FDA U.S. FOOD & DRUG ADMINISTRATION

LifeSmarts
Learn it. Live it.

BIOTECHNOLOGY

GENETIC ENGINEERING IN FOOD

The "Genetic Engineering in Food" module takes students on an exciting journey into the world of genetically modified organisms (GMOs) and their impact on the food we eat. This module provides a deep dive into the science of gene editing, showcasing how techniques like CRISPR are used to enhance crop yields, improve nutritional content, and combat pests. Students will gain valuable insights into the ethical considerations and safety regulations surrounding GMOs, directly connecting to LifeSmarts topics such as consumer rights, food safety, and environmental stewardship. Developed by educators and FDA experts, the lesson is enriched with informative videos, thought-provoking discussion questions, and real-world case studies that make the complex science of genetic engineering accessible and engaging. Teachers will find the vocabulary and enrichment activities particularly useful for helping students prepare for LifeSmarts competitions and fostering a deeper understanding of the technology shaping our food systems.

DISCUSSION QUESTIONS

- How does genetic engineering differ from traditional selective breeding in agriculture, and what advantages does it offer for modern food production?
- Discuss the role of CRISPR-Cas9 in genetic engineering. How has this technology revolutionized the field, and what are its potential applications in agriculture?

CHALLENGE QUESTION

- Research a recent advancement in the use of CRISPR technology in agriculture. What are the implications of this advancement for food production and sustainability?

See this lesson and more at LifeSmarts U.

This lesson was developed by educators and experts in conjunction with the U.S. Food & Drug Administration

VOCABULARY

- Genetic Engineering
- CRISPR-Cas9
- Transgene
- Plasmid
- Genome Editing
- Selective Breeding
- Recombinant DNA (rDNA)
- Transformation
- Agrobacterium Tumefaciens
- Gene Silencing

ACTIVITIES

- Genetic Engineering: Intro
- Targeted Genome Editing (CRISPR-Cas - A Genome Editing System)

VIDEOS

* See reverse side for list

FDA U.S. FOOD & DRUG ADMINISTRATION

LifeSmarts Learn it. Live it.

VIDEO LINKS

Soybean Genetic Modification (6:30)
www.youtube.com/watch?v=wTraZwHDHXk

Herbert W. Boyer & Stanley N. Cohen (6:49)
www.youtube.com/watch?v=G3H-Uzts108

Transferring genes into plants (1:35)
www.youtube.com/watch?v=HOLaRq6ahzo

Bacterial Transformation 3D Animation (2:01)
https://vimeo.com/170630548

Gene editing yields tomatoes that flower and ripen weeks earlier (2:50)
www.youtube.com/watch?v=Jem3hP734uA

CRISPR Gene Editing Explained (2:11)
https://video.wired.com/watch/crispr-gene-editing-explained

CRISPR Explained (Mayo Clinic) (1:38)
www.youtube.com/watch?v=UKbrwPL3wXE

CRISPR – A Word Processor for Editing the Genome (6:09)
www.ibiology.org/genetics-and-gene-regulation/crispr

Who Wants to Be a Genetic Engineer - Crop Genetic Engineering Simulation
https://mediahub.unl.edu/media/10740

Transformation in Bacteria
www.youtube.com/watch?v=dKD19cXkWBw

How to Make a Genetically Modified Plant
www.youtube.com/watch?v=JtkhHIG3nx4&t=365s

CRISPR – History of Discovery
www.youtube.com/watch?v=RKh2mi3tsmc

Nature Video: CRISPR Gene Editing and Beyond
www.youtube.com/watch?v=4YKFw2KZA5o

New Gene Editing Tool May Yield Bigger Harvests
www.youtube.com/watch?v=UUo6IxLRbQ4

What is CRISPR-Cas?
www.youtube.com/watch?v=52jOEPzhpzc

Future Predictions – Food Technology and Science
www.youtube.com/watch?v=GCXhdAGx3NI

OTHER WEB LINKS

Why Gene Editing Is the Next Food Revolution
https://www.nationalgeographic.com/environment/future-of-food/food-technology-gene-editing

A Visual Guide to Genetic Modification
https://blogs.scientificamerican.com/sa-visual/a-visual-guide-to-genetic-modification

DISCUSSION QUESTIONS (SAMPLE ANSWERS)

- A: Genetic engineering differs from traditional selective breeding in that it allows scientists to directly modify the DNA of an organism, rather than relying on the slow process of crossbreeding over multiple generations. This technique can target specific genes responsible for desired traits, such as drought resistance or enhanced nutritional value, and introduce these genes into the organism's genome. The advantage of genetic engineering is that it is faster and more precise, reducing the likelihood of unwanted traits being passed on (a problem known as linkage drag in selective breeding). This precision also enables the creation of crops that are better suited to meet the challenges of modern agriculture, such as climate change, pests, and food security.

- A: CRISPR-Cas9 is a powerful tool in genetic engineering that allows scientists to make precise edits to an organism's DNA. Unlike earlier methods of genetic modification, CRISPR-Cas9 can target specific locations in the genome, making it possible to add, remove, or alter DNA sequences with high accuracy. This technology has revolutionized the field by enabling the development of crops with enhanced traits, such as improved resistance to diseases, increased yield, and better nutritional profiles. The potential applications in agriculture are vast, including the creation of crops that can withstand extreme weather conditions, reduce the need for chemical pesticides, and address global food security challenges.

CHALLENGE QUESTION (SAMPLE ANSWER)

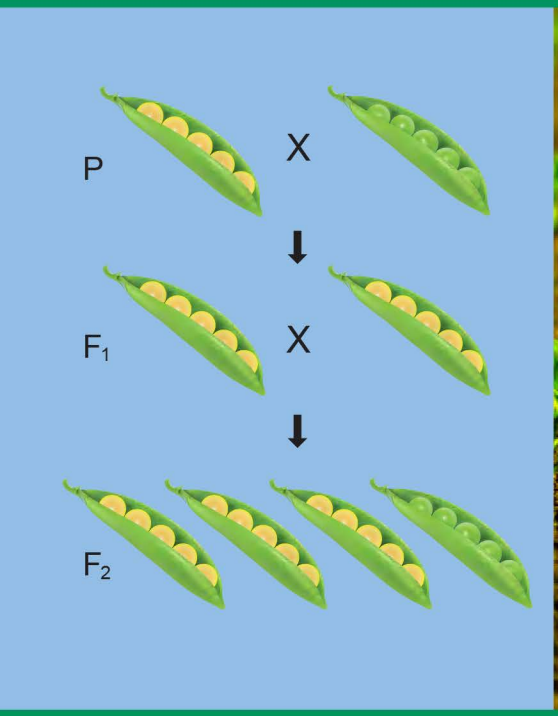
- **A recent advancement is the use of CRISPR technology to create drought-resistant rice.**
  https://www.youtube.com/watch?v=ItEL8fOMkRw

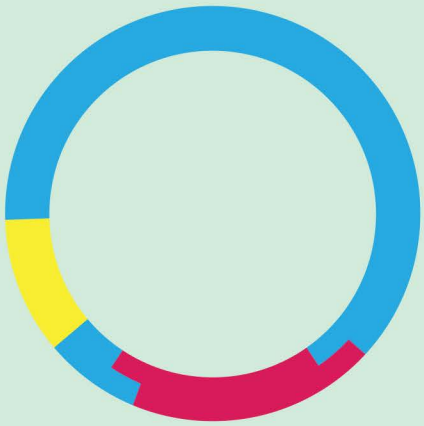
  Key Points:
  Details: Scientists used CRISPR to edit the genome of rice plants, enhancing their ability to survive in low-water conditions. This modification is particularly significant for regions where water scarcity threatens food security.

  Implications: The development of drought-resistant rice has the potential to improve food production in arid regions, contributing to sustainability by reducing the need for irrigation. This advancement could help stabilize food supplies in areas affected by climate change, ensuring that communities can continue to produce staple crops despite increasingly erratic weather patterns.

SCIENCE AND OUR FOOD SUPPLY

Biotechnology - Genetic Engineering in Food





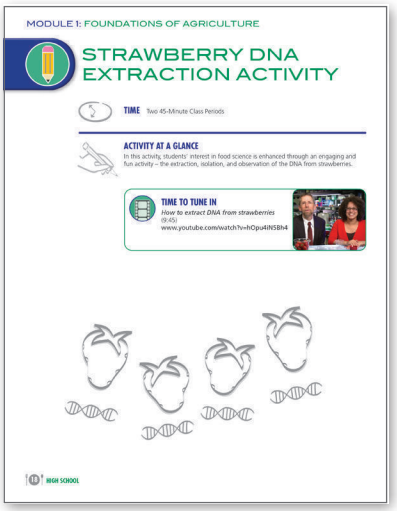
Teacher's Guide for High School Classrooms

1st Edition

FDA

OVERVIEW OF ACTIVITIES

The activities are written in this easy-to-understand format.


MODULE 1: FOUNDATIONS OF AGRICULTURE

STRAWBERRY DNA EXTRACTION ACTIVITY

TIME Two 45-Minute Class Periods

ACTIVITY AT A GLANCE

In this activity, students' interest in food science is enhanced through an engaging and fun activity – the extraction, isolation, and observation of the DNA from strawberries.

TIME TO TUNE IN

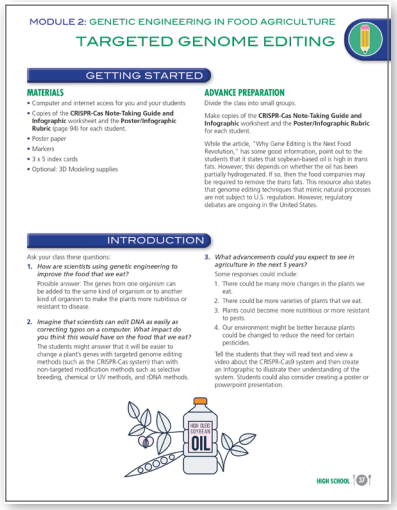
How to extract DNA from strawberries (9:45)
www.youtube.com/watch?v=hOpu4iN5Bh4

HIGH SCHOOL

TIME: The approximate amount of time needed to perform the activity.

ACTIVITY AT A GLANCE: Briefly summarizes the activity.

TIME TO TUNE IN: Shows the URL for an online video related to that activity.


MODULE 2: GENETIC ENGINEERING IN FOOD AGRICULTURE

TARGETED GENOME EDITING

GETTING STARTED

MATERIALS

- Computer and internet access for you and your students
- Copies of the **CRISPR-Cas Note-Taking Guide and Infographic** worksheet and the **Poster/Infographic Rubric** (page 94) for each student.
- Poster paper
- Markers
- 3 x 5 index cards
- Optional: 3D Modeling supplies

ADVANCE PREPARATION

Divide the class into small groups.

Make copies of the **CRISPR-Cas Note-Taking Guide and Infographic** worksheet and the **Poster/Infographic Rubric** for each student.

While the article, "Why Gene Editing is the Next Food Revolution," has some good information, point out to the students that it states that soybean-based oil is high in *trans* fats. However, this depends on whether the oil has been partially hydrogenated. If so, then the food companies may be required to remove the *trans* fats. This resource also states that genome editing techniques that mimic natural processes are not subject to U.S. regulation. However, regulatory debates are ongoing in the United States.

INTRODUCTION

Ask your class these questions:

1. ***How are scientists using genetic engineering to improve the food that we eat?***
   Possible answer: The genes from one organism can be added to the same kind of organism or to another kind of organism to make the plants more nutritious or resistant to disease.
2. ***Imagine that scientists can edit DNA as easily as correcting typos on a computer. What impact do you think this would have on the food that we eat?***
   The students might answer that it will be easier to change a plant's genes with targeted genome editing methods (such as the CRISPR-Cas system) than with non-targeted modification methods such as selective breeding, chemical or UV methods, and rDNA methods.
3. ***What advancements could you expect to see in agriculture in the next 5 years?***
   Some responses could include:
   1. There could be many more changes in the plants we eat.
   2. There could be more varieties of plants that we eat.
   3. Plants could become more nutritious or more resistant to pests.
   4. Our environment might be better because plants could be changed to reduce the need for certain pesticides.

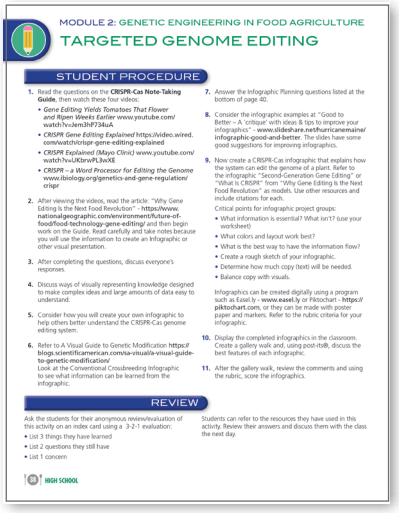
Tell the students that they will read text and view a video about the CRISPR-Cas9 system and then create an infographic to illustrate their understanding of the system. Students could also consider creating a poster or powerpoint presentation.

HIGH SCHOOL

MATERIALS: Includes the items needed to perform the activity.

ADVANCE PREPARATION: Indicates what you need to do before conducting the activity.

INTRODUCTION: Provides fun, innovative suggestions for introducing the activity. Where provided, suggested teacher dialogue is indicated by ***boldface italics***.


MODULE 2: GENETIC ENGINEERING IN FOOD AGRICULTURE

TARGETED GENOME EDITING

STUDENT PROCEDURE

1. Read the questions on the **CRISPR-Cas Note-Taking Guide**, then watch these four videos:
   - *Gene Editing Yields Tomatoes That Flower and Ripen Weeks Earlier* www.youtube.com/watch?v=Jem3hP734uA
   - *CRISPR Gene Editing Explained* https://video.wired.com/watch/crispr-gene-editing-explained
   - *CRISPR Explained (Mayo Clinic)* www.youtube.com/watch?v=JKbrvePLJvXE
   - *CRISPR – a Word Processor for Editing the Genome* www.ibiology.org/genetics-and-gene-regulation/crispr
2. After viewing the videos, read the article: "Why Gene Editing Is the Next Food Revolution" – **https://www.nationalgeographic.com/environment/future-of-food/food-technology-gene-editing/** and then begin work on the Guide. Read carefully and take notes because you will use the information to create an infographic or other visual presentation.
3. After completing the questions, discuss everyone's responses.
4. Discuss ways of visually representing knowledge designed to make complex ideas and large amounts of data easy to understand.
5. Consider how you will create your own infographic to help others better understand the CRISPR-Cas genome editing system.
6. Refer to A Visual Guide to Genetic Modification **https://blogs.scientificamerican.com/sa-visual/a-visual-guide-to-genetic-modification/**
   Look at the Conventional Crossbreeding Infographic to see what information can be learned from the infographic.
7. Answer the Infographic Planning questions listed at the bottom of page 40.
8. Consider the infographic examples at "Good to Better – A 'critique' with ideas & tips to improve your infographics" – **www.slideshare.net/hurricanemaine/infographic-good-and-better**. The slides have some good suggestions for improving infographics.
9. Now create a CRISPR-Cas infographic that explains how the system can edit the genome of a plant. Refer to the infographic "Second-Generation Gene Editing" or "What Is CRISPR" from "Why Gene Editing Is the Next Food Revolution" as models. Use other resources and include citations for each.
   Critical points for infographic project groups:
   - What information is essential? What isn't? (use your worksheet)
   - What colors and layout work best?
   - What is the best way to have the information flow?
   - Create a rough sketch of your infographic.
   - Determine how much copy (text) will be needed.
   - Balance copy with visuals.

   Infographics can be created digitally using a program such as Easel.ly – **www.easel.ly** or Piktochart – **https://piktochart.com**, or they can be made with poster paper and markers. Refer to the rubric criteria for your infographic.
10. Display the completed infographics in the classroom. Create a gallery walk and, using post-its®, discuss the best features of each infographic.
11. After the gallery walk, review the comments and using the rubric, score the infographics.

REVIEW

Ask the students for their anonymous review/evaluation of this activity on an index card using a 3-2-1 evaluation:

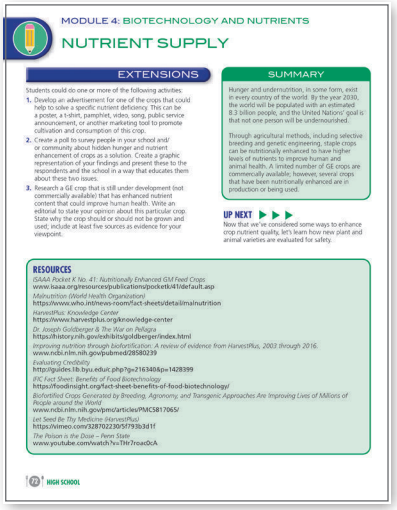
- List 3 things they have learned
- List 2 questions they still have
- List 1 concern

Students can refer to the resources they have used in this activity. Review their answers and discuss them with the class the next day.

HIGH SCHOOL

STUDENT PROCEDURE: Gives the step-by-step process for the activity.

REVIEW: Uses interesting questions to guide students through a review of what they learned in the activity.


MODULE 4: BIOTECHNOLOGY AND NUTRIENTS

NUTRIENT SUPPLY

EXTENSIONS

Students could do one or more of the following activities:

1. Develop an advertisement for one of the crops that could help to solve a specific nutrient deficiency. This can be a poster, a t-shirt, pamphlet, video, song, public service announcement, or another marketing tool to promote cultivation and consumption of this crop.
2. Create a poll to survey people in your school and/or community about hidden hunger and nutrient enhancement of crops as a solution. Create a graphic representation of your findings and present these to the respondents and the school in a way that educates them about these two issues.
3. Research a GE crop that is still under development (not commercially available) that has enhanced nutrient content that could improve human health. Write an editorial to state your opinion about this particular crop. State why the crop should or should not be grown and used, include at least five sources as evidence for your viewpoint.

SUMMARY

Hunger and undernutrition, in some form, exist in every country of the world. By the year 2030, the world will be populated with an estimated 8.3 billion people, and the United Nations' goal is that not one person will be undernourished.

Through agricultural methods, including selective breeding and genetic engineering, staple crops can be nutritionally enhanced to have higher levels of nutrients to improve human and animal health. A limited number of GE crops are commercially available; however, several crops that have been nutritionally enhanced are in production or being used.

UP NEXT ▶ ▶ ▶

Now that we've considered some ways to enhance crop nutrient quality, let's learn how new plant and animal varieties are evaluated for safety.

RESOURCES

ISAAA Pocket K No. 41: Nutritionally Enhanced GM Food Crops:
www.isaaa.org/resources/publications/pocketk41/default.asp

Malnutrition (World Health Organization)
https://www.who.int/news-room/fact-sheets/detail/malnutrition

HarvestPlus: Knowledge Center
https://www.harvestplus.org/knowledge-center

Dr. Joseph Goldberger & The War on Pellagra
https://history.nih.gov/exhibits/goldberger/index.html

Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016.
www.ncbi.nlm.nih.gov/pubmed/28580239

Evaluating Credibility
http://guides.lib.byu.edu/c.php?g=216340&p=1428399

IFIC Fact Sheet: Benefits of Food Biotechnology
https://foodinsight.org/fact-sheet-benefits-of-food-biotechnology/

Biofortified Crops Generated by Breeding, Agronomy, and Transgenic Approaches Are Improving Lives of Millions of People around the World
www.ncbi.nlm.nih.gov/pmc/articles/PMC5817065/

Let Seed Be Thy Medicine (HarvestPlus)
https://vimeo.com/328702230/5f7930d1f

The Poison is the Dose – Penn State
www.youtube.com/watch?v=THr7roaxDcA

HIGH SCHOOL

SUMMARY: Summarizes key concepts learned in the activity.

EXTENSIONS: Suggest activities to help students learn more about the topic.

RESOURCES: Provide references to online resources for the activity or for further study.

UP NEXT: Gives a preview of the next activity.

MODULE 2

GENETIC ENGINEERING IN FOOD AGRICULTURE

This module introduces students to laboratory methods used to alter genetic material and create organisms with desired traits.

For this module, it is recommended that teachers will have already taught students the following underlying key concepts: Cell structure and function, DNA structure, DNA replication, bacterial structure (prokaryotes), basic cell division and reproduction, transcription, translation, protein expression and synthesis, and general selective breeding.

BACKGROUND INFORMATION: PART 1

Genetic Engineering introduces some key milestones in the development of tools used in the laboratory to change DNA sequences. It also highlights select genetic modification processes.

ACTIVITY

Genetic Engineering introduces students to laboratory techniques developed to change an organism's genetic material to give it a new trait.

Time to Tune In

Soybean Genetic Modification (6:30)
www.youtube.com/watch?v=wTraZwHDHXk

Herbert W. Boyer & Stanley N. Cohen (6:49)
www.youtube.com/watch?v=G3H-Uzts108

Transferring genes into plants (1:35)
www.youtube.com/watch?v=HOLaRq6ahzo

Bacterial Transformation (2:04)
https://vimeo.com/170630548

BACKGROUND INFORMATION: PART 2

Targeted Genome Editing discusses cutting-edge technology now being used to "edit" DNA.

ACTIVITY

CRISPR-Cas – A Genome Editing System allows students to explore how one technology is used to target a specific DNA locus to delete, change, or insert DNA sequences. Genome editing systems can be either transgenic or work without inserting DNA from another organism.

Time to Tune In

Gene editing yields tomatoes that flower and ripen weeks earlier (2:50)
www.youtube.com/watch?v=Jem3hP734uA

CRISPR Gene Editing Explained (2:11)
https://video.wired.com/watch/crispr-gene-editing-explained

CRISPR Explained (Mayo Clinic) (1:38)
www.youtube.com/watch?v=UKbrwPL3wXE

CRISPR – a Word Processor for Editing the Genome (6:09)
www.ibiology.org/genetics-and-gene-regulation/crispr

BACKGROUND INFORMATION

PART 1
Genetic Engineering

For most of history, farmers had to wait several plant generations before crops had the traits they most desired. The farmers used selective breeding, the process of choosing parent plants with the best traits over many generations. Selective breeding resulted in dramatic genetic changes to the species. While earlier farmers had no concept of the science of genetics, selective breeding based on observable traits allowed them to use plants' DNA to solve agricultural challenges and to improve the food supply. This approach to selecting specific traits is exemplified by the apple activity in Module 1.

Although selective breeding is still widely used, there are more modern processes available to alter the genetics of microorganisms, plants, and animals. More modern techniques to alter an organism's genetics includes mutation breeding, molecular marker-assisted breeding, genetic engineering, and genome editing.

Genetic engineering (GE) refers to deliberately modifying the characteristics of an organism by altering its genetic material. GE techniques include particle bombardment, Agrobacterium-mediated transformation, and targeted genome editing (the most recent additions to the genetic engineer's toolbox). Using GE technology, scientists can bring us improved agricultural products and practices faster than in the past.

Why genetically engineer plants?

Plants are genetically engineered for many of the same reasons that selective breeding is used: Better nutrition, higher **crop yield** (output), greater resistance to insect damage, and immunity to plant diseases.

Selective breeding techniques involve repeatedly cross-breeding plants until the breeder identifies offspring that have inherited the genes responsible for the desired combination of traits. However, this method may also result in the inheritance of unwanted genes responsible for unwanted traits (called **linkage drag**), and it can result in the loss of desired traits.

GE techniques can be used to isolate a gene or genes for the desired trait, add a gene from another organism or edit chromosomal DNA in a single plant cell, and generate a new plant with the trait from that cell. By adding one desired gene from the donor organism or by editing the gene in the chromosomal DNA of the single cell, the unwanted traits

Key biotechnology events related to food agriculture

1901	Japanese biologist Shigetane Ishiwatari discovered *Bacillus thuringiensis* (Bt), which makes a natural pesticide, found in soil worldwide and used by farmers since the 1920s.
1919	Karoly Ereky introduced the new term *biotechnology* (i.e., using biological systems to create products).
1971	Paul Berg completed a landmark gene splicing experiment.
1973	Stanley Cohen and Herbert Boyer created the first modified organism using recombinant DNA (rDNA) technology.
1974	Rudolf Jaenisch and Beatrice Mintz created the first transgenic animal (a mouse).
1978	Herbert Boyer starts a new company, Genentec and produces recombinant insulin.
1983	Mary-Dell Chilton inserted an antibiotic-resistant gene into a tobacco plant creating the first GE plant.
1987	Calgene creates the FlavrSavr® tomato.
1989	Chymosin from GE microorganisms authorized as a food processing aid by FDA.
1994	FDA concludes the FlavrSavr® tomato is as safe as comparable non-GE tomatoes.
1995	EPA approves the use of a Bt toxin as a plant-incorporated pesticide in a GE crop.
1998	GE virus-resistant papaya was grown commercially in Hawaii.
2012	CRISPR-Cas9 is used as a programmable RNA-guided DNA cutting tool.
2015	Genetically modified salmon is the first GE animal approved for food use in the United States.
2017	GE apples are available for sale in the United States.
2019	FDA completes consultation of high oleic soybean oil, first food from a genome edited plant.

BACKGROUND INFORMATION

from the donor's other genes can be excluded. GE is used in conjunction with selective breeding to produce GE plant varieties that are on the market today.

Advanced Content

GE techniques can be used to add new DNA to code for the expression of a new protein or to suppress expression of a native plant protein. Protein suppression can be achieved though transcriptional or post-transcriptional gene silencing (PTGS). **RNA interference (RNAi)** is a form of PTGS that targets mRNA transcripts for cleavage, preventing their translation into protein. The two different ways to achieve a desired trait are important, because both have been used to create GE plants that are used in food today.

Development of GE Tools in Bacteria

Throughout the past 100 years, several developments have led to current GE methods. After early geneticists were able to identify the gene locus for specific plant traits, various methods were used to try to transfer the specific DNA sequence from one plant to another. One method was injecting the DNA from the donor plant directly into the recipient plant cell to see if it would integrate into the recipient cell's genome. Unfortunately, the DNA was degraded, and the method was unsuccessful. It was like trying to send an envelope through the mail with only a zip code; the postal service wouldn't know where to deliver it. Scientists eventually used bacteria to transfer new DNA to the recipient plant cell.

Transformation is the changing of the cell's genetic makeup through the addition of new DNA. The DNA can come from the environment surrounding the cell via "**horizontal gene transfer**" or be added in a laboratory through GE methods. The laboratory method developed to combine genetic sequences that would not otherwise be found in the genome is called **recombinant DNA (rDNA)** technology.

In 1973, Herbert Boyer and Stanley Cohen produced the first successful GE organism. Boyer had expertise using **restriction endonucleases** (enzymes that cut DNA at specific nucleotide sequences), and Cohen studied **plasmids** (small rings of DNA) in bacteria. They were able to use a restriction enzyme to cut open a plasmid loop from one bacterial species, insert a gene from a different bacterial species, and close the plasmid, which combined the genes from different bacteria into one rDNA molecule. An enzyme called ligase was used to help join the cut DNA strand. Then they transformed this rDNA plasmid into the bacterium *Escherichia coli (E. coli)* and showed that the bacteria could utilize the rDNA. In Boyer and Cohen's experiment, one gene coded for tetracycline resistance and the other for kanamycin resistance. Tetracycline and kanamycin are antibiotics that kill bacteria that do not have resistance genes. It was possible to see which of the *E. coli* in their experiment had successfully acquired the new genes by culturing them in the presence of the antibiotics, where only the successfully transformed bacteria could grow. These experiments showed that bacterial transformation could be used to deliver the desired DNA to a useful site, just as the postal service delivers mail to the correct address.

Restriction enzymes are like scissors that cut DNA at specific sequences. Some restriction enzymes leave blunt DNA ends while others leave short, single-stranded overhangs called sticky ends.

Ligase enzymes are like the glue or tape for connecting DNA sequences in GE, or molecular biology, procedures.

Bacterial transformation still serves as the basis for a number of DNA technologies. Bacteria are used extensively in the laboratory for rDNA research. There are even some species of bacteria that go through the transformation process naturally, but most bacteria needs manipulation to become **competent** (able to take up the plasmid). Using the techniques from bacterial transformation, scientists have learned how to change the genome of plants, including plants that we use for food.

Scientists worldwide continue to use the Boyer and Cohen techniques to improve GE tools that develop, modify, and improve consumer products, including many of the food products we eat.

Nature's Own Genetic Engineer

A widely used method of transferring a transgene to a plant is to use the soil bacterium *Agrobacterium tumefaciens* (*A. tumefaciens*). This bacterium has a natural ability to enter a plant cell and insert its own DNA into a plant's genome. A plasmid is constructed to include *A. tumefaciens* genes needed for transferring DNA into the recipient plant cell, the transgene of interest, and a selectible marker, such as a gene conferring antibiotic-resistance or herbicide tolerance. Scientists now use the bacterium's natural behavior to insert the transgene into a plant's genome.

BACKGROUND INFORMATION

Simplified Steps of Plasmid Development

Application of GE Tools in Plants

Plants can be genetically engineered to be resistant to pests and herbicides, to increase crop yield, or to tolerate adverse weather conditions using a process similar to bacterial transformation. Plants can also be engineered to produce fruits and vegetables that have longer and more stable shelf-lives in the grocery store. These GE uses have potential trickle-down benefits from the farmer to consumers, animals, and the environment. Because plants are eukaryotic and contain a nucleus, a slightly different method than the one used for bacterial transformation is used to insert the gene of interest.

For example, if scientists find a gene for enhanced drought resistance in a plant, and they want to use the gene to make another plant more drought resistant, an advantage of GE over selective breeding is that less time is required and linkage drag is avoided. The desired gene to be transferred and added to the genome of the recipient plant is often referred to as a **transgene**.

Genetic Engineering

- Allows the direct transfer of one or just a few genes between either closely or distantly related organisms
- Achieves crop improvement in a shorter time compared to conventional breeding
- Allows plants to be modified by adding, removing, or switching off particular genes

Adapted from: Agricultural Biotechnology (A Lot More than Just GM Crops).
www.isaaa.org/resources/publications/agricultural_biotechnology/download/Agricultural_Biotechnology.pdf

The technologies used to clone or synthesize genes are changing and evolving. The three major methods currently used are:

- Traditional cloning – isolating DNA directly from the genome of the donor organism and inserting it into a plasmid for later use
- Subcloning the gene of interest – copying the gene from an existing collection of DNA clones ("DNA library")
- *De novo* gene synthesis – building a gene from scratch, using single nucleotides or short oligonucleotide strands without the need for a physical template

The techniques used by scientists to assemble and insert DNA pieces into the plasmid are also evolving along with the complexity of multi-gene DNA constructs. While simple restriction enzyme protocols can be used to create a single gene insert, multi-gene constructs such as those required for complex plant traits require more complex assembly strategies.

What is a DNA Library?

A DNA library is a collection of cloned DNA fragments that are stored in plasmids, which in turn are maintained and propagated in bacterial or yeast cells. The type of library is classified by the source of the DNA and the plasmid – referred to as a cloning vector – used to construct the library. Sources of DNA may be a single cell, a tissue, an organism, or an environmental sample containing multiple organisms. The DNA may be obtained from genomic sequences or from isolated mRNA and converted to complementary DNA (cDNA). Scientists use DNA libraries to find and study DNA encoding proteins or other functions of interest.

BACKGROUND INFORMATION

General Plasmid Preparation

Bacterial plasmids are used to store a ready supply of the gene of interest. In the case of Agrobacterium-mediated plant transformation, the plasmids are used to transfer the gene of interest to the genome of the recipient plant. To receive the gene of interest, the bacterial plasmids are treated with a restriction enzyme that is compatible with the gene. This way, the plasmid DNA will have the same sticky ends as the gene, so they will combine more easily. The gene and plasmid DNA preparations are mixed with DNA ligase to seal the sticky ends of the DNA molecules together.

Scientists may also modify the bacterial plasmid using a similar process to insert one or more **selectable marker** genes. The selectable marker genes will be important later in the GE process when bacteria or plant cells with the gene of interest are being isolated. There are many selectable markers used to screen for bacterial, as well as plant transformants.

Selectable markers include:

- Auxotrophy (selects for the ability to grow on certain carbon sources)
- Antibiotic resistance (selects for ability to grow in the presence of a specific antibiotic)
- Herbicide tolerance (selects for ability to grow in the presence of a specific herbicide)

This new bacterial plasmid is called a **transformation plasmid** and has the gene of interest as well as the selectable marker gene. The transformation plasmid is added to bacteria using a bacterial transformation method. Finally, the bacteria are plated onto a medium containing the selection factor that will inhibit the growth of bacteria that did not take up the plasmid. The Petri plates are incubated to encourage bacterial growth, and only the bacteria that have taken up the transformation plasmid with the selectable marker gene will grow. Bacteria without it will not grow, resulting in millions of bacteria with the gene of interest in their DNA.

The next step is to transfer the gene to the plant cells. Currently, the most frequently used technique is Agrobacterium-mediated transformation. Bombardment with a gene gun is less common and typically used in cases where Agrobacterium-mediated methods don't work. Agrobacterium is a plant pathogen that has the natural ability to transfer DNA to plant cells. GE methods use a version of the Agrobacterium plasmid that has been "disarmed": the modified plasmid still has the ability to transfer DNA into the plant's genome, but it's disease-causing genes have been removed. Agrobacterium that have been transformed with the plasmid carrying the gene of interest and selectable marker are mixed with the plant cells. The Agrobacterium enters the plant cells and inserts a segment of the plasmid DNA (containing the gene and selectable marker gene) into the plant's genome. Once the Agrobacterium has had time to transform the plant cells, the cells are placed on medium containing: (1) An antibiotic that kills the Agrobacterium, (2) the selection factor that will inhibit growth of plant cells that did not take up the plasmid DNA, and (3) plant hormones that encourage the transformed cells to grow into new plants.

After a gene has been successfully inserted into the plant's genome, the modified plant must be able to grow and reproduce with its newly modified genome. First, the genotype of the plant must be studied so that the scientists only grow plants in which the genome has been modified correctly. When this is done, the GE plants will be grown under controlled conditions in a greenhouse and then in field trials to make sure that the new plants possess the desired new trait and show no new undesired characteristics.

Food from GE Plants

The first GE plant evaluated by the FDA for human consumption was the FlavrSavr® tomato. FDA concluded that the FlavrSavr® tomato was as safe as comparable non-GE tomatoes. It was brought to market in 1994, but it was not sufficiently profitable to continue production. Although there are currently no GE tomatoes on the market, other GE food crops are commercially available. Most of these GE plants were engineered to increase resistance to disease or pests, or tolerance to specific herbicides.

As of 2023, there were 12 food crops available in the U.S. Of these, only a few GE crops in the grocery store are available as whole produce. Whole produce could include certain cultivars of apple, pineapple, potato, papaya, sweet corn, squash, and tomato. Ingredients derived from GE corn, soybeans, sugar beets, and canola (such as flour, oil, starch, and sugar) are used in a wide variety of foods including cereal, corn chips, veggie burgers, and more.

> The 12 GE crops today are: Alfalfa, apples, canola, corn (field and sweet), cotton, papaya, pineapple, potatoes, soybeans, squash, sugar beets, and tomato.

Animal food: In the United States, more than 95 percent of food-producing animals consume food containing ingredients from GE crops. GE plants can also be found in food for non-food producing animals, such as cats and dogs.

ACTIVITY 1: GENETIC ENGINEERING

TIME Three 45-Minute Class Periods

ACTIVITY AT A GLANCE

In addition to selective breeding, GE tools are used by plant breeders to solve agricultural challenges, such as producing enough food to feed a growing global population, or minimizing production impacts on our environment. Plants have been engineered to be more nutritious, more resistant to pests, drought tolerant, and more robust to remain intact during packing and transport. In this activity, students will review the process of bacterial transformation and then look at the processes involved in creating GE plants.

TIME TO TUNE IN

Soybean Genetic Modification (6:30)
www.youtube.com/watch?v=wTraZwHDHXk

Herbert W. Boyer & Stanley N. Cohen (6:49)
www.youtube.com/watch?v=G3H-Uzts108

Transferring genes into plants (1:35)
www.youtube.com/watch?v=HOLaRq6ahzo

Bacterial Transformation 3D Animation (2:01)
https://vimeo.com/170630548

GETTING STARTED

MATERIALS

- Computer and internet access for the teacher and students
- **Genetic Engineering** worksheet
- Set of **The Genetic Engineering Process** cards (10 cards with illustrations and 10 cards with GE process steps). See pages 32-33.
- Mailing Labels – 10 to a sheet; 2 sheets for each set of cards
- 3" x 5" index cards
- Chart paper
- Double-sided tape

ADVANCE PREPARATION

1. Divide the class into small groups.
2. Make a copy of the **Genetic Engineering** worksheet for each student.
3. Make **The Genetic Engineering Process** cards. To make one set of cards, copy the 10 steps in The Genetic Engineering Process on one sheet of 2" x 4" mailing labels and the illustrations for those steps on another sheet of labels. Attach the labels to 3" x 5" index cards. You could also copy the templates on card stock. Making sets in different colors helps keep the sets together. (Make one set of cards for each group.)

   Alternatively, print the card art and text boxes using only one side of each sheet of paper, and cut the sections out for students to compare and match up.

   Remember to mix (shuffle) the cards before handing them out.

GENETIC ENGINEERING

INTRODUCTION

Genetic engineering is often misunderstood, so it is important to determine what your students understand about bacterial transformation and, especially, genetic engineering. Use the KWL (What do you **K**now? – What do you **W**ant to know? – What did you **L**earn?) strategy to begin the activity.

Ask your students what bacterial transformation means to them. Refer them to the **Genetic Engineering** worksheet and ask them to record their thoughts and questions on the chart at the top of the worksheet.

When your students have completed their responses, ask them to share their thoughts with the class. Ask if they have heard the term "genetic engineering" and, if they have, what this term means.

Have them record their ideas and questions.

These questions will help you to assess your students' current understanding, so you can address any of their misconceptions.

Throughout this activity, students should refer back to the questions and comments on their worksheet.

Note: The steps in this activity can be adjusted to match the pace and content you want to emphasize with your students. The activity could follow a vocabulary review and be used to further review vocabulary. It could also be used as a post-assessment of the module's content.

STUDENT PROCEDURE

1. Everyone should have a copy of the **Genetic Engineering** worksheet (page 31). Complete the "What do I know?" and "What do I want to know?" columns of the KWL Chart.

2. Watch the video – *Herbert W. Boyer & Stanley N. Cohen* **www.youtube.com/watch?v=G3H-Uzts108** that explains how the two scientists, Herbert Boyer and Stanley Cohen, were the first to transform bacteria. Complete the "What did I learn" column.

3. Watch the two videos – *Soybean Genetic Modification* **www.youtube.com/watch?v=wTraZwHDHXk** and *Transferring genes into plants* **www.youtube.com/watch?v=HOLaRq6ahzo**. .

   Watch the 3D Animation – *Bacterial Transformation* **https://vimeo.com/170630548**. While watching the animation, answer the questions on the **Genetic Engineering** worksheet. When the worksheet is completed, discuss your responses as a class, so that everyone has a clear understanding of the process of bacterial transformation and the terminology used in the process. Refer to your KWL Charts to make any changes.

4. In the next part of the activity, you will work with a set of cards that represents the steps in the **Genetic Engineering Process**. Your task is to put the cards in the correct procedural order. There are two parts to the card set: one has descriptions of the steps; the other has diagrams that illustrate each step.

5. Each group should have a set of the Genetic Engineering Process cards. Distribute the cards with the text descriptions of the GE steps to your group. The challenge is to put the description cards in the order that reflects the steps in the GE process. As each card is read out, discuss where in the process this step takes place. When your group thinks they have the correct order, share this with the teacher. If the order is correct, match the illustrated card to the description card. Each group member should be able to explain how the group determined the order of the cards and which illustration went with each step.

6. Once your group has successfully arranged your cards, use double-sided tape to attach the cards to a piece of chart paper. Use the charts to explain the steps in the GE process; what information on the cards helped you to put them in the right order? In your own words, describe the GE Process.

GENETIC ENGINEERING

REVIEW

Show the video that was seen at the beginning of the activity: *Herbert W. Boyer & Stanley N. Cohen* **www.youtube.com/watch?v=G3H-Uzts108.** Have students return to their KWL Charts and review their comments and questions about GE. Ask them what further questions they might have, and use these as the basis for the review, addressing any misconceptions the students still have.

SUMMARY

Genetic engineering is the use of modern techniques, including recombinant DNA methods, to modify the genetic information in an organism. It allows for faster trait selection than selective breeding, and can enhance the development of plant cultivars to help address some environmental challenges. Some anticipated changes for the future include: A larger library of genes to choose from as scientists are rapidly sequencing the genomes of organisms, and the ability to modify increasingly complex traits as scientists learn more about the cellular and molecular biology of plants.

EXTENSIONS

Students could do one or more of the following activities:

1. Conduct the Who Wants to Be a Genetic Engineer - Crop Genetic Engineering Simulation **https://mediahub.unl.edu/media/10740**
2. Create an infographic on bacterial or plant transformation.
3. Conduct online research and create a chart of crops that have a specific GE-modified trait.

RESOURCES

- *Transformation in Bacteria* **www.youtube.com/watch?v=dKD19cXkWBw**
- *How to Make a Genetically Modified Plant* **www.youtube.com/watch?v=JtkhHIG3nx4&t=365s**
- *Who Wants to Be a Genetic Engineer - Crop Genetic Engineering Simulation* **www.labxchange.org/library/items/lb:LabXchange:b4d9f467:lx_simulation:1**
- *Changing the Blueprints of Life - Genetic Engineering: Crash Course Engineering #38* **www.youtube.com/watch?v=FY_ZUEKWhBc**

UP NEXT

In the next lesson, we will study another process used to change the genome of a plant – CRISPR.

STUDENT WORKSHEET

ACTIVITY 1: GENETIC ENGINEERING

Name _____ Date _____ Class/Hour _____

KWL CHART			
Exploration Question	**What do I know?**	**What do I want to know?**	**What did I learn?**
What is bacterial transformation?			
What is genetic engineering?			

Answer the questions below after viewing these videos: *Soybean Genetic Modification* www.youtube.com/watch?v=wTraZwHDHXk, *Restriction Endonucleases (enzymes)* https://www.youtube.com/watch?v=GJrAsW41a64, and *3D Animation – Bacterial Transformation 3D Animation* https://vimeo.com/170630548

1. What is bacterial transformation? _____
2. Can bacteria transform naturally? If so, how? _____
3. What are plasmids? _____
4. What is the role of plasmids in bacterial transformation? _____
5. What is the role of DNA ligase? _____
6. Explain how the calcium chloride bath is used to insert the foreign DNA into the bacterial cell. _____
7. What happens when DNA is transcribed and replicated? _____
8. What do the scientists do to confirm that transformation has taken place? _____

Obtaining the desired gene

Scientists use one of several methods to screen and isolate the cell with the library plasmid containing the desired gene.

Isolation of the bacteria with the desired gene

The bacteria are plated onto a selective medium. Only bacteria with the desired gene and the selection marker gene will survive. The bacteria serve as a ready supply of the desired gene for use by scientists.

Isolation of the desired gene

The library plasmids with the desired gene are placed in a test tube with a restriction enzyme. The enzyme cuts the DNA at specific sites and frees the desired gene from the library plasmid.

Separation of the desired gene

The transformation plasmid with the desired gene is separated from the bacterial cells and purified

Preparation of the transformation plasmid parts

The desired gene, a selection marker gene, and "empty" transformation plasmid are cut to make them compatible for ligation.

Transference of the desired gene

Scientists choose an appropriate insertion method to insert the desired gene into the plant cells they are studying.

Ligation of the transformation plasmid parts

The desired gene, selection marker gene, and the "empty" transformation plasmid are combined in a test tube with a DNA ligase to seal the sticky ends of the DNA molecules together. This new bacterial transformation plasmid has incorporated the desired gene and the selection marker gene.

Propagating the genetically engineered plants

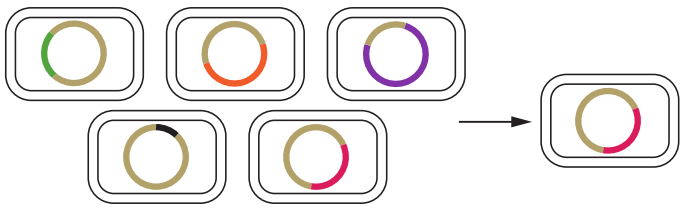
Plant cells are grown on selective media so that only the transformed cells carrying the new genes will grow. The media also contains substances that encourage the plant cells to grow into new plants.

Addition of desired gene to bacteria

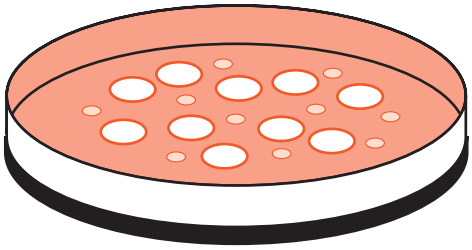
The transformation plasmid with the desired gene and the selection marker gene are added to bacterial cells.

Testing the genetically engineered plants

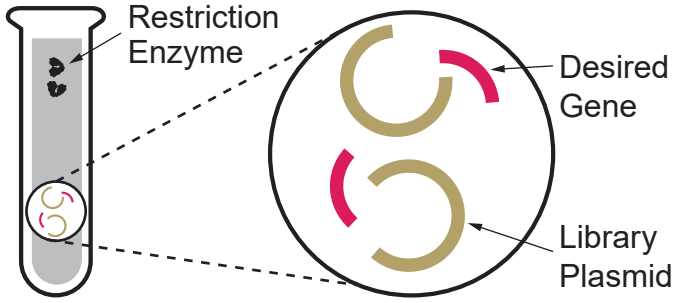
The plant is tested to determine if it incorporated the desired trait.

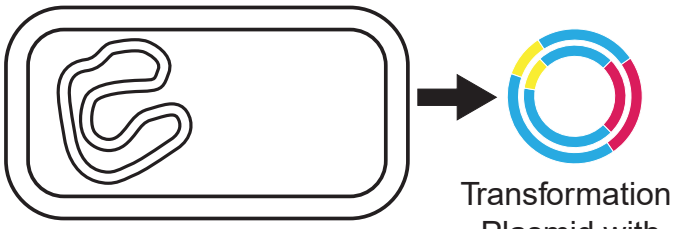


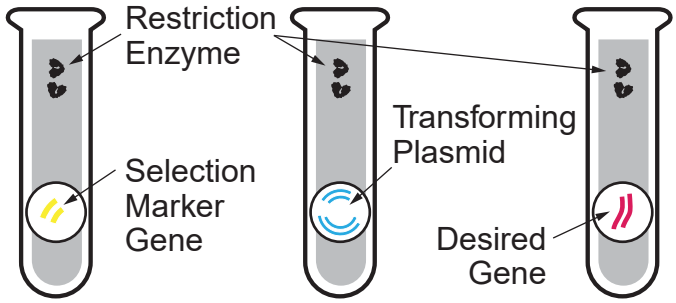
Screen Library and Isolate Cells

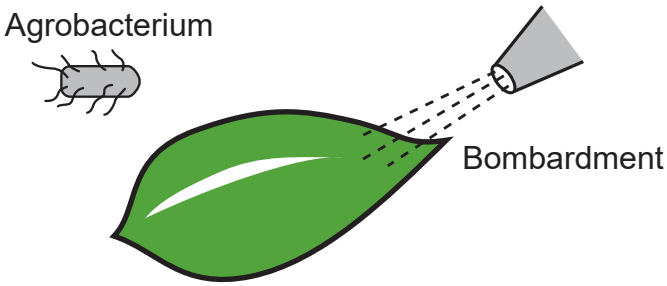


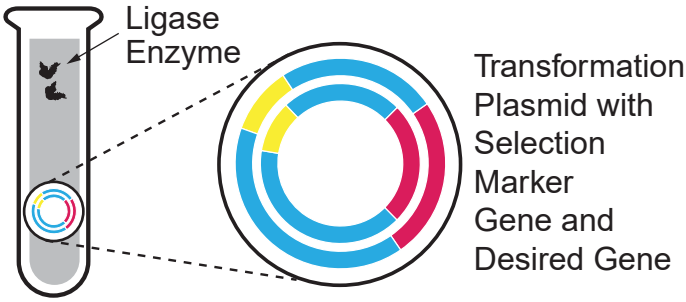
Bacterial Culture

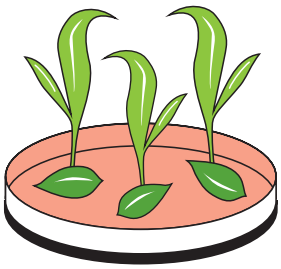




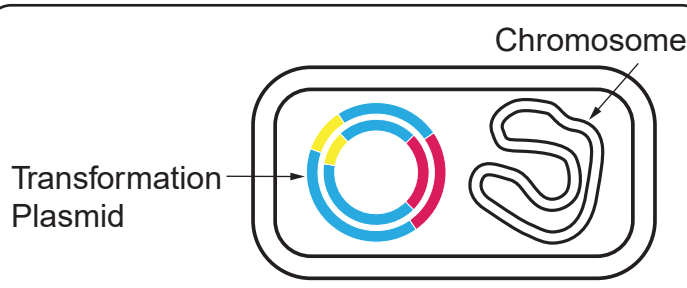








Culture of Genetically Engineered Plants



Plant with Desired Gene

BACKGROUND INFORMATION

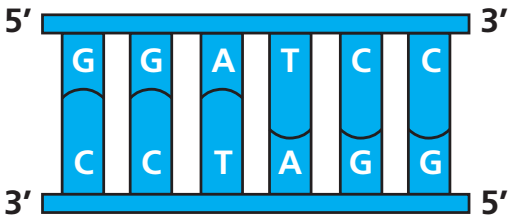
PART 2
Targeted Genome Editing

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.

BACKGROUND INFORMATION

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:

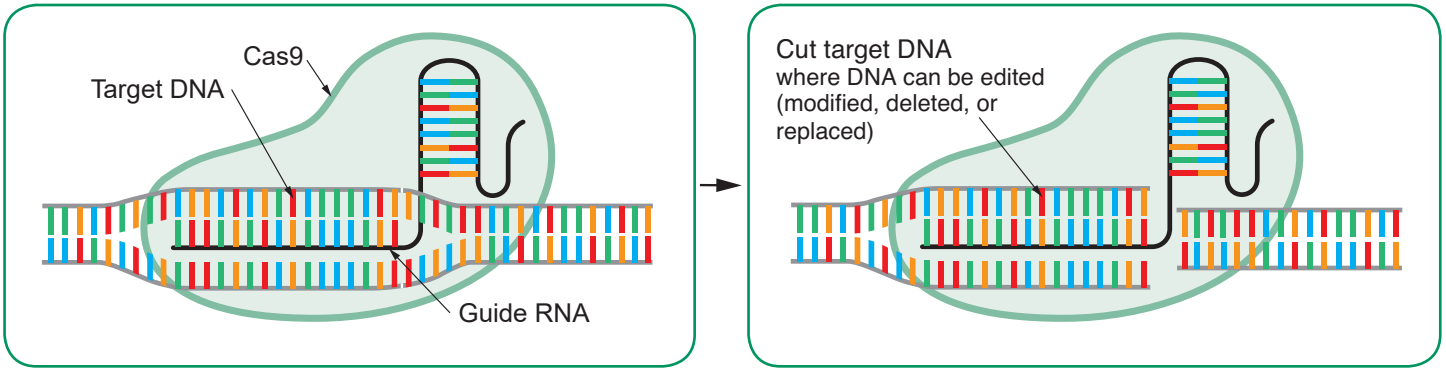
- a plant's yield performance
- nutritional value
- tolerance to biotic stress such as viral, fungal, and bacterial diseases
- tolerance to abiotic stress such as environmental conditions, including changes in water availability, temperature, and soil chemistry

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



ACTIVITY 2 - TARGETED GENOME EDITING

TIME Three 45-Minute Class Periods

ACTIVITY AT A GLANCE

In this activity, students develop an understanding of the CRISPR-Cas9 gene editing system and create an infographic (or poster or model) to demonstrate their understanding of the system.

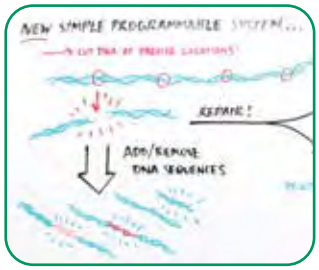
TIME TO TUNE IN

Gene Editing Yields Tomatoes That Flower and Ripen Weeks Earlier (2:50)
www.youtube.com/watch?v=Jem3hP734uA

CRISPR Gene Editing Explained (2:11)
https://video.wired.com/watch/crispr-gene-editing-explained

CRISPR Explained (Mayo Clinic) (1:38)
www.youtube.com/watch?v=UKbrwPL3wXE

CRISPR – A Word Processor for Editing the Genome (6:09)
www.ibiology.org/genetics-and-gene-regulation/crispr



TARGETED GENOME EDITING

GETTING STARTED

MATERIALS

- Computer and internet access for you and your students
- Copies of the **CRISPR-Cas Note-Taking Guide and Infographic** worksheet and the **Poster/Infographic Rubric** (page 94) for each student.
- Poster paper
- Markers
- 3 x 5 index cards
- Optional: 3D Modeling supplies

ADVANCE PREPARATION

Divide the class into small groups.

Make copies of the **CRISPR-Cas Note-Taking Guide and Infographic** worksheet and the **Poster/Infographic Rubric** for each student.

While the article, "Why Gene Editing is the Next Food Revolution," has some good information, point out to the students that it states that soybean-based oil is high in *trans* fats. However, this depends on whether the oil has been partially hydrogenated. If so, then the food companies may be required to remove the *trans* fats. This resource also states that genome editing techniques that mimic natural processes are not subject to U.S. regulation. However, regulatory debates are ongoing in the United States.

INTRODUCTION

Ask your class these questions:

1. *How are scientists using genetic engineering to improve the food that we eat?*

   Possible answer: The genes from one organism can be added to the same kind of organism or to another kind of organism to make the plants more nutritious or resistant to disease.

2. *Imagine that scientists can edit DNA as easily as correcting typos on a computer. What impact do you think this would have on the food that we eat?*

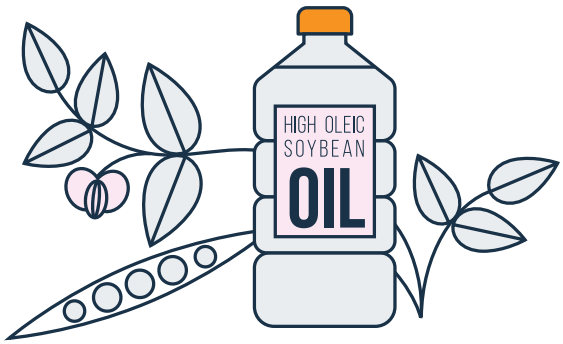
   The students might answer that it will be easier to change a plant's genes with targeted genome editing methods (such as the CRISPR-Cas system) than with non-targeted modification methods such as selective breeding, chemical or UV methods, and rDNA methods.

3. *What advancements could you expect to see in agriculture in the next 5 years?*

   Some responses could include:

   1. There could be many more changes in the plants we eat.
   2. There could be more varieties of plants that we eat.
   3. Plants could become more nutritious or more resistant to pests.
   4. Our environment might be better because plants could be changed to reduce the need for certain pesticides.

   Tell the students that they will read text and view a video about the CRISPR-Cas9 system and then create an Infographic to illustrate their understanding of the system. Students could also consider creating a poster or powerpoint presentation.



TARGETED GENOME EDITING

STUDENT PROCEDURE

1. Read the questions on the **CRISPR-Cas Note-Taking Guide**, then watch these four videos:
   - *Gene Editing Yields Tomatoes That Flower and Ripen Weeks Earlier* www.youtube.com/watch?v=Jem3hP734uA
   - *CRISPR Gene Editing Explained* https://video.wired.com/watch/crispr-gene-editing-explained
   - *CRISPR Explained (Mayo Clinic)* www.youtube.com/watch?v=UKbrwPL3wXE
   - *CRISPR – a Word Processor for Editing the Genome* www.ibiology.org/genetics-and-gene-regulation/crispr
2. After viewing the videos, read the article: "Why Gene Editing Is the Next Food Revolution" - **https://www.nationalgeographic.com/environment/future-of-food/food-technology-gene-editing/** and then begin work on the Guide. Read carefully and take notes because you will use the information to create an Infographic or other visual presentation.
3. After completing the questions, discuss everyone's responses.
4. Discuss ways of visually representing knowledge designed to make complex ideas and large amounts of data easy to understand.
5. Consider how you will create your own infographic to help others better understand the CRISPR-Cas genome editing system.
6. Refer to A Visual Guide to Genetic Modification **https://blogs.scientificamerican.com/sa-visual/a-visual-guide-to-genetic-modification/**
   Look at the Conventional Crossbreeding Infographic to see what information can be learned from the infographic.
7. Answer the Infographic Planning questions listed at the bottom of page 40.
8. Consider the infographic examples at "Good to Better – A 'critique' with ideas & tips to improve your infographics" - **www.slideshare.net/hurricanemaine/infographic-good-and-better**. The slides have some good suggestions for improving infographics.
9. Now create a CRISPR-Cas infographic that explains how the system can edit the genome of a plant. Refer to the infographic "Second-Generation Gene Editing" or "What Is CRISPR" from "Why Gene Editing Is the Next Food Revolution" as models. Use other resources and include citations for each.

   Critical points for infographic project groups:
   - What information is essential? What isn't? (use your worksheet)
   - What colors and layout work best?
   - What is the best way to have the information flow?
   - Create a rough sketch of your infographic.
   - Determine how much copy (text) will be needed.
   - Balance copy with visuals.

   Infographics can be created digitally using a program such as Easel.ly - **www.easel.ly** or Piktochart - **https://piktochart.com**, or they can be made with poster paper and markers. Refer to the rubric criteria for your infographic.
10. Display the completed infographics in the classroom. Create a gallery walk and, using post-its®, discuss the best features of each infographic.
11. After the gallery walk, review the comments and using the rubric, score the infographics.

REVIEW

Ask the students for their anonymous review/evaluation of this activity on an index card using a 3-2-1 evaluation:

- List 3 things they have learned
- List 2 questions they still have
- List 1 concern

Students can refer to the resources they have used in this activity. Review their answers and discuss them with the class the next day.

TARGETED GENOME EDITING

EXTENSIONS

Students could do one or more of the following activities:

1. Review the video *CRISPR Gene Editing Explained*, which uses the analogy of a toy train to explain the CRISPR-Cas system, and design an infographic that uses a similar analogy to explain the system.
   https://video.wired.com/watch/crispr-gene-editing-explained
2. View the video *CRISPR – History of Discovery* **www.youtube.com/watch?v=RKh2mi3tsmc** and create an infographic about the history of the development of the CRISPR-Cas9 system.
3. Research multiple genome editing techniques, such as CRISPR-Cas, TALEN, and Zinc Finger Nucleases, and compare and contrast their characteristics and advantages in a chart.

SUMMARY

Genome editing techniques like CRISPR-Cas are powerful tools that scientists can use to target specific locations in the genome for editing (add, remove, or modify a gene to increase or decrease its expression) and thus change the traits of that organism. The promise and challenges that genome editing systems hold for agriculture are currently unknown. But, based on the results we have now, it is exciting to think about crops of the future and what they might be able to do.

UP NEXT

Now that you know about some of the tools farmers and scientists have to select or alter plant traits, let's take a look at some of the environmental factors that can challenge or help plants grow in the field.

RESOURCES

- *A Visual Guide to Genetic Modification*
  https://blogs.scientificamerican.com/sa-visual/a-visual-guide-to-genetic-modification/
- *CRISPR – A History of Discovery*
  www.youtube.com/watch?v=RKh2mi3tsmc
- *HHMI Biointeractive: CRISPR-Cas9 Mechanisms & Applications*
  media.hhmi.org/biointeractive/click/CRISPR/
- *Nature Video: CRISPR Gene Editing and Beyond*
  www.youtube.com/watch?v=4YKFw2KZA5o
- Science Magazine (more technical option)
  CRISPR-Cas guides the future of genetic engineering
  http://science.sciencemag.org/content/361/6405/866
- *New Gene Editing Tool May Yield Bigger Harvests*
  www.youtube.com/watch?v=UUo6IxLRbQ4
- *What is CRISPR-Cas?*
  www.youtube.com/watch?v=52jOEPzhpzc
- *Future Predictions – Food Technology and Science*
  www.youtube.com/watch?v=GCXhdAGx3NI

CRISPR-CAS NOTE-TAKING GUIDE AND INFOGRAPHIC WORKSHEET

Name _____ Date _____ Class/Hour _____

Directions: Watch the following four (short) videos and read the article about gene editing; then answer the questions.

- *Gene Editing Yields Tomatoes That Flower and Ripen Weeks Earlier* **www.youtube.com/watch?v=Jem3hP734uA**
- *CRISPR Gene Editing Explained* **https://video.wired.com/watch/crispr-gene-editing-explained**
- *CRISPR Explained (Mayo Clinic)* **www.youtube.com/watch?v=UKbrwPL3wXE**
- *CRISPR – a Word Processor for Editing the Genome* **www.ibiology.org/genetics-and-gene-regulation/crispr**

Why Gene Editing Is the Next Food Revolution - **www.nationalgeographic.com/environment/future-of-food/food-technology-gene-editing**, complete the following questions.

1. Why do scientists want to be able to edit DNA? _____
2. What is CRISPR and how do scientists use it? _____
3. In what type of organism was CRISPR first discovered? _____
4. What does the acronym CRISPR stand for? _____
5. What is Cas? _____
6. How did scientists harness or program the CRISPR-Cas9 system they identified in bacteria? _____
7. Describe the steps in the CRISPR-Cas system. _____
8. List some potential benefits/applications of CRISPR technology for our food. _____

Infographic Planning

Remember: An infographic: (1) Is an explanation that helps you more easily understand something, (2) integrates words and pictures, (3) is self-explanatory, (4) makes for faster understanding of a concept, and (5) is understandable.

1. Review "A Visual Guide to Genetic Modification" **https://blogs.scientificamerican.com/sa-visual/a-visual-guide-to-genetic-modification**
2. After reviewing the first infographic "Conventional Crossbreeding," consider the following questions:
   - What makes this infographic interesting – the content, the design, or both?
   - How was the information arranged and presented? Were there sections, titles, and/or graphs?
   - How are fonts, color, and graphics used?
   - Did the design contribute to how you felt about the information?
   - What did you like about the infographic?
   - What would you change in the infographic to make it better?
3. As you design your infographic, consider the following questions:
   - What is your goal?
   - Who is your audience?
   - What information do you want to include?
   - What information is essential? What information is not?
   - Did you create an outline to organize your information?
   - How will you arrange your flow of information?
   - What colors and layout work best?
   - Have you streamlined your information?
4. Use the back of this page or another sheet of paper to design a rough sketch of your infographic.

FROM MODULE 2

STUDENT WORKSHEET
SOME ANSWERS
ACTIVITY 1: GENETIC ENGINEERING

Name _____ Date _____ Class/Hour _____

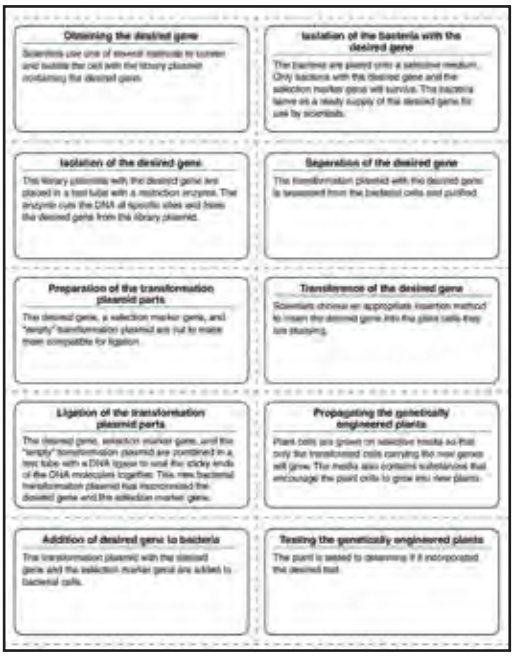
KWL CHART			
Exploration Question	**What do I know?**	**What do I want to know?**	**What did I learn?**
What is bacterial transformation?			
What is genetic engineering?			

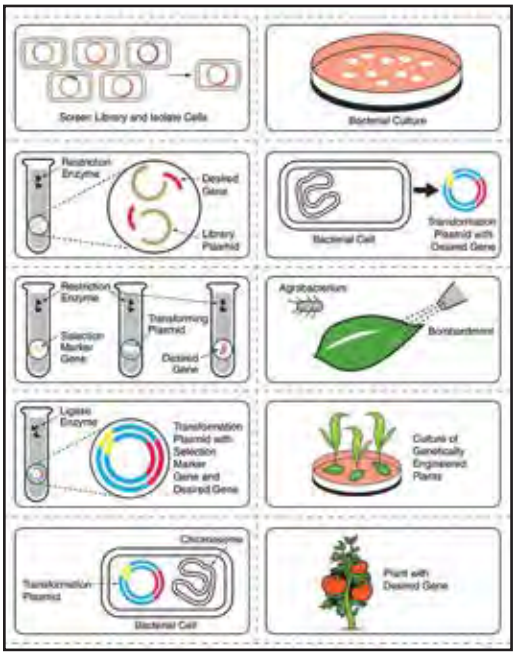
Answer the questions below after viewing these videos: *Soybean Genetic Modification* **www.youtube.com/watch?v=wTraZwHDHXk**, *Restriction Endonucleases (enzymes)* **www.youtube.com/watch?v=5hgbcdQPISI**, and *3D Animation – Bacterial Transformation* **https://vimeo.com/170630548**

1. What is bacterial transformation?
   Transformation is the process of introducing foreign DNA into a bacteria cell which results in newly acquired genetic trait(s) for that cell.
2. Can bacteria transform naturally? If so, how?
   Yes, they can take up DNA from the environment.
3. What are plasmids?
   Plasmids are small, circular, extrachromosomal DNA molecules found in bacteria cells. They can replicate independently of the bacteria genome and frequently carry antibiotic-resistance genes.
4. What is the role of plasmids in bacterial transformation?
   Plasmids are DNA loops that are modified and used to carry the new gene into the bacterial cell.
5. What is the role of DNA ligase?
   DNA ligase is an enzyme that joins the new gene to the plasmid.
6. Explain how the calcium chloride bath is used to insert the foreign DNA into the bacterial cell.
   Both the plasmid and the cell membrane are negatively charged, and this prevents the plasmid from entering the cell. The calcium ions are positively charged and attracted to the cell membrane and plasmid. When the cell is subjected to heat shock, the plasmid can enter the cell.
7. What happens when DNA is transcribed and replicated?
   When DNA is transcribed, it is copied into RNA. When DNA is replicated, it is copied into DNA.
8. What do the scientists do to confirm that transformation has taken place?
   A selectable marker (such as a gene for resistance to a particular antibiotic) is added to the transformation plasmid so that when the cells are plated on the selective medium with the antibiotic, only those that took up the plasmid with the marker will survive.

MODULE 2 ACTIVITY 1 ANSWERS

GENETIC ENGINEERING PROCESS CARDS





CRISPR-CAS NOTE-TAKING GUIDE

1. Why do scientists want to be able to edit DNA?

Scientists want to edit DNA to better understand the function of specific genes. DNA editing also allows them to modify a plant genome in a very targeted way to give it favorable traits, e.g., disease-resistance, drought-tolerance.

2. What is CRISPR and how do scientists use it?

A CRISPR is a sequence of nucleotides in the bacterial genome where the bacterium keeps a record of a previous infection by a virus and later uses it to identify and fight subsequent attacks by the same or similar virus. Scientists can use these sequences, CRISPR, to change an organism's DNA.

3. In what type of organism was CRISPR first discovered?

CRISPR was discovered in bacteria.

4. What does the acronym CRISPR stand for?

Clustered Regularly Interspaced Short Palendromic Repeats

5. What is Cas?

Cas are genes that are close to CRISPR (Cas stands for "CRISPR-associated"). They code for the proteins necessary for the CRISPR system to work. Cas9 (CRISPR associated protein 9) is a protein (more specifically an enzyme) with an important role in a bacterium's immunological response to a viral infection. It is used in genetic engineering to induce targeted double-stranded breaks in DNA.

6. How did scientists harness or program the CRISPR-Cas9 system they identified in bacteria?

They discovered that during the process, the two RNAs pair up and recruit Cas9 protein and direct it to bind to the target DNA and cut it. They then designed a guide RNA molecule that could recruit Cas9 and cut DNA in plasmids where they wanted, insert a gene, and allow the cell to close the loop.

7. Describe the steps in the CRISPR-Cas system.

1. Identify the gene responsible for the desired trait and design a piece of guide RNA and an enzyme to target that gene. 2. Introduce the guide RNA and the restriction enzyme into a cell. 3. The guide RNA locates and binds to the DNA sequence, and its Cas9 enzyme then cuts the DNA at the targeted location. 4. Add or remove a target section of DNA depending on the desired new trait. 5. Allow the cell to naturally repair itself. 6. Remove the guide RNA and Cas9. 7. The resulting plant can be crossed with the original one, and the change is then passed on to the offspring.

8. List some potential benefits/applications of CRISPR technology for our food.

Increase yields, reduce allergens such as gluten, make food more nutritious or enhance flavor, make plants more impervious to drought and pests, or change the growing season of crops

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FDA would also like to thank the Professional Development Program in Food Science middle level and high school teachers from 2018 and 2019 who gave valuable feedback on the piloted activities for this Guide.