

# CRISPR Gene Editing in Strawberry<sup>1</sup>

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Recent technology has been developed to precisely engineer genes for traits of interest. This approach is known as "CRISPR" gene editing. Gene editing is distinct from other forms of genetic engineering, such as transgenic technology, often colloquially referred to as "GMO." With gene editing, the final product can match that obtained by conventional plant breeding, but in a much shorter timeframe. CRISPR has been applied in many agronomic crops and is poised to make contributions in strawberry. We anticipate that, over the next decade, CRISPR and other gene editing techniques will be used to rapidly develop elite strawberry varieties with improved disease resistance, fruit quality, and other valuable attributes.

## What is CRISPR gene editing?

One of the great disappointments in the pursuit of improved varieties is the discovery of a new advanced selection that would be valued by the industry except for one critical flaw. Gene editing technology can be used to almost surgically adjust the gene or genes behind that deleterious trait. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is one form of gene editing that can be used to precisely modify a gene of interest without otherwise compromising the favorable traits of an elite variety (Rani et al. 2016; Bortesi and Fischer 2015). For example, instead of breeding for many years to move a disease resistance gene from a wild strawberry into a modern strawberry, gene editing allows a direct introduction of the genetic information. Think of it as a "cut and paste" mechanism. This is particularly useful for cultivated strawberries because they are genetically complex, making conventional breeding difficult. The UF/IFAS strawberry breeding program has identified several important gene regions controlling disease resistance traits that are directly relevant to Florida growers. By using CRISPR technology, these genes or gene variants can be moved into desirable genetic backgrounds that can be further moved via conventional crossing in later generations. We can utilize established DNA marker-assisted breeding tools to track the edited

genes in subsequent generations, adding to the speed of new variety development. Regulatory oversight of gene-edited crops in the U.S. has shifted in recent years: USDA-APHIS's 2020 SECURE Rule, which had streamlined review for many gene-edited plants, was vacated by a federal court in December 2024, returning the process to the pre-2020 "Am I Regulated" letter system. As of early 2026, APHIS has proposed a new rule ("Regaining Lost Efficiencies for Products of Biotechnology") intended to restore simplified pathways for low-risk gene-edited plants, with publication expected sometime in 2026. These tools remain expected to be valuable in the long-term efforts of every strawberry breeding program, though the exact regulatory pathway for edited strawberry varieties is still evolving.

## Does CRISPR gene editing = transgenic technology?

"Transgenic technology" refers to the transfer of a genetic material from one species to another. CRISPR, on the other hand, can be used to precisely change DNA sequence, switching it from one naturally occurring variant to another naturally occurring variant. Using this new technique, we can cut a strawberry's genome at a desired location so that existing genes can be removed or added. Historically, USDA took the position that many gene-edited crops should not require the same regulation as transgenic crops, since the final product can match what conventional breeding could produce — for example, in 2016 the agency stated it would not regulate a CRISPR-edited mushroom (Waltz 2016; Hoffman 2021). However, following the 2024 vacatur of the SECURE Rule, growers and breeders currently need to go through USDA's reinstated legacy review process for new gene-edited events, pending the outcome of APHIS's anticipated 2026 rulemaking. Countries like Sweden and Argentina have made similar proclamations, indicating that the finished CRISPR-edited varieties do not fall under certain regulations because they do not contain "foreign" DNA. We published another article about CRISPR gene editing in crop improvement, [HS1334: Current Status of](#)

## Development of a CRISPR System in Strawberry

While the finished varieties do not contain DNA from other organisms, the process introduces genetic information that orchestrates the desired genetic change. The first step is to develop and optimize a tissue culture and transformation system (protocols to introduce foreign DNA to new plants) for UF strawberry lines, so that new plants can be regenerated from cells containing introduced DNA. However, just as each cultivar has different traits and qualities, they also behave differently with respect to introduction of new genes.

The major strawberry cultivars grown in Florida such as Florida Brilliance™, Florida Encore™, and Florida Ember™ were used for tissue culture optimization. As shown in Figure 1, UF strawberry breeding program uses two approaches for gene editing in octoploid strawberry. In the first approach, explants are transformed with *Agrobacterium*, followed by selection of transformed calli and regeneration of shoots from the transformed explants to produce gene-edited plants. In the second approach, protoplasts are isolated from strawberry tissues and transfected with gene-editing reagents to edit the target genes. The edited protoplasts are then regenerated into whole plants carrying the desired target gene edits.

### No "Foreign" DNA Sequences

Once the genetically engineered gene product is ready for CRISPR gene editing, transformation, where the new genetic material is delivered to a single strawberry cell, is the first step in the genetic engineering process. For DNA delivery, *Agrobacterium tumefaciens*-mediated transformation is widely used for CRISPR gene editing. *Agrobacterium tumefaciens* is a widespread, naturally occurring soil bacterium that causes crown gall in many plant species and has the ability to introduce new genetic material into plant cells (Gelvin 2003). This bacterium works as a natural genetic engineer and is used in labs for plant transformation. Gene-edited plants using *Agrobacterium*-mediated transformation will contain foreign bacterial DNA sequences. It is not an easy process to remove the bacterium-derived DNA sequences through breeding.

Recently, the development of gene editing using protoplasts and regeneration of plants from protoplasts has been demonstrated in other plant species (Woo et al. 2015; Kanchiswamy 2016; Dutt et al. 2015). This method is known as a completely "DNA-free" gene editing system. Briefly, the protein/genetic material complex for gene editing will be assembled in vitro and the complex mixed with strawberry protoplast isolated from embryogenic calli and polyethylene glycol, which allows direct transfer by endocytosis into protoplasts. The gene edited with protoplasts is cultured (cell suspension culture) into calli, and mature plants can be regenerated using the tissue culture protocol outlined above (Figure 1). We have since successfully established this protocol: a stable, rapid protoplast-based CRISPR/Cas9-ribonucleoprotein (RNP) screening system for cultivated octoploid strawberry, achieving DNA-free gene editing without regeneration through tissue culture from *Agrobacterium*-transformed callus. This system optimized protoplast isolation and PEG-mediated RNP delivery, and was validated by targeted editing of disease resistance and fruit quality gene, confirming that "DNA-free" CRISPR editing is now achievable in commercial octoploid strawberry cultivars (Lee et al., 2026).

### Gene Editing for Disease Resistance

The UF strawberry breeding program provides a direct commercialization path for disease-resistant varieties to reach Florida growers. New varieties with better resistance will bring increased profitability to the Florida strawberry industry. The Florida Strawberry Growers Association estimates that diseases cost the Florida industry at least \$15 million each year. In the last five years, the UF strawberry breeding program has identified regions of strawberry chromosomes that control fruit flavor and multiple disease resistance (Barbey et al. 2021; Chandra et al. 2021; Nelson et al. 2021; Noh et al. 2018; Oh et al. 2021; Oh et al. 2020; Oh et al. 2019; Salinas et al. 2020). Neopestalotiopsis resistance has remained the first priority given its severity: a genome-wide association study of UF breeding germplasm found that fewer than 12% of elite lines carry resistance and identified two resistance loci (Alam et al. 2024). Our goal is to add Neopestalotiopsis resistance to Florida Brilliance™,

Sweet Sensation® 'Florida127', and newer cultivars such as Florida Medallion™ and Florida Encore™, which remain highly susceptible to this disease — in contrast to Florida Ember™, which was released in 2023 in part for its improved Neopestalotiopsis resistance. Evaluations of the gene-edited lines will be performed in concert with crosses to integrate the changes into other major varieties and advanced selections with conventional hybridization.

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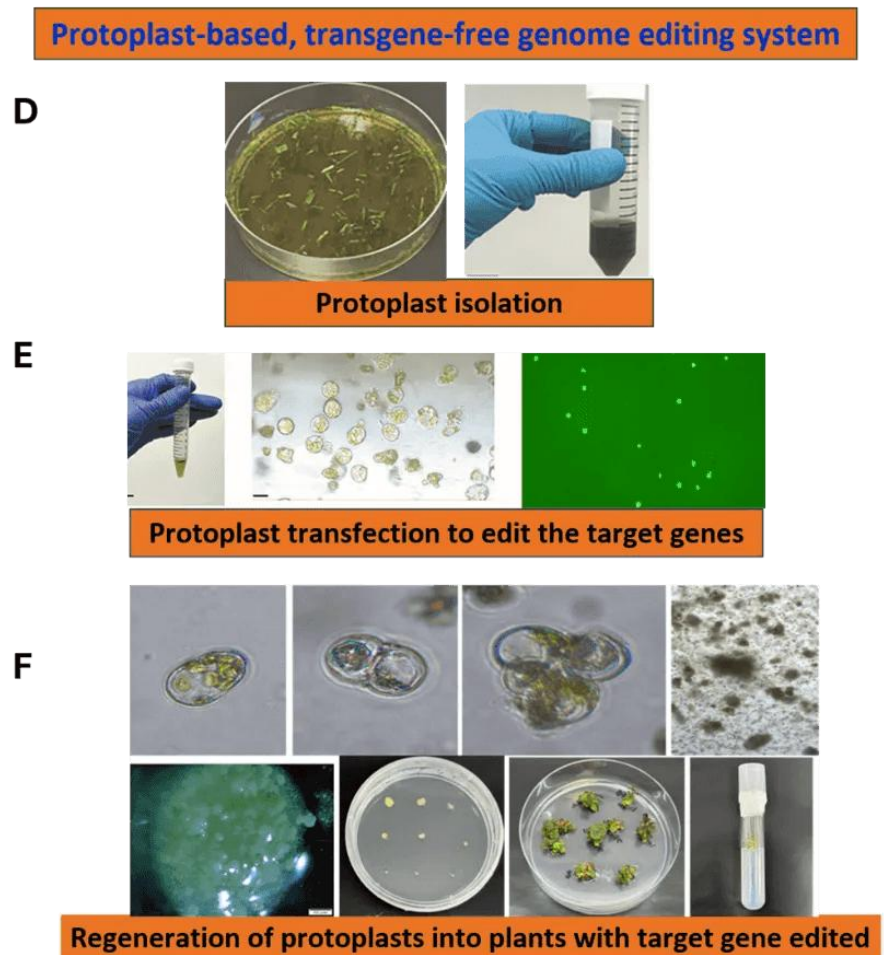
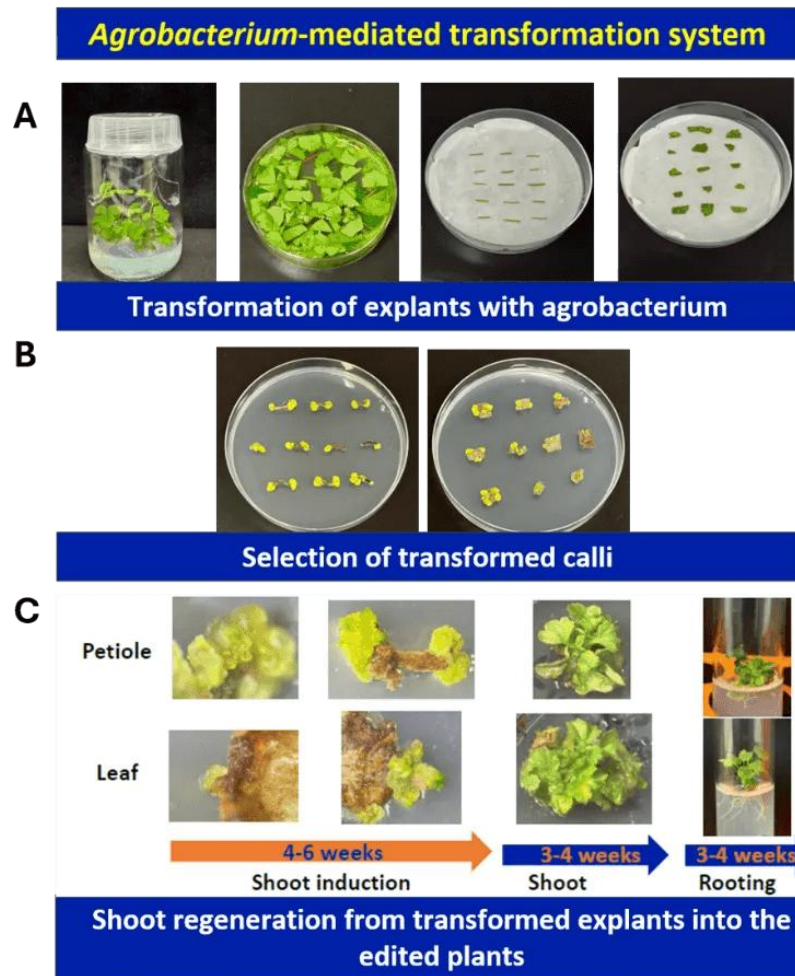


Figure 1. Two genome editing systems for cultivated strawberry: *Agrobacterium*-mediated transformation and protoplast-based, transgene-free genome editing. A. Transformation of explants with *Agrobacterium*; B. Selection of transformed calli; C. Shoot regeneration and rooting of transformed explants; D. Isolation of strawberry protoplasts; E. Transfection of protoplasts for target gene editing; F. Regeneration of gene-edited plants from protoplasts. (long description)

Credit: Antt Htet Wai, UF/IFAS

## Long Description of Figure 1

A two-column scientific infographic compares two plant genetic engineering workflows. The left column, titled “*Agrobacterium*-mediated transformation system,” contains panels A–C showing explant transformation, transgenic shoot initiation in calli, and regeneration of edited plants. The right column, titled “Protoplast-based, transgene-free genome editing system,” contains panels D–F showing protoplast isolation, transfection for target-gene editing, and regeneration into plants.

In panel A, photographs show green plant explants in a culture vessel and petri dishes at successive stages of transformation. Panel B shows two petri dishes containing small yellow-green callus pieces selected on culture medium. Panel C presents a progression from petiole and leaf explants through shoot induction, shoot

development, and rooting, illustrated with close-up images of green tissue and plants in culture containers. Colored arrows indicate stages lasting 4–6 weeks, 3–4 weeks, and 3–4 weeks.

In panel D, a petri dish contains plant tissue in liquid, and a gloved hand holds a tube containing a dark green suspension, illustrating protoplast isolation. Panel E shows a tube, microscope images of rounded protoplasts, and a green fluorescence image with scattered bright points, illustrating transfection. Panel F shows sequential microscope images of developing cells or cell clusters, followed by images of green tissue growing in culture dishes and a culture tube.

Orange and blue banners identify the processes and stages.

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