BACKGROUND INFORMATION

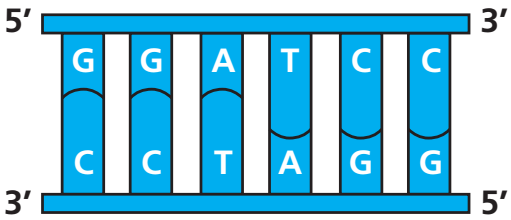
PART 2
Targeted Genome Editing

While original rDNA techniques would often result in random integration of the desired gene(s), newer **genome editing** techniques use tools to target the desired gene or the "edit" to a precise locus in the genome. One genome editing technique currently used by plant scientists is the CRISPR-Cas system. It's part of a natural bacterial defense system that scientists are using to cut and modify DNA more precisely than any previous GE method.

What is CRISPR and how is it used by bacteria?

CRISPR stands for **Clustered Regularly Interspaced Short Palindromic Repeats**. CRISPRs are sequences of nucleotides in the bacterial genome where bacteria keep a record of previous infections by a virus and later use it to identify and fight subsequent attacks by the same virus. When a bacterial cell is infected by a virus, the cell incorporates pieces of the viral DNA into the CRISPR sequence, which then produces small, non-coding RNAs that act like virus detectors. This is a form of **adaptive immunity**.

Sample Palindromic Sequence



The sequence read in one direction on one strand matches the sequence read in the opposite direction on the complementary strand.

Acronym Alert

Early genetic engineering (GE) began about half a century ago, while genome editing is a more recent technique. Although both two-word phrases begin with a G and an E, in this curriculum, genome editing will always be spelled out, and GE refers to the broader category of genetic engineering techniques.

Close to the CRISPRs are **CRISPR-associated (Cas)** genes that encode for Cas proteins. In bacteria, Cas proteins are part of the adaptive immune system. Some Cas proteins help the bacterial cell to capture small pieces of invading viral DNA for insertion into the CRISPR sequences during the initial infection; others silence the attacking virus' DNA during subsequent infections to protect the bacteria. For example, the small RNAs made from the CRISPR sequence containing the previously captured pieces of viral DNA (from the first infection) bind to the Cas9 endonuclease enzyme and target it to cut the viral DNA of repeat invaders.

Developing CRISPR-Cas as a New GE Tool

In 2012-2013, several scientific teams tested whether they could adapt the bacterial CRISPR-Cas immune system for use as a genome editing tool. First, they determined which specific components of the system were needed: The Cas9 enzyme and a guiding RNA. Next, they showed that they could target the Cas9 enzyme to cut a specific locus of their choosing simply by changing part of the guiding RNA sequence to match the targeted genome sequence. Collectively, multiple scientific teams showed CRISPR-Cas9 could be used as a programmable RNA-guided DNA cutting tool in bacteria, plant, mouse, and human cells.

This discovery was important because it meant that scientists could now cut and "edit" genomic DNA at a specific location of their choice. When the cell tries to repair the broken DNA strand by joining the pieces back together, scientists could take advantage of this process to add or remove specific DNA sequences. They could also include a repair template (with a mutation or a new gene entirely) to guide a specific repair by the cell's own mechanisms. In agriculture, genome editing using CRISPR-Cas, or one of several other available DNA targeting and cutting tools, can be used to create plants that produce higher yields, are more nutritious, and have characteristics that will help them endure extreme weather conditions.

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Here's the CRISPR-Cas9 process:

1. The scientist first identifies the precise location for the desired edit in the plant's genome.
2. A small piece of guide RNA is designed to target the DNA sequence at that location.
3. The guide RNA and Cas9 can be introduced into the plant cell as either DNA, RNA, or an RNA-protein complex called a ribonucleoprotein.
4. The guide RNA locates and binds to the targeted plant genomic DNA sequence. Its associated Cas9 enzyme then cuts the DNA at the targeted location.
5. The plant cell's own repair machinery re-attaches the cut DNA ends. During the process, nucleotides may be removed from or added onto the cut DNA ends. This can result in the loss of an undesirable trait or the expression of a new desired trait.
6. The cells are grown into mature plants with edited DNA.
7. The edited DNA is now heritable and can be passed on to the offspring.

Note: Depending on the method by which the guide RNA and Cas9 were introduced, they may not be present in the mature plant.

If the scientist includes a repair template during the plant transformation process (step 3), the repair template will direct the repair of the genomic DNA at the cut site (step 5).

CRISPR-Cas Delivery

There are several possible CRISPR-Cas delivery methods. Plasmid-mediated delivery transforms the cell with a plasmid or plasmids carrying the genes for the guide RNA and Cas protein, similar to rDNA technology. Alternatively, direct delivery of the Cas9 protein with guide RNA into plant cells can be used. The choice of delivery method depends on several factors, including which method is most efficient for the type of plant being edited and whether the scientist's goal is transient or stable expression of the CRISPR-Cas components.

In 2013, scientists discovered how to use the CRISPR-Cas system to edit a plant's genome. Since this discovery, many scientists throughout the world have been working to improve our food supply through genome editing using CRISPR-Cas as well as other targeted DNA cutting systems like TALEN and Zinc Finger Nucleases. These genome editing tools are being used to improve:

- a plant's yield performance
- nutritional value
- tolerance to biotic stress such as viral, fungal, and bacterial diseases
- tolerance to abiotic stress such as environmental conditions, including changes in water availability, temperature, and soil chemistry

The most studied crops are rice, corn, tomato, potato, barley, and wheat. Specific examples of researchers and their projects include scientists at Pennsylvania State University who used genome editing to extend the shelf-life of white mushrooms by disabling an enzyme that causes the mushrooms to brown, and scientists in Spain who used genome editing to modify the genome of wheat strains to be significantly lower in gluten.

The first food produced from a genome-edited crop became commercially available in 2019: High oleic soybean oil is lower in unhealthy fats than original soybean oil. Scientists are continually testing the potential of genome editing techniques to solve a range of food-related problems, such as:

- producing bananas that are resistant to a fungal disease that destroys the crop
- providing a solution to the citrus greening disease that is threatening U.S. orange trees
- protecting the world's chocolate supply by improving the cacao plant's ability to fight a virus that is destroying the crop in West Africa

CRISPR-Cas9