Genetic Engineering: Journey of a Gene Teacher Resources

Summary

Genetic Engineering: Journey of a Gene is a standards-aligned, 5-E life science unit that adapts the Journey of a Gene online learning modules (https://ge.unl.edu/journey-of-a-gene/) created by Dr. Don Lee for the high school biology classroom. Throughout this unit, students will explore the process of creating genetically engineered organisms and examine their real world applications. The online learning environment provide students with an authentic agricultural scenario with real scientists explaining how genetic engineering is used to solve a disease problem in soybeans. In addition, students will conduct a series of hands-on, minds-on activities including extracting strawberry DNA, carrying out an inquiry-based flower dissection to explore structure and function, and conducting a simulation of backcross breeding. Students will use their learning to address socioscientific problems by designing transgenes to solve a number of real-world food and health issues.

Grade Level

9-12

Contents

Lesson 1 Design	ing a Genetically Engineered Organism	2
Lesson 2 Gene I	nsertion	. 25
Lesson 3 Flower	Anatomy and Plant Breeding	. 36
Lesson 4 Backer	oss Breeding with Transgenic Plants	. 45

Contents address the following Next Generation Science Standards

- HS-LS1-1. Construct an explanation based on evidence for how the structure of DNA determines
 the structure of proteins which carry out the essential functions of life through systems of
 specialized cells.
- HS-LS1-2. Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.
- HS-LS3-3. Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.
- HS-ETS1-3 Evaluate a solution to a complex real-world problem based on prioritized criteria and trade-offs that account for a range of constraints, including cost, safety, reliability, and aesthetics, as well as possible social, cultural, and environmental impacts.

Authors

Caitlin Falcone, Life Science Teacher, Lourdes Central Catholic School, Nebraska City, Nebraska Dr. Don Lee, Professor of Agronomy and Horticulture, University of Nebraska-Lincoln Erin Ingram, Curriculum Development Specialist, University of Nebraska-Lincoln, IANR Science Literacy Molly Brandt, Graduate Research Assistant, University of Nebraska-Lincoln, IANR Science Literacy





Lesson 1 | Designing a Genetically Engineered Organism **Background**

Purpose

This lesson utilizes resources from passel unledu to review the concepts of DNA, genes and proteins through animation, activities, and discussion. This lesson introduces the first three steps in crop genetic engineering (extracting DNA, locating and cloning a gene, and modifying a gene). Students will perform a sample extraction of DNA. In addition, students are introduced to gene regions, their functions, and their application in genetically engineering organisms. Students will practice designing a transgene through inclass activities. Lastly, a quiz option is available to assess retention of ideas.

Standards

Next Generation Science Standards

HS-LS1-1Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

Common Core

RST.9-10.4 Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 9-10 texts and topics.

Estimated Time

Three 45-minute class periods

Student Materials

- Student Worksheets
- Scissors
- Glue or Tape
- Computer and Internet Access

For strawberry DNA extraction each student group needs:

- 1 strawberry
- mortar and pestle
- 10 mL (2 tsp) dish detergent
- 125 mL (1/2 cup) water
- 5 g (1 tsp) salt (non-iodized)
- rubber band
- coffee filter
- 2 plastic cups
- tray of ice
- masking tape and marker for labeling
- 91% cold isopropanol (rubbing alcohol)
- popsicle stick or coffee stir stick





Teacher Materials

- PowerPoint Presentation
- Student Worksheet Answer Key
- Lesson 1 Quiz (optional)
- Lesson 1 Quiz Answer Key (optional)

Vocabulary

- amino acids: the basic building blocks of proteins
- coding region: the section of a gene that contains the instructions about how to make a specific protein. This region is found between the promoter and the termination sequence in the DNA
- codon: three consecutive nucleotides in an RNA molecule that code for a single amino acid. This is what the ribosome reads when it translates RNA instructions to make a protein.
- **DNA:** the instruction manual for a cell. Sections of the DNA called 'genes' can include instructions for building proteins. DNA is a double-stranded molecule held together by weak bonds between base pairs of nucleotides.
- gene: a sequence of DNA that provides the instructions for making a protein.
- gene cloning: locating and copying a gene responsible for a specific trait
- genetic engineering: the process of manually adding DNA with a specific function to the genome (DNA) of an organism
- **nucleotides:** the subunits that are linked together to make DNA or RNA.
- **promoter:** the first region of a gene that determines when and where the gene is 'turned on.'
- **protein:** molecules that 'do the work' of a cell. DNA provides the instructions to build these large molecules which are made of folded chains of amino acids. Once built, proteins have jobs that involve the structure, function, or regulation of the organism's cells, tissues, and organs. Each protein has a unique function determined by its shape.
- termination sequence: the sequence of DNA which signals the transcription (photocopying of the gene to make RNA) to stop.
- transgenic: an organism that has new a new DNA sequence found in every one of its cells

Key STEM Ideas

This lesson introduces students to a very complex subject of genetic engineering by thoroughly examining the first three steps: extracting DNA from an organism with a desired trait, locating and cloning the responsible gene, and modifying the gene to express in a particular way in the organism. Students learn the three regions that comprise a gene. They will design a transgene to address a pest problem and apply a genetic engineering solution to address real-world agriculturally-based issues.

Students' Prior Knowledge

Students should be familiar with DNA as a means of passing hereditary information and understand that DNA provides instructions, a blueprint, or a recipe for cells to read when making proteins necessary for survival of an organism.





Genetic engineering is employed regularly as a tool by plant geneticists to solve agricultural crop production issues such as managing pest, parasite, or pathogen problems, reducing our reliance on chemical inputs, and improving crop productivity. Genetic engineering has been successfully employed to develop crops with a wide array of desirable traits including insect and disease resistance, herbicide tolerance, and resistance to bruising or browning. This lesson teaches students the foundation of how genetic alteration is accomplished and how genetic engineering can be applied to solve real-world problems.

Essential Links

- "Overview of Crop Genetic Engineering":
 http://passel.unl.edu/pages/animation.php?a=overview genetic engineering.swf&b=990818777
- "Gene Cloning"
 http://passel.unl.edu/pages/animation.php?a=genecloning.swf&b=990819293
- "Gene Regions"
- http://passel.unl.edu/pages/animation.php?a=GeneRegions.swf&b=1011727433

Sources/Credits

The unit is adapted for the high school biology classroom from the web-based learning portal "Genetic Engineering: Journey of a Gene" found at https://ge.unl.edu/journey-of-a-gene/.

- PPT slides and Activity, Designing Transgenes to Solve Real-World Issues adapted from Dr. Donald Lee resources (passel.unl.edu)
- Strawberry DNA Extraction Lab adapted from Strawberry Breeding and Genetics lesson available at the National Ag in the Classroom curriculum matrix (agclassroom.org)

Additional credits

- List of genetically engineered organisms: https://gmoanswers.com/sites/default/files/Genetic traits GMOS expressed US.PDF
- European corn borer estimated loss costs from: http://www.ent.iastate.edu/pest/cornborer/insect
- European corn borer images from UNL Entomology, David Keith, and Jim Kalisch (all from University of Nebraska-Lincoln, Department of Entomology)

http://contentdm.unl.edu/cdm/singleitem/collection/pestscor/id/219/rec/88 http://contentdm.unl.edu/cdm/singleitem/collection/pestscor/id/36/rec/91 http://contentdm.unl.edu/cdm/singleitem/collection/pestscor/id/86/rec/94





Lesson Procedures

Engage

Part 1: Introduction to Genetic Engineering

- 1. Distribute the student worksheet. Display slide 2 of the PowerPoint presentation. Ask students to discuss the following questions with a partner and write down their answers on the worksheet. Discuss answers a whole class to gain a sense of student understanding of genetic engineering, its applications, and its goal.
 - What is genetic engineering? Genetic engineering is the process of adding foreign DNA to the genome of an organism.
 - What organisms can you think of that have been genetically engineered? Students may come up with a variety of answers. A list of genetically engineered foods can be found here: https://gmoanswers.com/sites/default/files/Genetic traits GMOS expressed US.PDF
 - What do you think is the goal of genetic engineering? The goal is to add one or more new traits that are not already found in that organism.

Explore and Explain

How would I genetically engineer an organism?

- 2. Using slide 3, ask students to brainstorm with a partner how they would go about genetically engineering an organism. Have students write down their steps or draw a picture.
- Facilitate a student discussion of potential steps in the process. Consider discussing the following questions:
 - a. How do scientists select a trait to incorporate?
 - b. Can traits be synthesized or must they exist in an organism already?
 - Where do these traits come from? What is the source of the trait?
 - d. How does the gene get inserted into a new organism?
- 4. Students do not need to have accurate conceptions at this time. It is simply useful to take note of student conceptions of how this process might work in order to address misunderstandings or gaps in student understanding.

Steps in the Process of Crop Genetic Engineering

- Provide students with access to computers with internet. Have students view the interactive module "Overview of Crop Genetic Engineering" (http://passel.unl.edu/pages/animation.php?a=overview_genetic_engineering.swf&b=990818777)
- 6. Instruct students to focus on the overall process of genetic engineering. The focus should not be on the specifics or details of each step at this time. Have students answer the follow-up questions and compare the real process of genetic engineering to their previous conceptions.
- 7. Ask students to discuss if any of their steps were included in the actual process. Were any steps missing?
- Using slides 4-6, work as a class to map out the basic steps of genetic engineering on a white board.
- 9. Explain to students that this lesson will focus on the first three steps of genetic engineering.

Step 1: Identifying an Organism with a Desired Trait and Extracting the DNA

10. Refer students back to the class map of the steps and explain that the class will be practicing the first step by extracting DNA from a strawberry. Be sure students understand that this process would be similar when extracting DNA from any organism from humans to bacteria.





- 11. Use slides 7-8. Split students into groups of 3-4 and provide each group with access to the necessary materials. Read through the lab procedures as a whole class before groups begin.
- 12. Instruct students to follow the lab procedures and answer the lab reflection and extension questions.
- 13. When all students are done, have groups discuss what they observed during the extraction. Were there any similarities or differences between groups?
- 14. Discuss with students the purpose for each step in the DNA extraction process. Answers are provided in the teacher notes.

Step 2: Gene Cloning

- 15. Discuss with students the step which comes after DNA extraction. Referring back to the class map may be useful.
- 16. Present slide 9. Have students view the gene cloning animation found here: http://passel.unl.edu/pages/animation.php?a=genecloning.swf&b=990819293.
- 17. Instruct students to answer the follow-up questions.
- 18. Have students discuss their answers with a partner. If students are unsure of the process, it may be useful to create a diagram of this process as a whole class.

Step 3: Understanding Gene Regions and Modifying the Gene

- 19. Refer back to the class map of the genetic engineering process. Explain to students that they will be moving onto the third step, modifying the gene, but first they need to understand the regions of the gene.
- 20. Use slides 10-18 to provide students with direct instruction about the gene being made up of a promoter, coding region, and termination sequence. Alternatively, students can view an animation found at http://passel.unl.edu/pages/animation.php?a=GeneRegions.swf&b=1011727433.
- 21. Direct students to fill out the follow-up questions on their student worksheet. Answers can be checked as a group. If misunderstandings are observed, a review of gene regions can be conducted.

Extend

Designing a Transgene

- 22. Direct students to read the problem European corn borer causes for corn growers (slide 20).
- 23. Using slides 19-26 for support, have students act as plant breeders to design a transgene using what they have just learned about gene regions. They must select an appropriate promoter, coding region, and termination sequence to solve the issue presented.
- 24. Discuss student answers to this problem. Have students divide into groups that selected 35S or PEP Carboxylase as their promoter. Both of these answers are acceptable so have students discuss how they made their choice.
- 25. Explain that different choices are acceptable in this case. Both promoters have the protein expressed in part of the corn plant that is potentially eaten by ECB. Depending on the promoter selected, the plant may be more or less protected from ECB feeding. There are trade-offs; the plant expressing only in green tissue may have more amino acids available to make proteins that result in higher productivity.
- 26. Now have students describe which coding region they selected. Both Bt. CRY1A and Bt. CRY9 are effective against ECB. Discuss why it would be a benefit for breeders to have access to multiple coding regions that result in the same trait. A choice of multiple coding regions gives a breeder options if the pest becomes resistant to a toxin (possibly through random mutation) in the future.
- 27. Discuss with students that since the termination sequence just signals the end of the gene, it does not need to be altered, generally.





28. Discuss as a class what trait has been added to the corn plant. Some students may mention the plants' toxicity specifically to European corn borer. More simply, the plant now possesses a trait conferring resistance to ECB.

Part 3: Application of Genetic Engineering to Real-World Issues

- 29. Tell students that they will be addressing real agriculturally-based issues and designing potential genetic engineering solutions to these scenarios.
- 30. Explain to students that they will be designing transgenes from a pool of possible promoters and coding regions. These solutions provide one way of addressing these 4 different issues.
- 31. Read through the four scenarios with students to ensure students understand the problems.
- 32. Distribute scissors, and glue or tape.
- 33. Potentially work through first example as a class. Have students cut out the appropriate promotor and coding region and paste on each specific scenario.
- 34. Have students complete the remaining three scenarios on their own.
- 35. Check and discuss student answers. All of the problems have actually already been genetically engineered. For more information on genetically engineered goats that produce spider silk in their milk, please see the enriching activities below.

Evaluate

36. As an optional evaluation of student understanding of concepts presented in this lesson, students can take the quiz provided.





Answer Key

Part 1: Introduction to Genetic Engineering

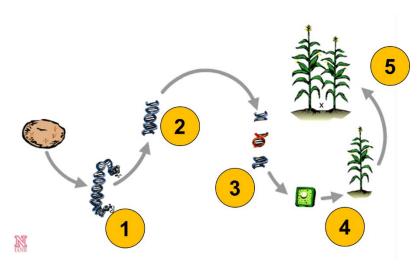
Discuss each of the questions below with a partner and provide your answer.

- What is genetic engineering? Genetic engineering is the process of adding foreign DNA to the genome of an organism.
- What organisms can you think of that have been genetically engineered? Students may come up with a variety of answers. A list of genetically engineered foods can be found here: https://gmoanswers.com/sites/default/files/Genetic_traits_GMOS_expressed_US.PDF
- What do you think is the goal of genetic engineering? The goal is to add one or more new traits that are not already found in that organism.

Steps in the Process of Crop Genetic Engineering

View the interactive module "Overview of Crop Genetic Engineering" (http://passel.unl.edu/pages/animation.php?a=overview_genetic_engineering.swf&b=990818777) As you click through the module, focus on understanding the overall process of genetic engineering. You will not need to understand the specifics or details of each step yet. Answer the follow-up questions.

1. What are the five basic steps in the process of genetically engineering a crop?



- 1. Extract DNA
- 2. Clone a single gene
- 3. Design the gene
- 4. Transformation
- 5. Backcross breeding





- 2. How are genes, proteins, and traits related? Small sections of DNA are called genes. The genes code for the proteins which are responsible for the trait.
- 3. What action is taken after the single gene that codes for the desired protein is located and copied out of all the DNA?
 - Genetic engineers modify the gene to express in a specific way inside the new plant.
- 4. How is the desired gene cut into pieces? Enzymes are used to cut the gene apart.
- 5. What is recombinant DNA?
 - When one or more of the gene regions are replaced or modified and pasted back together to function as a normal gene, this new combination of DNA is called recombinant DNA.
- 6. What two methods can be used to insert the new gene (also called the transgene) into a single plant cell?

Gene gun Agrobacterium

- 7. How does the transgene become incorporated into all of the cells of the transgenic plant? The transgene is inserted into a plant cell nucleus (where the chromosomes that are made of DNA are located). Once the transgene is incorporated into one of the chromosomes the plant cell can pass it on to offspring. As the plant cell is totipotent, it can divide and multiply into another complete plant. With each cell replication, the chromosomes including the transgene are copied.
- 8. Why is backcross breeding necessary? The transgenic plant is lacking other desirable traits such as the ability to produce a high yield (yield can be described as a large amount of the crop for consumption). Backcross breeding moves the transgene into another variety of the crop to produce a high quality hybrid. This hybrid will include the new transgene and traits that make the crop marketable such as high yield.





Exploring Early Steps in Crop Genetic Engineering

You have learned that genetic engineering is the process of manually adding new DNA to an organism. The purpose of this process is to add one or more new traits that are not already found in that organism.

This lesson will focus on the <u>first three steps</u> of the genetic engineering process:

- **Step 1**: Identifying an organism with the desired trait and extracting its DNA.
- Step 2: Locating the gene responsible for the desired trait and cloning (or copying) that gene.
- **Step 3**: Modifying the gene to express in a particular way in the engineered plant.

Step 1: Identifying an Organism with a Desired Trait and Extracting the DNA

Once a scientist has identified an organism with a desirable trait, the DNA of that organism needs to be extracted. DNA carries the genetic code for all living organisms. Each cell in a plant or animal has a nucleus with multiple chromosomes. Each chromosome is made up of DNA. Genes are short segments of this DNA that code for proteins resulting in the organism's traits. We will practice extracting DNA from a common food crop (strawberries); however, DNA could be extracted from cells from any organism from bacteria to humans.

Materials Needed

- 1 strawberry
- mortar and pestle
- 10 mL (2 tsp) dish detergent
- 125 mL (1/2 cup) water
- 5 g (1 tsp) salt (non-iodized)
- rubber band

- coffee filter
- 2 plastic cups
- tray of ice
- masking tape and marker for labeling
- 91% cold isopropanol (rubbing alcohol)
- popsicle stick or coffee stir stick

Lab Procedures

- 1. Place one strawberry into the mortar and grind it with the pestle.
- 2. In a cup, mix the water, dish detergent, and salt. Add the solution to the strawberry in the mortar. Continue to grind the mixture.
- 3. Label a second cup with your name. Place a coffee filter inside the cup and use a rubber band to hold it in place.
- 4. Pour the strawberry mixture into the filter and place the cup in the tray of ice. **It's important to** keep the mixture COLD while it slowly filters. Wait several minutes for the mixture to filter.
- 5. After the mixture has filtered, **SAVE** the filtered liquid (which contains the DNA) in the cup. Discard the coffee filter and strawberry remains in the trash.
- 6. Gently add an amount of isopropanol (rubbing alcohol) equal to the amount of filtered liquid to the cup. Remember to layer the isopropanol on top of the clear liquid rather than mixing the two layers together. Watch and wait. Bubbles will begin to form and a white stringy substance will become visible. This precipitate (the solid that forms when a chemical is added to a solution) is the DNA!
- 7. Place the cup back into the ice tray and check on it in 10 minutes. If you don't stir the layers, a large "glob" of strawberry DNA will form. (Leave the cup on ice for as long as possible.)
- 8. Pick up the DNA using a popsicle stick or coffee stir stick.





Lab Reflection

- 1. Why did you have to crush up the strawberry to get out the DNA? Crushing the strawberries breaks open the strawberry cells, where the DNA is stored.
- 2. Why did you need to add dish detergent and salt? The dish detergent breaks down the cells, allowing the DNA to be released. The salt in the makes the DNA molecules stick together, and the salt also helps separate the proteins that are being released from the cells.
- 3. What did you observe when you added alcohol to the filtered liquid? Bubbles start to form. A glob of milky white goo gradually becomes visible.
- 4. What did the DNA look like? It looks like milky or translucent goo, mucus, phlegm, etc.
- 5. If you would have cut your strawberry in half and performed two separate DNA extractions with each half, would the resulting DNA be the same in both extractions? Explain. (Hint: Remember that strawberry started out as one fertilized cell.) Yes, all cells in the plant contain the same DNA because they originated from replication of a single fertilized cell. All of the DNA in the cell nucleus was copied during the cell replication.

Lab Extension

6. The next step in genetic engineering is to locate the gene responsible for the trait and clone (or copy) them. Predict what might happen if you skipped this step and simply inserted all of the DNA from the strawberry into a new plant.

Answers may vary. We encourage acceptance of all answers that show reasoning and application of inheritance concepts.

Sample answer: If all DNA from the strawberry was inserted into a new plant rather than a single gene, the new plant would have all the traits from the original plant rather than the single desirable trait.





Developing a genetically engineered crop variety, or transgenic, is a long and complicated process. The first step in this process involves a genetic engineer identifying an organism with a desirable trait. Some traits might be especially valuable to a farmer such as a plant being able to defend itself against an insect pest or tolerate poor environmental conditions such as drought or flooding. Other traits might be selected for their value to a consumer, such as a long shelf-life or added nutritional value.

Next, the genetic engineer extracts DNA from the organism with the desired trait. Then, the engineer locates the specific gene responsible for the wanted trait and makes copies of the gene. This step is known as **gene cloning**.

To learn more about gene cloning, view the animation found here: http://passel.unl.edu/pages/animation.php?a=genecloning.swf&b=990819293

- 1. In the animation example, the desired trait was found in what organism?

 A bacterium
- 2. What is a restriction enzyme? How is it used?

 A restriction enzyme acts like scissors. It is added to a test tube with the extracted DNA and cuts the DNA into gene-sized pieces. It is also added to another test tube of bacterial plasmids.
- 3. Bacterial plasmids are small circles of DNA in bacterial cells that are naturally present in addition to the bacteria's other DNA. A plasmid is capable of storing and transporting cloned DNA segments. What happens to the cut plasmids and gene-sized pieces of DNA when they are mixed together? The gene-sized pieces join and bond with one another to make recombinant plasmids. Some cut plasmids will bond with themselves (they do not contain any extracted DNA).
- 4. How are recombinant plasmids inserted into bacterial cells?

 Bacterial cells are added to the test tube and electroporation is performed to create small holes in the bacterial cell walls. The plasmids enter the bacteria through these small holes and make the bacterium express the genes.
- 5. The transformed bacteria are placed on a media that contains an antibiotic. Why is this done? Those bacterial cells that take up a plasmid will have antibiotic resistance. The antibiotic media will eliminate those bacterial cells that are not transformed (do not include a plasmid).
- 6. Will all bacterial cells that are grown on the antibiotic plates have the desired gene (the gene of interest)? Why or why not?
 No, only one colony of bacteria will include the gene of interest because the rest of the colonies only include other gene-sized segments of the extracted DNA.





Step 3: Understanding Gene Regions and Modifying the Gene

Once a gene has been located and cloned, the genetic engineer can modify or replace regions of the gene to make it express in a particular way in the plant.

To learn more about the regions of a gene and what functions they perform, view the animation found here: http://passel.unl.edu/pages/animation.php?a=GeneRegions.swf&b=1011727433.

- 1. What are three regions that make up a gene and what do they do?
 - Promoter:
 - o Acts like an on/off switch for the gene
 - o Signals how much, where, and when a protein is made
 - Coding region:
 - o Signals which protein to make based on the sequence of nucleotides, which make codons, which code for amino acids
 - Termination sequence:
 - o Signals the end of the gene
 - o Prevents the cell from reading the next gene on the chromosome
- 2. What is a codon and what does it do? A codon is a sequence of 3 nucleotides. A codon codes for a specific amino acid.
- 3. How are amino acids related to which protein is produced by a gene? The order of amino acids produced determines the protein.





Part 2: Designing a Transgene

According to the United States Department of Agriculture's Economic Research Service (USDA ERS), corn accounts for more than 95% of total feed grain production in the U.S. Feed grain is used to feed livestock. It is also made into a variety of food and industrial products and is used to produce ethanol for fuel.



European corn borer larva feeding on corn stalk

In order to protect this valuable agricultural crop, growers must minimize damage from pests, pathogens, and parasites. A major pest of corn is a moth known as the European corn borer, (ECB). During the larval stage, it feeds on various parts of the corn plant including the leaves, stalk, and ears of corn. Feeding damage results in poor ear development, broken stalks, and dropped ears. Yield losses and control expenses associated with European corn borer cost farmers in the U.S. more than 1 billion dollars.

Plant breeders can use genetic engineering to develop improved lines of corn that incorporate desirable traits, such as resistance to pests like the European corn borer. Imagine you are a plant breeder. You are responsible for designing a transgene to combat European corn borer damage of corn. Use the information provided below to accomplish your task.

Select and circle your choice of promoter, coding region, and termination sequence from the choices in the table.

Gene Region Choices					
Promoter Coding Region		Termination Sequence			
35S Expresses in all tissues	Bt. CRY1A Encodes a protein toxic to European Corn Borer	ACCGATACGTTACA Signals the end of the gene			
PEP Carboxylase Expresses in green tissue	PAT Encodes for a protein that breaks down the herbicide, Liberty				
	Bt. CRY 9 Encodes a protein toxic to European Corn Borer				
	EPSPS+CTP Encodes for a protein that breaks down the herbicide, Roundup				

Note: Multiple answers are acceptable. Depending on the promoter selected, the plant may be more or less protected from ECB feeding. There are trade-offs; the plant expressing only in green tissue may have more amino acids available to make proteins that result in higher productivity. Either promoter region would be appropriate. Two coding regions would be appropriate for control of ECB. A choice of multiple coding regions gives a breeder options if the pest becomes resistant to the toxin (possibly through random mutation) in the future. Only one choice is available for a termination sequence. Generally, these sequences are not altered.





What trait will your transgenic corn plant have?

The transgenic corn plant will be resistant to ECB feeding and damage.

Part 3: Designing Transgenes to Solve Real-World Issues

Instructions: Read each of the four real-world issue scenarios provided. Examine the list of promotors and coding regions in the tables on pages 5 and 6. Select one promoter and one coding region to potentially solve each scenario. Using scissors, cut apart the promoters and coding regions. Paste your selected promoter and coding region with the corresponding scenario.

Scenario #1

Although rice plants naturally produce beta-carotene (Vitamin A), none is present in the endosperm, the part of the plant that is consumed. In many countries where rice is a staple food, people are vitamin A deficient, which may lead to blindness or susceptibility to other diseases.

By increasing the nutritional value of rice, scientists hope to prevent millions from suffering these afflictions.

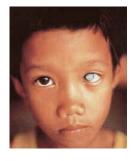


Photo source: www.goldenrice.org

Promoter:

Rice Seed Specific Promoter

Coding Region:

Beta-carotene protein

Scenario #2

The herbicide, Roundup, can stop enzyme productivity in plants which halts the plant's growth and development. The plant eventually dies from starvation. Agrobacteria, a naturally occurring soil bacteria, has a resistance to Roundup and can function in its presence.

By creating soybean plants that can tolerate being sprayed with Roundup herbicide, plant breeders hope to improve soybean production by reducing competition with weeds.

Promoter:

Leaf and Stem Specific Promoter

Coding Region:

Round-up digestion enzyme



Photo source: extension.missouri.edu





Scenario #3

Pigs are unable to digest 50-75% of the phosphorous in their food. In areas of swine production, the soil builds up phosphorous content. After heavy rains, the phosphorus leaches into freshwater ponds, streams, and rivers. This causes algae blooms which result in fish deaths due to low dissolved oxygen content and reduced water quality.

By creating a transgenic pig capable of digesting phosphorous, breeders hope to avoid the problem of phosphorous impacting quality of freshwater ponds, streams, and rivers.

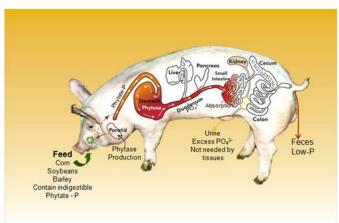


Photo source: http://www.uoguelph.ca/enviropig

Promoter:

H. Salivary gland specific promoter

Coding Region:

Phosphate digestion enzyme

Scenario #4

Spiders produce silk that has long impressed engineers as it is stronger than steel yet thinner than a human hair. If scientists could use this silk in technology we could revolutionize bridges, medical sutures, car bodies, and Kevlar. However, spider silk production is time-consuming and expensive due to spiders' small size and the complexity of the process. Initially attempts were made to use bacteria to produce the silk proteins. These efforts failed; however, it has been found that goats have very similar mammary glands to spiders' silk glands.

Promoter:

Goat mammary gland specific promoter

Coding Region:

Silk fiber protein



Photo source: en.wikipedia.org





Be sure this page is printed on one-side only!

Using scissors, cut along the dotted lines to separate the promotor choices.

Promoter Choices	
Promoters	Purpose
A. Weak Promoter	A. Expresses few copies of protein
B. Flower Petal Specific Promoter	B. Expresses protein in a flower's petals
C. Leaf and Stem Specific Promoter	C. Expresses protein in leaf and stem tissue
D. Weak Promoter	D. Expresses few copies of protein
E. Spider Silk Gland Specific Promoter	E. Expresses protein in the spider silk
	gland
F. Rice Seed Specific Promoter	F. Expresses protein in the rice seed
G. Goat Mammary Gland Specific	G. Expresses protein in goat mammary
Promoter	glands
H. Salivary Gland Specific Promoter	H. Expresses protein in salivary glands





Be sure this page is printed on one-side only!

Using scissors, cut along the dotted lines to separate the coding region choices.

Coding Region Choices	
Coding Region	Purpose
A. Round-up digestion enzyme	A. Codes for protein that digests Round-up herbicide
B. Vitamin A enzyme	B. Codes for protein that digests vitamin A
C. Vitamin C enzyme	C. Codes for protein that digests vitamin C
D. Phosphate digestion enzyme	D. Codes for protein that digests phosphate
E. Silk protein fiber	E. Codes for a silk fiber protein
F. Starch enzyme	F. Codes for a protein to digest starch
G. Milk protein enzyme	G. Codes for an enzyme to digest milk protein
H. Steroid binding protein	H. Codes for a protein which binds steroids





Teacher Materials

Lesson 1 Quiz Answer Key

Instructions: Read each question carefully. Circle the letter corresponding to the best answer.

- 1. What is the term used to describe the segment of DNA that will be designed, copied and then introduced into a genetically engineered plant?
 - a. trapped gene
 - b. transgene
 - c. mutant gene
 - d. recombinant protein
- 2. Why does the geneticist need to include the promoter in their transgene design work?
 - a. The promoter allows the gene to actually insert into the plant cell.
 - b. The promoter is only needed in genes that are not naturally occurring in the plant otherwise the plant will not be able to copy the gene when the cell is ready to divide.
 - c. The promoter is the gene's on and off switch which all genes need to have so they can be expressed in specific cells to control plant cell function.
 - d. The promoter carries all the new gene coding instructions for the plant cell. Otherwise, the plant would lack the ability to ready the rest of the gene to make a protein encoded by introduced gene.
- 3. Which part of the gene encodes the amino acid sequence of the protein?
 - a. coding region
 - b. promoter
 - c. both the promoter and coding region
 - d. neither the promoter or the coding region
- 4. What is the on/off 'switch' region of a gene?
 - a. amino acids
 - b. termination sequence
 - c. coding region
 - d. promoter
- 5. What is the protein coding part of the gene?
 - a. amino acid
 - b. termination sequence
 - c. coding region
 - d. promoter





- 6. The ______ of the protein will determine the function that protein will have inside the body.
 - a. Length
 - b. Shape and size
 - c. Weight and location
- 7. Amino acids fit together to produce larger particles called
 - a. Living cells
 - b. Proteins
 - c. Genes
 - d. Tissue
- 8. The promoter region...
 - a. Determines where the gene turns on.
 - b. Signals how much protein to produce.
 - c. Both of the above
- 9. The coding region...
 - a. Encodes the information for which protein to produce.
 - b. Signals how much protein to produce.
 - c. Prevents the cell from reading the next gene on the chromosome.
- 10. Each gene has three regions called...
 - a. Starter region, Coding region, Ending sequence
 - b. Termination sequence, Coding region, Prompter
 - c. Promoter, Coding region, Termination sequence
- 11. Promoters are very specific. For a soybean plant, this could mean:
 - a. Some promoters will only turn on in green tissue.
 - b. Some promoters will turn on when a specific infection is present.
 - c. Some promoters will turn on in all areas of the plant.
 - d. All of the above.
- 12. Which of the following statements is most true?
 - a. Genes in all living things are written in the same basic DNA language.
 - b. Each species will have a different DNA language.
- 13. The termination sequence...
 - a. Signals where the gene finishes copying.
 - b. Determines what is actually copied and gives the trait.
 - c. Prevents the cell from reading the next gene on the chromosome.
 - d. Both A and C
 - e. Both B and C





- 14. If the goal is to create a transgene that will work to fight an infection of the soil borne fungus, Fusarium virguliforme, should the gene be active all the time?
 - a. No, the promoter should trigger the expression of the gene only when the infection is present so the plant doesn't waste energy.
 - b. Yes, the promoter should always be active so that it is ready to be expressed at any time the infection shows up so the plant is always protected.
- 15. If my breeding goal is to create a corn plant which will target European corn borer at all stages of growth, which gene has the best promoter to meet my goal?
 - a. Gene #1, cloned from that cauliflower mosaic virus. This gene is expressed in all plant tissues and encodes the '35S' ribosome that the virus needs to replicate in plant tissues.
 - b. Gene #2, cloned from a bacillus Thuringiensis (Bt) bacteria and encodes a protein the bacteria uses to kill some caterpillars such as European corn borer larvae.
 - c. Gene #3, cloned from a corn plant. The gene encodes a protein the plant needs for photosynthesis and the gene is only expressed in green tissue that has active photosynthesis.
- 16. If my breeding goal is to create a corn plant which will target European Corn Borer larvae at all stages of growth, which gene has the best coding region to meet my goal?
 - a. Gene #1, cloned from that cauliflower mosaic virus. This gene is expressed in all plant tissues and encodes the '35S' ribosome that the virus needs to replicate in plant tissues.
 - b. Gene #2, cloned from a bacillus Thuringiensis (Bt) bacteria and encodes a protein the bacteria uses to kill some caterpillars such as European Corn Borer larvae.
 - c. Gene #3, cloned from a corn plant. The gene encodes a protein the plant needs for photosynthesis and the gene is only expressed in green tissue that has active photosynthesis.
- 17. Genes are made of...
 - a. Amino acids
 - b. Chromosomes
 - c. Nucleotides
 - d. All of the above
- 18. Proteins are made of...
 - a. Amino acids
 - b. Chromosomes
 - c. Nucleotides
 - d. All of the above





Lesson 1 Quiz

Student Name

Instructions: Read each question carefully. Circle the letter corresponding to the best answer.

- 1. What is the term used to describe the segment of DNA that will be designed, copied and then introduced into a genetically engineered plant?
 - a. trapped gene
 - b. transgene
 - c. mutant gene
 - d. recombinant protein
- 2. Why does the geneticist need to include the promoter in their transgene design work?
 - a. The promoter allows the gene to actually insert into the plant cell.
 - b. The promoter is only needed in genes that are not naturally occurring in the plant otherwise the plant will not be able to copy the gene when the cell is ready to divide.
 - c. The promoter is the gene's on and off switch which all genes need to have so they can be expressed in specific cells to control plant cell function.
 - d. The promoter carries all the new gene coding instructions for the plant cell. Otherwise, the plant would lack the ability to ready the rest of the gene to make a protein encoded by introduced gene.
- 3. Which part of the gene encodes the amino acid sequence of the protein?
 - a. coding region
 - b. promoter
 - c. both the promoter and coding region
 - d. neither the promoter or the coding region
- 4. What is the on/off 'switch' region of a gene?
 - a. amino acids
 - b. termination sequence
 - c. coding region
 - d. promoter
- 5. What is the protein coding part of the gene?
 - a. amino acid
 - b. termination sequence
 - c. coding region
 - d. promoter





- 6. The ______ of the protein will determine the function that protein will have inside the body.
 - a. Length
 - b. Shape and size
 - c. Weight and location
- 7. Amino acids fit together to produce larger particles called
 - a. Living cells
 - b. Proteins
 - c. Genes
 - d. Tissue
- 8. The promoter region...
 - a. Determines where the gene turns on.
 - b. Signals how much protein to produce.
 - c. Both of the above
- 9. The coding region...
 - a. Encodes the information for which protein to produce.
 - b. Signals how much protein to produce.
 - c. Prevents the cell from reading the next gene on the chromosome.
- 10. Each gene has three regions called...
 - a. Starter region, Coding region, Ending sequence
 - b. Termination sequence, Coding region, Prompter
 - c. Promoter, Coding region, Termination sequence
- 11. Promoters are very specific. For a soybean plant, this could mean:
 - a. Some promoters will only turn on in green tissue.
 - b. Some promoters will turn on when a specific infection is present.
 - c. Some promoters will turn on in all areas of the plant.
 - d. All of the above.
- 12. Which of the following statements is most true?
 - a. Genes in all living things are written in the same basic DNA language.
 - b. Each species will have a different DNA language.
- 13. The termination sequence...
 - a. Signals where the gene finishes copying.
 - b. Determines what is actually copied and gives the trait.
 - c. Prevents the cell from reading the next gene on the chromosome.
 - d. Both A and C
 - e. Both B and C





- 14. If the goal is to create a transgene that will work to fight an infection of the soil borne fungus, *Fusarium virguliforme*, should the gene be active all the time?
 - a. No, the promoter should trigger the expression of the gene only when the infection is present so the plant doesn't waste energy.
 - b. Yes, the promoter should always be active so that it is ready to be expressed at any time the infection shows up so the plant is always protected.
- 15. If my breeding goal is to create a corn plant which will target European corn borer at all stages of growth, which gene has the <u>best promoter</u> to meet my goal?
 - a. Gene #1, cloned from that cauliflower mosaic virus. This gene is expressed in all plant tissues and encodes the '35S' ribosome that the virus needs to replicate in plant tissues.
 - b. Gene #2, cloned from a bacillus Thuringiensis (Bt) bacteria and encodes a protein the bacteria uses to kill some caterpillars such as European corn borer larvae.
 - c. Gene #3, cloned from a corn plant. The gene encodes a protein the plant needs for photosynthesis and the gene is only expressed in green tissue that has active photosynthesis.
- 16. If my breeding goal is to create a corn plant which will target European Corn Borer larvae at all stages of growth, which gene has the <u>best coding region</u> to meet my goal?
 - a. Gene #1, cloned from that cauliflower mosaic virus. This gene is expressed in all plant tissues and encodes the '35S' ribosome that the virus needs to replicate in plant tissues.
 - b. Gene #2, cloned from a *Bacillus thuringiensis* (Bt) bacteria and encodes a protein the bacteria uses to kill some caterpillars such as European corn borer larvae.
 - c. Gene #3, cloned from a corn plant. The gene encodes a protein the plant needs for photosynthesis and the gene is only expressed in green tissue that has active photosynthesis.
- 17. Genes are made of...
 - a. Amino acids
 - b. Chromosomes
 - c. Nucleotides
 - d. All of the above
- 18. Proteins are made of...
 - a. Amino acids
 - b. Chromosomes
 - c. Nucleotides
 - d. All of the above





Lesson 2 | Gene Insertion

Background

Purpose

Genetic engineering is a form of biotechnology in which one or more genes are added to an organism to produce a desired trait. This lesson introduces how a transgene is inserted into an organism in order to create a genetically engineered organism. With prior understanding of basic genetics (Mendelian principles of heredity, DNA structure & function, mitosis & meiosis) students will use an interactive online animation to learn about the process of engineering a plant. Video content from "Journey of a Gene" will be used to show how this process is applied in a real-world plant transformation lab.

Standards

Next Generation Science Standards

HS-ETS1-3 Evaluate a solution to a complex real-world problem based on prioritized criteria and trade-offs that account for a range of constraints, including cost, safety, reliability, and aesthetics, as well as possible social, cultural, and environmental impacts.

Common Core

CCRA.R.7 Integrate and evaluate content presented in diverse media and formats, including visually and quantitatively, as well as in words.

Estimated Time

One 45-minute class period

Student Materials

- Student Worksheet
- Computer with internet access
- Scissors

Teacher Materials

- Student Worksheet Answer Key
- Engineer a Plant cards (1 copy per student)

Vocabulary

- agrobacterium: bacteria that causes disease in plants and has the ability to transfer a portion of its genes into plant cells
- plasmid: small, circular section of DNA, usually found in bacteria that can act as a vector
- vector: DNA used to carry a new section of DNA into a cell (often a plasmid)
- transgenic: A cell that has incorporated new DNA within it
- **Bt:** Bacillus thuringiensis is a bacteria that lives in the soil and produces proteins that are toxic to many insects
- **cotyledon**: part of the embryo within the plant seed; after germination, this becomes the first leaves of the seedling





Key STEM Ideas

All living organisms have traits determined by the genes in their DNA. They inherit these genes from their parents. Scientists use their understanding of inheritance and genetics to develop novel biotechnology solutions to agricultural challenges including damage from insect pests and resource competition from weeds. Scientists can alter the genetic makeup of an organism through the insertion of a transgene with the ability to give the organism a desired trait such as pest resistance or herbicide tolerance. This lesson will explain how non-transgenic plants are transformed into transgenic plants and how genes can be inserted into plants.

Students' Prior Knowledge

Students should understand that all living organisms are made up of genes that determine their traits. They should also know that genes can be manipulated to create organisms with desirable traits to be more beneficial to humans. Students will learn the steps of the gene insertion process and how a transgenic plant such as an herbicide-resistant soybean plant are created and grown.

Connections to Agriculture

Farmers regularly face environmental challenges when growing crops such as insect damage, resource competition from weeds, and drought. Luckily, scientists and plant breeders are using their knowledge of genetics and inheritance to develop transgenic crops capable of surviving and thriving even in the face of these environmental challenges.

The process of developing a transgenic or genetically engineered organism involves designing a transgene that will allow an organism to express a desirable trait (such as producing a toxin that kills an insect pest, being resistant to a disease, or allowing for the plant to be sprayed by an herbicide without being damaged) and inserting this transgene into the organism. In the case of plants, the transgenic crops can then be crossed with locally-adapted and/or high yield plants to incorporate those desirable traits into a commercial soybean variety available for farmers to purchase.

In this lesson we focus on the creation of a transgenic soybean plant that will be resistant to a disease known as sudden death syndrome. The lesson introduces a farmer in Iowa who has struggled with the problem of soybeans suddenly dying in a field with more than 50% yield losses in some years. By adding some gene to make the plants more resistant to this problem, breeders can harness biotechnology as a potential solution to combat this real world agricultural problem.

Essential Links

- Journey of a Gene, Introductory Video: https://ge.unl.edu/journey-of-a-gene/
- Journey of a Gene- Step 2, Transformation: https://ge.unl.edu/journey-of-a-gene/step-2transformation/objectives/
- PBS Interactive Animation: Creating a transgenic plant: http://www.pbs.org/wgbh/harvest/engineer/transgen.html

Sources/Credits

The unit is adapted for the high school biology classroom from the web-based learning portal "Genetic Engineering: Journey of a Gene" found at https://ge.unl.edu/journey-of-a-gene/.





Lesson Procedures

Prior to starting the lesson...

Print out 1 copy of the Engineer a Plant cards per student

Engage

Introduction: Identifying an Agricultural Problem: Sudden Death Syndrome (SDS) in Soybeans

- 1. Have students discuss what environmental issues farmers face when growing crops (Diseases, pests, poor weather, etc.)
- 2. Discuss which of these problems are potentially addressed by creating a genetically engineered plant.
- 3. Hand out the student worksheet.
- 4. Play the introductory video (https://ge.unl.edu/journey-of-a-gene/) about sudden death syndrome (SDS) in soybeans.
- 5. Have students answer the questions on their student worksheet in pairs or individually.
 - a. What happens to soybean fields afflicted with Sudden Death Syndrome (SDS)?
 - b. In the video, one scientist indicates that developing a genetically engineered variety of soybean plants resistant to SDS in a lab is a good option. Why isn't traditional breeding considered a viable solution?
 - c. What are three criteria scientists might consider to be important when creating a new soybean plant through genetic engineering? Why?
- 6. Have students discuss their answers in small groups or as a whole class. Answers are provided in the teacher notes.

Explore

Part 1: Practicing Inserting a Gene

- 7. Have students work in pairs or individually and provide students with access to computers and internet.
- 8. Instruct students to follow the instructions for Practicing Inserting a Transgene.
- 9. Instruct students to visit the Journey of a Gene website (see essential links).
- 10. Explain that while there are multiple steps in engineering a plant, this lesson will focus on how to insert a transgene to transform a plant into a transgenic, therefore, students should click on "Step 2: Transformation".
- 11. Have students read the Learning Objectives and watch the video "Constructing a Plasmid" found under Gene Delivery.
- 12. Students will define the following terms from the video:
 - a. Agrobacterium
 - b.Plasmid
 - c. Sticky ends
 - d. Transgene
- 13. Discuss student definitions to check for understanding. Answers are provided in the teacher notes.
- 14. Instruct students that they will now practice genetically engineering a tomato plant using an interactive animation. Have students visit the PBS interactive animation called "Engineer a Crop" (see essential links).
- 15. Students should practice dragging and dropping the steps in the right order in order to insert a Bt transgene into a tomato plant in order to create a transgenic tomato plant resistant to certain insect pests. Students may need to click through the module several times to fully understand the process.





- 16. While completing the PBS interactive animation, encourage students to document the steps they take in the table on their student worksheet and to provide the reason why each action is taken. Students should complete this task individually.
- 17. Students should also use what they learned in the interactive animation to answer the follow-up questions. Answers are provided in the teacher notes.
 - a. What is Bt?
 - b. How does the Bt gene get into the plant's genome?
 - c. What is technically incorrect about Step 6?
 - d. How can you tell if you were successful in inserting the transgene?
 - e. Have students spend several minutes reviewing the steps of the plant engineering process.
- 18. Once students feel comfortable in explaining the process, students can visit the instructor's desk one at a time to place the steps in order and explain why this is the correct order. Answer is provided in the teacher key.
- 19. Explain to students that for this activity no notes can be used. If students do not correctly order the steps, send them back to their computer to revisit the animation or study the steps in more depth. Complete with all students until all can accurately explain the process of engineering the plant.

Extend

Part 2: Testing for Gene Insertion

- 20. Explain to students that simply inserting the transgene is not enough. Scientists must confirm that the insertion was successful.
- 21. Have students watch the video located under the tab entitled, *Marker Genes* (video length: 4:05) and answer the follow-up questions to check for understanding. Answers are provided in the teacher notes.
 - a. What three genes are inserted into the plant in the example shown and what is their purpose?
 - b. In your own words, describe how antibiotics and herbicide are used by scientists to ensure successful insertion of the transgene into the plant.

Part 3: Using Gene Insertion in Research

- 22. For students to connect this process to a real-world context, have students watch the video located under the tab entitled, *In the Lab* (video length: 6:44).
- 23. Facilitate a class discussion about any new information that students learned from watching the scientist, Shirley Sato, transform soybean plants in the lab. If students mention any unfamiliar terms from the video, take some time to have students look up these terms.
- 24. Have students answer the follow-up questions individually. Answers are provided in the teacher notes.
 - a. After the seeds have germinated on the growth medium, what does, Shirley, the scientist do to the plant? Why is this done?
 - b. How long is the agrobacterium given to incorporate the transgene into the cotyledon?
 - c. True or False: Scientists have control over where the transgene is inserted in plant's genome when using agrobacterium.
 - d. What steps might scientists take after the plant is transformed in the lab and grown in a greenhouse?
- 25. Have students discuss in greater depth their answers to the final questions about what steps might come next in the process.









Discussion and Reflection

- 26. Have students revisit their answers to the question of important criteria to consider when engineering a plant.
- 27. Discuss criteria they are considering such as cost, reliability, safety, and farmer or consumer acceptance.
- 28. As a whole class, discuss advantages and disadvantages according to these criteria of using the genetic engineering process they learned about in this lesson.





Introduction

Identifying an Agricultural Problem: Sudden Death Syndrome (SDS) in Soybeans

Watch the video found here: https://ge.unl.edu/journey-of-a-gene/ (video length: 2:37). Answer the following questions.

- 1. What happens to soybean fields afflicted with Sudden Death Syndrome (SDS)? The scientist indicates a loss of 30-40% of yield. The farmer has lost more than 50% (closer to 67% of yield in a bad year). The video doesn't discuss what SDS damage looks like.
- 2. In the video, one scientist indicates that developing a genetically engineered variety of soybean plants resistant to SDS in a lab is a good option. Why isn't traditional breeding considered a viable solution?
 - Resistance to SDS involves 14 or more genes and this is difficult and time-consuming to incorporate into a soybean variety via traditional breeding. Instead, the genes can be artificially inserted and an SDS-resistant transgenic soybean plant can be created in a cost-effective and timely way in the lab.
- 3. What are <u>three criteria</u> scientists might consider to be important when creating a new soybean plant through genetic engineering? Why?
 - Accept reasonable answers. Students may discuss cost, time needed, farmer or consumer acceptance of the technology, efficacy or reliability of the resistance trait, etc.

Part 1: Practice Inserting a Gene

Genetic engineering enables scientists to modify the DNA of living organisms by transferring a transgene - or modified gene - into a target organism. The genetically engineered organism is then called a transgenic. What steps are involved in the insertion of a transgene into an organism? Let's find out. Working either individually or in pairs:

- 1. Go to passel.unl.edu/ge/
 - 2. Click on "Step 2: Transformation"
 - 3. Read the *Learning Objectives* and watch the video "Constructing a Plasmid", found under *Gene Delivery* (video length: 2:47).
 - 4. Define the following terms from the video:
 - a. Agrobacterium A soil bacteria used to deliver genes into plant cells. Natural ability to insert genes into plants.
 - b. Plasmid A small circular piece of DNA found in bacteria.
 - c. Sticky ends Section where broken nucleotide pairs have a slight charge. This makes them attracted to binding with a complementary sequence and reforming hydrogen bonds.
 - d. Transgene A gene that has been spliced from another organism or created in the lab.
 - 5. Under the tab, *Practice Inserting a* Gene, visit the PBS interactive animation called "Engineer a Crop". Practice dragging and dropping the steps in the right order in order to insert a Bt transgene into a tomato plant in order to create a transgenic tomato plant resistant to certain insect pests.
 - 6. Complete the table below with the eight steps of engineering the crop from the animation. In one column, list the physical action you must take for each step and in the next column describe WHY you are doing that action what do you hope to get out of it?





Step #	Physical Action	Goal/Result
1	Add Bt gene to a vector	Make Bt DNA a part of vector DNA
2	Add vector to bacterium	Get Bt DNA into bacterial DNA
3	Grow bacterium	Increase number of Bt genes present
4	Add tomato leaf bits to bacterium	Integrate vector DNA into tomato plant DNA
5	Grow plant cells	Turn plant "cell" into "plant"
6	Spray herbicide	Remove plants that did not incorporate desired gene
7	Grow plants	Get a mature plant from cuttings
8	Expose plants to pest	See if your plant really does have the desired trait and rule out any with undesirable traits

- Answer the following questions based on what you learned from the animation.
 - a. What is Bt? A type of bacteria that is toxic to certain species of insects.
 - b. How does the Bt gene get into the plant's genome? The Bt gene gets into the plant's genome because Agrobacterium, a type of soil bacteria, is able to insert plasma DNA into plant cells that it infects.
 - c. What is technically incorrect about Step 6? The herbicide is a way of "double checking" to make sure the plants have the desired transgene. Occasionally, some non-transgenic plants are able to live on the ampicillin growth media even though they do not contain ampicillin resistance. Testing the plants with herbicides can reveal if the plants got the herbicide resistance gene that was also placed into the plant's genome during transformation with the transgene.
 - d. How can you tell if you were successful in inserting the transgene? Another gene, making the plants resistant to a specific herbicide was also added. So, if the plants don't die when sprayed with the herbicide, then you were successful!
- 8. Spend a few minutes reviewing the steps involved in engineering a plant. When you feel you understand the steps in the process, go to your instructor's desk. Eight cards representing the above steps will be available. (The Engineer a Plant Cards are found with the teacher materials.) Arrange the cards in the correct order AND explain why the cards are placed in that particular order. If you are successful, you can move on to the next task. No notes are allowed for this step.

If you can't put them in the right order or explain it yet, your instructor will tell you to come back to this practice exercise to hone your skills before you try again.









Part 2: Testing for Successful Gene Insertion

Watch the video located under the tab entitled, Marker Genes (video length: 4:05).

- 1. What three genes are inserted into the plant in the example shown and what is their purpose?
 - a. The transgene for the desired trait such as disease resistance
 - b. Marker gene #1- an antibiotic resistance gene
 - c. Marker gene #2- an herbicide resistance gene
- 2. In your own words, describe how antibiotics and herbicide are used by scientists to ensure successful insertion of the transgene into the plant.
 First, the cotyledons are grown on a growth medium containing an antibiotic, this should prevent growth from all cells that contain the inserted gene. As a final check, an herbicide can be applied. Plants with the herbicide resistant gene (inserted with the transgene) will be indicated by a lack of damage to the leaves.

Part 3: Using Gene Insertion in Research

Watch the video located under the tab entitled, *In the Lab* (video length: 6:44).

- 1. After the seeds have germinated on the growth medium, what does, Shirley, the scientist do to the plant? Why is this done?
 - The plant is wounded as a way to attract the agrobacterium when it is added.
- 2. How long is the agrobacterium given to incorporate the transgene into the cotyledon? The cotyledons are left for three (3) days on the filter paper while the agrobacterium incorporates its DNA into the plant tissues.
- 3. True or False: Scientists have control over where the transgene is inserted in plant's genome when using agrobacterium. The transgene is randomly inserted.
- 4. What steps might scientists take after the plant is transformed in the lab and grown in a greenhouse? Students should mention something about the plants being transplanted to a field and grown under normal environmental conditions. Yield or other measurements of plant health/productivity can be taken.





Teacher Materials

Engineer a Plant Cards

The following cards include all steps in genetically engineering a plant. They are for use in Part 1: Practice Inserting a Gene.

- 1. Make one copy of the cards per student.
- 2. Cut out these cards so that no lines are visible.
- 3. Have students arrange the cards in order on your desk and explain WHY each step is done. If they make a mistake they go back to their desk for more practice.
- 4. When students have completed this to your satisfaction, they can move on.

Add Bt gene to a vector	Grow plant cells on growth media
Add vector to bacterium	Apply herbicide
Grow bacterium on media	Grow plant cells into full plants
Add bits of tomato leaf to the media with the bacterium	Expose plants to pests





Lesson 3 | Flower Anatomy and Plant Breeding

Background

Purpose

Students will use this module to learn about the basic structure and function of flower anatomy as it relates to plant breeding.

Standards

Next Generation Science Standards

HS-LS1-2. Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.

Common Core

W.9-10.7 Conduct short as well as more sustained research projects to answer a question (including a self-generated question) or solve a problem; narrow or broaden the inquiry when appropriate; synthesize multiple sources on the subject, demonstrating understanding of the subject under investigation.

Estimated Time

Two 45-minute class periods

Student Materials

- 1 flower blossom
- 1 microscope or hand lens (5-10x)
- Dissecting tools (probes, tweezers, scalpel)
- Double-sided tape
- 2 pieces of paper
- Student worksheet
- Computer with internet access

Teacher Materials

- Digital microscope (optional)
- PowerPoint presentation
- Answer key





Vocabulary

- angiosperm: a flowering plant
- **sepals:** protective leaf-life enclosures for the flower buds, usually green but can be colored like petals
- **petals:** usually colorful modified leaves that make up the "flower", attracts a pollinator with color, scent, UV patterns
- stamen: male reproductive parts of a plant
- anther: part of stamen that produces the pollen
- filament: part of stamen that hold anther
- pollen: contains male genetic material, fertilizes the ovule
- pistil: female reproductive parts of a flower
- **stigma**: part of stigma that receives the pollen
- **style**: part of the pistil that connects to the ovary
- ovary: part of the pistil that contains the ovules; after ovules are fertilized, turns into the fruit
- ovules: contain female genetic material; after fertilization, develop into seeds

Key STEM Ideas

Understanding the structure and function of flower anatomy is essential to learning about sexual reproduction of flowering plants, known as angiosperms. This inquiry-driven lesson allows students to dissect flower blossoms and determine what characteristics of their structure helps them to provide reproductive function of plants. Sexual reproduction shares many similarities with human reproduction. Students will compare and contrast these systems to gain a better understanding of sexual reproduction in all organisms. A solid understanding of plant reproduction provides the foundation for plant breeding techniques. Finally, students will learn about what other skills are needed to be a plant breeder and explore historical impacts of Norman Borlaug, a world renowned plant breeder who won the Nobel Peace Prize in 1970 for his efforts to improve agriculture and feed millions of people.

Students' Prior Knowledge

Students should be familiar with the basics of human reproduction. Students will expand their understanding of sexual reproduction to include flowering plants. Some students may have prior understanding of correct angiosperm terminology, however, this lesson does not require a deep understanding of plant structure or function. Students should be familiar with dissection tools and how to use them safely.

Connections to Agriculture

Most students understand that animals such as humans reproduce sexually but have a tendency to think of physical sexual union when they see the term "sexual reproduction." Thus, they do not always understand that plants also reproduce sexually and require both male and female genetic material to make a new seed. Further confusing the issue is that plants can have both male and female parts on the same organism and self-pollinate!

It is important to reinforce for students the idea that sexual reproduction occurs in plants and is the foundation for our ability to breed plants with particular traits. The combining of genetics from two parent plants allows plant breeders to incorporate desirable traits into offspring by selecting and crossing specific male and female parent plants. With more genetic variation in plants, plant breeders can select for traits that





allow plants to survive and thrive in a variety of different environments while under different stresses such as pressure from diseases or pests.

Essential Links

- Time Lapse of Flowers Blossoming: https://vimeo.com/69225705
- Plant Reproduction in Angiosperms from the Ameoba Sisters: https://youtu.be/HLYPm2idSTE
- How to Cross Soybeans: https://vimeo.com/82420755
- A Student's Guide to Careers in Plant Breeding: https://youtu.be/pbRk64bc03c
- Norman Borlaug and the Green Revolution: https://youtu.be/Lg9-HTtgFOk

Sources/Credits

- Flower dissection activity modified from McIntosh, A. V., & Richter, S. C. (2007). Digital daisy: an inquiry-based approach to investigating floral morphology and dissection. Science Activities: Classroom Projects and Curriculum Ideas, 43(4), 15-21.
- Plant vs. Human Reproduction activity modified from www.mrl.ucsb.edu/





Lesson Procedures

Engage

- Have students view the video of flowers blossoming in time lapse: https://vimeo.com/69225705 (slide 1)
- 2. Ask students to discuss (1) What did they observe? and (2) Did they notice any similarities or differences between the different types of flowers? (Students can discuss structures that looked similar or different. While they may look a little different from flower to flower, the structures serve the same function no matter the flower.)

Explore

Activity 1: Dissection of a Flower

- 3. Have students work with a partner. Distribute the student worksheet and make materials available (slide
 - 2). Each pair will be given:
 - a. One flower blossom
 - b. Microscope or hand lens (5x–10x)
 - c. Assortment of dissecting tools (probes, tweezers, scalpel)
 - d. Double-sided tape
 - e. 2 pieces of paper (one for sketching, one for attaching flower parts)
- 4. Read through the procedures for the activity as a group.
 - a. Draw a sketch of the flower.
 - b. Gather dissection materials.
 - c. Begin carefully dissecting the flower.
 - d. As you gently remove parts of the flower, group them with similar parts.
 - e. When all parts of the flower have been taken apart, use double-sided tape to attach the parts to a piece of paper.
 - f. Write your prediction of what function each part serves in the plant.
- 5. (Optional) Before students start their dissection, you may wish to model the process of dissecting a flower under a digital microscope if students are unfamiliar with how to use their dissection tools or need more structured instruction.
- 6. Have each pair of students complete the activity.
- 7. Have students use their observations of flower structures to sketch a drawing of a flower on the white board. Write their observations of each structure and discuss how the characteristics of the different structures might help the plant to survive and thrive. Discuss students' function predictions as a class. After student own terms are discussed, introduce scientific terminology.
- 8. Ask students if they are familiar with proper terminology for the flower structures. Some students may already be knowledgeable of accurate terms for the various structures. Use slide 6 to introduce terms that are unfamiliar. Have students attempt to label the white board drawing with correct terms.
- 9. Use slide 7 to introduce accurate labeling of flower parts. Make corrections to the flower labeled on the white board.
- 10. Help students to connect the characteristics of the structures with their ability to perform a reproductive function. (Pollen is like a dust, it easily brushes off the anthers and can be transported from flower to flower by a pollinator or the wind, the stigma is sticky and allows pollen to adhere to it, the petals may smell nice or have markings that guide pollinators to the center of the flower, etc.)
- 11. Have students answer the post-activity reflection questions.





Activity 2: Flowering Plant vs. Human Reproduction

- 12. Have students make comparisons and contrasts between reproduction in flowering plants and humans by filling in the table for activity 2.
- 13. Using slide 8, watch the video "Plant Reproduction in Angiosperms" from the Ameoba Sisters (https://youtu.be/HLYPm2idSTE). Answer the follow-up questions and discuss as a group using slide 9.
 - a. According to the video, squash and green beans are considered fruit. Why is this?
 - b. Why are pollinators important for plant reproduction?
 - c. What is the purpose of fruit development in plant reproduction?

Extend

Activity 3: Controlling Plant Reproduction in Agriculture

- 14. Using slide 10, watch the video "How to cross soybeans" (https://vimeo.com/82420755).
- 15. Have students answer the follow-up questions and discuss as a group using slide 11.
 - a. What difference did they notice between the flower they dissected and the soybean flower dissected by a plant breeder?
 - b. Have students discuss the steps involved in plant breeders making a soybean cross.
 - c. Discuss why a plant breeder would perform this cross by hand rather than letting pollinators move the pollen.
 - d. Discuss why a plant breeder would want to control the parent plants of a soybean cross.

Activity 4: Careers in Plant Breeding

- 16. Read the introduction for activity 4. Discuss several ways that the food we eat is made better by the work of plant breeders.
- 17. Explain that the class will be watching two videos. Use slide 12 to watch the first video (A Student's Guide to Careers in Plant Breeding: https://youtu.be/pbRk64bc03c). This video will discuss what a plant breeder's job is and what skills are used to be successful in this career. Encourage students to think of one thing they would like about this job and one thing they would dislike.
- 18. Use slide 13 to watch the second video (Norman Borlaug and the Green Revolution: https://youtu.be/Lg9-HTtgFOk). This video will introduce Norman Borlaug, a world renown plant breeder who has won a Nobel Peace Prize for his improvements to agriculture that help feed the world. Discuss with students how a single plant breeder impacted human health and agriculture as a whole.

Evaluate

19. Use answers to questions on the student worksheet and class discussion/participation as means of evaluation.





Answer Key

Activity 1: Structure and Function of a Flower

Plant breeding requires the plant breeder to manipulate a flower to control a cross. In doing this, a breeder needs to have a good understanding of flower parts. As a review of flower anatomy, you will dissect a flower, separate the parts of the flowers, group them by similar structure, and predict their function for the plant.

Materials needed

- One flower blossom
- Microscope or hand lens (5x–10x)
- Assortment of dissecting tools (probes, tweezers, scalpel)
- Double-sided tape
- 2 pieces of paper (one for sketching, one for attaching flower parts)

Procedures

- 1. Draw a sketch of the flower.
- 2. Gather dissection materials.
- 3. Begin carefully dissecting the flower.
- 4. As you gently remove parts of the flower, group them with similar parts.
- 5. When all parts of the flower have been taken apart, use double-sided tape to attach the parts to a piece of paper.
- 6. Make observations about the flower structures based on what you can see, smell, and touch.
- 7. Write your prediction of what function each part serves in the plant.

Post-Activity Reflection

- 1. The parts of this flower make up what functional system for the plant? The reproductive system
- 2. What observations did you make about the flower petals? How do you think the petals contribute to plant reproduction?
 - They may be colorful, smell good, have lines on them, etc. They attract insects looking for a food reward.
- 3. What characteristics did you notice about pollen? Why do you think pollen is well-suited for moving genetic material between plants?
 - Pollen is dusty, easily brushes off the anthers. It can easily be trapped in the hairs of pollinators when they are gathering pollen and nectar for food and then transferred when multiple flowers are visited.
- 4. What did you observe about the stigma of the flower? How does this characteristic help the stigma to function?
 - The stigma is sticky and is good for helping to adhere pollen to the plant.





Activity 2: Flowering Plant vs. Human Reproduction

Now that you have explored the parts of a flower, it is time to compare and contrast flowering plant reproduction with human reproduction. Create a table that lists similarities and differences between these reproductive systems.

Similarities	Differences		
Ex) Both require fertilization of the egg by male genetic material.	Ex) The male genetic material is sperm in humans and pollen in plants.		
Both produce an egg.	Flowering plant must plant a seed for offspring to grow, fetus begins growing upon fertilization in humans.		
Both reproduce by sexual reproduction.	Female and male reproductive parts sometimes found in the same plant, separate females and males in humans.		
Offspring of both will share traits of the parents.	Time needed for reproduction is different.		
Both require male and female parts.	Plants are immobile and need a mobile vehicle (insect or wind) for reproduction; humans are mobile, no additional vehicle is needed.		
	Flowering plants can produce many more seeds at once; humans have a lower reproductive capacity.		

Watch the video "Plant Reproduction in Angiosperms" from the Ameoba Sisters (https://youtu.be/HLYPm2idSTE). Answer the following questions.

- 5. According to the video, squash and green beans are considered fruit. Why is this? All of these develop from the ovary of a fertilized flower so they are all considered fruit.
- 6. Why are pollinators important for plant reproduction? Since plants don't have the ability to move their own genetic material around, they rely on insects or wind to carry it around for them. Pollinators do this by accidently picking up pollen while feeding on nectar and pollen. Only after a plant is pollinated can the pollen fertilize the ovule in order to develop seeds.
- 7. What is the purpose of fruit development in plant reproduction? Fruits help the seeds travel far from the parent plant. They can be carried by wind, water, or an animal, or eaten by an animal and deposited elsewhere.





Activity 3: Controlling Plant Reproduction in Agriculture

Watch the video "How to cross soybeans" (https://vimeo.com/82420755) and answer the following questions.

- 8. What was one difference you noticed about the size of soybean flowers compared to the flower you dissected in activity 1?

 Soybean flowers are much smaller than the flower that was dissected.
- 9. Explain, in detail, what a plant breeder must do to pollinate a soybean plant. The breeder must know what crosses they want to make (which female and male parents they want to use).
 - Identify a female plant that has buds that will be opening soon.
 - Pull the sepals back to expose the petals.
 - Gently pull the petals out to expose the anthers and the stigma.
 - Clean (remove) the anthers out of the bud.
 - Find a male plant to pollinate the plant.
 - Collect the selected male parent flower to be used in the cross.
 - Open the flower to expose the anthers.
 - Pull out the anthers. Be sure that pollen is present.
 - Brush the pollen from the anthers onto the stigma.
- 10. What is the benefit of a plant breeder pollinating soybeans by hand rather than relying on an insect pollinator to move the genetic material from one plant to another? If the plant breeder pollinates the soybean plant by hand, this guarantees that specific male genetics of one plant are combined with the female genetics of another plant. The breeder has more control over the cross than if insects randomly moved pollen from one plant to another.
- 11. Why might a breeder want to control which parent plants are used in the reproduction of soybean seeds?

This allows breeders to select for desirable traits and keep track of which parents produce offspring with these particular traits.





Activity 4: Careers in Plant Breeding

You learned in Activity 3 why plant breeders need to understand flower anatomy and the mechanics of plant reproduction in order to breed new lines of soybeans. Plant breeders conduct research on plant characteristics, or traits, and work to create varieties of plants that can best survive and thrive in a particular environment. Important plant traits may include the ability to resist a disease or pest, to survive drought, to produce lots of fruit, or to mature quickly. Plant traits are controlled by the genes contributed by each parent plant and the breeder controls which plants contribute their genes to the seeds of the next generation of plants.

To learn more about careers in plant breeding and the impact plant breeding has had on the world, watch the two videos below.

A Student's Guide to Careers in Plant Breeding: https://youtu.be/pbRk64bc03c Norman Borlaug and the Green Revolution: https://youtu.be/Lg9-HTtgFOk

- 12. What skills do you have that could contribute to a career in plant breeding? Answers will vary.
- 13. What new skills would you need to learn in order to succeed as a plant breeder? Answers will vary.
- 14. What is one aspect of a career in plant breeding that you would like? What is one aspect you would dislike?

Answers will vary.

15. Who is Norman Borlaug and how did his work change agriculture around the world? Norman Borlaug was a plant breeder who helped farmers to improve their crops and increase their yields. In three years, he used plant breeding techniques to develop wheat that was resistant to stem rust fungus and crossed it with locally-adapted susceptible plants and selected for resistant lines. His team accelerated the breeding process by evaluating and selecting resistant lines twice every year. This strategy was known as shuttle breeding. He grew the wheat in two locations with different day lengths, making the wheat adaptable to many different areas. His wheat also responded well to fertilizer and so developed heavy seed heads. The heavy seed heads collapsed under the weight causing a problem called lodging. He used plant breeding techniques to solve this problem. Breeders had developed a line called semi-dwarf wheat which grew shorter, stronger stalks which could support the weight of the grain. Borlaug crossed this line into his breeding program to produce a wheat line that would not lodge and was adapted to growing conditions in Mexico. His wheat lines were then grown in other areas such as India and Pakistan increasing wheat yields dramatically. This dramatic increase in food production was called the green revolution.

His efforts to improve agriculture were recognized when he was awarded a Nobel Peace Prize in 1970.





Lesson 4 | Backcross Breeding with Transgenic Plants Background

Purpose

Students will learn the role of traditional plant breeding techniques in genetic transformations and perform a simulation of backcross breeding.

Standards

Next Generation Science Standards

HS-LS3-3. Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

Common Core

HSS.IC.A.2 Decide if a specified model is consistent with results from a given data-generating process, e.g., using simulation.

Estimated Time

One 45-minute class period

Student Materials

Each student will need:

- Computer and internet access
- Journey of a Gene online tutorial
- Student worksheet

Each group of 2-3 students will need:

- 3 clear plastic cups of the same size
- Two different colors of beads or other small items

Teacher Materials

- PowerPoint presentation
- Answer key

Vocabulary

- **breeding:** the activity of controlling the mating and production of offspring
- elite line: crop varieties with the most desirable traits such as high yield potential or drought resistance
- **donor parent:** the parent plant that has the new/desired gene
- recurrent parent: the parent plant from the elite line
- backcross breeding: the process of breeding a transgenic line of crops with the elite line over and over to get the best product possible: all of the elite line characteristics plus the new/desired gene





Key STEM Ideas

Students will learn about how plant breeding is a necessary step during the crop genetic engineering process. Students will use knowledge of DNA, inheritance patterns of genes and traits, and probability to simulate the process of backcross breeding. Backcross breeding is a commonly used procedure in the development of new crop varieties including a new/desired transgene and high quality agronomic traits such as high yield.

Students' Prior Knowledge

Students should be familiar with concepts of DNA, inheritance, and how traits are a result of genes. Students should be familiar with the beginning steps in crop genetic engineering including extracting DNA from an organism with a desired trait, cloning the gene, modifying the gene, and inserting the modified gene (the transgene) into a transgenic line. This lesson will continue on with an explanation of how plant breeding is important to the genetic engineering process by crossbreeding the transgenic plants with elite plants expressing traits that are desirable to plant breeders and producers/farmers.

Connections to Agriculture

Genetic engineering is NOT all about white lab coats, beakers, and high tech equipment. Although these things are required to get the desired gene in the plant, genetic engineers are dependent on the breeding process conducted in greenhouses and in the field.

Once the desired gene has been inserted, hundreds if not thousands of plants must be grown. Random genetic variation means that some of the plants grown will have desirable traits of the elite line but not the new gene, others will have the new gene but not all the desirable traits and only a certain percentage will have both the desired traits AND the new gene.

When those plants that have BOTH the desired traits AND the new gene are grown, they are tested for multiple generations to assess their productivity, resistance, presence of the gene, etc.

The bottom line is, the engineers can put the gene in, but that alone won't give producers a commercial variety with all of the desirable traits they want. The process of genetic engineering is fundamentally dependent on traditional breeding techniques.

Essential Links

- Who wants to be a genetic engineer? Game: http://passel.unl.edu/pages/animation.php?a=who_wants_to_be_ge.swf&b=1023486473
- Journey of a Gene tutorial: passel.unl.edu/ge/

Sources/Credits

- Crop genetic engineering process image and corn image from the University of Nebraska-Lincoln (passel.unl.edu)
- Video tutorials and Who wants to be a genetic engineer? game from the University of Nebraska Plant and Soil Sciences. (passel.unl.edu)





Engage

Activity 1: Five Steps of Genetic Engineering a Plant.

- 1. Distribute the student worksheet and provide students with computer and internet access.
- 2. Direct students to review the steps of the crop genetic engineering process by playing the game "Who wants to be a genetic engineer?" found here:

 http://passel.unl.edu/pages/animation.php?a=who wants to be ge.swf&b=1023486473. The game offers two options, to create a cinnamon-flavored apple or a Bt corn variety resistant to European corn borer. Instruct students to select the cinnamon-flavored apples and fill in the table on the student worksheet with the steps they took to create the crop.
- 3. In their own words, have students discuss the steps that must occur when creating a genetically engineered crop.
- 4. Show slide 2 of the PowerPoint presentation and explain to students that they will be focusing in this lesson on the final step in crop genetic engineering, backcross breeding.

Explore

Video 1: An Introduction to Backcross Breeding

- 5. To learn more about the process of backcross breeding, instruct students to visit the "Journey of a Gene" website at passel.unl.edu/ge/. Have students click on "Step 3: Breeding", read the learning objectives and watch the video "Backcrossing".
- 6. Once students have watched the video they should answer the follow-up questions. Discuss student answers as a whole class or in small groups.
- 7. Demonstrate the process of backcross breeding using slides 3-16 of the PowerPoint presentation.
- 8. It is important to discuss with students how the transgenic and elite gene percentages are calculated at each step.

Explain

Activity 2: Performing a Simulated Backcross

- 9. To illustrate the backcross breeding of a transgenic line with an elite line, students will work in small groups to complete a plant breeding simulation using cups filled with different colored beads to represent the mixing of different genetic backgrounds of the transgenic and elite lines.
- 10. See slides 17-23 of the PowerPoint presentation for illustrated instructions of this activity.
- 11. Divide students into groups of 2-3 and provide each group with three plastic cups and two different colors of beads. Students should have access to approximately 50 beads of one color to represent the transgenic genes and approximately 175 beads of another color to represent the elite genes.
- 12. Students should complete a total of 6 crosses during the simulation. As students complete each cross, they can fill in the offspring's genetic makeup (% transgenic genes and % elite genes) on the student worksheet.





- 13. Once students understand the concept of how the inheritance percentages change with each cross, use slide 25 to provide students with the equation to calculate the percentage of elite genes inherited by the offspring at each backcross. The equation is $\frac{2^n-1}{2^n}$, where *n* is the number of backcrosses.
- 14. Students can use the equation to double-check their answers on the student worksheet. Calculations for all crosses can be found on slide 26. In addition, answers are provided in the teacher notes.
- 15. Once students have completed the simulation, have them discuss their simulation. Did their simulation look similar to another group? Did all simulations look similar? Why is this? Facilitate a discussion of probability and variability between simulations.
- 16. Finish having students discuss the follow-up question: Do you think the final backcross would have more traits like the transgenic line or like the elite line?

Extend

- 17. Direct students to the Journey of a Gene website, where they will watch the video "In the field" featuring a plant breeder discussing the use of backcrossing in developing new varieties of soybeans. Have students answer the follow-up questions.
- 18. Reiterate to students that the major benefit of genetic engineering is that traits can be added that would otherwise be unavailable in the native population or would be exceedingly difficult to incorporate using traditional breeding techniques.
- 19. Have students reflect on what they have learned about backcross breeding and discuss the reflection questions with a partner. Acceptable answers are provided in the teacher notes but students may discuss other interesting aspects of the backcross breeding process.

Evaluate

20. Student worksheets and activities can be used as a means of evaluating student understanding.





Answer Key

Activity 1: Five Steps of Genetic Engineering a Plant

Play the game "Who wants to be a genetic engineer?" found here: http://passel.unl.edu/pages/animation.php?a=who wants to be ge.swf&b=1023486473. In your own words, fill in the table with the steps you took to create a cinnamon-flavored apple. Be specific!

Steps	in plant genetic engineering	Create a cinnamon-flavored apple
1.	The genetic engineer identifies an organism with a desired trait and extracts the organism's DNA . Somewhere in the DNA is a gene that codes for a protein that causes the trait.	Extract DNA from cinnamon
2.	The genetic engineer locates the gene that codes for the protein, removes it from the DNA, and clones the gene using a technique called Polymerase Chain Reaction (PCR).	Clone the cinnamon flavor gene
3.	A gene is made up of three coding regions: the promoter, coding region, and termination sequence. The genetic engineer can cut these regions apart and one or more of the three regions can be altered or replaced so that the gene is expressed in a particular way inside the plant.	Modify the cinnamon flavor gene
4.	The modified gene is a combination of regions and is called recombinant DNA. The new gene can then be inserted into plant cells using a transformation method such as agrobacterium. Once the gene is successfully inserted into a plant cell 's nucleus, it can be incorporated into one of the chromosomes. The plant cell will divide and multiple to make a new transgenic plant and all its cells will have the gene. The gene can also can be passed on to offspring.	Insert the cinnamon flavor gene into apple cells
5.	The final step is called backcross breeding where a plant breeder crosses the transgenic plant with a high-yielding or locally-adapted elite line to produce a hybrid. These crosses are performed multiple times over several years. The final result is a high-yielding transgenic hybrid that expresses the desired trait.	Breed the apple tree

For this lesson, we will focus on the fifth and final step in genetic engineering called backcross breeding.





Video 1: An Introduction to Backcross Breeding

Go to passel.unl.edu/ge/.

Click on "Step 3: Breeding".

Read the Learning Objectives and watch the video "Backcrossing".

- 1. What is a transgenic line?
 Plants from a standard line that have been transformed to contain the transgene.
- 2. What is an elite line?
 Plants that have high quality traits that are desired by plant breeders and producers/farmers.
- 3. What are two differences between a transgenic line and an elite line?

 The transgenic line contains the transgene (the Bt gene) and the elite line does not.

 The elite line is newer and has higher yield compared to the transgenic line which comes from a standard line that is older and has lower yield.
- 4. How are transgenic and elite inbred lines produced? Self-pollination of the transgenic and elite lines occurs meaning the male and female parts of the same plant are used for sexual reproduction.
- 5. What is the result of the inbred lines?

 The transgenic plants will produce seeds with two copies of the transgene (the Bt gene) while the elite line will contain only elite line genes.
- 6. What is the goal of backcrossing?

 The goal of backcrossing is to breed the transgene into a new line of plants that also have a large percentage of genes from the elite line that produce the high quality traits that are desired.

You can use slides 3-16 of the PowerPoint presentation to illustrate the process of backcrossing! This is good for students to see before moving onto Activity 2.

Activity 2: Performing a Simulated Backcross

To illustrate the backcross breeding of a transgenic line with an elite line, you will work in small groups to complete a plant breeding simulation using cups filled with different colored beads to represent the mixing of different genetic backgrounds of the transgenic and elite lines.

Materials needed:

- Three cups
- Two different colors of beads





- 1. Year 1: Begin by filling two cups about half full with one color of beads in each. These two cups represent plants with 100% transgenic genes and 100% elite genes.
- 2. To make the first cross, pour half of the beads from each cup into a third cup and mix the beads. This represents the F1 cross.
- 3. Empty the unused beads from the 100% transgenic cup. After each cross, refill the cup containing 100% elite genes.
- 4. Year 2: Pour half the beads from the F1 cross and 100% elite into an empty cup and mix the beads. This represents Backcross 1.
- 5. Years 3-6: Continue this backcross process, mixing ½ the beads from the resulting "offspring" with ½ the beads from the 100% elite cup.

Fill in table below with % transgenic and % elite genes comprising the offspring's genetic makeup. Remember, the amount of remaining genetic information from the transgenic parent is reduced by 50% with each backcross.

Time	Parent #1	Parent #2	Offspring Produced	Offspring's Genetic Makeup
Year 1	100% Transgenic	100% Elite	F1 Cross	50% Transgenic 50% Elite
Year 2	F1 Cross	100% Elite	Backcross 1	25% Transgenic 75% Elite
Year 3	Backcross 1	100% Elite	Backcross 2	12.5% Transgenic 87.5% Elite
Year 4	Backcross 2	100% Elite	Backcross 3	6.25% Transgenic 93.75% Elite
Year 5	Backcross 3	100% Elite	Backcross 4	3.125% Transgenic 96.875% Elite
Year 6	Backcross 4		Backcross 5	1.56% Transgenic 98.44% Elite





1. After completing the simulation, do you think the final backcross would have more traits like the transgenic plant or like the elite plant? Explain.

The final backcross has >98% of genes from the elite line and so the traits should be more like the elite plant. The exception is the addition of the transgene and a small percentage of other genes from the transgenic line.

Video 2: Applying Backcross Breeding in the Field

On the "Journey of a Gene" website, watch the video "In the field" and answer the following questions.

2. In your backcross breeding simulation, you bred a transgenic plant and an elite plant. In the video, George talks about a donor parent and a recurrent parent being used in the backcross. How are these terms related? Circle the word to best complete the sentence.

The transgenic plant is	the <mark>donor</mark> or recurrent	parent
The elite plant is the	donor or recurrent	parent.

- 3. Explain why transgenic plants do not speed up the availability of new varieties.

 Transgenic plants still need to go through a time-consuming backcross breeding process which takes years to incorporate the transgene into a line along with other desirable characteristics such as high yield.
- 4. If it is not faster, what is the benefit of transgenic plants?

 Transgenic plants can add new traits that are not available within the native plants.

Discussion and Reflection

- 5. Should the backcross breeding process occur in a greenhouse or the field? Explain your answer. Answers may vary. In general, backcross breeding needs to occur in the field because of the large number of plants involved and the plant breeder's need to select for high quality plants under normal environmental conditions.
- 6. Why don't genetic engineers just put the desired genes into the "elite" varieties that farmers really like?

There are a limited number of lines that are used for transformation and those are not elite varieties. Transformation is limited to standard lines that grow well on media or are relatively easy to transform.



