Multiplex Gene Editing

The Key to Unlocking the Full Potential of Seed — and the Future of Agriculture

We are at a crucial moment in history. The effects of climate change are undeniable, and agriculture is tied to more than 10% of greenhouse gas emissions in the U.S. alone. Farmers are of course inherently committed to conservation – after all, few businesses are as dependent on natural resources. But to sustainably address the changing environment while growing enough to keep up with a rapidly growing global population, farmers also need new technology and tools.

There is no silver bullet to solve the climate crisis. However, the recent confluence of major technological advances offers society a unique opportunity to take a major leap forward in improving the resilience and sustainability of our global food system.

This change starts with seed – seed that requires fewer natural resources while continuing to produce enough food and feed. The key is an approach called **multiplex gene editing**, a new and powerful breeding technique with the potential to meaningfully change how plants grow and have a nature-positive impact on the planet.

A NEW ERA OF PLANT BREEDING

Gene editing is a New Breeding Technology, or NBT – an advanced form of an activity humans have been refining for more than 12,000 years. The goals of plant breeding, defined as the manipulation of plants for human benefit, remain the same: make adjustments to a plant, recreate those adjustments and advance them as fast as possible.

Imagine corn that requires 10% less land, 40% less water and 40% less nitrogen than today's crops.

The impact of this work cannot be overstated. Crops and crop characteristics we take for granted today simply did not exist thousands, or even hundreds, of years ago. Just look at the modern produce aisle: Staple foods such as brussels sprouts, cauliflower, broccoli and kale all began as different parts of the same wild mustard plant before humans selectively bred them to amplify certain traits. Even today's corn is almost entirely the result of human cultivation, bred from a plant – teosinte – that few would mistake for its modern offspring.

While the historical focus of breeding has primarily been on **enhancing yield** (boosting crop productivity), **protecting harvests** (building up plants' tolerance to weed, insect, disease and environmental stresses) and/or **improving quality** (meeting consumer needs from seed to storage), multiplex gene editing is now enhancing our ability to also **address climate change**, arguably the most complex and greatest challenge of our lifetime.

Imagine corn that requires **10% less land, 40% less water and 40% less nitrogen** than today's crops. Multiplex gene editing not only makes these previously unimaginable enhancements possible, it does so with plants' natural DNA. It works like traditional breeding, but with greater precision, accuracy and speed.

A multiplex approach promises to deliver on the sustainability possibilities unlocked by gene editing – possibilities that are **crucial not only to the future of farming, but to our planet and people.**

A BRIEF HISTORY OF BREEDING

Multiplex gene editing is a crucial step forward in the 12,000-year history of breeding. To recognize the impact of this technology, it is important to understand what came before.[1]

BREEDING 1.0

Humans chose and crossed plants with specific features they liked. This incidental approach – effective, but also incredibly slow and painstaking – covered the first 10,000 years of breeding.

BREEDING 2.0

After Gregor Mendel's discovery of the law of genetic inheritance in the late 19th century, humans began to improve selection efforts through statistics and experiments. This work eventually led to breeder Norman Borlaug receiving the Nobel Peace Prize for increasing wheat yields by 70%.

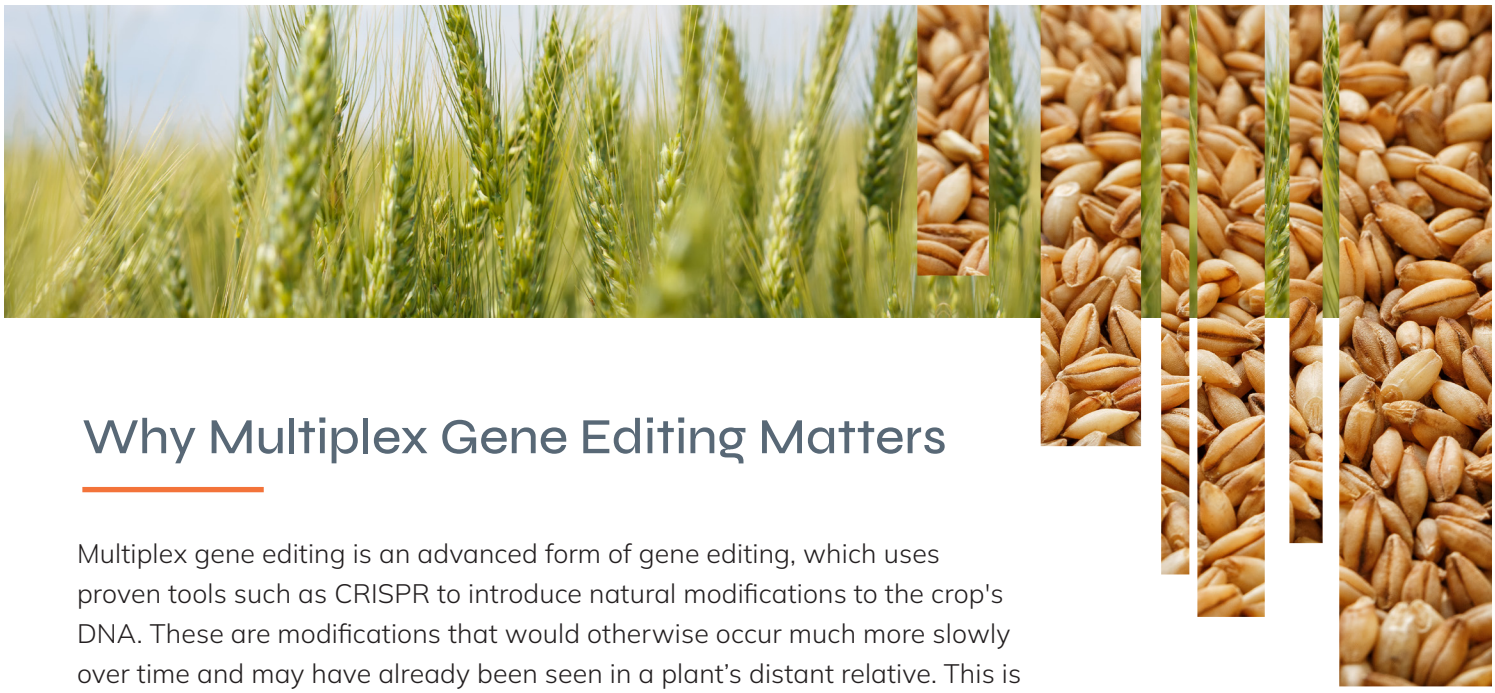
BREEDING 3.0

After the discovery of DNA's structure, breeders started to use genetic and genomic data to support and further accelerate breeding decisions. Markers enabled the ability to detect desired traits and predict the genetic value of untested plant populations.

BREEDING 4.0

Today, the convergence of A.I., genomics and multiplex gene editing make it possible to understand and address the complex crop systems that influence performance and resource use efficiency – opening the door to significant beneficial enhancements.

1. JG. Wallace, E Rodgers-Melnick, and ES. Buckler- 2018- Annual Review of Genetics, Vol. 52:421-444



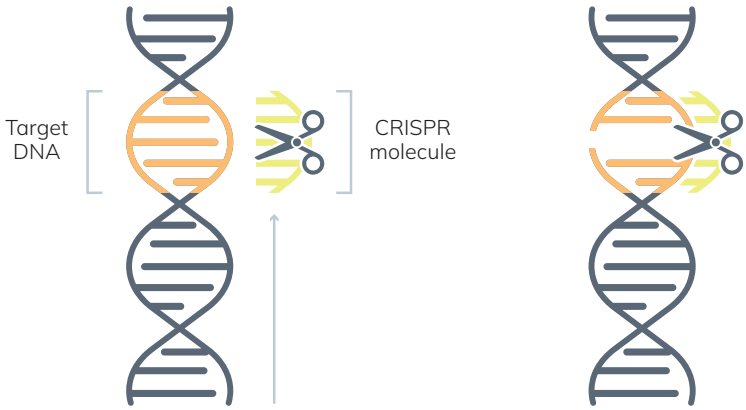
Why Multiplex Gene Editing Matters

Multiplex gene editing is an advanced form of gene editing, which uses proven tools such as CRISPR to introduce natural modifications to the crop's DNA. These are modifications that would otherwise occur much more slowly over time and may have already been seen in a plant's distant relative. This is similar to traditional breeding techniques but with **far more precision in far less time and with far fewer resources**.

HOW GENE EDITING WORKS

The most basic definition of gene editing, also called genome editing, is changing a DNA sequence in a living cell.[1] Although the concept dates back decades, its practical application was virtually impossible until 2012. That's the year eventual Nobel laureates Jennifer Doudna and Emmanuelle Charpentier discovered a method for precisely editing genomes today best known as CRISPR-Cas9.

Using a tool such as this, breeders and plant scientists can now "cut" a specific DNA sequence, then rely on cells' natural DNA repair mechanisms to introduce changes at that site. Put simply, this technology unlocks the ability to edit a living organism's DNA similar to how a word processor is used to edit written documents – adding, deleting and replacing letters in the cell's natural genetic code.



1 Search

A CRISPR molecule finds a precise location in the target DNA

2 Cut

The CRISPR enzyme cuts the target DNA at the point found by the guide

3 Edit

The existing sequence is rewritten

1. "What is CRISPR." Innovative Genomics Institute. https://innovativegenomics.org/education/digital-resources/what-is-crispr

SIMPLE EDITS ARE IMPACTFUL…

Editing even just a single gene can have a noticeable effect. In fact, nearly all gene editing work done to date in food and agriculture has been the result of simple edits like turning off, or "knocking out," a gene.

Some of the most prominent examples include:

Improved Soybean Oil
with significantly reduced saturated fatty acids

GABA-Enriched Tomatoes
with high levels of amino acid thought to lower blood pressure

Browning-Resistant Button Mushrooms
for potentially longer shelf life

…BUT MULTIPLEXING IS NEEDED

Simple edits alone are not sufficient in tackling the biggest challenges we face. Plants' genomes are incredibly complex. The wheat genome, for example, is five times bigger than the human genome. Meanwhile, characteristics such as water use efficiency are driven not by one or two native genes, but by a complex network in which many of these genes work together. Additionally, desired enhancements can require different types of edits. Sometimes a gene needs to be knocked out completely, while other times genes' expressions need to be amplified or suppressed.

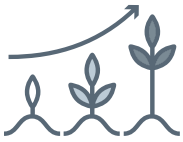
Enter multiplex gene editing, which involves executing a variety of edits and edit types to multiple genes – all at the same time. Today's gene editing tools already allow scientists to turn genes on and off, dial up or down a gene's expression, and make highly precise gene replacements.

New tools are constantly being developed. Cambridge, Massachusetts-based Inari, for example, has a leading multiplex gene editing toolbox that gives its plant scientists a range of abilities:

Turn Off/On		**Gene knockout**	Inactivates or removes a specific gene, thus disabling its effects
		**Gene insertion**	Inserts natural DNA at a target location to enable a specific effect
Regulate Up/Down		**Promoter fine tuning**	Adjusts a gene's expression to reduce a gene's effects
		**Enhancer insertion**	Adjusts a gene's expression to increase a gene's effects
High-Precision Replacements		**PRIDE™: P**recision, **R**eplacement **I**nsertion, **D**eletion, **E**diting	This tool enables very precise deletions, insertions and sequence modifications through a single tool. while acting as a sort of sewing machine – repairing visible seams that can occur around gene-edited sites

All this is happening at the same time that the latest advances in genomics and artificial intelligence are producing a monumental increase in new knowledge. As a result, breeders and scientists now have the opportunity to address biological complexity through a systems approach and pinpoint exactly which types and combinations of edits will have the most positive impacts at specific locations within a crop's DNA structure. The result will be accelerated crop improvement that address global needs.

The possibilities of multiplex gene editing include:



10-20%

Yield Increases

that enable farmers to grow more on less land

40%

Reductions in Nitrogen Use

by improving crops' resource use efficiency

40%

Reductions in Water Use

– reducing the need for an increasingly precious resource

Because of climate change, the farmers who feed our growing population will not have a choice but to operate with less land and fewer inputs. **With multiplex editing in hand, breeders and crop scientists can help farmers get the job done.**

FREQUENTLY ASKED QUESTIONS

How is gene editing different from what is often called "GMO"?

GMOs typically refer to plants with transgenic traits, desired characteristics such as herbicide resistance that result from the insertion of foreign DNA. Gene editing, on the other hand, works with a plant's natural DNA.

Are CRISPR and gene editing the same?

CRISPR is a tool in the multiplex gene editing toolbox. Scientists continue to discover and develop ever more effective and diverse tools.

If multiplex gene editing is the future, why aren't major agricultural organizations focused on this technology?

Multiplex gene editing is a new and complex approach. While several multinational and startup companies use genome editing tools for single-gene modifications, multiplex editing requires building the knowledge to determine exactly which genes and/or gene networks to simultaneously edit, currently an area of pioneering R&D.

Does multiplex gene editing face any roadblocks?

A clear regulatory path accessible to companies and organizations of all sizes is crucial to bringing multiplex gene edited products to market at a speed that keeps pace with both our changing climate and growing global population.

ABOUT INARI™

inari.com

Inari is the SEEDesign™ company, using new breeding technology to push the boundaries of what is possible by designing nature-positive seeds for a more sustainable food system. A combination of AI-powered predictive design and a pioneered multiplex gene editing toolbox enables Inari to unlock the full potential of seed and advance critical solutions with broad applications for growing more food with fewer resources. This includes products that will exponentially increase yield while reducing the environmental impact on land, water and nitrogen use – delivering a nature-positive impact while creating value for the entire value chain, starting with farmers.