grown methods, resulting in stable outputs. Astaxanthin, for example, is an antioxidant widely used in cosmetics and health products. It is naturally harvested from algae, but algae growth requires ample sunlight and is easily affected by climatic conditions. Currently, gene-edited yeast allows astaxanthin to be produced through fermentation, which is faster and more stable than the conventional system.

Figure 4: Applications on microorganisms boost animal health and crop health, which ultimately change farming practices



Source: CTCI 2018[7][8][9][10]

Conclusion

Gene-editing techniques are booming. With continued improvements, we can expect higher editing success rates and cheaper methods. The positive applications include higher crop and livestock production as a result of improved plant and animal genetics, as well as innovative new crop and livestock inputs, such as better probiotics to enhance animal gut health, and new biologicals for crop protection and nutrient uptake. The outlook for these three systems depends not only on the intrinsic nature of each organism, but also on the ethics debate and public perception. Microorganisms, in this regard, have the simplest genetics and an almost neutral perception among the public, suggesting that gene editing of microorganisms will play an important role in changing the way we farm.

[7] Shen, Wei-Chiang. "Application and development of gene-editing technology in agri-microbes" Development of genome editing technology on agriculture and energy, edited by Wang, Jin-Guu and Lur, Hur-Seng, CTCI (2018):119-126

[8] Liu, Je-Ruei. "Applications of gene-edited probiotics in livestock production" Development of genome editing technology on agriculture and energy, edited by Wang, Jin-Guu and Lur, Hur-Seng, CTCI (2018): 127-154

[9] Shen, Roa-Pu. "Application of gene-edited E. coli on bioenergy production" Development of genome editing technology on agriculture and energy, edited by Wang, Jin-Guu and Lur, Hur-Seng, CTCI (2018): 181-189

[10] Chin, Wei-Chih, Liu, Hsien-Lin, Huang, Chieh-Chen. "Application and development of yeast on alternative energy" Development of genome editing technology on agriculture and energy, edited by Wang, Jin-Guu and Lur, Hur-Seng, CTCI (2018): 195-199

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