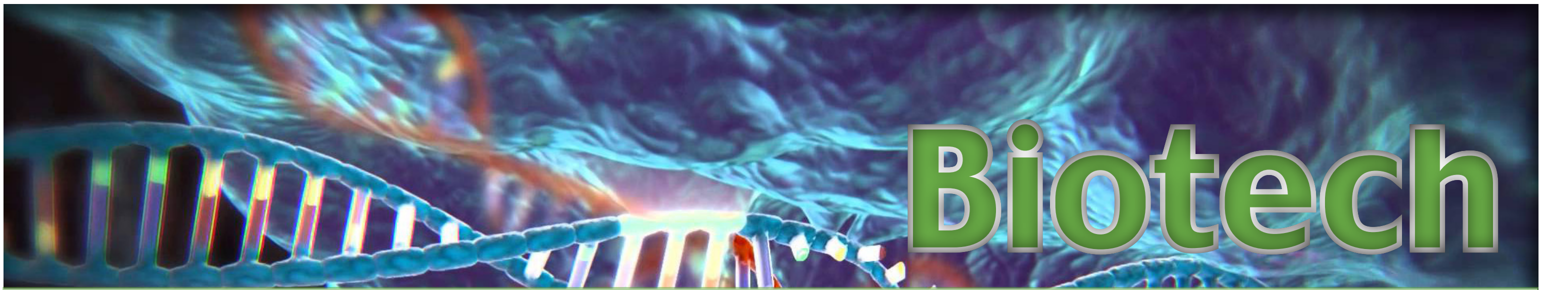
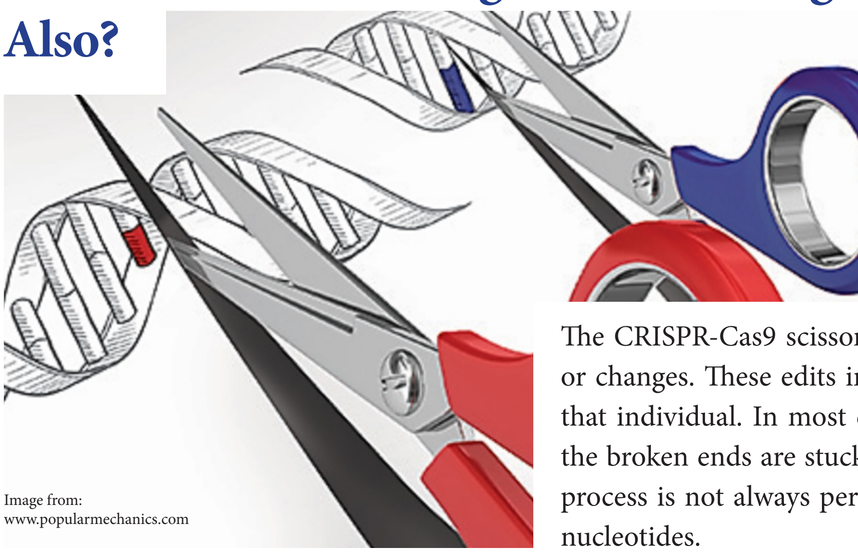


Biotech In focus

Cooperative Extension Service
Biotechnology Outreach Program
College of Tropical Agriculture and Human Resources
University of Hawaiʻi at Manoa

June 2016 Issue 51

CRISPR-Cas9: As Genetic Engineering Is Transformed, Will Regulations Change Also?



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In last month's bulletin, we introduced CRISPR-Cas9, a gene editing tool borrowed from a unique immune-like system discovered in bacteria. CRISPR DNA and Cas proteins allow bacteria to recognize invading viruses and target them for destruction. In the hands of researchers, CRISPR-Cas9 has become a "molecular scissor", that can cut the DNA of most living organisms with remarkable precision, snipping genes only at very specific DNA sequences.

The CRISPR-Cas9 scissors can be used on their own to cause targeted gene edits, or changes. These edits in the DNA sequence can be inherited by the offspring of that individual. In most cases, when a length of DNA is snipped into two pieces, the broken ends are stuck back together using a natural repair process. This repair process is not always perfect and often results in the loss of some DNA letters, or nucleotides.

We can think of a gene's DNA nucleotide sequence as the recipe for a protein, written in a language in which each three nucleotide letters (which actually represent quite complex molecules) of DNA represent a specific step in protein production. Mutations or mistakes that result from DNA repair can make the recipe impossible to read and follow. Providing unprecedented precision, CRISPR-Cas9 has become a popular tool for "knocking out" a gene, damaging the gene to prevent its protein from being made. Similar? mutations or repair mistakes occur naturally and have played a significant role in evolution.

As noted in previous bulletins, mutagens such as radiation or DNA-damaging chemicals can also be used to alter genes for breeding crops, though in a much less targeted way. Mutation breeding has produced many desirable crop traits. Some of the crop plants bred using mutagenesis can:

- retain color, because a protein that limits pigmentation isn't produced;
- resist disease, because a protein that makes the plant vulnerable to attack isn't produced;
- yield more food, because a protein needed for tall growth, for example, isn't produced. (Short plants spend less energy on making stems and are less likely to fall over.)

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Thank you to Jessica Radovich for graphics and Kathleen Vickers for text editing.

Temporary Agent

Of course, eating mutagenesis-bred ‘Rio Red’ grapefruit or ‘Calrose 76’ rice doesn’t cause us any DNA damage. The crops bred using radiation breeding aren’t radioactive, and crops bred using chemical mutagens aren’t poisonous. In mutation breeding, the crop DNA is permanently changed, but the agent that changes the DNA doesn’t become part of the plant. The same is true for CRISPR-Cas9 knock-out crops. The CRISPR DNA and the gene that produces Cas9 protein can be added to cells temporarily, without becoming part of the genome. The resulting mutations are passed down to the next generation of cells, but the CRISPR and Cas9 genes are not.

In the United States, crops are considered genetically modified (GM) if they contained new, foreign genes. These human-assembled transgenes typically include the DNA recipe for a protein that provides a new trait, such as herbicide tolerance or pesticide production, plus DNA sequences that instruct cells to produce the new protein abundantly and consistently. In most cases, a gene for antibiotic resistance is added to help researchers identify which of the treated cells has a working version of the new gene.

Applying Regulations

Because CRISPR-Cas9 can knock out a plant gene without adding a transgene to the plant’s genome, the regulations developed for GM crops may not apply to crops developed using this method. For example, a scientist recently used CRISPR-Cas9 to knock out a gene that normally causes white button mushrooms to turn brown, a color that many consumers find unappealing. The new CRISPR-bred mushrooms do not turn brown also have a longer life after harvest and are better suited to mechanical harvesting.

Crop Assesement

The scientist asked the U.S. Department of Agriculture to determine whether the new mushrooms would be regulated like earlier GM crops. This request is required as the USDA evaluates biotechnology. The USDA is required to assess crops that have been modified with genetic traits that change them in a way that they become weed-like.

Implications

In April 2016, USDA’s Animal and Plant Health Inspection Service (APHIS) responded to the scientist’s request for clarification. USDA APHIS accepted DNA evidence showing that no transgenes are present in the mushroom genome and concluded that the mushroom is not likely to become a pest. In a decision that could have important implications for U.S. food policy, USDA APHIS reported that the agency does not plan to regulate the CRISPR-bred mushrooms as a transgenic crop.

CRISPR-Cas9 is a remarkable way to delete genes, but it can also be used to add new genes to almost any living species more complex than bacteria. In our next bulletin, we will consider some of the ways in which this cut-and-paste gene editing might shape our future.

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