Biotechnology Unit Overview

Disclaimer: The charts included in this unit make one set of connections between the lessons outlined in the unit and the NGSS performance expectations. Other valid connections are likely; however, space and time restrictions prevent us from listing all possibilities.

This high school unit on Biotechnology exposes students to several methods and techniques of artificial selection and genetic modification as applied in agriculture. The Science and Engineering Practices covered in these lessons include: **Constructing Explanations and Designing Solutions**, **Asking Questions and Defining Problems**, **Developing and Using Models**, **Analyzing and Interpreting Data** and **Engaging in Argument from Evidence** in order to explain the nature of science and make decisions about important issues in the practice of science.

Much of this unit centers around the Nature of Science. These four aspects are closely aligned with the practices:

Scientific Investigations Use a Variety of Methods
Scientific Knowledge is Based on Empirical Evidence
Scientific Knowledge is Open to Revision in Light of New Evidence
Scientific Models, Laws, Mechanisms, and Theories Explain Natural Phenomena

While these four are more closely aligned with crosscutting concepts:

Science is a Way of Knowing
Scientific Knowledge Assumes an Order and Consistency in Natural Systems
Science is a Human Endeavor
Science Addresses Questions About the Natural and Material World

Lesson 1 *Selective Breeding vs Genetic Modification* introduces the phenomena of the unit through a big question, ***What are GMOS and why are we afraid of them?*** then encourages students to begin to compare two techniques used in modern agriculture, Constructing Explanations of each and determining the advantages and disadvantages of each. Lesson 2 *DNA Extraction* introduces students to the process of developing a protocol, with each group designing their own method for extracting DNA from whole kernel corn, then

presenting their protocol to the lab cohort with an explanation of their choices. Students learn the importance of making strong arguments in science as they use evidence and reasoning to support their claims. They also communicate, collaborate, and skeptically evaluate each other's claims. Lesson 3 *DNA Sentences* asks students to Construct an Explanation for transcription and translation in the synthesis of proteins from DNA using words for sentences as a model. Lesson 4 *Punnett Squares* utilizes a traditional prediction tool to compare to the actual outcome of a trait, thereby Asking Questions and Defining Problems of this method of predicting offspring outcomes. Lesson 5 *Moving Genes* asks students to put the steps of genetic modification in the proper order and encourages Constructing Explanations for their choice of order.

Lesson 6A *Plasmid Modeling*, asks students to make a model of a plasmid to mimic the actual process of developing a plasmid with a gene of interest. 6B *Pipetting Skills* and 6C *Plant Tissue Culture*, are skills-based lessons that give students an opportunity to practice the skills they need to be successful in completing the next two lessons. 6D *Transform Bacteria* and Lesson 7 *Green Fluorescent Protein (GFP) Purification Lab* are kits from BioRad Explorer™ and allow students to Analyze and Interpret Data and Engage in Argument from Evidence to support their conclusions. Lesson 8 *GMO Speed Dating* helps students to determine which genetic modifications have been made and how the donor and recipient are matched to create the genetic modification.

Biotechnology is a set of techniques that have been developed to help improve medicine, agriculture and fuel production. Taking natural traits of organisms and selecting for them has been a method used for centuries (a.k.a. selective breeding or artificial selection). New tools have been discovered in the past 30 years that have changed the way scientists can manipulate a species and have sped up the process, making it much more precise.

Science and Engineering Practices

Lesson 1: Selective Breeding vs Genetic Modification
Constructing Explanations and Designing Solutions

Lesson 2: DNA Extraction
Constructing Explanations and Designing Solutions

Lesson 3: DNA Sentences
Constructing Explanations and Designing Solutions

Lesson 4: Punnett Squares
Asking Questions and Defining Problems

Lesson 5: Moving Genes
Constructing Explanations and Designing Solutions

Lesson 6A: Plasmid Modeling
Developing and Using Models
Constructing Explanations and Designing Solutions

Lesson 6B: Pipetting Practice
Asking Questions and Defining Problems

Lesson 6C: Plant Tissue Culture
Developing and Using Models

Lesson 6D: Transform Bacteria
Asking Questions and Defining Problems
Analyzing and Interpreting Data

Lesson 7: GFP Purification Lab
Constructing Explanations and Designing Solutions

Lesson 8: GMO Speed Dating
Constructing Explanations and Designing Solutions

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Feed the World - HS Biotechnology Unit Storyline

Phenomenon: Biotechnology is a set of techniques that have been developed to help improve medicine, agriculture and fuel production. Taking natural traits of organisms and selecting for them has been a method used for centuries (a.k.a. selective breeding or artificial selection). New tools have been discovered in the past 30 years that have changed the way scientists can manipulate a species and have sped up the process, making it much more precise. This unit presents the overarching question: **What are GMOS and why are we afraid of them?** then helps students discover several of the skills and some of the techniques being used in biotechnology, related to agriculture.

Lesson/ Focus Question ***What are GMOS and why are we afraid of them?***	**Overview**	**What should students be able to explain?**	**How does this add to their explanatory model?**
Lesson 1 Selective Breeding vs Genetic Modification *"What are the advantages of selective breeding? How does selective breeding differ from genetic modification?"*	Students model selective breeding to achieve a model offspring. Brainstorm questions to drive investigations.	Selective breeding is better than random crossing. Selective breeding is done by plant breeders to achieve specific traits in offspring. Some traits are controlled by multiple genes.	Selective breeding has been done for multiple centuries. Technology has advanced to allow plant breeders to be much more precise.
Lesson 2 **DNA Extraction** *"How might we see the raw material of life, DNA?"*	Students create their own protocol to extract DNA from corn.	DNA can be extracted from corn or other plants, how to do it, and scientists work together to create protocols to follow.	DNA is present in all living things, and can be extracted. However, there is more to the story!
Lesson 3 **DNA Sentences** *"How are proteins coded for by DNA?"*	Students translate and transcribe DNA to model protein "sentences."	DNA communicates traits by coding for proteins. DNA works to produce visible traits through transcription and translation (may use this activity as formative assessment to assess student understanding of DNA).	Pieces of DNA that are transcribed and translated determine the traits (proteins) in living things.

Lesson 4 **Punnett squares** *"Why do scientists use biotechnology? What are the advantages?"*	Students use a model to learn about Punnett squares, then complete Punnett squares of crosses to show predictability in breeding.	There are limitations of conventional breeding. (Traditional breeding takes more time and results are not always specific to the traits desired.)	Traditional techniques have some disadvantages.
Lesson 5 **Moving Genes** *"What are the steps in genetic modification?"*	Students sequence arrange cards in order to outline of the steps of genetic modification.	Scientists use various tools to help sequence DNA, isolate the gene of interest, remove that gene, copy the gene and insert it into the DNA to transform the target species.	New tools and techniques can help overcome the disadvantages of traditional selective breeding methods.
Lesson 6A (the next 4 lessons address one set of performance expectations and should be done in this sequence) **Plasmid Modeling** *"How does genetic modification work? How does DNA work in bacteria? How might we use that to aid in genetic modification?"*	Students model a plasmid with a gene of interest and a way to select to determine if the plasmid was added to bacteria.	How DNA in the form of plasmids determine the traits of the bacteria. By using these plasmids, bacteria can be modified easily.	Students see that DNA can be modeled and show modifications easily.
Lesson 6B **Pipetting skills** *"How might we develop and practice our micropipetting skills? Why is micropipetting important?"*	Students create patterns using micropipetters and check their accuracy by checking their results through mass.	.Accuracy is important when measuring small amounts.	Microliters of material are all that are needed when working on the cellular level.

Lesson 6C **Plant tissue culture** *"How might we create a sterile growing environment? How important is a sterile environment? How is a plant clone produced?"*	Students practice plant tissue culturing in sterile environments. Plant tissue culturing is a breeding technique that can be used to propagate plants from traditional methods and more technological methods..	How can we maintain a sterile environment and prevent contamination of results?	This technique was used to grow resistant rainbow papaya once modified with a ringspot virus protein gene.
Lesson 6D **Transform Bacteria BioRad pGLO™ Lab** *"How can we genetically modify bacteria? What are the results of gene insertion?"*	Students "transform" bacteria to glow in UV light.	DNA gets moved from one organism to another through genetic engineering.	Students analyze their results to determine if they transformed bacteria and explain how the process worked.
Lesson 7 **Green Fluorescent Protein (GFP) Purification Lab (BioRad)** *"How can proteins be purified? What is the mechanism used in biotechnology to extract proteins of interest?"*	Students extract protein from bacteria they created in the previous lab. (from **BioRad pGLO™ Lab**.) How do we know that the protein is what causes the trait?	Extension of pGLO lab. Students observe that the protein is visible in UV light.	This method is used to extract insulin from bacteria that make it.
Lesson 8 **GMO Speed Dating** *"What do you know about GMOs?"*	Students match their recipient/donor card to another's card that explains why the organism might have been modified and how.	Which organisms have been genetically modified and for what reasons.	GMOs have been created for a variety of reasons.

Biotechnology Pre-/Post-Test

Choose the best answer

1. DNA extraction uses the _____ properties of cell parts and the _____ properties of common household materials to break down the cell and allow the DNA to clump together.
    a. physical/chemical
    b. biological/chemical
    c. chemical/biological
    d. chemical/physical

2. DNA transcription and translation results in the formation of:
    a. Amino acids from proteins
    b. mRNA and tRNA
    c. Proteins from amino acids
    d. New cells

3. Punnett squares are used to
    a. Predict offspring ratios from parent crosses
    b. Determine the dominance of a gene
    c. Create new genotypes
    d. Show recessiveness

4. A plasmid is:
    a. Only human-made and inserted in bacteria
    b. A piece of the cell membrane
    c. A tool used in selective breeding
    d. A circular piece of DNA that occurs naturally in bacteria

5. Micropipetting describes the process of:
    a. Transferring large amounts of liquid.
    b. Moving genes from organism to organism.
    c. Measuring and transferring very small amounts of liquid.
    d. Changing phases of a substance from solid to liquid.

6. Which of the following occurred due to a genetic transformation where a gene was transferred from one organism to another organism performed by humans?
    a. Resistance of papaya to ringspot virus
    b. Growth of square watermelons
    c. Jellyfish ability to fluoresce in UV light
    d. Resistance of bacteria to antibiotics

7. Green Fluorescent Protein can be purified and removed from the bacteria that produce it just like:
    a. Antibiotics
    b. Antidepressants
    c. Stimulants
    d. Insulin

Complete the sentences below…

8. Selective breeding is a process of breeding where:

9. Genetic modification differs from selective breeding in that:

10. A protocol is:

11. Sterile environments are critical to plant tissue culture since:

Biotechnology Pre-/Post-Test - Answers

Choose the best answer

1. DNA extraction uses the _____ properties of cell parts and the _____ properties of common household materials to break down the cell and allow the DNA to clump together.
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    b. Antidepressants
    c. Stimulants
    d. Insulin

Complete the sentences below…

8. Selective breeding is a process of breeding where:
- *Organisms of the same species (or those that can mate i.e. donkey and horses to get mules) with the preferred traits are crossed.*

9. Genetic modification differs from selective breeding in that:
- *Only the specific genes for a trait are isolated, and added to a new organism.*

10. A protocol is:
- *A specific procedure used to accomplish a certain outcome (i.e. "recipe" for a process to remove DNA from corn)*

11. Sterile environments are critical to plant tissue culture since:
- *Growing plant tissue can be easily overcome by fungi or pathogens*

Biotechnology Unit Vocabulary

Lesson 1

Genetics - a branch of biology that deals with the heredity and variation of organisms.
Traits - an inherited characteristic.
Drought - lack of rain or moisture
Meiosis - the process of chromosomal mixing that occurs when organisms create gametes for reproduction
Genetic modification - the process of changing genes either through mutation or selective breeding; can be natural, human-driven by selecting for certain traits or engineered by adding a gene for a trait from a specific organism.

Lesson 2

Protocol - a detailed plan of a scientific or medical experiment, treatment, or procedure.
Extraction - the act or process of removing something.

Lesson 3

DNA - deoxyribonucleic acid
mRNA - messenger RNA
tRNA - transfer RNA
CRISPR - is a family of DNA sequences found within the genomes of prokaryotic organisms such as bacteria and archaea
Transcription - when used to refer to gene expression, it is the process of making a complementary strand of mRNA to DNA
Translation - mRNA is decoded to determine an amino acid sequence to synthesize a protein

Lesson 4

Phenotype - observable traits of an organism
Genotype - set of genes in an organism
Alleles - different forms of a gene (i.e. a gene for smooth seed coat is an allele for seed coat, so is a gene for wrinkled seed coat)
Homozygous - a genotype in which both genes (one from each parent) is the same allele
Heterozygous - a genotype in which both genes are different alleles
Dominant - a gene that when present its trait will be expressed in the offspring
Recessive - both alleles for the trait must be present for the trait to be expressed
Monohybrid cross - a cross between two organisms where only one trait of interest is predicted
Dihybrid cross - a cross between two organisms in which two traits that are on two different chromosomes are predicted

Lesson 5

Plasmid vector - circular strand of DNA that is found in bacteria that makes many copies of genes

Gene of interest - foreign (transgenic) DNA that codes for a desirable trait
Heat shock - a technique where a bacterium is held on ice, then immersed in a heated solution to open up pores in the cell membrane that allow plasmid vectors to enter, then returned to ice
Promoter - region of DNA that initiates transcription of a particular gene
Restriction enzymes - proteins produced by bacteria that cleave or cut DNA at specific sites along the molecule

Lesson 6A
Plasmid - an extrachromosomal ring of DNA especially of bacteria that replicates autonomously.
Nucleotides - any of several compounds that consist of a ribose or deoxyribose sugar joined to a purine or pyrimidine base and a phosphate group and that are the basic structural units of nucleic acids (such as RNA and DNA).

Lesson 6B
Micropipetting - Micropipettes are the type of air displacement pipettes that are used to transfer small quantities of solutions measured in microliters, 1000 µl (microliters) equals 1 ml (milliliter) and is the maximum amount that is commonly dispensed with micropipettes.

Lesson 6C
Plant tissue - A mature vascular plant (any plant other than mosses and liverworts), contains several types of differentiated cells. These are grouped together in tissues.
Tissue culture - growth of tissues or cells in an artificial medium separate from an organism typically in a liquid or semi-solid agar, also called **nutrient medium.**
Explant - a sample from a plant that will grow and differentiate into a new plant
Aseptic technique - a set of specific practices and procedures performed to make equipment and areas free from all microorganisms and to maintain that sterility

Lesson 6D
Selective breeding - the process of modifying the characteristics of living things, especially to enhance one or more desirable traits by selection in breeding controlled by humans

Lesson 7
Hydrophobic interaction column chromatography - a separation technique that uses the properties of hydrophobicity to separate proteins from one another.
Lyse - to cause dissolution or destruction of cells
Supernatant - the liquid lying above a residue after centrifugation
Hydrophobic - hydrophobic molecules repel water
Hydrophilic - hydrophilic molecules mix easily with water

Lesson 8
Donor - The organism in which a gene of interest is found
Recipient - The organism that receives the gene of interest

Lesson 1

Selective Breeding vs Genetic Modification - Teacher

Focus Questions: *What are the advantages of selective breeding? How does selective breeding differ from genetic modification?*

Learning Target: *Students model selective breeding and evaluate the strengths and weaknesses.*

Vocabulary: genetics, trait, drought, meiosis, genetic modification

HS-LS1: From Molecules to Organisms: Structures and Processes

HS-ESS3: Earth and Human Activity

Performance Expectation	Connections to Activity
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-ESS3-4.** Evaluate or refine a technological solution that reduces impacts of human activities on natural systems.	Students are given three traits of corn. They are challenged to make the most drought resistant corn. DNA codes for the traits that help corn to be drought resistant. They construct an explanation of the best way to obtain offspring with the drought-resistant corn traits. Students evaluate the method of selective breeding.
Science & Engineering Practice	
Constructing Explanations and Designing Solutions • Construct an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future.	Students explain the process they followed in the activity, then describe another method that may be more precise (genetic modification).
Disciplinary Core Idea	
LS1.A: Structure and Function • All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain	Students realize that there are many traits and chromosomes that are involved in this particular characteristic (drought resistance). They are encouraged to determine how the

the instructions that code for the formation of proteins, which carry out most of the work of cells. **ESS3.C: Human Impacts on Earth Systems** • Scientists and engineers can make major contributions by developing technologies that produce less pollution and waste and that preclude ecosystem degradation.	process of meiosis may impact plant breeding and efficiency of obtaining the desired traits. Students see why GMO crops have been modified and gain a better understanding of the process.
Cross Cutting Concept	
Structure and Function • Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, the structures of different components, and connections of components to reveal its function and/or solve a problem. **Stability and Change** • Feedback (negative or positive) can stabilize or destabilize a system. **Influence of Science, Engineering, and Technology on Society and the Natural World** • Engineers continuously modify these technological systems by applying scientific knowledge and engineering design practices to increase benefits while decreasing costs and risks.	Students are asked to consider the costs and benefits of the modifications.

*Student handout information is indicated in light gray print. Answer keys are imbedded in the student handout section.

Teacher Background

Humans have been selectively breeding animals and plants for thousands of years, choosing the ones with the most favorable characteristics and breeding them to achieve amazing results. Look around at the various dog breeds that have resulted from domesticating wolves or watch the video *Popped Secret: The Mysterious Origin of Corn* at https://www.hhmi.org/biointeractive/popped-secret-mysterious-origin-corn. Up until the late 1970's, that was the only way to get desired traits, but many other traits come along due to the sorting of chromosomes and crossing over that takes place during meiosis during sexual reproduction. Plant breeders have been able to overcome some of these obstacles by using genetic modification to improve crop characteristics, making them insect resistant, herbicide resistant, less prone to enzymatic browning, disease resistant or provide them with a health benefit.

The lesson, ***Selective Breeding vs Genetic Modification***, and the accompanying slide deck, leads students through a modeling activity to allow them to see how trying to get a specific set of traits is not as easy as it seems, even when they are allowed to select for the traits by choosing the offspring they want to cross.

Materials

*Starburst® candies Red, Yellow and Pink - these have no allergens and are individually wrapped - 3 per student
Opaque cups (foam cups or colored plastic that students cannot see through) - 1 per every 2 students
Slide deck
Electronic device for researching information
***Each candy represents a trait or phenotype. This activity does not intend to model allele combinations.**

Prior knowledge

In order to successfully understand this activity, students need to have some background in the process of meiosis and a general familiarity with the idea of selective breeding. Additional material can be supplemented by showing Lesson 1 Environment or Genetics slide deck from the middle school Biotechnology unit.

Student Handout

The purpose of this activity is to simulate selective breeding as it is accomplished by plant scientists. The Starburst® candies in the cup represent different traits that are desirable in corn to provide drought resistance. A *strong root system* is important to drought resistance since strong roots support a healthy stem and plant and make it easier for water and nutrients to enter the plant. *Seedling disease resistance* will add to drought resistance if the corn has some natural immunity to fungi, bacteria, nematodes and root feeding insects. One of the root feeding insects is called rootworm, which becomes a beetle as an adult. The larvae feed on the roots during the early part of the growing season. If the corn has *rootworm resistance*, it is more likely to survive drought as well.

https://commons.wikimedia.org/wiki/File:Diabrotica_virgifera_virgifera_larvae.jpg

Student Handout

1. You have three Starburst® candies. Which characteristics does your corn plant have? Circle the combination of traits you have.

R	R	R	R	R	R	P	P	P	Y
R	R	Y	R	P	P	P	Y	P	Y
R	Y	Y	P	P	Y	Y	Y	P	Y
Strong root system	Strong root system Seedling diseases resistance	Strong root system Seedling diseases resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Root-worm resis-tance	Seedling diseases resistance

2. Combine your Starburst® with those of someone else at your table. Place your six traits in the cup.
3. Shake the cup.
4. Draw out three Starburst® (traits). This represents the offspring from your cross.
5. Which characteristics does your new corn plant have? Circle the combination of traits you have.

R	R	R	R	R	R	P	P	P	Y
R	R	Y	R	P	P	P	Y	P	Y
R	Y	Y	P	P	Y	Y	Y	P	Y
Strong root system	Strong root system Seedling diseases resistance	Strong root system Seedling diseases resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Root-worm resis-tance	Seedling diseases resistance

6. Select another corn plant at your table that has some or all of the desired traits. Cross your plant with that plant by repeating steps 2-4. How many offspring in the class, have all three of the desired traits? (Report as the number that do out of the total possible.)

- *There will be less than 100% that have all three of the desired traits (unless they choose while looking).*

7. Why didn't choosing the parents result in all of the offspring having the desired traits?

- *There will be six candies in the cups and the probability of drawing out one of each color will be determined by the number of each color and the mixing that will take place.*

8. How does meiosis affect the outcome? What are the limitations of this model?

- *Meiosis allows for different chromosomes from the parents to end up in different gametes, one from each parent, but not all gametes will have all the same information. Unless the parents are genetically identical, the offspring will show some traits from both parents.*
- *This model does not allow for the separation of these traits to show up on separate chromosomes (i.e. the colors represent different traits, but these traits are on different chromosomes or are influenced by multiple genes. The candies do not represent genes, just the resulting traits.*

9. How might plant breeders overcome these obstacles?

- *Research on which genes influence these traits can be done. Scientists may be able to isolate which genes and modify the genes through gene editing (CRISPR) or they may find the trait in different species, isolate and transfer those genes to plants to give them the traits to make them drought resistant.*

10. How much might it cost (in dollars and time) to make these modifications? What are the environmental costs and benefits?

- *Economic and time: On average, GMOs take 13 years and $130 million of research and development before coming to market*. See more info about the costs at: https://gmoanswers.com/ask/what-average-cost-associated-research-production-and-testing-single-genetically-modified.
- *Environmental costs are: 1) a monocrop with the same genetic modifications can be more susceptible to a disease due to the lack of genetic diversity in the crop, 2) some weeds in fields have become resistant to the use of herbicides after many years of use of that herbicide, 3) some crops that do not have resistance to an herbicide may be harmed if the herbicide is used in an adjacent field.*
- *Benefits: 1) the amount and concentration of harmful herbicides has decreased, 2) the amount of harmful insecticides is greatly reduced since the plants make their own insecticide, 3) some crops are disease resistant which saved the industry of rainbow papaya (research papaya and ringspot virus), and is also used in potatoes and zucchini, 4) health benefits have been added to some plants, such as, high oleic oil from*

soybeans is more similar to olive oil, but still have the ability to fry for longer periods with less residue than regular soybean oil (soybean oil is sold as vegetable oil).

Differentiation

Other ways to connect with students with various needs:

i. **Local community**: students may investigate local plants that resist drought for additional testing.

ii. **Students with special needs (language/reading/auditory/visual)**: students may plant different seeds and water with various amounts to determine the characteristics that lead to drought resistance.

iii. **Extra support**: Students might read: "Pioneer Research to Develop Drought-tolerant Corn Hybrids" https://www.pioneer.com/CMRoot/Pioneer/US/products/seed_trait_technology/see_the_difference/corn_drought.pdf or "Drought-Tolerant Corn Hybrids Yield More in Drought-Stressed Environments with No Penalty in Non-stressed Environments" https://www.frontiersin.org/articles/10.3389/fpls.2016.01534/full

iv. **Extensions**: Students can research the development of corn by viewing: *Popped Secret: The Mysterious Origin of Corn* from HHMI Biointeractive: https://www.hhmi.org/biointeractive/popped-secret-mysterious-origin-corn to determine how corn might be bred to be more drought resistant.

Rubric for Assessment

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-ESS3-4. Evaluate or refine a technological solution that reduces impacts of human activities on natural systems.

Skill	Developing	Satisfactory	Exemplary
Construct an explanation of selective breeding	Student can describe the activity but does not make a connection to selective breeding.	Student can describe the activity and explain how it connects to selective breeding	Student can describe the activity and explain how it connects to selective breeding. Student can describe limitations within the simulation.
Evaluate or refine a technological solution to reduce the impact of human activities on natural systems.	Student can explain that genetic modification is a technological	Student can explain how genetic modification is a technological	Student can explain how genetic modification is a technological

	advance but cannot describe the advantages over selective breeding nor the impact either has on natural systems.	advance over selective breeding and describe the impact of genetic modification on natural systems.	advance over selective breeding, describe the costs and benefits of genetic modification and the impact of genetic modification on natural systems.

Rubric for Student Self-Assessment

Skill	Description	Yes	No
Constructing Explanations	I constructed an explanation of the selective breeding process and the obstacles to it.		
Evaluate or refine a technological solution to reduce the impact of human activities on natural systems.	I can list the costs (both economic and time) and benefits of genetic modification		

Selective Breeding vs Genetic Modification

Case Study

- According to Pioneer, the yield loss due to drought ranges from 40–80 bu/acre in Nebraska, with higher ranges in the west.
- Economic effects of drought can be up to $9 billion in a drought year.
- Economic impacts may include farmers who lose money because drought destroyed or lowered the yield of their crops.
- These economic impacts can be both direct, such as decreases in corn production, and indirect, as seen by increases in the price of animal feed.
- The traits that will help reduce the effects of drought in corn include: a strong root system, resistance to root worm, and resistance to seedling diseases.

Selective Breeding Activity

- You have three Starburst® candies. These candies represent the traits that determine resistance to drought.
- Stack your three Starburst® and determine your trait combinations using the following key...
    - Red for strong root system
    - Pink for rootworm resistance
    - Yellow for seedling diseases resistance

Table of traits for drought resistance

Make note of what characteristics your plant has.

R	R	R	R	R	R	P	P	P	Y
R	R	Y	R	P	P	P	Y	P	Y
R	Y	Y	P	P	Y	Y	Y	P	Y
Strong root system	Strong root system Seedling diseases resistance	Strong root system Seedling diseases resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Root-worm resis-tance	Seedling diseases resistance

Find another person at your table and combine your Starburst®

- Place your six traits in the cup
- Shake the cup
- Draw out three Starburst® (traits)
- This represents the offspring from your cross

Table of traits for drought resistance

What characteristics does your offspring have?

R	R	R	R	R	R	P	P	P	Y
R	R	Y	R	P	P	P	Y	P	Y
R	Y	Y	P	P	Y	Y	Y	P	Y
Strong root system	Strong root system Seedling diseases resistance	Strong root system Seedling diseases resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Root-worm resis-tance	Seedling diseases resistance

Choose another person to cross plants with.

- Plant breeders do not rely on random combinations to see what happens.
- Find another plant with traits that you would like. Put the traits in the cup and see what the offspring results are.
- How many plants result with all of the desired traits?

- How might plant breeders increase the chances they will get the desired traits in their offspring?

Reflection

- How did the offspring from your cross differ from the “parents”?
- What traits did your offspring have?
- The first process we used is random and the offspring are not easily predictable. However, scientists can select for traits they desire and there are predictable results, but the ratios of offspring rarely produce 100% of the traits desired each time. The second time, we were more selective...did all of the offspring have the desired traits?
- Seed researchers can selectively breed for resistance.
- How long might it take to get the desired traits?

If it were only that easy...

- These traits (drought resistance) are controlled by many genes on different areas of several chromosomes.
- How might the process of meiosis affect the breeding of different plants to create the traits desired?
- How might plant breeders overcome these obstacles?
- How much might it cost (economically and time) to make these modifications? What are the environmental costs and benefits?

Which crops are GMO in the United States? (Only 10!)

- Corn (field and sweet)
- Alfalfa
- Canola
- Cotton
- Soybeans
- Sugar beets
- Rainbow Papaya
- Potatoes
- Summer squash (zucchini)
- Arctic Apples

What are the modifications?

- Corn (field and sweet) - drought and herbicide tolerance; insect resistance
- Alfalfa - herbicide tolerance
- Canola - herbicide tolerance
- Cotton - herbicide tolerance and insect resistance
- Soybeans - herbicide tolerance and insect resistance; health benefit
- Sugar beets - herbicide tolerance
- Rainbow Papaya - disease resistance
- Potatoes - insect and disease resistance; reduce food waste
- Summer squash (zucchini) - disease resistance
- Arctic Apples - reduce food waste

Costs and benefits...

What are the costs of making these genetic modifications (both economic and environmental)?

What are the benefits of these genetic modifications?

Lesson 1

Selective Breeding vs Genetic Modification

Focus Questions: *What are the advantages of selective breeding? How does selective breeding differ from genetic modification?*

Vocabulary: genetics, trait, drought, meiosis, genetic modification

The purpose of this activity is to simulate selective breeding as it is accomplished by plant scientists. The Starburst® candies in the cup represent different traits that are desirable in corn to provide drought resistance. A *strong root system* is important to drought resistance since strong roots support a healthy stem and plant and make it easier for water and nutrients to enter the plant. *Seedling disease resistance* will add to drought resistance if the corn has some natural immunity to fungi, bacteria, nematodes and root feeding insects. One of the root feeding insects is called rootworm, which becomes a beetle as an adult. The larvae feed on the roots during the early part of the growing season. If the corn has *rootworm resistance*, it is more likely to survive drought as well.

https://commons.wikimedia.org/wiki/File:Diabrotica_virgifera_virgifera_larvae.jpg

1. You have three Starburst® candies. Which characteristics does your corn plant have? Circle the combination of traits you have.

R	R	R	R	R	R	P	P	P	Y
R	R	Y	R	P	P	P	Y	P	Y
R	Y	Y	P	P	Y	Y	Y	P	Y
Strong root system	Strong root system Seedling diseases resistance	Strong root system Seedling diseases resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Root-worm resis-tance	Seedling diseases resistance

2. Combine your Starburst® with those of someone else at your table. Place your six traits in the cup.
3. Shake the cup.
4. Draw out three Starburst® (traits). This represents the offspring from your cross.
5. Which characteristics does your new corn plant have? Circle the combination of traits you have.

R	R	R	R	R	R	P	P	P	Y
R	R	Y	R	P	P	P	Y	P	Y
R	Y	Y	P	P	Y	Y	Y	P	Y
Strong root system	Strong root system Seedling diseases resistance	Strong root system Seedling diseases resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance	Strong root system Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Rootworm resistance Seedling diseases resistance	Root-worm resis-tance	Seedling diseases resistance

6. Select another corn plant at your table that has some or all of the desired traits. Cross your plant with that plant by repeating steps 2-4. How many offspring in the class, have all three of the desired traits? (Report as the number that do out of the total possible.)

7. Why didn't choosing the parents result in all of the offspring having the desired traits?

8. How does meiosis affect the outcome? What are the limitations of this model?

9. How might plant breeders overcome these obstacles?

10. How much might it cost (in dollars and time) to make these modifications? What are the environmental costs and benefits?

Rubric for Self-Assessment

Skill	Description	Yes	No
Constructing Explanations	I constructed an explanation of the selective breeding process and the obstacles to it.		
Evaluate or refine a technological solution to reduce the impact of human activities on natural systems.	I can list the costs (both economic and time) and benefits of genetic modification		

Lesson 2
DNA Extraction - Teacher

Focus Question: *How might we extract the raw material of life, DNA?*
Learning Target: *Students develop a protocol (uniform accepted method) to extract DNA from corn.*
Vocabulary: *protocol, extraction*

This activity is adapted from:
Chowning, J. T., Wu, R., Brinkema, C., Crocker, W. D., Bass, K., & Lazerte, D. (2019, February). A new twist on DNA extraction. *The Science Teacher.*
And can be found here:
https://libguides.fredhutch.org/SEP/sep/dna?_ga=2.38659875.1602666715.1550264665-1837730585.1550264665

HS-LS1: From Molecules to Organisms: Structures and Processes

Performance Expectation	Connections to Activity
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.	This activity is one step toward the accomplishment of this performance expectation. Making DNA visible helps students to begin to *construct the explanation.*
Science & Engineering Practice	
Constructing Explanations and Designing Solutions • Construct an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future.	Students design their own solutions to address the problem of how to extract DNA from cells. They then plan and carry out an investigation based on their experimental designs. Afterwards, they construct claims for the results they observe based on evidence from their results. They communicate information about their findings with other teams in a classroom "lab meeting" discussion where students respectfully question and critique each other's designs. Finally, students collaboratively argue for how the protocol could best be redesigned, based on evidence from class data.
Disciplinary Core Idea	

LS1.A: Structure and Function • All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells.	This activity reinforces that DNA is located in cells and is sequestered/protected within the nucleus.
Cross Cutting Concept	
Structure and Function • Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, the structures of different components, and connections of components to reveal its function and/or solve a problem.	Students base their solutions to the problem posed in the activity on the properties of different materials made available for use in their protocols and their potential effects on the structures and functions of cellular components.

*Student handout information is indicated in light gray print. Answer keys are imbedded in the student handout section.

Teacher Background

This activity allows students to practice creating a protocol to extract DNA from corn. A protocol, or established procedure, is important to assure replication and high quality results. Protocols consist of detailed step-by-step instructions. A current protocol may be revised if and when new technology or materials become available. Different labs researching the same thing may use different protocols based on the equipment and resources available.

Prior Knowledge

In order to successfully complete this activity, students should know:

- An organism's DNA is within the nuclei of its cells.
- DNA can be extracted from cells using mechanical and chemical means. Knowledge of the properties of cell components as well as the composition of materials used in the extraction can help in developing an effective procedure.
- Communication, collaboration, and skepticism are essential to the scientific research process.
- It is important to back claims with evidence and reasoning, and to use evidence and reasoning to evaluate the claims of others.

Students will be able to:

- Actively participate in a class discussion evaluating the varied methods and results of class approaches.
- Make claims and support them with evidence and reasoning.
- Critically and respectfully evaluate the claims of others.
- Revise methods in light of group discussion.

Suggested timing

Allow 2 periods for students to determine protocol for their own lab group and to carry it out
Allow 1 period to run lab meeting
Allow 1 period to redesign protocol
Allow 1 period for evaluation/assessment

Materials

Resource Cards - 1 set per group printed front to back so descriptions are on the back of each card
Student handout
Communication/Collaboration Check-In
Lab Meeting Data Sheet Post-Assessment
"Bag-In-A-Bag" Cell Model - 1 for the class
- Cardboard box = plant cell wall
- Large paper bag = cell membrane
- Shredded plastic bags (filler) = cytoplasm
- Smaller paper bag = nucleus
- Yarn = DNA

Test tube of extracted corn DNA
For protocol testing
- Gauze (filter) Funnels
- 15 ml test tubes
- Plastic bags
- Corn (fresh or frozen)
- Vegetable oil
- Baking soda
- Salt
- Vinegar
- Water
- Sugar
- Ethanol (refrigerated)
- Beakers
- Graduated cylinder
- Meat tenderizer

Teacher Preparation

Step-by-step guidelines are included on the student sheet to allow students some structure for the development of their protocols within their groups.

1. Make copies as described in the Materials section.
2. Set up an area in the classroom that students can access the different resources and measuring tools for DNA extraction.
3. Make the "Bag-In-A-Bag" cell model.

4. Perform a DNA extraction from corn to use as the investigative phenomenon for the class, using the procedure below:
5. Make the DNA Extraction Buffer by mixing the following ingredients:
    a. 10 ml dishwashing soap
    b. 90 ml water
    c. 1.5 g salt
    d. Pinch meat tenderizer
6. Place a cup of canned or frozen whole corn in a plastic baggie and mash it into a pulp.
7. Add 10 ml DNA Extraction Buffer to the bag. Carefully mix the contents. Avoid bubbles.
8. Place a gauze-lined funnel on top of a 15 ml collection tube and pour the contents of the bag through the filter. Try to collect 3 ml of the corn mixture.
9. Slowly add 5 ml of cold ethanol to the liquid along the side of the tube to create a layer of ethanol on top of the corn mixture.
10. The corn DNA should precipitate out of solution into the ethanol.

In this lesson, students are tasked with creating a protocol for extracting DNA from corn. The students will have to collaborate within their teams for the initial design. Resource Cards show the materials available and their properties. Some materials are decoys like baking soda and vinegar, while others are commonly found in DIY DNA extraction protocols. Students will need to create steps using their collective knowledge about cells and DNA.

For additional details and day by day instructions, specifically how to facilitate the protocol development and discussion sessions see:
https://libguides.fredhutch.org/ld.php?content_id=44113665

Student Handout

As a member of the lab, you will be expected to closely collaborate with the other members of your lab. Working with others is essential to improving the quality of the science done by the group. Your lab has been divided into working teams.

Use your team's knowledge about cells and DNA to collaborate on a protocol design.

1. Use your team's knowledge about cells and DNA to collaborate on a protocol design.
    a) Develop your own protocol for isolating DNA from whole corn.
        Include details about your use of:
        equipment,
        times,
        amounts, and
        temperatures.
    b) Try out your protocol and collect data on your success.

2. Present your team's findings during the lab meeting.

Lab meetings are focused on three main concepts:

- Communication – sharing your information with others.
- Collaboration – working together with others towards a goal.

• Skepticism – evaluating information critically and looking for evidence and reasoning behinds claims.

By using communication, collaboration, and skepticism, the lab will use the knowledge gained from all the teams' results to develop and test a final protocol.

Differentiation

Other ways to connect with students with various needs:

i. **Local community**: Students may visit a local plant pathology or university lab, or invite a technician from the lab, to discover how they do diagnostic or specific lab tests and how the method they use was decided.

ii. **Students with special needs (language/reading/auditory/visual)**: Students may participate in the activity in a number of ways: choosing the reagents to use, determining the quantity and the temperature requirements, gathering materials once decided upon, measuring the amounts, preparing the DNA by crushing the corn, etc.

iii. **Extra support**: If students are struggling, they may watch *Fantastic DNA! Extraction Steps* at https://www.youtube.com/watch?v=vpXsWaUrcJs

iv. **Extensions**: Students can try to extract DNA from other fruits or vegetables, or try to extract more DNA by increasing the amounts of selected materials.

Rubric for Assessment

Skill	Developing	Satisfactory	Exemplary
Construct an explanation extracting DNA	Student can describe the activity but does not make connections to the mechanics of extracting DNA.	Student can describe the activity and explain how materials used helped to extract the DNA.	Student can describe the activity and explain how materials used helped to extract DNA, and uses the method on various organisms with success.
Communication using evidence	Student shared no ideas or few ideas with the group providing little to no evidence for them, and had difficulty with the extraction protocol.	Student shared ideas with the group supported by evidence and contributed to the development of the protocol.	Student shared ideas supported by evidence, listened to shared ideas and helped to evaluate to focus the actions of the group to develop the protocol.

Rubrics for Self-Assessment

Communication/Collaboration Check-in
Your Current Ability to Communicate Using Evidence – indicate by putting an X on the line

a. Rating Scale: My current ability to communicate using evidence when talking in my lab group.

1 ----------5----------10 1 = very low, 10 = very high

b. Rating Scale: My current ability to communicate using evidence when talking in large class

"lab meeting". 1 ----------5----------10 1 = very low, 10 = very high

c. What is one way in which you improved in your ability to participate in a class discussion? Be specific. Give an example from the lab group work or meeting if possible.

I would rate our collaboration as: Non-existent OK Very Good The reason for my rating is:
One improvement I would suggest in order to improve our communication and collaboration is:

Final Assessment

Skill	Yes	No
Everyone had a chance to participate equally in our discussion.		
Everyone listened well to contributions.		
Someone in our group took over.		
I "kept up" and understood what our group was doing and why.		
We divided up the work fairly.		

BAKING SODA

DISHWASHING SOAP

MEAT TENDERIZER

OIL

SALT

SUGAR

VINEGAR

WATER

FILTER

Feed the World

MEAT TENDERIZER

pH: 6-8 (slightly alkaline)

DESCRIPTION:
Meat tenderizer is a solid, seasoning like compound added to meat to make it more tender. The active ingredient is papain.

FUNCTION:
Meat tenderizer interacts with the proteins, breaking them down so the meat becomes softer and easier to chew/digest.

TYPE OF MOLECULE:
papain—an enzyme derived from papaya functionally digests proteins

DISHWASHING SOAP

pH: 6-8 (slightly alkaline)

DESCRIPTION:
Dishwashing soap is often a liquid that is added to water to aid in washing dishes or other materials.

FUNCTION:
Removes grease (lipids) and disrupts the connections (bonds) between fat molecules .

TYPE OF MOLECULE:
surfactant— allows hydrophobic (water hating) molecules to be broken apart

BAKING SODA

pH: 9 (alkaline)

DESCRIPTION:
Baking soda is a salt composed of sodium ions and bicarbonate ions. It is a white solid that is usually a fine powder. It has a slightly salty, alkaline taste.

FUNCTION:
Baking soda is a well-known cooking ingredient used to raise soda breads, cookies and cakes. In addition, it has wide range of applications, including cleaning, deodorizing, maintaining pH, and fire extinguishing.

TYPE OF MOLECULE:
ionic, a chemical salt

SUGAR

pH: neutral

DESCRIPTION:
Sucrose is a disaccharide, meaning it is made of simple sugars joined together. It is 50% glucose and 50% fructose.

FUNCTION:
Sucrose is a naturally occurring sugar (carbohydrate) It is found primarily in plants, where it serves as a way to store energy. It is usually found in roots, fruits and nectars. Animals obtain sucrose by feeding on plants.

TYPE OF MOLECULE:
polar, covalent bonds, carbohydrate (disaccharide)

SALT

pH: 7-8 (mostly neutral)

DESCRIPTION:
Salt water is water containing salt (NaCl). The salt dissociates into charged sodium (Na+) and chlorine (Cl-) ions.

FUNCTION:
Salt in water allows free Na and Cl ions to easily interact with polar molecules. In salt solutions, polar molecules can form clumps.

TYPE OF MOLECULE:
Ionic compounds

OIL

pH: neutral

DESCRIPTION:
Triglycerides are the main component of most food fats and oils. A triglyceride is composed of glycerol and three fatty acids.

FUNCTION:
Assists in heat transfer in cooking. Add flavor and texture

TYPE OF MOLECULE:
non-polar, covalent bonds, lipid

FILTER

pH: N/A

DESCRIPTION:
A type of paper or cloth that often has very small pores (openings) or a fine mesh.

FUNCTION:
Filters are used to collect large piece of materials or debris that can be found in liquids—separating large pieces from small pieces—letting small pieces through the filter while large pieces stay on top of the filter.

TYPE OF MOLECULE:
N/A

WATER

pH: 7 (ideal, neutral)

DESCRIPTION:
A polar molecule made of hydrogen and oxygen, participates in hydrogen bonding, important for all life

FUNCTION:
Water acts as a solvent, allow salts to dissolve and providing a pH buffer in chemical reactions.

TYPE OF MOLECULE:
polar, covalent bonds, hydrogen bonds

VINEGAR

pH: ~2.4 (acidic)

DESCRIPTION:
Vinegar is a liquid that is produced from the fermentation of ethanol into acetic acid. The fermentation is carried out by bacteria. Vinegar consists of acetic acid (CH_3COOH), water and trace amounts of other chemicals.

FUNCTION:
A cooking ingredient also used for pickling

TYPE OF MOLECULE:
Polar, covalent bonds

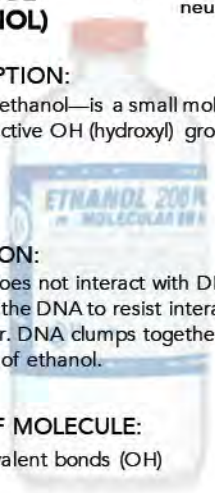
PINEAPPPLE JUICE

ETHANOL

Feed the World

ALCOHOL (ETHANOL)

pH: 7.33 (mostly neutral)



DESCRIPTION:
Alcohol—ethanol—is a small molecule with a reactive OH (hydroxyl) group present.

FUNCTION:
Ethanol does not interact with DNA and will allow the DNA to resist interaction with water. DNA clumps together in the presence of ethanol.

TYPE OF MOLECULE:
polar, covalent bonds (OH)

PINEAPPLE JUICE (BROMELAIN)

pH: 3.5 (acidic)

DESCRIPTION:
Pineapple plants contain bromelain. Bromelain is a proteolytic enzyme (an enzyme that digests proteins) found in fresh pineapple.

FUNCTION:
Bromelain is one of the most popular enzymes used to break down proteins in food items, like gelatin and meat.

TYPE OF MOLECULE:
Protein, peptide

Lesson 2
DNA Extraction

Focus Question: *How might we see the raw material of life, DNA?*
Vocabulary: *protocol, extraction*

Background

Protocols are established procedures adopted by a lab or group of labs for producing quality results that can be replicated by others. These detailed step-by-step instructions insure that scientists are doing all the actions necessary to get good results. Protocols are constantly evolving based on new technology and/or scientific discoveries. Different labs researching the same thing may use different protocols based on the equipment and resources available.

As a member of a within your lab, you will be expected to closely collaborate with the other members of your team. Working with others is essential to improving the quality of the science done by the group. Your lab has been divided into working teams.

1. Use your team's knowledge about cells and DNA to collaborate on a protocol design.
   a) Develop your own protocol for isolating DNA from whole corn.
      Include details about your use of equipment,
      times,
      amounts, and
      temperatures.

b) Try out your protocol and collect data on your success.

2. Present your team's findings during the lab meeting.

Lab meetings are focused on three main concepts:

- Communication – sharing your information with others.
- Collaboration – working together with others towards a goal.
- Skepticism – evaluating information critically and looking for evidence and reasoning behind claims.

By using communication, collaboration, and skepticism, the lab will use the knowledge gained from all the teams' results to develop and test a final protocol.

Develop and test your protocol

1. In your lab notebook, record any information about cells and DNA that might be important know when extracting DNA.
2. Record the question: "How can we use collaboration to figure out how to get DNA out of cells?"
3. Collaborate with your team to determine what resources you will need and how/in what order you will use them.
4. Once all members of your team have agreed on a method, record your planned protocol.
5. Your teacher will sign off on your initial protocol confirming that all members of your team agree.

6. Fill out the Communication/Collaboration Check-In before you begin testing.
7. Obtain all the materials you need. Work through your initial protocol for the rest of the class time. Write notes on any changes, observations, or clarifications in your lab notebook.
8. Once finished, record your results. Do you believe that you extracted DNA? If so, what does it look like? If not, why do you think you did not get it?
9. Label your test tube with your group name and date. Store in the refrigerator. Keep your results to compare with the other groups' results during the next meeting.
10. For homework, consider changes you would make to your protocol and why you would make those changes.

Lab Meeting

Prepare an informal presentation of your findings during the lab meeting. Communicate a summary of your protocol and how well your method of extraction worked out. In your group, discuss the following questions and record your answers in your lab notebook:

a. What was your protocol?
b. What can you conclude about the effectiveness of your method? (claim)
c. How do your results support your conclusion and why? (evidence and reasoning)
d. What worked with your protocol? What did not?

2. All members of your lab team must take part in the presentation of your protocol and findings.
3. After each presentation, be prepared to ask clarifying questions of the presenting team.
4. As each team presents their findings, consider whether the team used a "scientific" approach to developing their protocol.

a. Was their design informed by their understanding of the cell?
b. Is their protocol detailed enough for anyone to be able to repeat it and obtain the same results? This characteristic is called "being replicable".

5. Consider how other groups' protocols differ from yours. What can you learn from the other groups that you did not know or did not consider?
6. Using the Lab Meeting Data Sheet, make notes on how each team's protocol differed. During the discussion, write down any interesting questions, responses, or comments that you feel would be important to note.
7. After all groups have presented, the class will collaborate in developing a new protocol using the knowledge gained from each team's initial testing. Record the new protocol in your lab notebook.

New Protocol Testing

1. Return to your team and run the new class-developed protocol.
2. In your lab notebook, write down any observations.
3. Store the lab results in the refrigerator with your team name and date.
4. Compare the results from the class-developed protocol with your initial results.
5. Complete the Post-Assessment.
6. Discuss your final results as a class.

Rubrics for Self-Assessment

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Your Current Ability to Communicate Using Evidence – indicate by putting an X on the line

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"lab meeting". 1 ----------5----------10 1 = very low, 10 = very high

c. What is one way in which you improved in your ability to participate in a class discussion? Be specific. Give an example from the lab group work or meeting if possible.

I would rate our collaboration as: Non-existent OK Very Good The reason for my rating is:
One improvement I would suggest in order to improve our communication and collaboration is:

Final Assessment

Skill	Yes	No
Everyone had a chance to participate equally in our discussion.		
Everyone listened well to contributions.		
Someone in our group took over.		
I "kept up" and understood what our group was doing and why.		
We divided up the work fairly.		

Lesson 3
DNA Sentences - Teacher

Focus Question: *How are proteins coded for by DNA?*
Vocabulary: *deoxyribonucleic acid (DNA), messenger RNA (mRNA), transfer RNA (tRNA), CRISPR, transcription, translation*

LS1: From Molecules to Organisms: Structures and Processes

Performance Expectation	Connections to Activity
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.	Students use DNA, codons and anticodons to transcribe and translate sentences related to information about corn.
Science & Engineering Practice	
Constructing Explanations and Designing Solutions • Construct an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future.	Students *construct an explanation* by writing out the processes of transcription and translation for proteins after completing their groups' four sentences (proteins) from words (amino acids) correctly using the terms: DNA, mRNA and tRNA. (Students will need to correct the sentences if they are incorrect before constructing their explanation). Students *design solutions* by modeling "genetically modified" DNA to make a different sentence using the transcription and translation rules they followed; describing that the sentence they make will represent a different protein. Extension: Model CRISPR by "knocking out" a portion of a gene to make a different protein.
Disciplinary Core Idea	
LS1.A: Structure and Function • All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the	DNA is shown as the guide for the creation of proteins; changes in DNA create changes in the mRNA and tRNA, therefore the words (amino acids) of the sentences (proteins).

formation of proteins, which carry out most of the work of cells.	
Cross Cutting Concept	
Structure and Function • Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, the structures of different components, and connections of components to reveal its function and/or solve a problem.	Students determine a new DNA combination to change the composition of the words (amino acids) and sentence (protein).

*Student handout information is indicated in light gray print. Answer keys are imbedded in the student handout section.

Materials

DNA strand cards and tRNA cards with words on the back
Transcription/Translation Data Sheet
Large sheet of paper for each group
Markers

Prior Knowledge

In order to successfully complete this activity, students must know that DNA bases match according to the following table:

DNA	mRNA	tRNA
A	U	A
C	G	C
T	A	U
G	C	G

In addition, those three letter codes actually match up with amino acids to make proteins just as the words in this activity make sentences. The order and number of the amino acids determine the protein.

Teacher preparation

1. Copy the DNA strips and cut into separate strands.
2. Copy the tRNA cards front to back so anticodons are on one side with words on the other.
3. Set out DNA strands at a central location in the room. (This location represents the nucleus; remember DNA does not leave the nucleus.)
4. Set out tRNA cards around the perimeter of the room, grouped by first letters of the anticodons (A's together, C's together, etc)
5. Group students by four's; provide each group with a copy of the **Transcription/Translation data sheet** copied front to back so each group has enough for the number of sentences assigned. **Note**: Each box in the grid is for a codon or anticodon (three-letter code).

6. Assign sentences to groups. (If all students will practice all roles, each group of four should have four sentences assigned.)
   a. Students will send their transcriber to the nucleus to transcribe the DNA to mRNA.
   b. After transcription, the student returns to the group table and hands the transcription to the translator (tRNA).
   c. After translation, the runners go to find the matching translated tRNA anticodons around the room and once they find the match, they copy the word to their data sheet. STUDENTS MUST NOT TAKE THE tRNA CARDS as they are used in multiple sentences.
   d. Together, the group determines the sentence looking for errors.
   e. Students switch roles (transcriber becomes translator, translator becomes a runner, one runner becomes the transcriber)
   f. Repeat these steps for the number of sentences assigned.

6. Have students *construct an explanation* (orally, on video, or written) of the process they used to transcribe and translate the sentences correctly using the words: DNA, mRNA, tRNA, amino acids and proteins.

7. Students in groups or individually *design a solution* by creating their own DNA sentence that will code for a different sentence (using the words in the simulation). Students exchange their new DNA strand with another group/student to check their "genetic engineering."

Student Handout

Background

Deoxyribonucleic acid (DNA) is the molecule of life. DNA is one of the most recognizable nucleic acids, a double-stranded helix. The process by which DNA codes for proteins involves enzymes and additional single-stranded nucleic acids, specifically messenger ribonucleic acid (mRNA) and transfer ribonucleic acid (tRNA). The steps in protein synthesis, transcription from DNA to mRNA, and translation from mRNA to tRNA can be demonstrated by modeling. In this activity, each triplet code of DNA will represent a word in a sentence rather than a code for an amino acid. Introns and exons are omitted. The words can be found by transcribing the DNA into mRNA, then translating mRNA into tRNA.

1. Choose one person to be the transcriber. Find the DNA strand assigned to your group located at the table in the center of the room. (This will represent the nucleus.)

Note: The first triplet code is a "start" code, which in eukaryotes, represents the amino acid methionine. For this activity, it is the same triplet code for all and is denoted by the word "START." Each punctuation symbol is represented by a different "stop" anticodon.

2. On the data sheet provided, transcribe the mRNA codons from the DNA strand (without moving the DNA).
3. At your group's table, choose a different person to translate the mRNA codons to tRNA anticodons.
4. Write these anticodons on your data sheet.
5. Choose two people to go find the tRNA anticodons around the room. Turn over the anticodons to find the words of the sentence and write the words on your data sheet.
6. Write your sentence in large print/script on the large sheet or white board at your table.

7. Switch roles and repeat for each additional DNA strand you are assigned.

Extension:

After your group has translated your assigned sentences, research each statement to find evidence to support or refute the statement.

Create a new "sentence" (protein) using DNA triplets to create a different set of words (amino acids).

Reflection

1. How are chromosomes, DNA, genes and proteins related?
   - *Chromosomes are strands of DNA, they contain genes, which are portions of the DNA strands. The bases of DNA that are contained in a gene code for amino acids in a specific order to create proteins that create a trait.*

2. What area of the cell does the table holding DNA represent in this modeling activity?Why can't the DNA strand be brought back to your group?
   - *The nucleus is where DNA is located in a cell. DNA does not leave the nucleus.*

4. What area of the cell does your table represent?
   - *The table represents the ribosome. The ribosome is the location of protein synthesis.*

5. What do the words represent? The completed sentences?
   - *The words represent amino acids, and the completed sentences represent the proteins.*

6. What do you think the consequences might be if an error occurred in the cell as it goes through the process of protein synthesis?
   - *If an error occurs in the transcription or translation process, different codons and anticodons would be produced. This might result in different amino acids that might change the function of the protein and/or prevent a protein from being formed at all.*

Differentiation

Other ways to connect with students with various needs:

i. **Local community**: Students may visit a local ethanol plant or invite an extension person or member of the state corn board to their classroom to hear about the benefits of ethanol and the uses of corn.

ii. **Students with special needs (language/reading/auditory/visual)**: Cards can be enlarged for the visually impaired, or spoken so one can participate in the activity.

iii. **Extra support**: If students are struggling, they may watch *Transcription and Translation: From DNA to Protein* https://www.youtube.com/watch?v=bKIpDtJdK8Q

iv. **Extensions**: After completing transcription and translation of the assigned sentences, students may research the evidence for or against the statement. Additionally, students could model CRISPR-Cas9 by "knocking out" a portion of a gene to make a different sentence (protein). Follow the CRISPR HHMI Biointeractive Click and Learn to see the mechanism used.

Rubric for Constructing Explanations and Designing Solutions

Skill	Beginning	Satisfactory	Exemplar
Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins	Participated in the DNA sentence activity. Explanation of the function of DNA and the roles DNA, mRNA and tRNA in transcription and translation of DNA into proteins does not accurately or completely explain the process.	Participated in the DNA sentence activity. Explanation of the function of DNA and the roles DNA, mRNA and tRNA in transcription and translation of DNA into proteins is complete and accurate; new DNA describes a different sentence (protein).	Participated in the DNA sentence activity. Explanation of the function of DNA and the roles DNA, mRNA and tRNA in transcription and translation of DNA into proteins is complete and accurate; new DNA strand describes a different "sentence" that describes an actual protein that will carry out a particular function. (i.e. GFP, insulin, etc)

Rubric for Self-Assessment

Skill	Yes	No
I participated in each role during the DNA activity.		
I can explain the function of DNA and the roles of DNA, mRNA and tRNA in transcription and translation of DNA into proteins.		
I created a new "sentence" (protein) using DNA triplets to create a different set of words (amino acids)		

1. Ethanol decreases CO2 production.
   TAC AGT CCG TAG TGA ATT
2. Ethanol is clean energy from corn.
   TAC AGT TCC GAC ATC ATG AGG ATT
3. GMOs improve nutrition.
   TAC AAA CCC AGC ATT
4. DNA is the molecule of life.
   TAC TTA TCC TCG TGG TTT TAA ATT
5. Biotechnology increases yield.
   TAC CGG CCC CGT ATT
6. Biotechnology decreases pesticide use.
   TAC CGG CCG AAC AGA ATT
7. GMOs will meet the increasing demand for food.
   TAC AAA GCG CGA TCG CCC ATA TAT CAA ATT
8. GMOs decrease poverty and increase sustainability.
   TAC AAA CCG TGC GAG CCC AAG ATT
9. GMOs can grow on marginal land
   TAC AAA ACG TGT TTT GCA CCT ATT
10. Some corn is drought tolerant.
    TAC ACT AGG TCC CAC TTC AAT
11. One bushel of corn used for ethanol makes 17.5 pounds of distiller's dried grains.
    TAC CTT GAT TTT AGG AGA TAT AGT CAG TTG ACC TTT CCA ATT
12. Corn ethanol meets the demand for fuel and food.
    TAC AGG AGT CGA TCG ATA TAT ATC GAG CAA ATT
13. One bushel of corn equals 56 pounds.
    TAC CTT GAT TTT AGG CAG TTG ACC ATT
14. One bushel of corn makes 2.8 gallons of ethanol.
    TAC CTT GAT TTT AGG CAG TTG ACC TTT AGT ATT
15. GMOs grow more food on less land.
    TAC AAA TGT CCC CAA TTT CCG CCT ATT
16. Biotechnology decreases energy use.
    TAC CGG CCG ATC AGA ATT
17. Biotechnology decreases CO2 production.
    TAC CGG CCG TAG TGA ATT
18. Biotechnology improves water and soil quality.
    TAC CGG CCC GTT GAG AAT GCT ATT
19. 40% of corn is used to produce ethanol.
    TAC TTG ACC TTT AGG TCC AGA CAT TGA AGT ATT
20. Dent corn produces most corn products.
    TAC GAA AGG TGA ACA AGG TGA ATT

AAA

AAC

AAG

AAU

ACA

ACC

ACG

ACU

Feed the World

DRAFT

PESTICIDE

GMOs

SOIL

SUSTAINABILITY

POUNDS/GALLONS/
PERCENT

MOST

SOME

CAN

AGA

AGC

AGG

AGU

AUA

AUC

AUG

AUU

Feed the World

DRAFT

NUTRITION

USE(D)/USING

ETHANOL

CORN

ENERGY/FUEL

DEMAND

FROM

Feed the World

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CAA

CAC

CAG

CAU

CCA

CCC

CCG

CCU

Feed the World

DRAFT

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DROUGHT

FOOD

TO

MAKES/EQUALS

INCREASE(ING)/
IMPROVE(ING)/
MORE

DISTILLER'S
DRIED GRAINS

LAND

DECREASE(S)/
DECREASING/
LESS

CGA

CGG

CGU

CUU

GAA

GAC

GAG

GAU

Feed the World

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BIOTECHNOLOGY

MEET(S)

ONE

YIELD

CLEAN

DENT

BUSHEL

AND

GCA

GCG

GCU

GUA

GUU

UAA

UAC

UAG

WILL

MARGINAL

OUR

QUALITY

LIFE

WATER

CO_2

START

UAU

UCC

UCG

UGA

UGC

UGG

UGU

UUA

Feed the World

DRAFT

IS/ARE

FOR

PRODUCE(S)/
PRODUCTS/
PRODUCTION

THE

MOLECULE

POVERTY

DNA

GROW

UUC

UUG

UUU

17.5/56/2.8/40

TOLERANT

OF/ON

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TAC AGT CCG TAG TGA ATT

TAC AGT TCC GAC ATC ATG AGG ATT

TAC AAA CCC AGC ATT

TAC TTA TCC TCG TGG TTT TAA ATT

TAC CGG CCC CGT ATT

TAC CGG CCG AAC AGA ATT

6

TAC AAA GCG CGA TCG CCC ATA TAT CAA ATT

7

TAC AAA CCG TGC GAG CCC AAG ATT

8

TAC AAA ACG TGT TTT GCA CCT ATT

9

TAC ACT AGG TCC CAC TTC ATT

10

TAC CTT GAT TTT AGG AGA TAT AGT CAG TTG ACC TTT CCA ATT

12 TAC AGG AGT CGA TCG ATA TAT ATC GAG CAA ATT

13 TAC CTT GAT TTT AGG CAG TTG ACC ATT

14 TAC CTT GAT TTT AGG CAG TTG ACC TTT AGT ATT

15 TAC AAA TGT CCC CAA TTT CCG CCT ATT

DRAFT

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Feed the World

16 TAC CGG CCG ATC AGA ATT

17 TAC CGG CCG TAG TGA ATT

18 TAC CGG CCC GTT GAG GTT GCT ATT

19 TAC TTG ACC TTT AGG TCC AGA CAT TGA AGT ATT

20 TAC GAA AGG TGA ACA AGG TGA ATT

Transcription/translation group data sheet

Sentence number: _____

Transcriber name: _____

mRNA codons:

Translator: _____

tRNA codons:

Sentence: _____

tRNA runner name: _____

tRNA runner name: _____

Transcription/translation group data sheet

Sentence number: _____

Transcriber name: _____

mRNA codons:

Translator: _____

tRNA codons:

Sentence: _____

tRNA runner name: _____

tRNA runner name: _____

Lesson 3
DNA Sentences

Focus Question: *How are proteins coded for by DNA?*
Vocabulary: *deoxyribonucleic acid (DNA), messenger RNA (mRNA), transfer RNA (tRNA), CRISPR, transcription, translation*

Background

Deoxyribonucleic acid (DNA) is the molecule of life. DNA is one of the most recognizable nucleic acids, a double-stranded helix. The process by which DNA codes for proteins involves enzymes and additional single-stranded nucleic acids, specifically messenger ribonucleic acid (mRNA) and transfer ribonucleic acid (tRNA). The steps in protein synthesis, transcription from DNA to mRNA, and translation from mRNA to tRNA can be demonstrated by modeling. In this activity, each triplet code of DNA will represent a word in a sentence rather than a code for an amino acid. Introns and exons are omitted. The words can be found by transcribing the DNA into mRNA, then translating mRNA into tRNA.

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7. Switch roles and repeat for each additional DNA strand you are assigned.

Extension:

After your group has translated your assigned sentences, research each statement to find evidence to support or refute the statement.
Create a new "sentence" (protein) using DNA triplets to create a different set of words (amino acids).

Reflection

1. How are chromosomes, DNA, genes and proteins related?

2. What area of the cell does the table holding DNA represent in this modeling activity? Why can't the DNA strand be brought back to your group?

4. What area of the cell does your table represent?

5. What do the words represent? The completed sentences?

6. What do you think the consequences might be if an error occurred in the cell as it goes through the process of protein synthesis?

Rubric for Self-Assessment

Skill	Yes	No
I participated in each role during the DNA activity.		
I can explain the function of DNA and the roles of DNA, mRNA and tRNA in transcription and translation of DNA into proteins.		
I created a new "sentence" (protein) using DNA triplets to create a different set of words (amino acids)		

Lesson 4

Punnett Squares - Teacher

Focus Question: *How do breeders predict which traits will be in offspring? How might biotechnology methods improve the process?*

Lesson target: Students complete Punnett squares to compare theoretical ratios to actual ratios on corn cobs and determine the amount of time it might take to get a desired outcome.

Vocabulary: *genotype, phenotype, alleles, homozygous, heterozygous, dominant, recessive, monohybrid, dihybrid*

HS-LS3: Heredity: Inheritance and Variation of Traits

Performance Expectation	**Connections to activity**
HS-LS3-1. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.	Punnett squares are used to predict the offspring ratios from various crosses. Students complete the squares, determine the genotype and phenotype ratios and compare to a real plant.
Science and Engineering Practice	
Asking Questions and Defining Problems • Ask questions that arise from examining models or a theory to clarify relationships.	Students discover that Punnett squares can help to determine ratios of offspring, but in reality those predictions are not always accurate.
Disciplinary Core Ideas	
LS1.A: Structure and Function • All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins. (secondary) (Note: This Disciplinary Core Idea is also addressed by HS-LS1-1.) **LS3.A: Inheritance of Traits** • Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no as-yet known function.	Students see the result of crossing genes and the resulting genotypes and phenotypes that result from the different proteins that are produced based on the gene combinations.

Cross Cutting Concept	
Cause and Effect • Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects.	The different gene combinations in the Punnett squares result in different seed color and shape on the corn cob.

*Student handout information is indicated in light gray print. Answer keys are imbedded in the student handout section.

Materials*
Carolina Biological Supply 17-6810 Monohybrid cross
Carolina Biological Supply 17-6900 Dihybrid cross

*The activity can be completed without the Carolina corn, however, having the actual cobs allow students the added check to see that sometimes, the expected ratio is not what results in reality. (For example: although there is a 50-50 chance that human offspring will be male or female, there are many instances where there are families of all girls or all boys.) The percent chance resets each time a cross occurs. Each kernel of corn is a separate offspring that results from a single silk.

Prior Knowledge
Students need to understand how to show a cross using a Punnett square. This should be part of a middle school science course. Students will need to know the definition of alleles, the difference between a genotype and a phenotype, homozygous and heterozygous and dominant and recessive traits. Mendel's Law of Independent Assortment is also applied for these two traits.

Teacher Preparation
Make copies of the Student handout

Have corn from Carolina on hand for students to examine or make copies of a photo like this:

By Sam Fentress, CC BY-SA 2.0, https://commons.wikimedia.org/w/index.php?curid=348910

Student Handout

Monohybrid Cross - a cross looking at one gene for a trait

1. Cross Colored Aleurone with the genotype (RR) corn with Colorless Aleurone, genotype (rr), in the Punnett square below to show the F_1 results.
   a. Circle the correct words below: The **R R** genotype is homozygous / hetrerozygous dominant / recessive.

R R x r r

i. What are the resulting genotypes? *100% **R r***

ii. What are the resulting phenotypes? *100% colored aleurone*

2. When you cross two of the offspring from above, what will be the result in the F_2 generation?
   b. Circle the correct word in the sentence below: The **R r** genotype is homozygous / heterozygous.

R r x R r

a. What are the resulting genotypes? What are the percentages?
*25% **R R**; 50% **R r**; 25% **r r***
b. What are the resulting phenotypes? What are the percentages?
75% colored aleurone; 25% colorless aleurone

3. Count the kernels on the cob to determine the actual ratio.

Dihybrid cross - looking at two genes that are on two different chromosomes

4. Cross Colored Aleurone (RR), Starchy (SU/SU) corn with Colorless Aleurone (rr), Sweet Endosperm (su/su), Il in the Punnett square below to show the F_1 results.

	r su	r su	r su	r su
R Su				
R Su				
R Su				
R Su				

R R SU / SU x r r su / su

a. What are the resulting genotypes? What are the percentages?

*100% **R r Su / su***

b. What are the resulting phenotypes? What are the percentages?

100% colored aleurone, starchy corn

5. When you cross two of the offspring from above, what will be the result in the F2 generation?

	R SU	R su	r SU	r su
R SU				
R su				
r SU				
r su				

6. How might you get 100% colorless (**r r**) and sweet (**su su**) offspring?

- *From this cross, only 1 of the 16 possibilities will be colorless and sweet. If those offspring are crossed, 100% of the offspring will be colorless and sweet*

Reflection

1. How long would it take for a farmer to accomplish this amount of crossing on his/her own?
   - *Considering time for growing and the generations, it might take up to 3 years before the corn with the desired traits would be able to be harvested.*

2. What are some traits that farmers may value that could be a result of two different corn varieties?

- *Traits of value include: drought resistance, strong root systems (to prevent wind blowing the plants down (lodging) and to increase nutrient uptake), disease resistance, pest resistance, etc...*

3. Seed chipping is a technique discovered by Monsanto. How much time does that take off of the typical hybridization cycle?

- *Plants can be chipped, the genomes sequenced to find the genes of interest, then bred within one season, cutting the time in half or more compared to that of traditional breeding.*

4. Look for other techniques being used to cut the time it takes to make new hybrids (i.e. DNA markers and genome mapping). Describe how these methods are helping reduce the time to create new lines that will have increased resistance to weed pressure, pests and drought.

- *Answers will vary. They might research TALEN, gene silencing, CRISPR, etc.*

Differentiation

Other ways to connect with students with various needs:

i. **Local community**: Students may visit a local plant breeder at a greenhouse (horticulture related or produce based) to learn how the breeder chooses different varieties for breeding.

ii. **Students with special needs (language/reading/auditory/visual)**: Students may use different colored chips to represent the genes and add the chips to an enlarged Punnett square, making combinations and moving the chips.

iii. **Extra support**: If students are struggling, they may watch *Learn Biology: How to Draw a Punnett Square* https://www.youtube.com/watch?v=prkHKjfUmMs
Students may read the Khan Acaadmey article that explains Independent Assortment https://www.khanacademy.org/science/high-school-biology/hs-classical-genetics/hs-introduction-to-heredity/a/the-law-of-independent-assortment

iv. **Extensions**: Students may talk with a corn breeder (or other plant breeder) to see what other traits are of value to a farmer or grower of other plants (poinsettias, hydrangeas, lilies or other plants that are sold during different seasons give students several applications to their lives.)
This video: *DEKALB® Breeding HQ: Seed Chipping Technology* https://www.youtube.com/watch?v=gCb9TSpuxUU&list=PL-4ublK0Dtz73kHbrffkkgGxjWhtV0dIr shows how technology is improving plant breeding.

Rubric for Assessment

Skill	Developing	Satisfactory	Exemplary
Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.	Student can complete a monohybrid cross using a Punnett square, but struggles with the dihybrid cross. Student cannot explain how the process of meiosis and independent assortment affects results	Student can complete monohybrid and dihybrid crosses. Student can clarify the relationship of independent assortment of chromosomes on the outcome of multiple crosses.	Student can complete monohybrid and dihybrid crosses. Student can clarify the relationship of independent assortment of chromosomes on the outcome of multiple crosses. Student can also explain the impact of technology on traditional breeding.

Rubric for Self-Assessment

Skill	Yes	No
I correctly completed a Punnett square for a monohybrid cross.		
I correctly completed a Punnett square for a dihybrid cross.		
I understand Mendel's Law of Independent Assortment and its impact on the traits in these crosses.		
I discovered at least one new technique in breeding that has impacted the development of new hybrids.		

Lesson 4

Punnett squares

Focus Question: *How do breeders predict which traits will be in offspring? How might biotechnology methods improve the process?*

Vocabulary: *genotype, phenotype, alleles, homozygous, heterozygous, dominant, recessive, Law of Independent Assortment*

Use Punnett squares to show results from a hybrid cross and determine the genotypic ratios and phenotypic ratios of a hybrid. If a plant breeder is interested in creating a better corn plant, one with some traits from one variety and some traits from another, the traditional method is to cross these two varieties and look for the plants that exhibit the combination of traits desired.

Instructions

Monohybrid Cross - a cross looking at one gene for a trait

Use the model (Carolina Biological Supply 17-6810 Monohybrid cross) to complete the activity below.

1. Cross Colored Aleurone with the genotype (RR) corn with Colorless Aleurone, genotype (rr), in the Punnett square below to show the F_1 results.

   a. Circle the correct words below: The **R R** genotype is homozygous / hetrerozygous dominant / recessive.

	R	**R**
R		
r		

R R x r r

a. What are the resulting genotypes? What is the percent?

b. What are the resulting phenotypes? What is the percent?

2. When you cross two of the offspring from above, what will be the result in the F2 generation?

   b. Circle the correct word in the sentence below: The **R r** genotype is homozygous / heterozygous.

	R	**r**
R		
r		

R r x R r

a. What are the resulting genotypes? What are the percents?

b. What are the resulting phenotypes? What are the percents?

3. Count the kernels on the cob to determine the actual ratio.

Dihybrid cross - looking at two genes that are on two different chromosomes

Use the model (Carolina Biological Supply 17-6900 Dihybrid cross) to complete the activity below.

4. Cross Colored Aleurone (RR), Starchy (SU/SU) corn with Colorless Aleurone (rr), Sweet Endosperm (su/su), Il in the Punnett square below to show the F1 results.

	r su	**r su**	**r su**	**r su**
R Su				
R Su				
R Su				
R Su				

R R SU / SU x r r su / su

a. What are the resulting genotypes?

b. What are the resulting phenotypes?

5. When you cross two of the offspring from above, what will be the result in the F2 generation?

	R SU	**R su**	**r SU**	**r su**
R SU				
R su				
r SU				
r su				

R R SU / SU x r r su / su

a. What are the resulting genotypes?

b. What are the resulting phenotypes?

6. How might you get 100% colorless and sweet offspring?

Reflection

1. How long would it take for a farmer to accomplish this amount of crossing on his/her own?

2. What are some traits that farmers may value that could be a result of two different corn varieties?

3. Seed chipping, a technique discovered by Monsanto. How much time does that take off of the typical hybridization cycle?

4. Look for other techniques being used to cut the time it takes to make new hybrids (i.e. DNA markers and genome mapping). Describe how these methods are helping reduce the time to create new lines that will have increased resistance to weed pressure, pests and drought.

Rubric for Self-Assessment

Skill	Yes	No
I correctly completed a Punnett square for a monohybrid cross.		
I correctly completed a Punnett square for a dihybrid cross.		
I understand Mendel's Law of Independent Assortment and its impact on the traits in these crosses.		
I discovered at least one new technique in breeding that has impacted the development of new hybrids.		

Lesson 5
Moving Genes - Teacher

Focus Question: *How do we use plasmids to genetically modify bacteria?*
Learning Target: *Students will demonstrate their understanding of the steps in genetic modification by properly sequencing the cards.*
Vocabulary: *phenotype, genotype, plasmid vector, gene of interest, heat shock, promoter, restriction enzymes*

LS1: From Molecules to Organisms: Structures and Processes

Performance Expectation	Classroom Connection
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.	Students sequence the steps of creating a genetically modified organism using cards with the steps on them.
Science and Engineering Practice	
Constructing Explanations and Designing Solutions • Construct an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future.	Students will use the cards to construct an explanation for how organisms are genetically modified.
Disciplinary Core Idea	
LS1.A: Structure and Function • All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells.	Students will see the tools utilized in genetic modification and explain how they are used in sequence.
Cross Cutting Concept	
Structure and Function • Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, the structures of different components, and	Various tools are used and students can explain how each tool is important to the process of genetic modification.

connections of components to reveal its function and/or solve a problem.	

*Student handout information is indicated in light gray print. Answer keys are imbedded in the student handout section.

Teacher Background

Genetic modification is different than crossing different varieties of the same plant species. It is taking a gene from one species and inserting it into the genetic material of another, different species. This is what makes it so specific. We have been able to modify bacteria to produce insulin for humans that is genetically identical to the insulin produced in a human pancreas. How is this possible?

Scientists have been able to isolate genes that code for specific traits through genome studies (discovering the DNA sequence of an entire organism). Proteins, called restriction enzymes, have been discovered and used to cut out the desired gene from the strand of DNA. Scientists have also used various bacteria to act as vectors that can carry genetic material.

Bacteria are ideal species for these vectors, as they contain chromosomal DNA and plasmid DNA. A plasmid is a circular piece of DNA that is found naturally in bacteria. These plasmids can replicate when the bacterium replicates and may be composed of as few as 1,000 or up to 20,000 nucleotides. The genes on the plasmid are part of the traits expressed by the bacterium, perhaps helping it to resist antibiotics, or produce a toxin.

Teacher preparation

This activity may be used to introduce and/or review the topic of "bacterial transformation."

1. Copy Steps in Bacterial Transformation cards onto card stock, laminate, and cut.
2. Pass out to students so that the cards are in random order.
3. Ask students to place the cards into an order that "makes sense." Students may work individually, in pairs, or in small groups.
4. Ask questions as you circulate around the room
5. Remind students to use the vocabulary words as they ask questions of you and discuss the cards with their classmates. Encourage them to make a list of unfamiliar terminology and use favorite techniques to learn this new vocabulary.
6. Review the correct order with them.
7. Students may then take notes from the cards.

The correct order is: B, D, C, E, A

Card explanations

B: **Identify a phenotype of interest** - Researchers knew that *Bacillus thuringiensis* was a bacteria that produced a toxin

that was lethal to European corn borers. Farmers had been using *Bt* toxin on their crops for many years, but the toxin had to be applied with a sprayer. The larval stage of the borer causes damage to crops. They are dirty white, often having a pinkish tinge. The skin is smooth and free of hairs. There are numerous dark spots scattered over the sides and top of the body. The head is dark brown to black.

D: **Identify a gene that codes for the phenotype** - Researchers sequenced the gene that created the toxin, then were able to isolate it.

C: **Gene of interest is engineered into a plasmid vector** - Using restriction enzymes, scientists removed the gene of interest and created a plasmid that would contain the gene and any promoters to activate the gene.

E: **Agrobacterium are transformed by the addition of the plasmid vector** - bacteria are soaked in a low concentration of a salt solution then heat shocked. The salt solution sets up a charge across the cell membrane, while the heat opens small pores in the membrane (just as human skin pores are opened when exposed to heat). The plasmids are attracted by the opposite charge and slip inside the pores in the bacterial membrane. The bacteria is cooled quickly closing the pores to ensure the plasmid stays inside the bacteria.

A: **Replication of plasmid vector by bacteria** - Once the bacteria is tested to see if it has taken the plasmid in, (usually the plasmid contains an antibiotic resistance gene so the bacteria that have taken in the plasmid will live in the presence of an antibiotic) the bacteria are encouraged to replicate in large numbers producing many bacteria with the ability to make the toxin. The bacteria may then be inserted into the leaf, stem or root tissue of the plant and grown through a process called plant tissue culture (see lesson 6C to see a related activity) to grow into a mature plant that will produce the toxin.

Student handout

The set of cards contain the basic steps in using plasmid vectors to transform bacteria. Arrange the illustrated cards in order to show your understanding of how genes are moved.

List the order of the cards your group decided is correct. Take notes below on the process. Provide an explanation for why this is the arrangement by describing the steps in your own words.

Differentiation

Other ways to connect with students with various needs:

i. **Local community**: Students may do a search to see what genetic modification resources are available in their community. Medical labs are using genetic modification techniques to target specific diseases, agriculture companies (Corteva, Bayer, Syngenta, BASF) may have education and outreach departments that would send a speaker to your class. The county extension service or land grant university in your area may also offer speakers or programs to help consumers understand genetic modification. See

also: GMOs 101 from Michigan State University: https://msutoday.msu.edu/feature/2018/gmos-101/

ii. **Students with special needs (language/reading/auditory/visual)**: Students may need larger formatted cards, or "talking cards." Using augmented reality, students could take a photo of an attached qr code that would take them to an audio file that would read the text and explain the diagram. (This adaptation does not currently exist.)

iii. **Extra support**: If students are struggling, they may visit Engineer a Crop, an interactive activity at: http://www.pbs.org/wgbh/harvest/engineer/transgen.html where they may alter a crop traditionally or using transgenic methods.

iv. **Extensions**: New technology is available that will change the way genetic modification works. The technique is called CRISPR and there are various videos and articles about it. To learn the science behind CRISPR, visit HHMI BIointeractive: Click and Learn *CRISPR-Cas 9: Mechanisms and Applications* at https://www.hhmi.org/biointeractive/crispr-cas-9-mechanism-applications

Rubric for Assessment

Skill	Developing	Satisfactory	Exemplary
Structure and Function: Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, the structures of different components, and connections of components to reveal its function and/or solve a problem.	Student is able to explain portions of the following, but in a partial way: the structure of a plasmid, the mechanism of creating a new plasmid, the process of moving the plasmid into a cell and manipulating the resulting copies of the plasmid.	Student is able to explain the structure of a plasmid, the mechanism of creating a new plasmid, the process of moving the plasmid into a cell and manipulating the resulting copies of the plasmid.	Student is able to explain the structure of a plasmid, the mechanism of creating a new plasmid, the process of moving the plasmid into a cell and manipulating the resulting copies of the plasmid; and can make a prediction of the impact of newer technologies on this process.

Rubric for Self-Assessment

Skill	Yes	No
My group was able to properly sequence the steps in moving genes.		
I still have questions about the process of moving genes.		

Steps in bacterial transformation

Student instructions

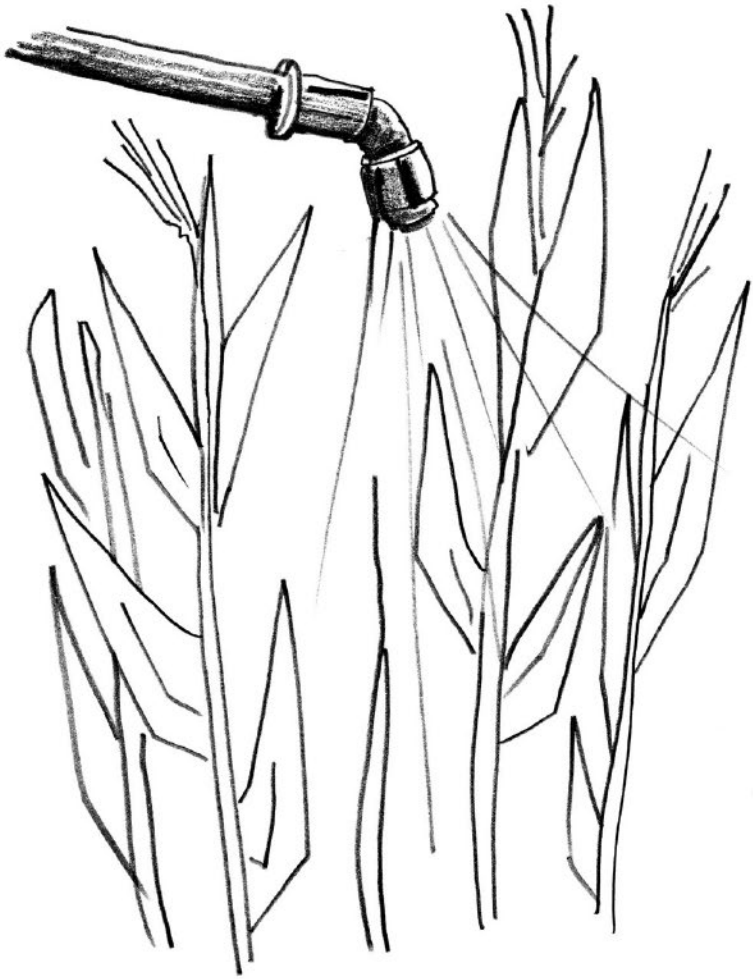
These cards contain the basic steps in using plasmid vectors to transform bacteria. Sort them into the correct order.
Be sure to learn and understand the meanings of any words that are unfamiliar to you.

Teacher instructions

1. Copy these cards onto card stock, laminate, and cut.
2. Pass out to students so that the cards are in random order.
3. This activity may be used to introduce and/or review the topic of "bacterial transformation."
4. Ask students to place the cards into an order that "makes sense." Students may work singly, in pairs, or in small groups.
5. Ask questions as you circulate around the room:
   - What do you need to start?
   - What is the final product you want?
   - Why did you put this card in this place?
   - What made you think that?
   - Which would come first: a few, or a lot?
6. Remind students to use the vocabulary words as they ask questions to you and discuss the cards with their classmates. Encourage them to make a list of unfamiliar terminology and use favorite techniques to learn this new vocabulary.
7. Review the correct order with them.
8. Students may then take notes from the cards.

Identify a phenotype of interest

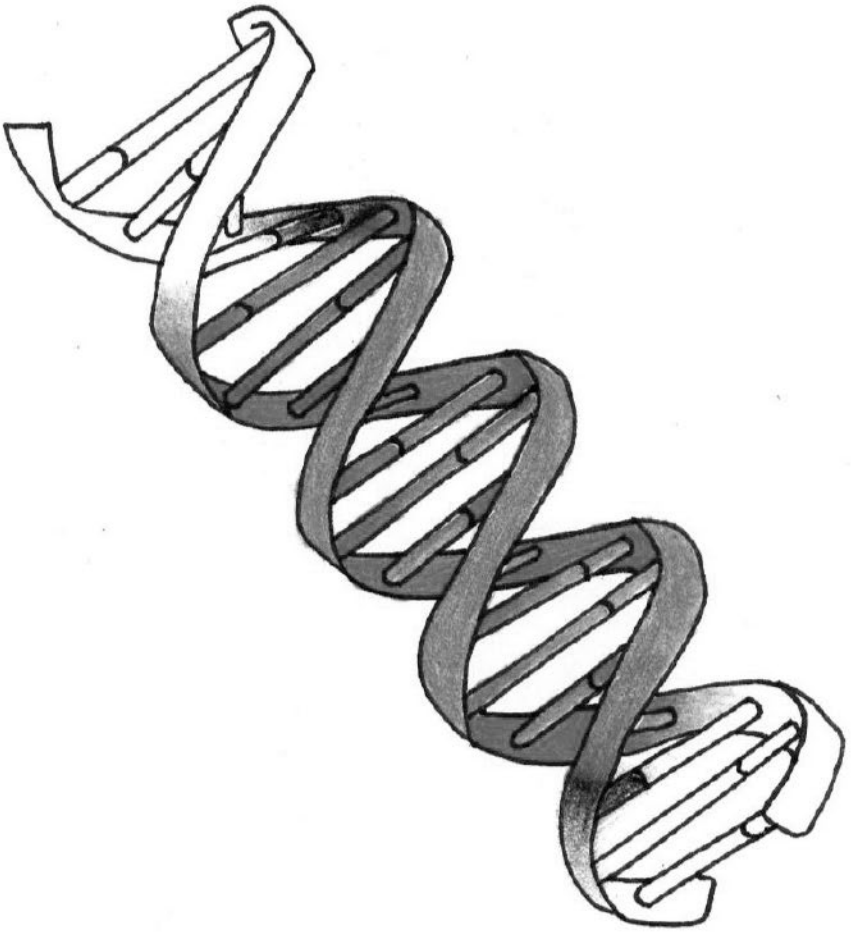
B



Example: *Bacillus thuringiensis* produces a toxin that is lethal to corn borers.

Identify a gene that codes for the desired phenotype

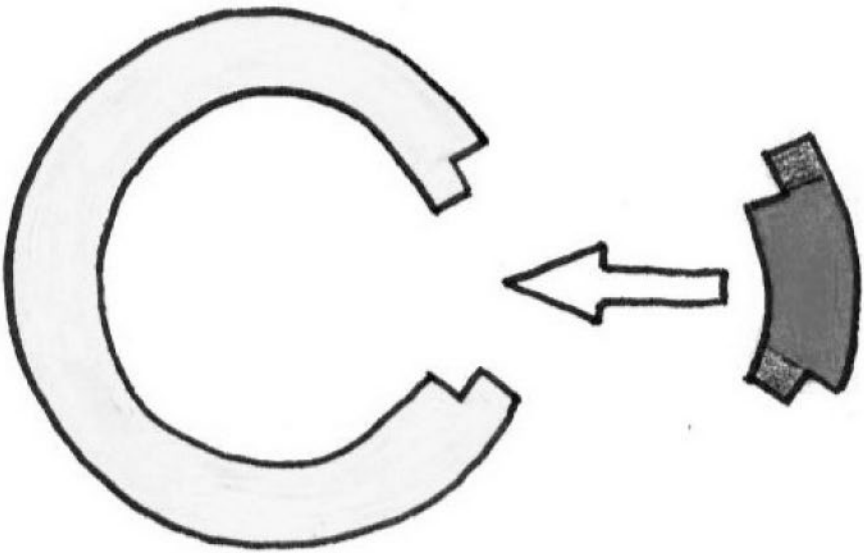
D



Example: Through experimental research, the gene for *Bt* toxin was identified and isolated

Gene of interest is engineered into a plasmid vector

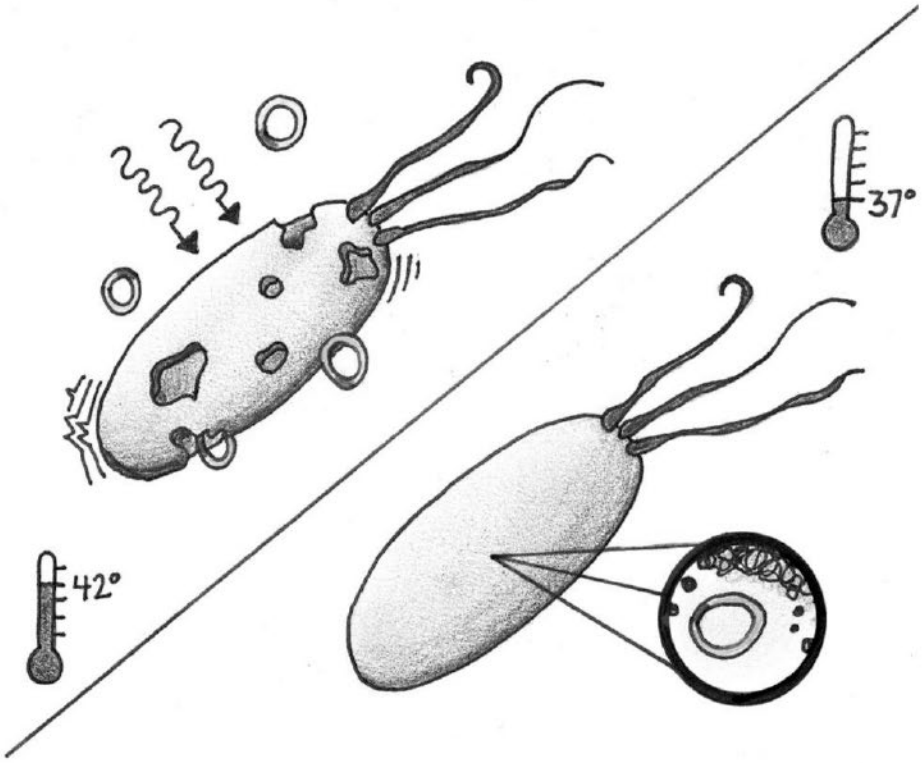
C



- Plasmid vector may be engineered by an individual lab or purchased from a supplier
- Restriction enzymes cut DNA, opening the plasmid
- Gene of interest is mixed with open plasmids
- Plasmid reforms with gene of interest within

Agrobacterium are transformed by the plasmid vector

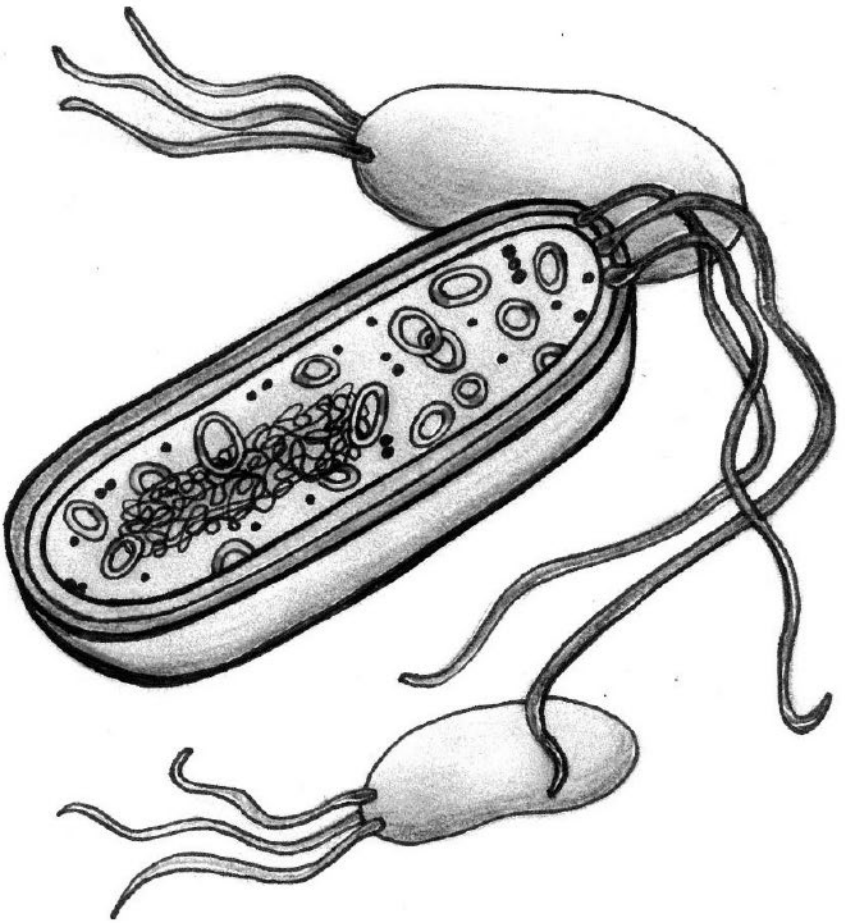
E



Bacteria uptake the plasmid when heat-shocked.
Placing bacterial cells in a hot water bath disrupts the cell membranes and makes them more permeable. The plasmids are now able to cross the disrupted membrane.

Replication of plasmid vector by bacteria

A



Agrobacterium cell machinery is harnessed as a way to multiply the plasmid vector containing the desired genes.

Lesson 5
Moving Genes

Focus Question: *How do we use plasmids to genetically modify bacteria?*
Vocabulary: *phenotype, genotype, plasmid vector, gene of interest, heat shock, promoter, restriction enzymes*

The set of cards contain the basic steps in using plasmid vectors to transform bacteria. Arrange the illustrated cards in order to show your understanding of how genes are moved.

List the order of the cards your group decided is correct. Take notes below on the process. Provide an explanation for why this is the arrangement by describing the steps in your own words.

Rubric for Self-Assessment

Skill	Yes	No
My group was able to properly sequence the steps in moving genes.		
I still have questions about the process of moving genes.		

Lesson 6A

Plasmid modeling - Teacher

Focus Questions: *How does genetic modification work? How does DNA work in bacteria? How might we use that to aid in genetic modification?*

Learning Target: *Students will model the process of plasmid creation and uptake by bacteria.*

Vocabulary: *restriction enzymes, plasmid vectors, nucleotides*

LS1: From Molecules to Organisms: Structures and Processes

Performance Expectation	Classroom Connection
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.	Students create a model of a plasmid that contains genes to synthesize a specific protein for a desired trait.
Science and Engineering Practice	
Developing and Using Models • Use a model based on evidence to illustrate the relationships between systems or between components of a system. **Construct an Explanation** • Construct an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future.	Student models will show the process of finding a gene of interest, cutting DNA with restriction enzymes, inserting the gene of interest into a plasmid and the process of DNA repair to add that gene to a plasmid. Students will then *construct an explanation* to show how that plasmid will be "inserted" into a bacterium and how the bacteria will express those genes.
Disciplinary Core Idea	
LS1.A: Structure and Function • All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells.	Plasmids are circular pieces of DNA; students will insert a gene of interest to show how the plasmid can be engineered. Students will *design a solution* to overcome the barriers of bacteria to successfully insert the plasmid.
Cross Cutting Concept	
Structure and Function • Investigating or designing new systems or structures requires a detailed	Enzymes have various functions; restriction enzymes can be used to remove DNA or insert DNA into plasmids.

examination of the properties of different materials, the structures of different components, and connections of components to reveal its function and/or solve a problem.	

*Student handout information is indicated in light gray print. Answer keys are imbedded in the student handout section.

pGLO plasmid. Sequence and map are available at http://explorer.bio-rad.com under "Teaching Resources"

Potential final model: http://www.bio-rad.com/webroot/web/pdf/lse/literature/1660033.pdf (page 10)

Teacher Background

Genetic modification is different than crossing different varieties of the same plant species. It is taking a gene from one species and inserting it into the genetic material of another, different species. This is what makes it so specific. We have been able to modify bacteria to produce insulin for humans that is genetically identical to the insulin produced in a human pancreas. How is this possible?

Scientists have been able to isolate genes that code for specific traits through genome studies (discovering the DNA sequence of an entire organism). Proteins, called restriction enzymes, have been discovered and used to cut out the desired gene from the strand of DNA. Scientists have also used various bacteria to act as vectors that can carry genetic material.

Bacteria are ideal species for these vectors, as they contain chromosomal DNA and plasmid DNA. A plasmid is a circular piece of DNA that is found naturally in bacteria. These plasmids can replicate when the bacterium replicates and may be composed of as few as 1,000 or up to 20,000 nucleotides. The genes on the plasmid are part of the traits expressed by the bacterium, perhaps helping it to resist antibiotics, or produce a toxin.

Prior Knowledge

If students have completed **Lesson 5 Moving Genes**, they should have a beginning conception of the steps in the process of genetic modification. This activity asks students model the creation of the plasmid, and describes how to illustrate a potential method of uptake by the plasmid by bacteria. Students need to know about the structures of bacteria: chromosomal DNA and plasmid DNA, the composition of the cell membrane, and how heat may affect it. This lesson will help prepare them for **Lesson 6D Transform Bacteria**

Materials

Pop beads
Mini marshmallows and toothpicks
Blocks
Or other similar materials for making sets of nucleotides and a strand of DNA

Teacher Preparation

Gather materials for student use to make models.

PART I

In this activity, you will create a model to show

1) how a gene may be removed from a strand of DNA, then
2) inserted into a plasmid, to be taken into bacteria.

This will demonstrate the process that was used to make the plasmid in the Bio-Rad pGLO™ Bacterial Transformation Kit.

Use beads or other materials (mini marshmallows, blocks, toothpicks, etc.) to create a model to show the process of genetic modification. Your model must include:

- the gene or genes of interest from the original species within the DNA strand;
- a gene to allow for the selection of the transformed cells (often this is an antibiotic resistance gene);
- the action of the restriction enzyme to "cut" out the gene(s) of interest;
- the addition of the gene or genes of interest into the existing DNA of the organism you are trying to modify; and
- the action of the DNA ligase that "attaches" the DNA nucleotides together in place.

Note: A process called PCR (polymerase chain reaction) makes hundreds of thousands of copies of the desired gene(s) to create copies that are inserted into bacteria or another vector. The transformed cells can be selected for replication (for example, another gene for antibiotic resistance is added to the plasmid along with the gene of interest; when the cells grow on a plate that has the antibiotic, the transformed cells are the only ones that grow.) These transformed vectors are used to transfer the gene to the desired species. (We will not include this aspect in this model.)

Ask students to connect their model to the phenomenon by explaining how genetic modification may allow for the species that is modified to have better survival chances.

PART II

Now that you have your model, how might we get the bacteria to uptake the plasmid you made?

1. If a plastic bottle or balloon acts as the bacteria model, how might we get the genes inside the bottle?

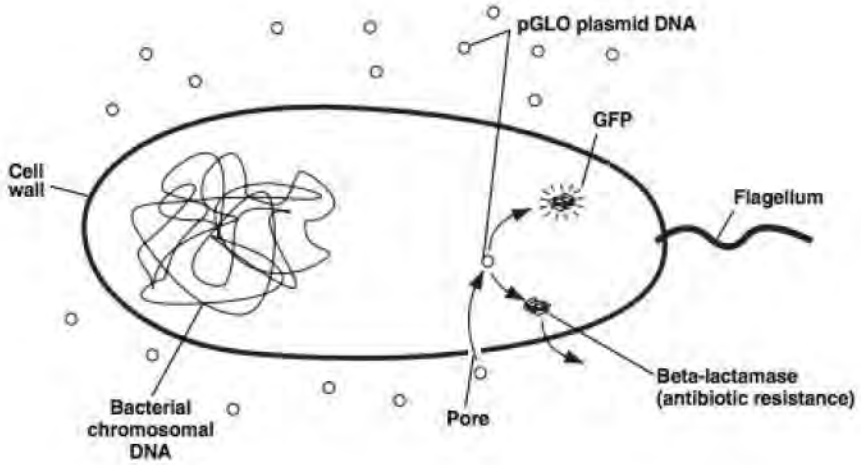
- *Allow students to discuss in their lab group, then ask for ideas. If students are struggling, remind them that they might need to use the physical properties of the cell materials (membrane, cytoplasm) and they might need to be reminded of the processes they have learned (osmosis, diffusion, charge differential, etc)*

2. What will happen to the cell membrane if we heat the bacteria a bit? (Think of yourself in a hot tub or sauna.) What will happen to the cell membrane if we put it in ice water? (Think of yourself on a chilly day without a coat on.)

- *Although this may seem like a silly analogy, this is exactly what scientists are doing to the bacteria: warming it to allow the pore spaces to open, then chilling it to encourage them to close.*

3. How could the conditions in #2 help us get the plasmid in the bottle or balloon?

- *Help students visualize what might happen to the pore spaces when warm and then when cold.*



http://www.bio-rad.com/webroot/web/pdf/lse/literature/1660033.pdf (page 35)

This diagram shows the bacteria and what is happening during the transformation. This helps students visualize how a plasmid may enter a bacterium and how many plasmids there are inside their vials during the experiment.

Differentiation

i. **Local community**: Students may do a search to see what genetic modification resources are available in their community. Medical labs are using genetic modification techniques to target specific diseases, agriculture companies (Corteva, Bayer, Syngenta, BASF) may have education and outreach departments that would send a speaker to your class. The county extension service or land grant university in your area may also offer speakers or programs to help consumers understand genetic modification. See also: *GMOs 101* from Michigan State University: https://msutoday.msu.edu/feature/2018/gmos-101/

ii. **Students with special needs (language/reading/auditory/visual)**: Since this lesson is primarily tactile and/or kinesthetic, it could be modified to have students draw the model in a series of comic book frames. Or one student could verbally describe the action while another creates the model and takes it through the steps of the process.

iii. **Extra support**: Students may watch *Genetic Engineering* at: https://www.youtube.com/watch?v=nfC689ElUVk and *Plasmid Rap* at: https://www.youtube.com/watch?v=QFfUFyUvP-w

iv. **Extensions**: New technology is available that will change the way genetic modification works. The technique is called CRISPR and there are various videos and articles about it. To learn the science behind CRISPR, visit HHMI BIointeractive: Click and Learn *CRISPR-Cas 9: Mechanisms and Applications* at https://www.hhmi.org/biointeractive/crispr-cas-9-mechanism-applications or watch *What is CRISPR-Cas?* at https://www.youtube.com/watch?v=52jOEPzhpzc&t=2s

Rubric for Assessment

Skill	Beginning	Satisfactory	Exemplar
Apply scientific ideas, principles, and/or evidence to provide an explanation of phenomena and solve design problems, taking into account possible unanticipated effects.	Explanation for how the model represents the plasmid contains only some of the parts to be transferred to a bacteria to make the proteins of interest.	Explanation for how the model represents the plasmid contains all the parts to be transferred to a bacteria to make the proteins of interest.	Explanation for how the model represents the plasmid contains all the parts to be transferred to a bacteria to make the proteins of interest and the reason why those proteins are of interest.

Rubric for Self-Assessment

Skill	Yes	No
My model showed all five aspects of the plasmid required.		

I was able to explain how my model operated in writing or orally to my classmates.		

Lesson 6A
Plasmid modeling

Focus Questions: *How does genetic modification work? How does DNA work in bacteria? How might we use that to aid in genetic modification?*
Vocabulary: *restriction enzymes, plasmid vectors, nucleotides*

Background

Genetic modification is different than crossing different varieties of the same plant species. It is taking a gene from one species and inserting it into the genetic material of another, different species. This is what makes it so specific. Scientists have been able to modify bacteria to produce insulin for humans that is genetically identical to the insulin produced in a human pancreas. How is this possible?

Scientists have been able to isolate genes that code for specific proteins that determine traits through genomic studies (discovering the DNA sequence of an entire organism). Proteins, called restriction enzymes, have been discovered and have been used to cut out the desired gene from a strand of DNA. Scientists have also used various bacterial species to act as vectors that can carry genetic material.

Bacteria are ideal species for these vectors, as they contain chromosomal DNA and plasmid DNA. A plasmid is a circular piece of DNA that is found naturally in bacteria. These plasmids can replicate when the bacterium replicates and may be composed of as few as 1,000 or up to 20,000 nucleotides. The genes in the plasmid are expressed by the bacterium, perhaps helping it to resist antibiotics, or produce a toxin.

PART I

In this activity, you will create a model to show

1) how a gene may be removed from a strand of DNA, then
2) inserted into a plasmid, to be taken up by bacteria.

This will demonstrate the process that was used to make the plasmid in the Bio-Rad pGLO™ Bacterial Transformation Kit.

Use beads or other materials (mini marshmallows, blocks, toothpicks, etc.) to create a model to show the process of genetic modification. Your model must include:

- the gene or genes of interest from the original species within the DNA strand;
- a gene to allow for the selection of the transformed cells (often this is an antibiotic resistance gene);
- the action of the restriction enzyme to “cut” out the gene(s) of interest;
- the addition of the gene or genes of interest into the existing DNA of the organism you are trying to modify; and
- the action of the DNA ligase that “attaches” the DNA nucleotides together in place.

Your model is just that. There is no need to make strings of genes with the exact numbers of bases, otherwise, you would need many more beads (materials).

Use your model to explain how genetic modification (by inserting a trait from another organism) may allow for the species that is modified to have better survival chances.

PART II

Now that you have your model, how might we get the bacteria to uptake the plasmid you made?

1. If a plastic bottle or balloon acts as the bacteria model, how might we get the genes inside the bottle?

2. What will happen to the cell membrane if we heat the bacteria a bit? (Think of yourself in a hot tub or sauna.) What will happen to the cell membrane if we put it in ice water? (Think of yourself on a chilly day without a coat on.)

3. How could the conditions in #2 help us get the plasmid in the bottle or balloon?

Rubric for Self-Assessment

Skill	Yes	No
My model showed all five aspects of the plasmid required.		
I was able to explain how my model operated in writing or orally to my classmates.		

Lesson 6B

Pipetting skills*

Focus Question: *How might we develop and practice micropipetting skills? Why is micropipetting important?*

Learning Target: Students practice micropipetting and check their accuracy.

Vocabulary: *micropipetting*

*Adapted from: "Pipetting by Design" www.cpet.ufl.edu

LS3: Heredity: Inheritance and Variation of Traits

Performance Expectation	Classroom Connections
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-LS3-1**. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.	This activity helps students practice skills that will be useful in the transformation of bacteria lab (Lesson 6D). Students practice micropipetting small amounts of liquids which is a necessary skill in a biotechnology lab.
Science and Engineering Practice	
Asking Questions and Defining Problems • Ask questions that arise from examining models or a theory to clarify relationships. (HS-LS3-1) **Constructing Explanations and Designing Solutions** • Construct an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future. (HS-LS1-1)	Students will micropipette colored water into cell well plates to make patterns. The patterns will encourage them to *ask questions and define problems*. Students will create the pattern assigned to them and *construct an explanation* to connect the pattern to biotechnology.
Disciplinary Core Idea	
LS3.A: Inheritance of Traits • Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. All cells in an organism	Students will practice moving small amounts of liquids to develop skills for transforming bacteria with the pGLO™ protein (Lesson 6D).

have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no as-yet known function.	
Cross Cutting Concepts	
Structure and Function • Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, the structures of different components, and connections of components to reveal its function and/or solve a problem.	Students will compare using micropipettors to 1 mL disposable pipettes on the accuracy of transferring small amounts.
Cause and Effect • Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects.	Using micropipettors instead of disposable pipettes results in more accuracy for these activities.
Connections to Nature of Science **Science is a Human Endeavor** • Technological advances have influenced the progress of science and science has influenced advances in technology. • Science and engineering are influenced by society and society is influenced by science and engineering.	Micropipettors are a technological advance that has influenced the progress of science.

*Student handout information is indicated in light gray print. Answer keys are imbedded in the student handout section.

Prior Knowledge

Students may have used a disposable 1mL pipette before. It is difficult to control accuracy when using those pipettes. This activity shows students that micropipettors can transfer much smaller amounts of liquid and are much more accurate, if the proper technique is used. This activity helps students prepare for the BioRad pGLO™ Transformation Lab. It is possible to do the lab with the sterile pipettes that are included in the kit from BioRad. This lesson will help students with other biotechnology related labs as micropipetting is a valued skill by folks in the industry.

Teacher Preparation

In order to develop a skill with accuracy, students need to practice. This activity allows students to practice with micropipettors and check their accuracy by measuring the mass of their results. This also gives students the opportunity to determine a percent error measurement.

1. Copy protocol instructions
2. Organize students in groups of 3-4, with two pipettes each of different sizes to work together to create the pattern. From the protocols (attached on additional pages) there are multiple amounts called for and each student will have ample opportunity to pipette. If a check for accuracy is desired after each student adds their assigned color/amounts, they can determine the mass at that point and measure to see where they are in terms of accuracy.

Final totals for each protocol
A = DNA = 5045µl = 5.045g
B = Corn = 5841µl = 5.841g
C = pGLO = 5217µl = 5.217g
D = a plasmid = 1932µl = 1.932g
E = GMO = 4694µl = 4.694g
F = KCC = 4104µl = 4.104g
G = DDG = 4800µl = 4.800g

Student Handout

Background

Micropipettes are used to transfer small measured volumes of liquid in the lab. Micropipettes can be fixed rate or adjustable, and can vary in the increment measured as well, full microliters (µl) or fractions of a µl. On an adjustable micropipette, the volume can be changed by twisting the handle or top of the plunger, depending on the brand/type you have. The readout dial will show the volume the micropipette is adjusted to transfer. The micropipette is limited to the volume range that is pre-set on the instrument. The most common ranges include: 0.5–10µl, 2–20µl, 10–100µl and 100– 1,000µl. A pipette tip must be used with the micropipettor. The tip is where the liquid is stored as it is being transferred. A new pipette tip should be used for each different liquid. The tip is ejected by using the ejector mechanism on the micropipette; some tips must be manually removed, depending on the micropipette.

Before pipetting, practice!

Practice feeling the “soft ” stop several times; taking the sample from the center of the container holding the liquid to be transferred; transferring the liquid while the pipette is upright; releasing the liquid into the appropriate microtube or cell well by touching the tip to the side and depressing the plunger, first to the “soft ” stop, next to the “hard” stop; changing tips for each liquid.

When ready to draw up a liquid, obtain a clean tip. Press the pipette into the open end of the tip and tap gently to “seat” the tip on the pipette. Place the tip in the liquid, just below the level of the liquid, in the center of the sample, but not to the base of the container. Depress the plunger on the pipette to the “soft ” or first stop. The position of this stop will vary depending on the volume set to transfer (since the point of initial resistance is determined by the desired volume of solution being transferred). Release the plunger to draw up the liquid. Once the desired

volume is drawn into the tip, remove the pipette, and take the liquid to its destination. Release the liquid along the side of the tube then depress the plunger, first to the “soft ” stop, then one second later to the second or “hard” stop to remove all of the contents. Remove the tip from the liquid before the plunger is released. Discard the tip by ejecting into a proper waste receptacle. If you need additional direction, watch this pipetting video: youtube.com/watch?v=bex0itUMxmI

Materials

Micropipettors of various sizes
96-well plate 1 per group
Colored water in 10mL amounts (color needed dependent upon which protocol)
Micropipetting protocol colors:

a. Red and Blue water
b. Yellow and Green water
c. Green and Blue water
d. Blue, Red and Green water
e. Red and Yellow water
f. Yellow and Green water
g. Blue and Red water

Procedure

1. Follow your assigned protocol to practice pipetting.
2. Mass your empty 96-cell well plate on a scale that measures to 0.001 g. What is the mass of your cell well plate?

3. Use a disposable pipette to accurately transfer the correct amount of liquid to the cell plate. Follow the first 5 instructions, then check your accuracy by measuring the mass of your plate. Is it possible to transfer the correct amounts with a disposable pipette?

4. Empty your cell well plate. Measure the mass again on the scale (it will be impossible to remove all of the water, so this will be a different amount than above). What is the mass of your plate?

5. Use the micropipettors to measure the amounts for your protocol. Add up the total of the amounts you have added to the cell well plate. Measure periodically (after 3-5 additions) to see how accurate your are. Be sure to subtract the amount from #4.
6. If the mass is equal to the pipetted amounts you have added (1000µl = 1g), then you are being accurate in your measurements. Take turns with others in your group adding the amounts called for, and continue checking your accuracy as you go along.
7. What is your pattern?

Assessment

1. Check your accuracy by using a mass scale that measures to the thousandths.

a. Add up the volume of the liquid added.
b. Convert volume to mass (1000µl = 1mL; 1mL = 1 g)
c. Zero a scale with an empty 96-well plate
d. Calculate the mass of the completed design

2. Check your percent error by doing the following:
   a. calculate the expected mass
   b. subtract your experimental mass from the expected mass
   c. take the absolute value of the answer
   d. divide the difference by the expected mass
   e. multiply by 100 to get the percent error

Example:
Expected mass = 4789µL or 4.789g
Experimental mass = 5 g
4.789 - 5 = - 0.211
0.211/4.789 = 0.044 x 100 = 4.4%

Because the amounts are so small, even a low percent error can result in major difficulties if completing a protocol in a lab. You want to be as close to 1% error as possible.

Differentiation

i. **Local community**: Students may do a search to see what genetic modification resources are available in their community. Medical labs are using genetic modification techniques to target specific diseases, agriculture companies (Corteva, Bayer, Syngenta, BASF) may have education and outreach departments that would send a speaker to your class. Lab techs in hospitals and veterinary clinics often use micropipetting skills, and may be interested in sharing experiences with your class.

ii. **Students with special needs (language/reading/auditory/visual)**: This lesson requires fine motor coordination and may result in frustration among students who are not detail-oriented or able to be precise. They may be employed as accuracy assessors. They could travel around the class to check progress and measure the mass of the plates to see how accurate each group is being.

iii. **Extra support**: Watch *Basic Pipetting* at: youtube.com/watch?v=bex0itUMxmI

iv. **Extensions**: Students may want to do more than one protocol, or work on their own to check their accuracy.

Assessment

Have students determine their percent error.

Rubric for Self-Assessment

Skill	Yes	Not Quite	No

Our group pipetted the correct amounts into the cell well plate and we calculated our percent error.			
Our pattern helped me to ask questions and define problems related to biotechnology.			
We were able to connect our pattern to a biotechnology related topic.			

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Protocol A

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the red water

20 µl: B1, B2, B3, B11

26 µl: D1, D3, D10, D11, D12

27 µl: E1, E3, E10, E12

28 µl: F1, F2, F10, F12

29 µl: C1, C3, C10, C12

Using the blue water

38 µl: B5, B8

36 µl: D5, D7, D8

48 µl: E5, E7, E8

59 µl: F5, F8

67 µl: C5, C6, C8

Using the red water

90 µl: B1, B2, B3, B11

132 µl: D1, D3, D10, D11, D12

127 µl: E1, E3, E10, E12

103 µl: F1, F2, F10, F12

112 µl: C1, C3, C10, C12

Using the blue water

126 µl: B5, B8

102 µl: D5, D7, D8

96 µl: E5, E7, E8

138 µl: F5, F8

114 µl: C5, C6, C8

Protocol B

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the yellow water

20µl: E5, E6, E9, E12

27µl: F4, F7, F9, F10, F12

45µl: G4, G7, G9, G11, G12

38µl: H5, H6, H9, H12

Using the green water

119µl: A2, A3, A7, A8

48µl: B1, B4, B7, B9

67µl: C1, C7, C8

76µl: D2, D3, D4, D7, D9

55µl: E2, E3, E4

Using the yellow water

70µl: E5, E6, E9, E12

110µl: F4, F7, F9, F10, F12

116µl: G4, G7, G9, G11, G12

93µl: H5, H6, H9, H12

Using the green water

129µl: A2, A3, A7, A8

118µl: B1, B4, B7, B9

107µl: C1, C7, C8

96µl: D2, D3, D4, D7, D9

88µl: E2, E3, E4

Protocol C

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using green water

20µl: B7, C7

47µl: D1, D2, D7

28µl: E1, E3, E7

59µl: F1, F2, F7, F8, F9

35µl: G1, H1

Using blue water

28µl: B5, B11

62µl: C4, C6, C10, C12

51µl: D4, D10, D12

90µl: E4, E5, E6, E10, E12

79µl: F5, F11

Using green water

70µl: B7, C7

116µl: D1, D2, D7

110µl: E1, E3, E7

85µl: F1, F2, F7, F8, F9

93µl: G1, H1

Using blue water

118µl: B5, B11

96µl: C4, C6, C10, C12

88µl: D4, D10, D12

199µl: E4, E5, E6, E10, E12

107µl: F5, F11

Protocol D

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using blue water

20µl: A6, A7

39µl: B5

28µl: C4, D4, E4

42µl: F5

51µl: G6, G7

Using red water

100µl: A8

111µl: B9

120µl: C10, D10

Using green water

140µl: E10

176µl: F9

115µl: G8

Using blue water

70µl: A6, A7

81µl: B5

76µl: C4, D4, E4

92µl: F5

101µl: G6, G7

Protocol E

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using red water

20µl: B2, B3, B4, B11

35µl: C1, C10, C12

47µl: D1, D3, D4, D10, D12

28µl: E1, E4, E10, E12

59µl: F2, F3, F11

Using yellow water

88µl: B6, B8

26µl: C6, C7, C8

45µl: D6, D8

79µl: E6, E8

27µl: F6, F8

Using red water

70µl: B2, B3, B4, B11

116µl: C1, C10, C12

110µl: D1, D3, D4, D10, D12

135µl: E1, E4, E10, E12

179µl: F2, F3, F11

Using yellow water

118µl: B6, B8

96µl: C6, C7, C8

89µl: D6, D8

129µl: E6, E8

107µl: F6, F8

Protocol F

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using green water

22µl: B1, B3, B10

31µl: C1, C2, C9, C11

47µl: D1, D9

29µl: E 1, E2, E 9, E11

40µl: F1, F3, F10

Using yellow water

32µl: B6, F6

23µl: C5, C7

74µl: D5, E6, E7

Using green water

122µl: B1, B3, B10

131µl: C1, C2, C9, C11

147µl: D1, D9

129µl: E1, E2, E9, E11

140µl: F1, F3, F10

Using yellow water

162µl: B6, F6

143µl: C5, C7

174µl: D5, E6, E7

Protocol G

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using blue water

119µl: B1, B2, B10, B11, B12

102µl: C1, C3, C9

127µl: D1, D3, D9, D11, D12

115µl: E1, E3, E9, E12

132µl: F1, F2, F10, F11

Using red water

20µl: B5, B6

34µl: C5, C7

89µl: D5, D7

44µl: E5, E7

100µl: F5, F6

Using blue water

22µl: B1, B2, B10, B11, B12

31µl: C1, C3, C9

29µl: D1, D3, D9, D11, D12

22µl: E1, E3, E9, E12

40µl: F1, F2, F10, F11

Using red water

74µl: B5, B6

158µl: C5, C7

37µl: D5, D7

196µl: E5, E7

88µl: F5, F6

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

DRAFT

Feed the World

Lesson 6B
Pipetting skills

Focus Question: *How can we develop and practice micropipetting skills? Why is micropipetting important?*
Vocabulary: *micropipetting*

Background
Micropipettes are used to transfer small measured volumes of liquid in the lab. Micropipettes can be fixed rate or adjustable, and can vary in the increment measured as well, full microliters (µl) or fractions of a µl. On an adjustable micropipette, the volume can be changed by twisting the handle or top of the plunger, depending on the brand/type you have. The readout dial will show the volume the micropipette is adjusted to transfer. The micropipette is limited to the volume range that is pre-set on the instrument. The most common ranges include: 0.5–10µl, 2–20µl, 10–100µl and 100– 1,000µl. A pipette tip must be used with the micropipettor. The tip is where the liquid is stored as it is being transferred. A new pipette tip should be used for each different liquid. The tip is ejected by using the ejector mechanism on the micropipette; some tips must be manually removed, depending on the micropipette.

Before pipetting, practice!
Practice feeling the "soft " stop several times; taking the sample from the center of the container holding the liquid to be transferred; transferring the liquid while the pipette is upright; releasing the liquid into the appropriate microtube or cell well by touching the tip to the side and depressing the plunger, first to the "soft " stop, next to the "hard" stop; changing tips for each liquid.

When ready to draw up a liquid, obtain a clean tip. Press the pipette into the open end of the tip and tap gently to "seat" the tip on the pipette. Place the tip in the liquid, just below the level of the liquid, in the center of the sample, but not to the base of the container. Depress the plunger on the pipette to the "soft " or first stop. The position of this stop will vary depending on the volume set to transfer (since the point of initial resistance is determined by the desired volume of solution being transferred). Release the plunger to draw up the liquid. Once the desired volume is drawn into the tip, remove the pipette, and take the liquid to its destination. Release the liquid along the side of the tube then depress the plunger, first to the "soft " stop, then one second later to the second or "hard" stop to remove all of the contents. Remove the tip from the liquid before the plunger is released. Discard the tip by ejecting into a proper waste receptacle. If you need additional direction, watch this pipetting video: youtube.com/watch?v=bex0itUMxml

Materials
Micropipettors of various sizes
96-well plate 1 per group
Colored water in 10mL amounts (color needed dependent upon which protocol)
Micropipetting protocol colors:

a. Red and Blue water
b. Yellow and Green water
c. Green and Blue water
d. Blue, Red and Green water
e. Red and Yellow water
f. Yellow and Green water
g. Blue and Red water

Procedure

1. Follow your assigned protocol to practice pipetting.
2. Mass your empty 96-cell well plate on a scale that measures to 0.001 g. What is the mass of your cell well plate?

3. Use a disposable pipette to accurately transfer the correct amount of liquid to the cell plate. Follow the first 5 instructions, then check your accuracy by measuring the mass of your plate. Is it possible to transfer the correct amounts with a disposable pipette?

4. Empty your cell well plate. Measure the mass again on the scale (it will be impossible to remove all of the water, so this will be a different amount than above). What is the mass of your plate?

5. Use the micropipettors to measure the amounts for your protocol. Add up the total of the amounts you have added to the cell well plate. Measure periodically (after 3-5 additions) to see how accurate your are. Be sure to subtract the amount from #4.
6. If the mass is equal to the pipetted amounts you have added (1000µl = 1g), then you are being accurate in your measurements. Take turns with others in your group adding the amounts called for, and continue checking your accuracy as you go along.
7. What is your pattern?

Assessment

1. Check your accuracy by using a mass scale that measures to the thousandths.
    a. Add up the volume of the liquid added.
    b. Convert volume to mass (1000µl = 1mL; 1mL = 1 g)
    c. Zero a scale with an empty 96-well plate
    d. Calculate the mass of the completed design
2. Check your percent error by doing the following:
    a. calculate the expected mass
    b. subtract your experimental mass from the expected mass
    c. take the absolute value of the answer
    d. divide the difference by the expected mass
    e. multiply by 100 to get the percent error

Example:

Expected mass = 4789µL or 4.789g
Experimental mass = 5 g

4.789 - 5 = - 0.211

0.211/4.789 = 0.044 x 100 = 4.4%

Because the amounts are so small, even a low percent error can result in major difficulties if completing a protocol in a lab. You want to be as close to 1% error as possible.

Rubric for Self-Assessment

Skill	Yes	Not Quite	No
Our group pipetted the correct amounts into the cell well plate and we calculated our percent error.			
Our pattern helped me to ask questions and define problems related to biotechnology.			
We were able to connect our pattern to a biotechnology related topic.			

Lesson 6C
Plant Tissue Culture

Focus Questions: *How might we create a sterile growing environment? How important is a sterile environment? How is a plant clone produced?*

Learning Target: Students will maintain a sterile growing environment. Students will propagate many plants of the same genetic background.

Vocabulary: *plant tissue, tissue culture, sterile/aseptic technique, explant, nutrient medium*

**This lesson was adapted from Plant Tissue Culture:*
https://www.apsnet.org/edcenter/K-12/TeachersGuide/PlantBiotechnology/Documents/PlantTissueCulture.pdf

LS1: From Molecules to Organisms: Structures and Processes

Performance Expectation	Classroom Connections
HS-LS1-4. Use a model to illustrate the role of cellular division (mitosis) and differentiation in producing and maintaining complex organisms.	Students will attempt to grow new plant tissue from existing plants. This will model cloning and the role of cell division and differentiation.
Science and Engineering Practice	
Developing and Using Models • Use a model based on evidence to illustrate the relationships between systems or between components of a system.	Students will choose a plant "model" to use as a tissue sample and clone it by growing it in culture.
Disciplinary Core Idea	
LS1.B: Growth and Development of Organisms • In multicellular organisms individual cells grow and then divide via a process called mitosis, thereby allowing the organism to grow. The organism begins as a single cell (fertilized egg) that divides successively to produce many cells, with each parent cell passing identical genetic material (two variants of each chromosome pair) to both daughter cells. Cellular division and differentiation produce and maintain a complex organism, composed of systems of tissues and organs that work together to meet the needs of the whole organism.	Students will choose a plant tissue, research the media needed to grow that plant tissue in culture, prepare the media, sterilize the plant tissue and grow the plant in the culture media.
Cross Cutting Concept	
Systems and System Models • Models (e.g., physical, mathematical, computer models) can be used to	Students will model a sterile environment, determine the importance of the sterile environment when plants are trying to grow and

simulate systems and interactions — including energy, matter, and information flows — within and between systems at different scales.	examine the results of a less than sterile environment.

Teacher Background

Watch *Tissue Culture Propagation: Class 101* before beginning: https://www.youtube.com/watch?v=qDOGrEhUe8A It may be helpful to share this video with students as well. Advanced students may be able to help with preparation of agar. There are many parts of this lab, but all require sterile technique. On the student handout, the procedure for **Plant preparation** and **Transfer of plant material to tissue culture medium** are included along with the materials needed for that procedure.

Plant research often involves growing new plants in a controlled environment. These may be plants that we have genetically altered in some way or multiple copies of cloned plants. This can be accomplished through the tissue culture of small tissue pieces from the plant of interest. These small pieces may come from a single mother plant or they may be the result of a genetic transformation of single plant cells, which are then encouraged to grow and to ultimately develop into a whole plant. Tissue culture techniques are often used for commercial production of plants as well as for plant research.

Tissue culture involves the use of small pieces of plant tissue (explants), which are cultured in a nutrient medium under sterile conditions. Using the appropriate growing conditions for the explant type, plants can be induced to rapidly produce new shoots and new roots. These plantlets can also be divided, usually at the shoot stage, to produce large numbers of new plantlets. The new plants can then be placed in soil and grown in the normal manner.

Note: Many types of plants are suitable for use in the classroom. Cauliflower florets particularly give excellent results since they can be grown into a complete plant in the basic tissue culture media, without the need for additional growth or root hormones. Green shoots are generally observable within three weeks, and roots develop within six weeks. If other plants are used, different materials will need to be added to the growth media to encourage root growth.

The most important part of this activity, however, is to maintain as sterile an environment as possible. Even one fungal spore or bacterial cell that comes into contact with the growth media will rapidly reproduce and soon completely overwhelm the small plant.

Prior knowledge

Students should be familiar with mitosis and may be familiar with cloning. The process of tissue culturing eventually results in differentiation of plant tissue into roots and shoots. The plant is very vulnerable to any type of fungal growth during this time, however. Discuss what is meant by sterile environment to help students understand the myriad of organisms that can contaminate the growth environment they are attempting to create.

Materials

1 Vial of Murashige Skoog (MS) media
1 L sterile distilled water
9 g of agar/L
30 g sucrose/L
The first container should have BAP added at the rate of 2.0mg/l. The second container should have the NAA hormone added at the rate of 0.1 mg/L. To do this it is necessary to make concentrated solutions of both BAP (2.0mg/ml) and NAA (1.0mg/ml) and filter sterilize them (see: https://www.youtube.com/watch?v=vcxy6FpfSuw for a demonstration). Add 1ml of the concentrated BAP stock or 100µl of the NAA concentrated stock to each 1 liter of media that you prepare.
1.5 L or 2 L flask in which to prepare the growth medium
Sterile culture tubes with lids
Small amounts of 1M NaOH and 1M HCl to adjust the pH of the media
pH paper (5.0–7.0 by tenths)
Aluminum foil
Glass aquarium or plastic oven bag or Laminar flow hood
Plastic sheet to cover the top of the aquarium (if preferred over plastic bag)
Adhesive tape
Hot plate with stir bar
70% alcohol (ethanol) in a spray bottle
Sterilized forceps or tweezers
Sterile petri dishes (for cutting)
Gloves
Sterile cutting equipment such as a scalpel blade, razor blade or cork borer
2 gallons of sterile distilled water
Cauliflower florets (or other plant tissue or fresh leaves)
Lidded containers (or beakers) to wash the plant material
Detergent-water mixture: 1mL detergent per liter of water
Sterilizing solution: 10% bleach solution (250 mL household bleach + 2250 ml water)
2 or 3 beakers or jars for sterile water
A well-lit area away from direct sunlight or use full-spectrum grow-light

Teacher Preparation

Agar Preparation (About 1 hour) - These steps will make 500 mL of growth medium, which is enough to prepare about 65 growing tubes.
1. Add 400 mL distilled water to a 1 L flask. Dissolve the Murashige-Skoog (MS) powder in the water. Stir the water continuously while adding the salt mixture using a magnetic spin bar in the flask while on the hot plate.
2. Add 15 g of sugar and continue to stir to dissolve. Adjust pH to 5.8 using drops of 1M NaOH or 1M HCl as necessary while gently stirring.
3. Add distilled water to make the total volume up to 500 ml.

4. Mass 4.5 grams of agar and add it to the MS solution.
5. Cover the flask with foil and heat on high and stir on the hot plate until the liquid is just boiling. Agar will boil over quickly.
6. Simmer the liquid for 30 minutes on low heat and let cool to touch (to about 50°C).
7. Pour the still warm medium into the culture tubes until one-third full. Cover immediately to cool.
8. Store culture tubes in sets of 6–8 in test tube racks.
9. Place the culture tubes in an autoclave to sterilize.

Sterilize transfer chamber and equipment preparation
(Students could be assigned to make their own sterile chambers if desired, or there can be one for class use that will need to be closely monitored as groups cycle through.)
If a laminar flow hood is available, this should be used to maintain a sterile environment. If not, follow the steps below to create a sterile working environment for the transfer of materials to the growth media.
1. A classroom transfer chamber can be made from a clean glass aquarium turned on its side. Scrub the aquarium thoroughly with a 10% bleach solution in a chemical hood if possible, making sure that you wear gloves and do not inhale the fumes. Rinse with sterile distilled water, turn upside down on a clean counter or paper towels and allow to dry.
2. Cut holes in a clean plastic sheet to allow arms to reach into the chamber and reinforce the cut edges with tape if necessary. Tape the clean plastic sheet over the open side of the aquarium making sure that the armholes are located at a convenient height. Plastic sleeves could also be fitted to these holes if you wish to make it easier to prevent the entry of airborne spores into the chamber. The finished aquarium chamber can be sterilized by spraying with 10% bleach solution just prior to each use and drying with sterile paper towel.
3. Alternatively, a plastic oven bag can be used. Plastic bags are sterile until opened since they are heated when formed. Before use, the bag can be sterilized by spraying with 10% bleach solution just prior to each use and drying with a sterile paper towel.
4. Wrap the forceps, scalpels, razor blades, and/or cork borer, paper towels and gloves (rubber or surgical) in aluminum foil, seal with tape and sterilize by placing in an oven at 350°F for 15 minutes. You can wrap each item separately or put together a “kit” so that each student will have their own sterile equipment to use.
5. Alternatively, the forceps and blades can be sterilized by dipping in 10% bleach solution and then rinsing in sterile water. If you choose to dip in bleach and rinse in sterile water, it is best if fresh solutions are available for each student group since the water can easily be contaminated if care is not used. These liquid containers should only be opened when they are inside of the sterile chamber.

Plant preparation (Assign to students for their own groups to use.)
You will be sterilizing the plant material to remove any bacteria or fungal spores that are present. The aim is to kill all microorganisms, but at the same time not cause any adverse damage to the plant material.

1. Cut cauliflower into small sections of florets about 1 cm across on a sterile surface within the sterile transfer chamber. If using plant leaves, cut into disks with a sterilized cork borer.
2. Wash the prepared plant material in a detergent-water mixture for about 20 minutes. This will help remove fungi etc., and the detergent will help wet the material and remove air bubbles that may be trapped between tiny hairs on a plant.
3. Transfer the washed plant material to the sterilizing bleach solution. Shake the mixture for 1 minute and then leave to soak for 10–20 minutes. Carefully pour off the bleach solution using the lid to keep the plant tissue from coming out and then carefully cap the container.
**Note 1*: At this point, the tissue is considered sterile. All subsequent rinses should be done with sterile water and all manipulations of the tissue performed with sterile instruments and supplies. Open one container at a time and never leave the lid off of any container longer than necessary.

***Teacher Note:** Many students will not fully appreciate the importance of carefully sterilizing explants and so there will be some cultures that become infected with bacterial or fungal growth. If you do not wish to emphasize this aspect of the laboratory students can be provided with plant materials that the instructor has already sterilized prior to use by the class.

Transfer of plant material to tissue culture medium
Use sterile gloves and equipment for all of these steps.
1. Spray the outside surfaces of the containers, the capped tubes and the aluminum wrapped supplies with 70% alcohol before moving them into the chamber.
2. Place the plant material still in the bleach sterilizing container, the containers of sterile water, the sterilized forceps and blades, some sterile paper towels to use as a cutting surface and enough tubes containing sterile medium into the sterile area.
3. Spray the gloves with a 70% alcohol solution and hands rubbed together to spread the alcohol just prior to placing hands into the chamber. Once gloves are on and sprayed they must not touch anything that is outside of the sterile chamber.
4. Carefully open the container with the plant material and pour in enough sterile water to half fill the container. Replace the lid and gently shake the container to wash tissue pieces (explants) thoroughly for 2–3 minutes to remove the bleach. Then let sit for 15 minutes. Pour off the water.
5. Remove the sterilized and washed plant material from the container, place on the paper towel or sterile petri dish. Cut the cauliflower into smaller pieces about 2 to 3 mm across. Be sure to avoid any tissue that has been damaged by the sterilizing solution, which is apparent by its pale color. If using a disk of plant leaf no additional cutting is necessary.
6. Take a prepared section of plant material in sterile forceps and place into the medium in the culture tube. Cauliflower pieces should be partly submerged in the medium, flower bud facing up. If using another plant leaf, be sure the disk is in contact with the agar.
7. Replace the lid on the culture tube.

Growing the plants
1. Place plant sections in a well-lit area of the classroom although not in direct sunlight. The shoots will grow more quickly if the explants are placed under fluorescent or grow-lights to provide at least 12 hours of light per day. The aquarium can be used as a growth chamber with

the lighting about 8–10" overhead. This will also help maintain a more regular and warm temperature. Ensure that the temperature does not go over 82.4°F. New shoots should develop within 2 weeks and should be well advanced in 3 to 4 weeks. Check the tubes daily and discard any that show signs of infection (before discarding, add bleach into the tube). Roots can appear within 6 weeks on cauliflowers.

2. Working inside the sterile aquarium chamber, remove the lid from the culture dish. There will usually be several shoots that have arisen from each explant. These shoots should be carefully separated by gently removing the whole explant from the media with sterile forceps and then separating the shoots by gently pulling them apart using two pairs of forceps. Each shoot should then be placed into a tube of rooting media and the bottom of the shoot pushed into the media so that good contact is made. The cap is replaced and the shoots are then allowed to grow as in step 1 until roots are formed, usually within 2–3 weeks.

Differentiation

i. **Local community**: Students may do a search to see if there is a plant genetics lab nearby. Searching for "agriculture research and development" may give a list of facilities in your area.

ii. **Students with special needs (language/reading/auditory/visual)**: This lesson includes kinesthetic activities as well as reading and following directions. Students in cooperative groups can rotate tasks and utilize all students' strengths.

iii. **Extra support**: Watch *Tissue Culture Propagation: Class 101*: https://www.youtube.com/watch?v=qDOGrEhUe8A

iv. **Extensions**: Students may want to try additional plant cuttings. Students will need to research what growth media/nutrients are required for different plants to grow.

Rubric for Assessment

Skill	Beginning	Satisfactory	Exemplary
Use a model to illustrate the role of cellular division (mitosis) and differentiation in producing and maintaining complex organisms.	Student used a plant model of his/her choice to show cellular division through plant tissue culture.	Student used a plant model to show cellular division through plant tissue culture. Student can explain how the process of mitosis leads to plant tissue culture.	Student used a plant model to show cellular division through plant tissue culture. Student can explain how the process of mitosis leads to plant tissue culture. Student researched additional nutrients to use with specific plant tissue.
	Student used sterile technique.	Student used sterile technique and the explants began to grow.	Student used sterile technique and helped to prepare additional materials to test.

Rubric for Self-Assessment

Skill	Yes	No
I used sterile technique to sterilize and transfer explants to culture tubes.		
The explants grew into healthy plants.		

Lesson 6C
Plant Tissue Culture

Focus Questions: *How might we create a sterile growing environment? How important is a sterile environment? How is a plant clone produced?*
Vocabulary: *plant tissue, tissue culture, sterile/aseptic technique, explant, nutrient medium*

Background

Plant research often involves growing new plants in a controlled environment. These may be plants that we have genetically altered in some way or multiple copies of cloned plants. This can be accomplished through the tissue culture of small tissue pieces from the plant of interest. These small pieces may come from a single mother plant or they may be the result of a genetic transformation of single plant cells, which are then encouraged to grow and to ultimately develop into a whole plant. Tissue culture techniques are often used for commercial production of plants as well as for plant research.

Tissue culture involves the use of small pieces of plant tissue (explants), which are cultured in a nutrient medium under sterile conditions. Using the appropriate growing conditions for the explant type, plants can be induced to rapidly produce new shoots and new roots. These plantlets can also be divided, usually at the shoot stage, to produce large numbers of new plantlets. The new plants can then be placed in soil and grown in the normal manner.

The most important part of this activity, however, is to maintain as sterile an environment as possible. Even one fungal spore or bacterial cell that comes into contact with the growth media will rapidly reproduce and soon completely overwhelm the small plant.

Materials

Sterile distilled water
10% bleach solution (Mix 100 mL household bleach + 900 mL water)
70% alcohol
Cutting equipment such as a scalpel blade or razor blade (Sterile or dip in 10% bleach then rinse in sterile water)
Forceps or tweezers (Sterile or dip in 10% bleach then rinse in sterile water)
Gloves
Cauliflower florets
Sterile paper towels or petri dishes for cutting
Lidded containers in which to wash the plant material
Detergent-water mixture (Mix 1ml detergent per liter of water)
Sterilizing solution: 1–2% bleach Solution (Mix 5–10 ml household bleach + 500 ml water)
2 or 3 beakers or jars for sterile water
2 spray bottles

Procedure

1. Mix 10% bleach solution, detergent-water mixture and sterilizing solution using amounts given.
2. Add 10% bleach to a spray bottle and add some to a beaker or jar for rinsing
3. Add 70% alcohol to the other spray bottle

Plant preparation

You will be sterilizing the plant material to remove any bacteria or fungal spores that are present. The aim is to kill all microorganisms, but at the same time not cause any adverse damage to the plant material.

1. Cut cauliflower into small sections of florets about 1 cm across on a sterile surface within the sterile transfer chamber. If using plant leaves, cut into disks with a sterilized cork borer.
2. Wash the prepared plant material in the detergent-water mixture for about 20 minutes. This will help remove fungi etc., and the detergent will help wet the material and remove air bubbles that may be trapped between tiny hairs on a plant.
3. Transfer the washed plant material to the sterilizing solution. Shake the mixture for 1 minute and then leave to soak for 10–20 minutes. Carefully pour off the bleach solution using the lid to keep the plant tissue from coming out and then carefully cap the container.

Note: At this point, the tissue is considered sterile. All subsequent rinses should be done with sterile water and all manipulations of the tissue performed with sterile instruments and supplies. Open one container at a time and never leave the lid off of any container longer than necessary.

Transfer of plant material to tissue culture medium

Use sterile gloves and equipment for all of these steps.

1. Spray the outside surfaces of the containers, the capped tubes and the aluminum wrapped supplies with 70% alcohol before moving them into the chamber.
2. Place the plant material still in the bleach sterilizing container, the containers of sterile water, the sterilized forceps and blades, some sterile paper towels to use as a cutting surface and enough tubes containing sterile medium into the sterile area.
3. Spray the gloves with a 70% alcohol solution and rub hands together to spread the alcohol just prior to placing hands into the chamber. Once gloves are on and sprayed they must not touch anything that is outside of the sterile chamber.
4. Carefully open the container with the plant material and pour in enough sterile water to half fill the container. Replace the lid and gently shake the container to wash tissue pieces (explants) thoroughly for 2–3 minutes to remove the bleach. Then let sit for 15 minutes. Pour off the water.
5. Remove the sterilized and washed plant material from the container, place on the paper towel or sterile petri dish. Cut the cauliflower into smaller pieces about 2 to 3 mm across. Be sure to avoid any tissue that has been damaged by the sterilizing solution, which is apparent by its pale color. If using a disk of plant leaf no additional cutting is necessary.
6. Take a prepared section of plant material in sterile forceps and place into the medium in the culture tube. Cauliflower pieces should be partly submerged in the medium, flower bud facing up. If using another plant leaf, be sure the disk is in contact with the agar.

7. Replace the lid on the culture tube.

Growing the plants

1. Place plant sections in a well-lit area of the classroom although not in direct sunlight. The shoots will grow more quickly if the explants are placed under fluorescent or grow-lights to provide at least 12 hours of light per day. The aquarium can be used as a growth chamber with the lighting about 8–10" overhead. This will also help maintain a more regular and warm temperature. Ensure that the temperature does not go over 82.4°F. New shoots should develop within 2 weeks and should be well advanced in 3 to 4 weeks. Check the tubes daily and discard any that show signs of infection (before discarding, add bleach into the tube). Roots can appear within 6 weeks on cauliflowers.

Rubric for Self-Assessment

Skill	Yes	No
It was easy to use sterile technique to sterilize and transfer explants to culture tubes.		
The explants grew into healthy plants.		

Lesson 6D

Transform Bacteria*

Focus Questions: *How can we genetically modify bacteria? What are the results of gene insertion?*

Learning Target: *Students will genetically modify (transform) bacteria by inserting a plasmid and examine the results of the transformation.*

Vocabulary: *selective breeding, plasmid, genetic modification, sterile/aseptic technique*

*This lesson uses Bio-Rad pGLO™ Bacterial Transformation Kit, Catalog #166-0003EDU available from explorer.bio-rad.com

LS1: From Molecules to Organisms: Structures and Processes
LS3: Heredity: Inheritance and Variation of Traits

Performance Expectation	Classroom Connection
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.	Students genetically modify *E. coli* bacteria to glow in the presence of arabinose under a UV light. They make observations about which bacteria grow best in which condition determined by the plasmid DNA that is contained within the bacteria.
HS-LS3-1. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.	Students *ask questions* and explain the results in each plate related to the role of DNA in coding the differences in the treated vs untreated *E. coli*.
EXTENSION: HS-LS3-3. Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.	Students determine the transformation efficiency of their experiment.
EXTENSION: HS-ESS3-4. Evaluate or refine a technological solution that reduces impacts of human activities on natural systems.	Students learn one technique to genetically modify an organism (*E. coli*), which is an example of a technological solution then evaluate whether genetic modification reduces impacts of human activities on natural systems.
Science and Engineering Practices	
Asking Questions and Defining Problems • Ask questions that arise from examining models or a theory to clarify relationships. (HS-LS3-1)	Students analyze their results to determine which bacteria showed change and why.
Analyzing and Interpreting Data • Apply concepts of statistics and probability (including determining function fits to data, slope, intercept, and correlation coefficient for linear fits) to scientific and engineering questions and	Students use their data to calculate transformation efficiency in their bacteria.

problems, using digital tools when feasible. (HS-LS3-3) **Constructing Explanations and Designing Solutions** • Construct an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future. (HS-LS1-1) • Evaluate or refine a solution to a complex real-world problem, based on scientific knowledge, student-generated sources of evidence, prioritized criteria, and trade-off considerations. (HS-ESS3-4)	Students obtain information about the process they used to transform bacteria and find information from other sources to *explain* how the protein the bacteria are now producing results in glow, and to communicate that with support from *evidence*. Students will be able to explain why selective breeding would not have created this bacterial trait. Students *evaluate and refine a solution* to determine if genetic modification results in reduced impacts of human activities on natural systems.
Disciplinary Core Ideas	
LS3.B: Variation of Traits • Environmental factors also affect expression of traits, and hence affect the probability of occurrence of traits in a population. Thus, the variation and distribution of traits observed depends on both genetic and environmental factors. **LS1.A: Structure and Function** • All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins. (secondary) (Note: This Disciplinary Core Idea is also addressed by HS-LS1-1.) **LS3.A: Inheritance of Traits** • Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or	Transformed bacteria will grow on plates that non-transformed bacteria will not. The bacteria that incorporated the plasmid will express genes that control bacteria will not.

structural functions, and some have no as-yet known function.	
Cross Cutting Concepts	
Structure and Function • Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, the structures of different components, and connections of components to reveal its function and/or solve a problem.	Genetic modification is possible as a result of various discoveries about enzymes that can cut DNA at specific points and pastes DNA back together again. These methods use the physical and chemical properties of several structures in a cell in order to accomplish the transformation.
Cause and Effect • Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects.	When actions are applied to a cell, certain outcomes are expected (heat shock will open pore spaces in the bacterial membrane and salt solution in the cell environment will attract the plasmid into the cell using differential charge).
Stability and Change • Feedback (negative or positive) can stabilize or destabilize a system.(HS-ESS3-4)	
Connections to Engineering, Technology, and Applications of Science **Influence of Engineering, Technology, and Science on Society and the Natural World** • Engineers continuously modify these technological systems by applying scientific knowledge and engineering design practices to increase benefits while decreasing costs and risks. (HS-ESS3-4)	New techniques are used to genetically modify organisms including: TALEN, Meganucleases and a genome-editing technique called CRISPR-Cas9.
Scale, Proportion, and Quantity • Algebraic thinking is used to examine scientific data and predict the effect of a change in one variable on another (e.g., linear growth vs. exponential growth).	Extension: Determining the transformation efficiency uses this thinking.
Connections to Nature of Science **Science is a Human Endeavor** • Technological advances have influenced the progress of science and science has influenced advances in technology. • Science and engineering are influenced by society and society is influenced by science and engineering.	Humans will need to determine if the use of these new technologies will be accepted.

Teacher Background

This activity was designed to demonstrate in one lab period how bacteria can be genetically modified. (The previous lessons were designed to be used to prepare students for this lab.) Genetic modification is a powerful tool wherein DNA from one organism is added to the DNA of

a different species. The results of genetic modification in agriculture have resulted in plants that are resistant to diseases, can make their own pesticides and resist herbicides. In this lesson, green fluorescent protein (GFP) is inserted into non-virulent *E. coli* bacteria in the form of a plasmid.

The source of GFP is a jellyfish, *Aequorea victoria*. GFP allows the jellyfish to fluoresce and glow in UV light. The mechanism to transform the bacteria is by the use of a plasmid. The kit comes with a plasmid that contains the gene for GFP, a gene for antibiotic resistance and a gene regulation system that turns the production of the protein on in the presence of arabinose, a monosaccharide. The plasmid is added to the bacteria in solution, then conditions are created to encourage the bacteria to uptake the plasmid. The bacteria is then grown overnight on various media that act as controls, and/or that select for transformed cells.

As always when using bacteria in the lab, **sterile technique** should be used when transferring materials by sterile pipette or sterile loops. The condition for uptake of the plasmid by the bacteria is critical for success in this lab. The process, called heat shock, can be compared to how skin pores close in extreme cold, open in warmth and close again quickly when exposed to cold once more. Bacterial membranes also have pore spaces that will be closed in the cold, opened in the heat and closed again tightly to hold in the plasmid if it crossed the membrane. The transformation solution used with the bacteria also sets up a charge differential across the membrane that attracts the plasmid to cross the membrane. The colonies in suspension will be held on ice, then placed in a water bath at 42°C for 50 seconds, then immediately back on ice.

Teachers are encouraged to read the manual for the kit before using this activity and during preparation for the lab. **This teacher document is intended to supplement the manual.** The lab manual is available here: http://www.bio-rad.com/webroot/web/pdf/lse/literature/1660033.pdf

Prior knowledge

Students are expected to practice sterile technique and clean their lab stations after the experiment. They need to be able to open sterile pipettes without contaminating them and use sterile loops. They need to minimize the time the pipettes and loops are out of the package before using them. It is helpful to practice these actions before lab day. Agar plates can be easily contaminated. Emphasize that the lids should be left on the plates except when transferring liquid to them.

If the previous lessons were used before this lab, it will help students to understand the methods and procedures that are used.

In order to explain the results of this lab, students should know how plasmids are made and what enzymes can be used to do that. They should be able to use the terms genotype and phenotype to describe the changes that result from the insertion of the plasmid.

Teacher preparation

1. Review safety procedures and lab skills

Safety procedures (see pages 5-6 in the lab manual)

The Escherichia coli bacteria HB101 K-12 strain contained in this kit is not a pathogenic organism like the E. coli strain O157 H7 that has sometimes been implicated in food poisoning. HB101 K-12 has been genetically modified to prevent its growth unless grown on an enriched medium. However, handling of the E. coli K-12 strain requires the use of standard Microbiological Practices. These practices include, but are not limited to, the following. Work surfaces are decontaminated once a day and after any spill of viable material. All contaminated liquid or solid wastes are decontaminated before disposal. All persons must wash their hands: (i) after they handle material containing bacteria, and (ii) before exiting the laboratory. All procedures are performed carefully to minimize the creation of aerosols. Mechanical pipeting devices are used, mouth pipetting is prohibited; eating, drinking, smoking, and applying cosmetics are not permitted in the work area; wearing protective eyewear and gloves is strongly recommended.

If an autoclave is not available, all solutions and components (loops and pipets) that have come in contact with bacteria can be placed in a fresh 10% bleach solution for at least 20 min for sterilization. A shallow pan of this solution should be placed at every lab station. No matter what you choose, all used loops and pipets should be collected for sterilization. Sterilize petri dishes by covering the agar with 10% bleach solution. Let the plate stand for 1 hr or more, and then pour excess plate liquid down the drain. Once sterilized, the agar plates can be double bagged and treated as normal trash. Safety glasses are recommended when using bleach solutions.

Ampicillin may cause allergic reactions or irritation to the eyes, respiratory system, and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Ampicillin is a member of the penicillin family of antibiotics. Those with allergies to penicillin or to any other member of the penicillin family of antibiotics should avoid contact with ampicillin.

Lab skills (See pages 6-7 in the lab manual)

- Sterile technique
- Use of the pipette
- Decontamination and disposal
- Incubation

2. Prepare agar plates - At least 5 days before planning to do the lab in class, the plates should be prepared. **See pages 11-17 in the lab manual for this preparation procedure**. Total preparation time for "cooking" the agar and pouring the plates is about 2 hours.

Materials

1000 mL flask
500 mL distilled water
Hot plate with magnetic stir plate
Magnetic stir bar

Long thermometer
1 package of LB agar (included in kit)
1 vial ampicillin (included in kit)
1 vial arabinose (included in kit)
Transformation solution (included in kit)
Sterile pipettes (included in kit)
Sterile petri dishes (included in kit)

Tips for agar preparation:

- Boiling the water and agar is easiest on a hot plate with a magnetic stir bar added, even though the procedure suggested is using a microwave.
- It is important to attend to the flask as once boiling it can easily boil over.
- Simmer the agar and water for 5-10 minutes after boiling to sterilize.
- Once the agar has boiled, remove it from heat as it needs to cool to 55°C. This gives enough time to re-hydrate the ampicillin and arabinose.
- Once you have added the transformation solution to the ampicillin and arabinose vials, begin to label the bottom of the plates with a permanent marker LB, LB/AMP and LB/AMP/ARA. The number needed for 8 lab groups is 8 LB, 16 LB/AMP and 8 LB/AMP/ARA. The manual calls for a starter LB plate for each lab group, but that is not really necessary. Two to four starter plates should give enough colonies, for all groups to use. The teacher or a lab assistant can distribute the colonies for groups.
- Begin to pour plates once agar is cooled adequately (you can safely handle the flask). The agar will go far if you pour just enough in each plate to cover the bottom of the dish. Quickly replace the lid.

*Note: Stretching materials for 10 groups (2 classes) is doable. You will need to provide additional sterile petri dishes for the starter plates if you choose to do this, but you should have enough agar if the plates are all the small size that come with the kit.

- Store the agar plates on the benchtop for a full day, then invert (bottom side up) to reduce the amount of condensation. Plates should be stored in the refrigerator until use.

3. Streak the starter plates - At least 24-36 hours before the lab, re-hydrate the *E. coli* according to the directions on **page 16 of the lab manual**. Streak the starter plates (LB). Incubate the streaked plates at 37°C overnight or on a countertop for 2-3 days.

4. Prepare lab solutions (4 students per station)
Fill one microtube per station with 1mL of transformation solution.
Fill one microtube per station with 1mL of LB broth.
Refrigerate solutions until lab day.

5. Prepare lab stations - Day of the lab prepare each lab station with the following materials:
1 foam microtube holder/float with the following 4 microtubes:
1 empty microtube labeled +
1 empty microtube labeled -

1 microtube with 1mL of transformation solution labeled TS
1 microtube with 1mL of LB broth labeled LB
1 permanent marker
Package of sterile pipettes
Package of sterile loops
Gloves (optional)
4 agar plates:
1 LB
2 LB/Amp
1 LB/Amp/Ara
Cup of crushed ice
For the class:
LB starter plates
Remaining transformation solution and LB broth

The student portion of the lab manual begins on **page 32** with Lesson 1 and contains several pieces that are not included in this unit. However, teachers may use these to assess student understanding. (It is suggested by this author, that you use *Lesson 6A Plasmid Modeling* to help students visualize the process that was used to prepare the plasmid.) Detailed step-by-step instructions for the lab are included if preferred over the quick guide.

Once students complete the procedure, they should complete the table with their predictions about bacterial growth and fluorescence.

Differentiation

i. **Local community**: Students may do a search to see if there is a plant genetics lab nearby. Searching for "agriculture research and development" may give a list of facilities in your area. Few of these facilities will be doing genetic modification, but they may be doing PCR and gel electrophoresis, looking for specific genomes or gene sequences to indicate pathogen resistance or the presence of pathogens in soil to diagnose problems farmers have with various diseases.

ii. **Students with special needs (language/reading/auditory/visual)**: Students in cooperative groups can rotate tasks and utilize all students' strengths.

iii. **Extra support**: Watch *pGLO Transformation Lab* https://www.youtube.com/watch?v=M6Uxrnpy3FM

iv. **Extensions**: Students may complete Lesson 3 on Data Collection and Lesson 4 Determining the transformation efficiency. This efficiency may be influenced by the procedure used during the lab.

Rubric for Assessment

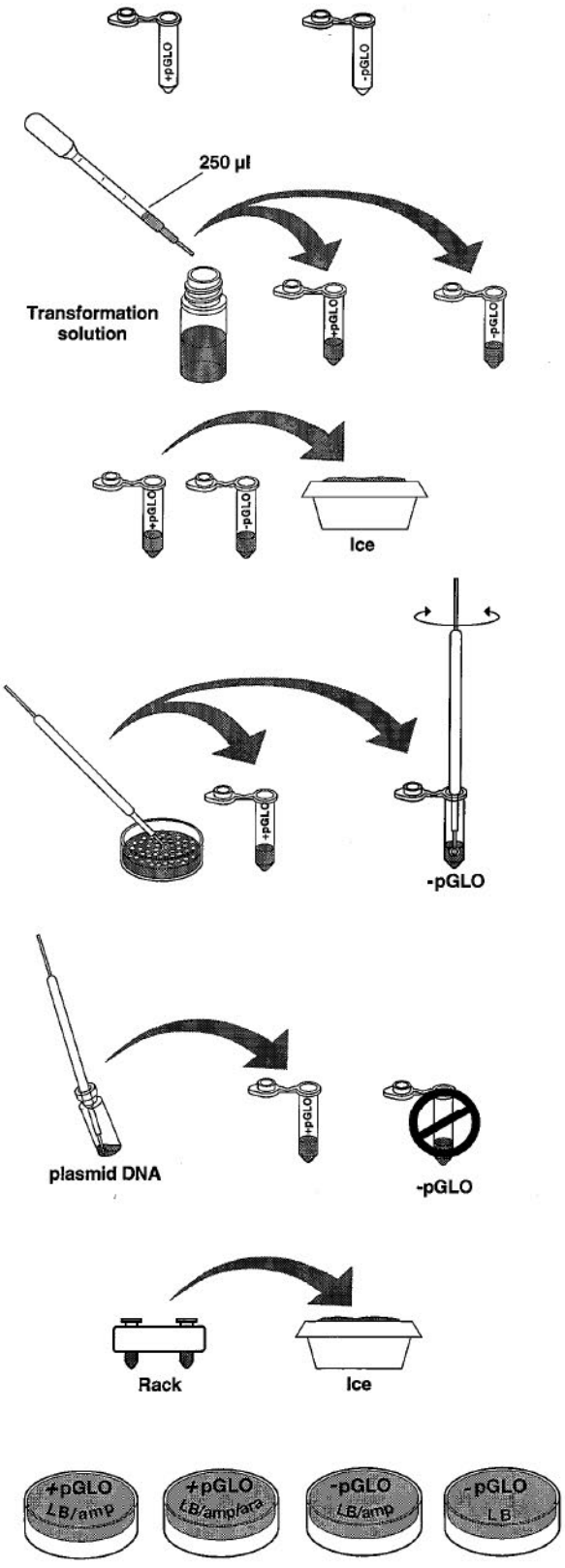
Skills	Developing	Satisfactory	Exemplary
Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.	Students complete the lab procedure but have no glowing colonies or they have contamination on their agar plates.	Students genetically modify *E. coli* bacteria to glow in the presence of arabinose under a UV light. They make observations about which bacteria grow best in which condition determined by the plasmid DNA that is contained within the bacteria.	Students genetically modify *E. coli* bacteria to glow in the presence of arabinose under a UV light. They make observations about which bacteria grow best in which condition determined by the plasmid DNA that is contained within the bacteria and explain their observations.
Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.	Students *ask questions* but are unable to explain the results in each plate related to the role of DNA in coding the differences in the treated vs untreated *E. coli*.	Students *ask questions* and explain the results in each plate related to the role of DNA in coding the differences in the treated vs untreated *E. coli*.	Students *ask questions* and explain the results in each plate related to the role of DNA in coding the differences in the treated vs untreated *E. coli*. Students can suggest a use for the GFP protein in a mobile organism.
EXTENSION: Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.		Students determine the transformation efficiency of their experiment.	
EXTENSION: Evaluate or refine a technological solution that reduces impacts of human activities on natural systems.		Students learn one technique to genetically modify an organism (*E. coli*), which is an example of a technological solution then evaluate whether genetic modification reduces impacts of human activities on natural systems.	

Rubric for Self-Assessment

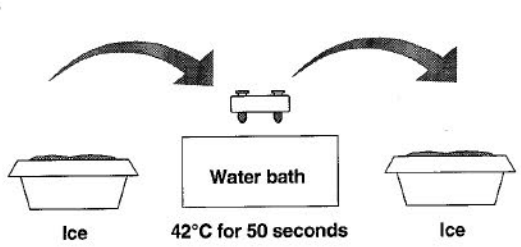
Skill	Yes	No
My group completed the lab and had glowing colonies.		
I can explain the pattern of growth and glow that we observed in our plates.		
I can explain the process used in this lab to transform bacteria.		
Extension: I can calculate the transformation efficiency we obtained during this lab.		

Transform Bacteria

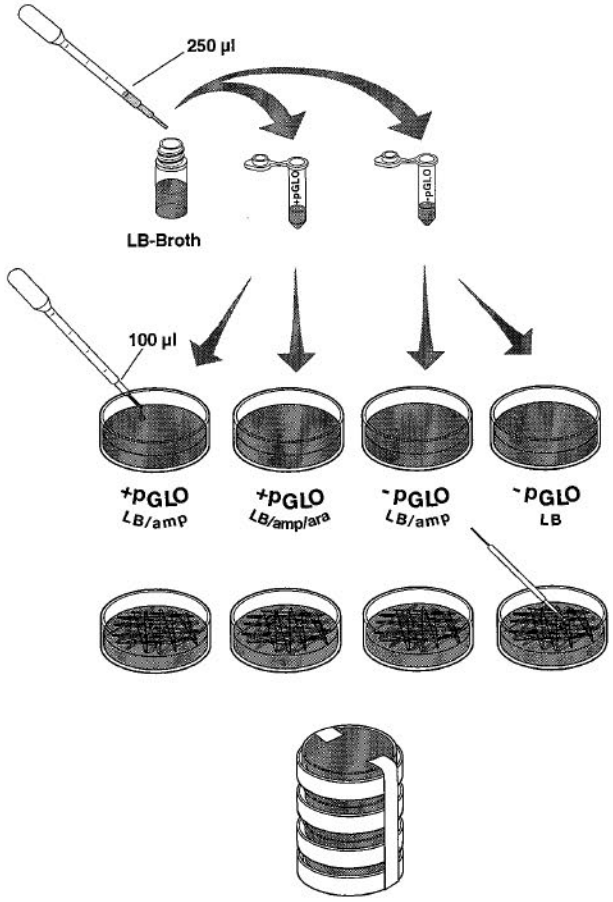
1. Label one closed micro test tube +pGLO and another -pGLO. Label both tubes with your group's name. Place them in the foam tube rack.
2. Open the tubes and using a sterile transfer pipet, transfer 250 µl of transformation solution ($CaC1_2$).
3. Place the tubes on ice.
4. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Place the tube back in the tube rack in the ice. Using a new sterile loop, repeat for the -pGLO tube.
5. Examine the pGLO plasmid DNA solution with the UV lamp. Note your observations. Immerse a new sterile loop into the plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles. Mix the loopful into the cell suspension of the +pGLO tube. Close the tube and return it to the rack on ice. Also close the -pGLO tube. Do not add plasmid DNA to the -pGLO tube. Why not?
6. Incubate the tubes on ice for 10 minutes. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.
7. While the tubes are sitting on ice, label your four agar plates on the bottom (not the lid) as follows: Label one LB/amp plate: +pGLO;
   Label the LB/amp/ara plate: +pGLO;
   Label the other LB/amp plate: -pGLO;
   Label the LB plate: -pGLO.



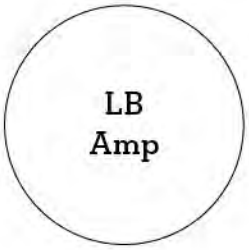
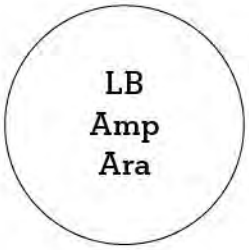
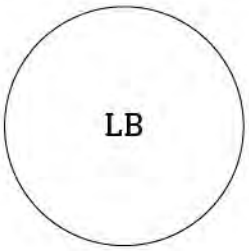
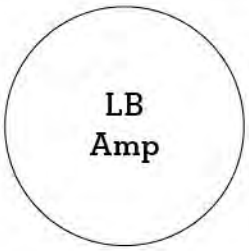
the (+) pGLO and (-) pGLO tubes into the water bath, set at 42 °C, for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. When the 50 seconds are done, place both tubes back on ice. For the best transformation results, the change from the ice (0°C) to 42°C and then back to the ice must be rapid. Incubate tubes on ice for 2 minutes.



9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipet, add 250 µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the other tube. Incubate the tubes for 10 minutes at room temperature.
10. Tap the closed tubes with your finger to mix. Using a new sterile pipet for each tube, pipet 100 µl of the transformation and control suspensions onto the appropriate plates.
11. Use a new sterile loop for each plate. Spread the suspensions evenly around the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface.
12. Stack up your plates and tape them together. Put your group name and class period on the bottom of the stack and place the stack upside down in the 37°C incubator until the next day.



- Research one of the genetic modifications that have been made to dent corn in the past 30 years. Create a presentation with data and photographs that explains how this genetic modification has improved any of the following areas:
  - Yield increase
  - Decrease of nutrient requirements for growth
  - Increase of insect resistance
  - Drought Tolerance
  - Resistance to soil pests (nematodes)
  - Decrease of herbicide use
  - Changes in the nutritional composition (protein, moisture, starch, etc.)

- pGLO	+ pGLO
 Prediction: Reason: Observed result:	 Prediction: Reason: Observed result:
 Prediction: Reason: Observed result:	 Prediction: Reason: Observed result:

Rubric for Self-Assessment

Skill	Yes	No
My group completed the lab and had glowing colonies.		
I can explain the pattern of growth and glow that we observed in our plates.		
I can explain the process used in this lab to transform bacteria.		
Extension: I can calculate the transformation efficiency we obtained during this lab.		

Lesson 7
GFP Purification*

Focus Questions: *How can proteins be purified? What is the mechanism used in biotechnology to extract proteins of interest?*

Learning Target: *Students remove a colony of transformed bacteria that results from the pGLO™ lab and treat it to remove and purify the green fluorescent protein (GFP) produced by the gene.*

Vocabulary: *hydrophobic interaction column chromatography, lyse, supernatant, hydrophobic, hydrophilic*

*This lesson uses Bio-Rad pGLO™ Bacterial Transformation Kit, Catalog #166-0005EDU available from explorer.bio-rad.com

ETS1.C Optimizing the Design Solution

Performance Expectation	Classroom Connections
HS-ETS1-2. Design a solution to a complex real-world problem by breaking it down into smaller, more manageable problems that can be solved through engineering.	Students are given the challenge to remove a protein from bacteria that produced it.
Science and Engineering Practice	
Constructing Explanations and Designing Solutions • Design a solution to a complex real world problem, based on scientific knowledge, student-generated sources of evidence, prioritized criteria and trade-off considerations	The kit comes with specific instructions for how to purify and remove the protein. Students experiment with the buffers to determine the order in which to use each to purify the protein.
Disciplinary Core Idea	
ETS1-C. Optimizing the Design Solution • Criteria might need to be broken down into simpler ones that can be approached systematically, and decisions about the priority of certain criteria over others (trade-offs) may be needed.	Students research to determine the best way to extract and purify the protein.
Cross Cutting Concept	
Structure and Function • Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, the structures of different components, and connections of components to reveal its function and/or solve a problem.	Students will use the known structures of bacteria and the known structures of GFP to determine how to break down the bacteria to remove the protein and purify it through hydrophobic interaction column (HIC) chromatography.

*Student handout information is indicated in light gray print. Answer keys are imbedded in the student handout section.

Student Background (This lesson is a continuation of the BioRad pGLO™ Transformation kit.) Proteins such as insulin, can be created by bacteria in labs, purified, then used as medicine.

> Genes can be cut out of human, animal, or plant DNA and placed inside bacteria. For example, a healthy human gene for the hormone insulin can be put into bacteria. Under the right conditions, these bacteria can make authentic human insulin. When allowed to multiply in gigantic vats (fermenters) these bacteria can be used to mass produce the human insulin protein. This genetically engineered insulin is purified using protein chromatography and used to treat patients with the genetic disease, diabetes, whose insulin genes do not function normally.
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> A common problem in purifying genetically engineered "designer" proteins from transformed bacteria is contamination by endogenous bacterial proteins. Chromatography is a powerful method used in the biotechnology industry for separating and purifying proteins of interest from bacterial proteins. Proteins purified in this manner can then be used, for example, as medicines to treat human disease, or, for household agents such as natural enzymes to make better laundry detergents.
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> The cloning and expression of the GFP gene (pGLO™ Bacterial Transformation kit), followed by the purification of its protein in this kit, is completely analogous to the processes used in the biotechnology industry to produce and purify proteins with commercial value. (http://www.bio-rad.com/webroot/web/pdf/lse/literature/4006099.pdf page 1)

This lab will use one technique, hydrophobic interaction column (HIC) chromatography to separate GFP from the bacteria that produced it. GFP is being used as a model protein to show a process of purification.

Methods to teach protein purification:

Option 1: Students begin by using one colony of transformed bacteria from each condition in the pGLO™ transformation lab. The colonies are added to nutrients to grow, then incubated. After 24-48 hours, the bacteria is centrifuged, resuspended, then an enzyme is added to lyse the bacterial membranes. The samples are frozen to complete the breakdown of the bacteria, then centrifuged again to remove the bacterial debris. The remaining protein samples are then added to the chromatography columns which contain a "bed" of microbeads, these columns are treated with buffers of high salt concentrations that retain the hydrophobic (water-hating) protein (GFP). The final treatment rinses the protein from the column. Students can be given the instructions to follow and get the results. This is the traditional method of using the lab, wherein students follow the instructions.

Option 2: Students are given a challenge to determine how to isolate and purify the GFP protein produced by the bacteria in the previous lab. Students must research to determine how to break down the bacteria then how to purify it using the columns and materials provided. The instructions provided still have the steps for breaking down the bacteria and purifying the

protein, but have the chemical and buffer names removed. If desired, no instructions might be given, except the challenge. The first page of the student handout for Option 2, does not reference the instructions.

Prior knowledge

Students need to have completed the BioRad pGLO™ Lab and have bacteria available from each of the conditions tested in that lab. Students who complete this lab should have advanced lab skills to be able to follow the multiple steps over multiple days of this lab. In addition, the combination of salts used are specifically sequenced to remove the protein and purify it. A good understanding of hydrophobic and hydrophilic properties is helpful.

Timeline (see page 4 of the lab manual)

Lesson 1	Introduction to Purification
Lesson 2	Picking Colonies and Inoculating Cell Cultures
Lesson 3	Purification Phase 1—Bacterial Concentration and Lysis
Lesson 4	Purification Phase 2—Removing Bacterial Debris
Lesson 5	Purification Phase 3—Protein Chromatography

- Each lesson takes a portion of the class period over 5 days.

Materials

Hot plate with magnetic stir option
Magnetic stir bar
250 mL Flask
55 mL distilled water
LB tablet (included in kit)
TE buffer (included in kit)
Arabinose (included in kit)
Ampicillin (included in kit)
UV lights (included in kit)
Sterile loops (included in kit)
Sterile pipettes (included in kit)
Test tube racks
Test tubes
Cups
Rocking table or shaking incubator or Incubator (set at 32°C)
Centrifuge

Teacher Preparation

Lesson 1 - Review of the principles used in the pGLO™ transformation lab. This includes a discussion of growth media, antibiotic selection and gene regulation (**see pages 9-10 of the lab manual**). At this point it is recommended to describe the process or ask students to research the steps that will be used to purify the protein and each can be reviewed as the step is completed. Divide students into groups they were in for the pGLO™ lab.

Lesson 2 - Choosing colonies and inoculating cell cultures (**see pages 6-8 in the lab manual**). Rehydrate the ampicillin and arabinose using TE buffer. Prepare the liquid media by heating distilled water and dissolving the LB tablet in the water.

- Again, the recommendation is to use a hot plate and stir bar, even though the manual suggests using a microwave.
- Allow the media to cool to below 55°C.
- Add 0.5 mL of arabinose and 0.5 mL of ampicillin to the flask of LB media.
- Mix by swirling.
- Aliquot 2 mL of the culture media into the 2 culture tubes per station. (Store culture tubes in refrigerator until ready to use.)

Students inoculate the tubes with a colony from their pGLO™ lab. Provide sterile loops and pipettes. The colonies should be shaken during incubation if possible. If not, students need to vigorously shake the culture tubes for at least 30 seconds, before placing in the incubator set at 32°C on their sides. (BE SURE LIDS ARE SEALED.)

Lesson 3 - Purification Phase 1 (**see page 8 in the lab manual**)

Rehydrate the vial of lyophilized lysozyme with TE buffer. Mix gently to aid in the resuspension. Keep the vial of lysozyme on ice or in a refrigerator until use.

Set up lab stations with a microtube containing 1 mL of TE solution and pipettes for each group. Multiple centrifuges may be needed as students need to use for 5 minutes at max speed.

Students will use 1 drop of lysozyme.

Tubes will be stored in the freezer overnight.

Lesson 4 - Purification Phase 2 (**see page 8 in the lab manual**)

Set up lab stations with pipettes, chromatography columns, equilibration buffer and binding buffer for each group.

Students will prepare chromatography columns as they centrifuge their samples.

Students centrifuge lysed cells for 10 minutes.

Lesson 5 - Purification Phase 3 (**see page 8 in the lab manual**)

Set up lab stations with test tubes, previously prepared chromatography tubes, pipettes, wash buffer and TE buffer.

Students load their lysed cells into the chromatography columns and run the protein through them.

Differentiation

Other ways to connect with students with various needs:

i. **Local community**: Students may visit a local biotech lab or look for a virtual tour of a biotech lab where proteins are extracted and purified.

ii. **Students with special needs (language/reading/auditory/visual)**: The directions for the lab are both visual and written. Students who need additional support may watch *GFP Purification* at https://www.youtube.com/watch?v=H7wv1hpfc_M (skip the Edvotek portion of the video from 1:18-3:47, unless you want them to run this protocol without using the GFP, but only food coloring/dye)

iii. **Extra support**: If students are struggling, they may watch *Protein Purification GFP* at https://www.youtube.com/watch?v=VkdU2RryFOM. The narrator is a fast talker, but the overview is good.
iv. **Extensions**: Students may complete Option 2 with support of the directions or without.

Rubric for Assessment

Skill	Developing	Satisfactory	Exemplary
Design a solution to a complex real-world problem by breaking it down into smaller, more manageable problems that can be solved through engineering.	Student was unable to extract and/or purify GFP.	Student could break the process of GFP purification into smaller steps; extracting GFP from the bacteria, then purifying GFP using HIC chromatography.	Student researched and successfully carried out the procedure of extracting GFP from bacteria based on the physical properties of bacterial membranes.
Prediction	Student incorrectly predicted the outcome of the buffers.	Student correctly predicted the outcome of using each of the buffers in the columns.	Student determined the proper buffers to use in the proper order to purify GFP using the properties of GFP.
			Student successfully carried out the procedure to purify and collect GFP.

Rubric for Self-Assessment

Skill	Yes	No
My group's predictions about the effects of each of the buffers were correct.		
I am able to explain the function/role of each buffer in the process of chromatography.		
We were successful in isolating and purifying GFP from the bacterial cells.		

Lesson 7
GFP Purification* (Option 1)

Focus Questions: *How can proteins be purified? What is the mechanism used in biotechnology to extract proteins of interest?*

Vocabulary: *hydrophobic interaction column chromatography, lyse, supernatant, hydrophobic, hydrophilic*

*This lesson uses Bio-Rad pGLO™ Bacterial Transformation Kit, Catalog #166-0005EDU available from explorer.bio-rad.com

Background (This lesson is a continuation of the BioRad pGLO™ Transformation kit.)

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This lab will use one technique, hydrophobic interaction column (HIC) chromatography to separate GFP from bacteria that produced it. GFP is being used as a model protein to show a process of purification.

Buffers

Use these descriptions.

Equilibration buffer (used in preparation of the column): raises the salt concentration of the column to match the GFP lysate

Binding buffer: raises the salt concentration of GFP to cause a conformational change in GFP (change in the tertiary structure of the protein), exposing the hydrophobic region of GFP

Wash buffer: washes away less hydrophobic, contaminating proteins from the column

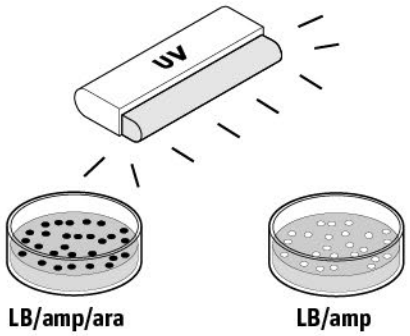
TE (elution) buffer: removes GFP from the column

Rubric for Self-Assessment

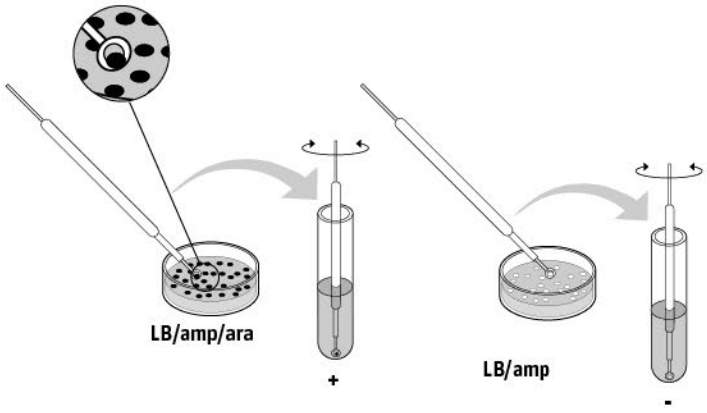
Skill	Yes	No
I am able to explain the function/role of each buffer in the process of chromatography.		
My group's predictions about the effects of each of the buffers were correct.		
We were successful in isolating and purifying GFP from the bacterial cells.		

Inoculation: growing cell cultures

1. Remove the transformation plates from the incubator and examine using the UV light. Identify several green colonies that are not touching other colonies on the LB/amp/ara plate. Identify several white colonies on the LB/amp plate.



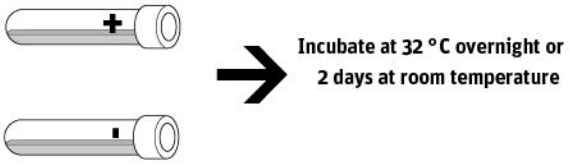
2. Obtain two culture tubes containing the growth media LB/amp/ara. Label one "+" and one "-". Using a sterile loop, lightly touch the loop to a green colony and immerse it in the "+" tube. Using a new sterile loop, repeat for a white colony and immerse it in the "-" tube (it is very important to pick only a single colony). Spin the loop between your index finger and thumb to disperse the entire colony.



3. Cap the tubes and place them in the shaking incubator or on the shaking platform and culture overnight at 32 °C or 2 days at room temperature.

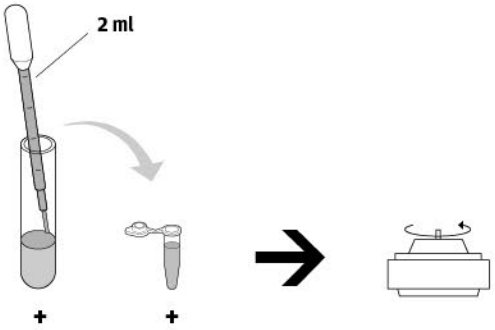
   or

   Cap the tubes and shake vigorously by hand. Place in the incubator horizontally at 32 °C for 24–48 hours. Remove and shake by hand periodically when possible.



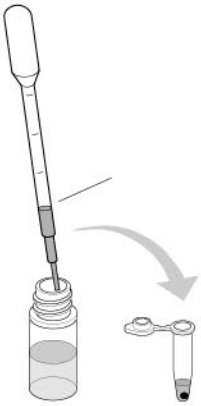
Purification phase 1: bacterial concentration

1. Label one microtube "+" with your name and class period. Remove your liquid cultures from the shaker and observe with the UV light. Note any color differences between the two cultures. Using a new pipette, transfer 2 ml of "+" liquid culture into the "+" microtube. Spin the microtube for 5 minutes in the centrifuge at maximum speed. The pipette used in this step can be repeatedly rinsed in a beaker of water and used for all following steps of this laboratory period.

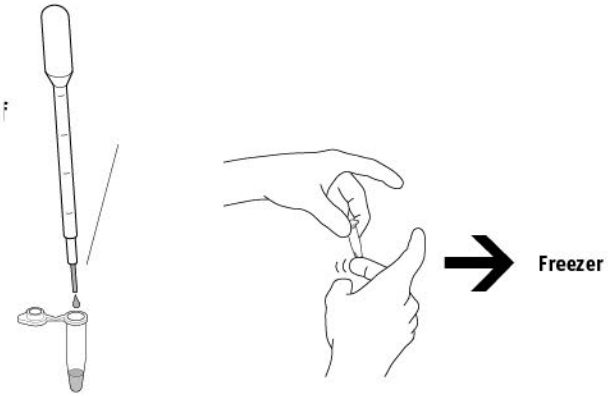


2. Pour out the supernatant and observe the pellet under UV light.

3. Using a rinsed pipette, add 250 μl of _____solution to the tube. Resuspend the pellet thoroughly by rapidly pipetting up and down several times.



4. Using a rinsed pipette, add 1 drop of _____ to the resuspended bacterial pellet to initiate enzymatic digestion of the bacterial cell wall. Mix the contents gently by flicking the tube. Observe the tube under the UV light.

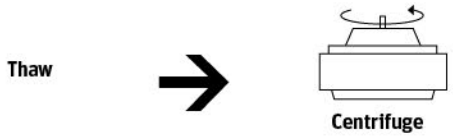


5. Place the microtube in the freezer until the next laboratory period. The freezing causes the bacteria to rupture completely.

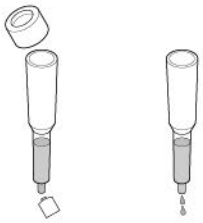
Content designed by EducationProjects.org on behalf of the farmer boards at Ohio Corn & Wheat and the Nebraska Corn Board.

Purification phase 2: bacterial lysis

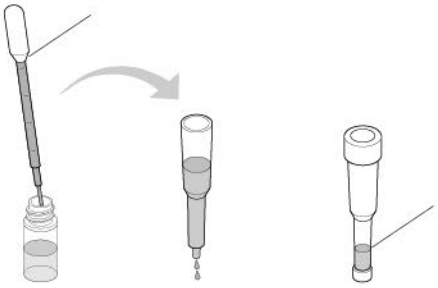
1. Remove the microtube from the freezer and thaw using hand warmth. Place the tube in the centrifuge and pellet the insoluble bacterial debris by spinning for 10 minutes at maximum speed.



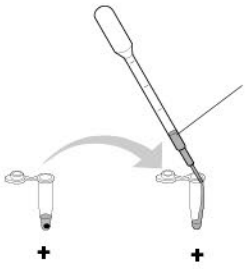
2. While your tube is spinning, prepare the chromatography column. Remove the cap and snap off the bottom from the prefilled HIC column. Allow all of the liquid buffer to drain from the column (~3–5 minutes).



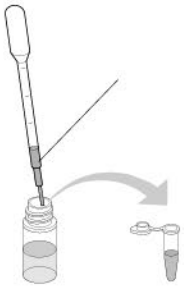
3. Prepare the column by adding 2 ml of _____ _____ to the top of the column. This is done by adding two 1 ml aliquots with a rinsed pipette. Drain the buffer to the 1 ml mark on the column. Cap the top and bottom and store the column at room temperature until the next laboratory period.



4. After the 10 minute spin, immediately remove your tube from the centrifuge. Examine the tube with the UV light. Using a new pipette, transfer 250 µl of the "+" supernatant into a new microtube labeled "+". Again, rinse the pipette well for the rest of the steps of this lab period.



5. Using a well rinsed pipette, transfer 250 µl of _____ _____ to the "+" supernatant. Place the tube in the refrigerator until the next laboratory period.



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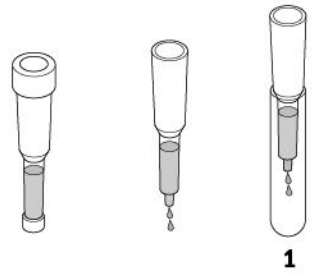
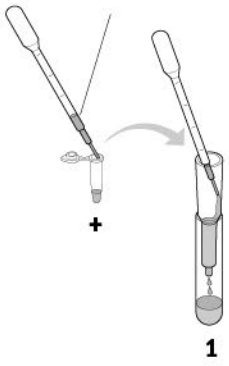
Wash buffer: washes away less hydrophobic, contaminating proteins from the column

TE (elution) buffer: removes GFP from the column

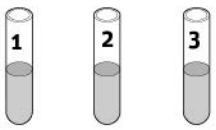
Collection tube number	Prediction	Observations under UV light (column and collection tube)
Tube 1 Sample in binding buffer		
Tube 2 Sample with wash buffer		
Tube 3 Sample with elution buffer		





Purification phase 3: protein chromatography

1. Label 3 collection tubes 1–3 and place the tubes in the foam rack or in a rack supplied in your laboratory. Remove the caps from the top and bottom of the column and place the column in collection tube 1. When the last of the buffer has reached the surface of the HIC matrix proceed to the next step below.
2. Using a new pipette, carefully and gently load 250 µl of the "+" supernatant onto the top of the column. Hold the pipette tip against the side of the column wall, just above the upper surface of the matrix and let the supernatant drip down the side of the column wall. Examine the column using a UV light. Note your observations. After it stops dripping transfer the column to collection tube 2.
3. Using the rinsed pipette, add 250 µl of _____ _____ and let the entire volume flow into the column. Examine the column using the UV light. Note your observations. After the column stops dripping, transfer it to tube 3.
4. Using the rinsed pipette, add 750 µl of _____ _____ and let the entire volume flow into the column. Examine the column using the UV light. Note your observations.
5. Examine all three collection tubes and note any differences in color between the tubes. Parafilm or Saran Wrap the tubes and place in the refrigerator until the next laboratory period.



Lesson 7
GFP Purification* (Option 2)

Focus Questions: *How can proteins be purified? What is the mechanism used in biotechnology to extract proteins of interest?*

Vocabulary: *hydrophobic interaction column chromatography, lyse, supernatant, hydrophobic, hydrophilic*

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This lab will use one technique, hydrophobic interaction column (HIC) chromatography to separate GFP from bacteria that produced it. GFP is being used as a model protein to show a process of purification.

Your challenge: Remove the GFP protein from the bacteria that made it.

1) The materials you will use include the following:
    a) Centrifuge, freezer, lysozyme, various buffers (see list below)
2) Determine how to break down the bacteria to release the GFP protein, then use the column to help you purify it. Take notes on your process of thinking and completing the extraction and purification.
3) Research Hydrophobic Interaction Column Chromatography (see http://www.bio-rad.com/en-us/applications-technologies/introduction-hydrophobic-interaction-chromatography-hic?ID=MWHB53MNI)

Buffer Descriptions

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TE (elution) buffer: removes GFP from the column

Rubric for Self-Assessment

Skill	Yes	No
I was able to break down the process of extracting GFP from bacteria and purifying the protein into smaller steps to accomplish the goal.		
My group worked together to determine the process for extracting and purifying the GFP.		
I am able to explain the function/role of each buffer in the process of chromatography.		

Lesson 8
GMO Speed Dating

Focus Question: *What do you know about GMOs?*

Learning Target: *Students try to find a match to their card and learn about the various genetic modifications that have occurred.*

Vocabulary: *donor, recipient*

*This resource has been adapted from an original activity ©OCR: http://www.ocr.org.uk/qualifications/as-a-levelgce-biology-a-h020-h420-from-2015/delivery-guide/Images/123-241420-le-dating-game-learner-activity-v1.doc

LS3: Heredity: Inheritance and Variation of Traits

Performance Expectation	Classroom Connection
HS-LS3-2. Make and defend a claim based on evidence that inheritable genetic variations may result from: … mutations caused by environmental factors.	Students are given a card with either a donor or recipient organism. Students read about the organism, then try to find a match for themselves.
Science and Engineering Practice	
Engaging in Argument from Evidence • Make and defend a claim based on evidence about the natural world that reflects scientific knowledge and student-generated evidence.	Students *make a claim and defend it based on evidence* provided on the cards or based on their own research.
Disciplinary Core Idea	
LS3.B: Variation of Traits • Environmental factors also affect expression of traits, and hence affect the probability of occurrences of traits in a population. Thus the variation and distribution of traits observed depends on both genetic and environmental factors.	In this case, the environmental factors are genetic modifications made by humans.
Cross Cutting Concept	
Cause and Effect • Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects.	Donors and recipients match up, then students discuss the benefits and risks of genetic modification.

Background

Genetic modification is a confounding topic to many people. In reality, humans have been genetically modifying plants and animals for thousands of years. Selective breeding and selective mutagenesis have resulted in many of the different crops and breeds of animals we

take for granted today. Teosinte is the most closely related plant to what we call corn or maize today; it is a tall grass that has wide branching lateral leaves and an ear with only 5–12 kernels tightly sealed in a hard casing. This plant still grows in southern Mexico.

There are many methods of genetic modification and more are being utilized every day. They include: selective breeding and hybridization, stacked traits, mutagenesis, transgenesis (transfer of a gene from one to another of a different species), TALEN, gene silencing and CRISPR. The most common method of genetic modification up to now, has been transgenesis. Donor genes have given recipient organisms new traits to add beneficial traits, or protection from disease, pests, drought, or flood. This activity is a review of some of those transgenic matches.

This activity came from the United Kingdom and has been adapted for this use. Some of the pairings are for human medical treatment, while some are for animals and crops.

Several of these pairings have been discussed, but not completed (i.e.strawberries with flounder genes to resist frost have not been released due to consumer perception that the strawberries may taste “fishy”; tomatoes with PG were the first GMO available to consumers, but are no longer available; carrots are not GMO in the U.S.; although bananas and potatoes have been suggested as a method for developing countries to grow and deliver vaccines, that has not become a reality yet.) On the other hand, fast-growing salmon (AquaBounty) were just approved in 2018 to be grown at indoor facilities, golden rice was finally approved for use in Bangladesh in 2019.

Materials
Donor cards
Recipient cards

Prior Knowledge
Students need to know vocabulary related to the donors and recipients (see list below):

protease	EPSP synthase
lactoferrin	CP4 EPSPS
cystic fibrosis	Polygalacturonase (PG)
Leber’s congenital amaurosis	zucchini yellow mosaic (poty)virus
papaya ringspot virus	cucumber mosaic cucumovirus
watermelon mosaic (poty)virus	anti-thrombin
chymosin	blight
glucosidase	antigen
Glyphosate	AFGP gene

Students need to be creative. All donors do have matches, however, some may be matched with more than one. Students should read the information on the backs of their cards, and be willing to make a match that they think makes sense. Whether correct or not, the match students make should be justified by evidence or benefit of the potential match, even if it has not been done in reality.

Teacher Preparation

Teachers need to copy the cards on cardstock front to back and cut them apart. Each student receives one card, either a donor or recipient card. If there are extra cards for your classroom, you can remove them so that all students have a match. The answer key is below. There is no student handout for this activity.

Procedure

1. Each participant gets one card. The donor card holders sit around the room. The recipient card holders rotate around the room spending 30-60 seconds with each donor card holder.
2. After 30-60 seconds, the recipient card holders move to a new donor card holder. Repeat until each recipient has found their match (and they step out of the rotation) or all recipients have spoken with all donors.
3. Remaining unmatched participants choose a match. Each matched pair stands and shares their match with the large group.
4. Have students discuss the benefits of the match and describe any risks.

Differentiation

Other ways to connect with students with various needs:

i. **Local community**: Students may ask a local farmer which, if any, genetically modified crops she/he uses and why. If a local farmer is not available, contact the county extension service, the state corn or soybean check-off program or the state department of agriculture for more information about the local use of GM crops.

ii. **Students with special needs (language/reading/auditory/visual)**: The cards for the activity have supportive photos on one side and the details on the other. Students could pair up with one card to determine the most likely match with two other students.

iii. **Extra support**: If students are struggling, they may research one of the modifications to learn more about it.

iv. **Extensions**: Students may research the story of one or more of the failed attempts at genetic modification and determine how they might have been more successful (i.e. Flavr Savr Tomato, freeze protected strawberries with flounder gene, etc)

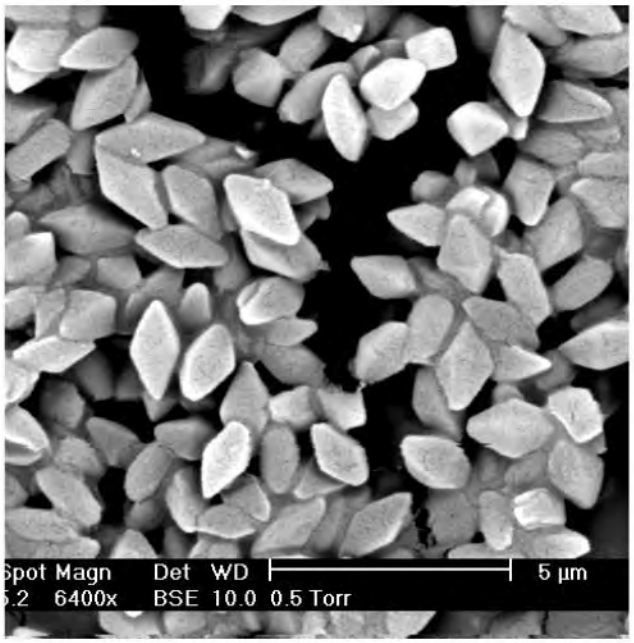
Rubric for Assessment

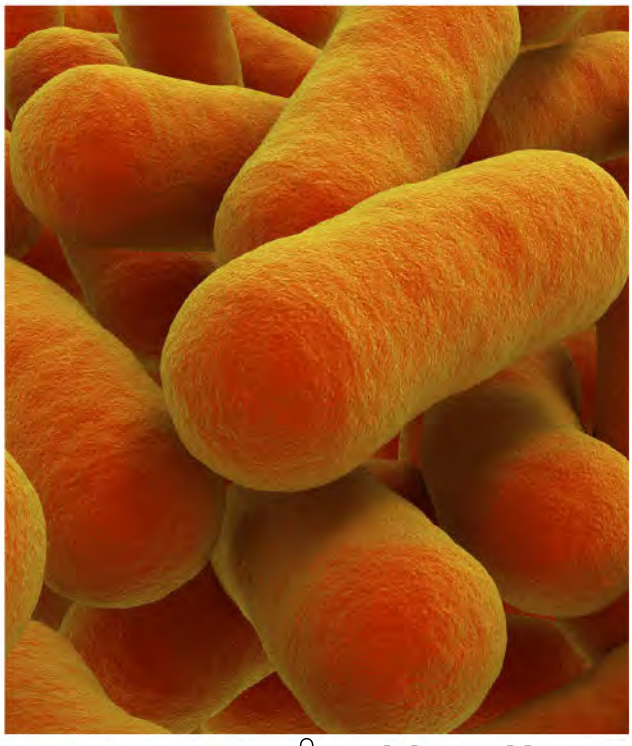
Skill	Developing	Satisfactory	Exemplary
Make and defend a claim based on evidence that inheritable genetic variations may result from: … mutations caused by environmental factors.	Student found a match but the claim that the match was possible did not contain evidence.	Student found a match and the claim that the match was possible contained evidence to support the match.	Student found a match and the claim that the match was possible contained evidence to support the match and the evidence was discussed in terms of benefits and risks.

Rubric for Self-Assessment

Skill	Yes	No
I found a match and made a claim about the possibility of the match.		
I provided evidence to back up the claim.		
I was able to discuss the benefits and risks of the match.		

Bacillus thuringensis	Maize	insect resistant crop
Agrobacterium sp. C4		herbicide resistant crop
Bacillus subtilis		drought resistant crop
Bacillus thuringensis	Cotton	insect resistant crop
Agrobacterium sp. C4		herbicide resistant crop
Bacillus thuringensis	Soybean	insect resistant crop
Agrobacterium sp. C4		herbicide resistant crop
Giant leaf frog	Potato	disease resistant crop
Hepatitis B virus		vaccine production
Human	Carrot	pharmaceutical product for Gaucher's disease patients
Human	Rice	lactoferrin-containing rice treats children with diarrhoea
Erwinia uredovora Maize		Golden Rice 2 with β-carotene to prevent vitamin A deficiency
Golden orb weaver spider	Goat	strong silk fibres for medical and military uses
Human		pharming of anti-thrombin III
Human	Sheep	pharming of factor IX for haemophilia B sufferers
Human	Mouse	mouse cancer models
Jellyfish		NeonMice
Human	Human	gene therapy for recessive genetic disorders like cystic fibrosis and Leber's congenital amaurosis
Jellyfish	Zebrafish	Glo-Fish™
Cow	*Escherichia coli*	GM rennet (chymosin) for cheese-making
Human		insulin for diabetics
Scorpion	Cotton	insect-resistant crop
Papaya ringspot virus	Papaya	resistance to PRSV
Ocean pout	Salmon	antifreezing compounds
	Strawberry	
Antisense Polygalacturonase (PG) enzyme from tomato	Tomato	stops formation of enzyme that breaks down pectin; keeps fresh longer
Zucchini yellow mosaic virus (ZYMV) and *watermelon mosaic virus (WMV2)*	Squash	resistance to SMV
Delta-12 oleate desaturase enzyme silencing from soybean	Soybean	stops conversion of oleic acid into linoleic acid; health benefit





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Bos primigenius
Cattle

Key gene
Cym

Properties of gene product
Chymosin is a protease enzyme that curdles milk

GM use
GM bacteria produce the enzyme which is purified and used to make cheese. Previously chymosin was extracted from the stomachs of calves so cheese made in this way was not acceptable to vegetarians. 80–90% of the cheese sold in Britain is made with GM bovine chymosin.

Phyllomedusa bicolor
Giant Leaf Frog

Key gene
DRS B1

Properties of gene product
B1dermaseptin protein kills bacteria and fungi.

GM use
To prevent blight and bacterial diseases in potato crops.

DONOR

Name
Agrobacterium sp
C4 strain

Key gene
C4 EPSPS

Properties of gene product
EPSP synthase performs a crucial metabolic step in plant chloroplasts. The bacterial version is undamaged by glyphosate.

GM use
To make crops resistant to glyphosate so it can be used as a weed killer without harming the maize, cotton or soya bean crops.

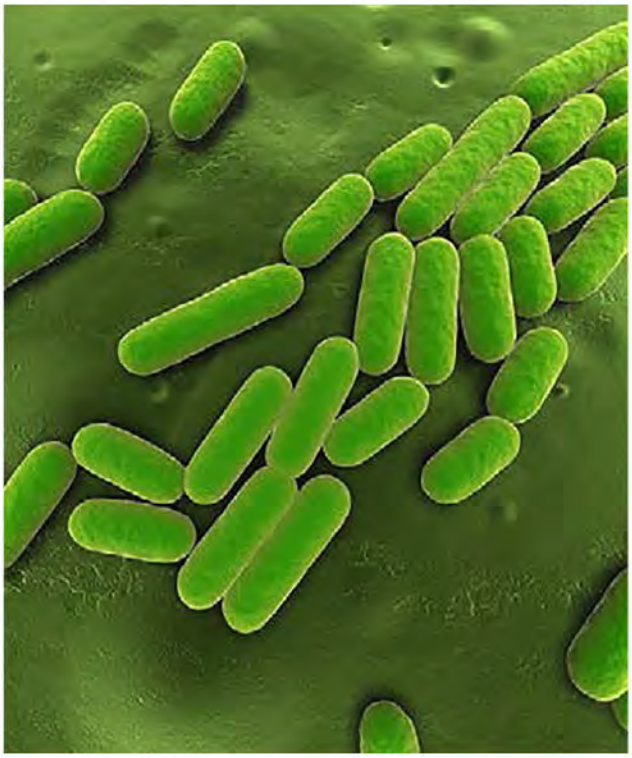
DONOR

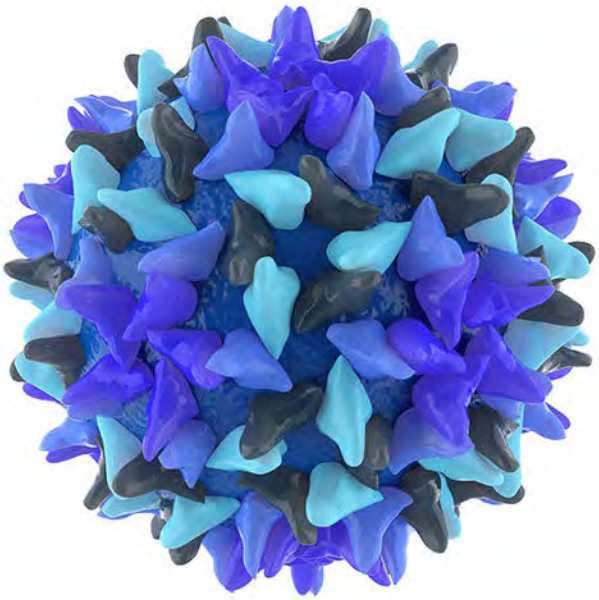
Name
Bacillus thuringensis

Key gene
Cry

Properties of gene product
Crystal protein kills caterpillars, maggots and beetles that eat the protein.

GM use
To make crops such as maize, cotton and soya bean resistant to herbivorous insects.





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Nephila clavipes
Golden Orb Weaver

Key gene
MaSp

Properties of gene product
High-strength silk fibre for webs.

GM use
Gene is switched on in mammary glands of GM goats to mass-produce the silk fibre for artificial tendons and ligaments and for bullet-proof vests and parachutes.

Bacillus subtilis

Key gene
cspB

Properties of gene products
Cold shock protein B helps organisms metabolise normally during abiotic stress.

GM use
To produce higher yields for maize crops and produce a higher yield under drought conditions.

DONOR

Name
Aequorea Victoria
Jellyfish

Key genes
GFP

Properties of gene products
Green Fluorescent Protein glows under UV light.

GM use
The gene is extensively used as a marker to reveal which organisms have taken up a foreign gene and in which tissues the gene is switched on. Spin-offs include Glo-Fish™ and NeonMice sold as pets in the USA.

DONOR

Name
Hepatitis B virus

Key gene
HBsAg

Properties of gene product
Surface antigen of virus stimulates an immune response in humans if injected or given orally

GM use
GM potatoes eaten raw in small quantities boost immunity to hepatitis B. This is an inexpensive and efficient way to deliver vaccines in developing countries.

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Homo sapiens
Human

Key genes
Normal alleles coding for insulin, lactoferrin, Factor IX, anti-thrombin III and glucosidase.

Properties of gene products
Insulin controls blood glucose concentration.
Lactoferrin is an antimicrobial found in colostrum and milk.
Factor IX helps blood clot.
Anti-thrombin III stops blood clotting.
Glucosidase in lysosome function.

GM use
Pharmaceutical drugs
Insulin from GM bacteria treats diabetics.
Lactoferrin in GM rice treats diarrhoea in children. Factor IX from GM sheep's milk treats people with haemophilia B.
Anti-thrombin III from GM goats' milk is used as an anti-coagulant in surgical procedures.
Glucosidase from GM carrot cells in culture treats people with Gaucher's disease.

DONOR

Name
Androctonus australis hector
Scorpion

Key genes
AaHIT1

Properties of gene products
Toxic to insects but not harmful to mammals.

GM use
To kill insects on GM cotton crops.

Homo sapiens
Human

Key genes
Mutated version of *BRCA1* and activated *Ras* oncogene

Properties of gene products
Cause cancer. The products of the normal versions of the genes repair DNA mutations and suppress tumours.

GM use
Creating cancer research models
GM mice engineered to carry the mutant alleles are used to study cancer and treatments for cancer.

DONOR

Name
Homo sapiens
Human

Key genes
CFTR
RPE65

Properties of gene products
CFTR protein allows normal mucus production in lungs and gut.
RPE65 protein is needed in rods and cones for normal vision.

GM use
Gene therapy
Normal CFTR allele is introduced into lung epithelial cells of cystic fibrosis patients.
RPE65 inserted into retinal cells of blind patients with Leber's Congenital Amaurosis restored sight.

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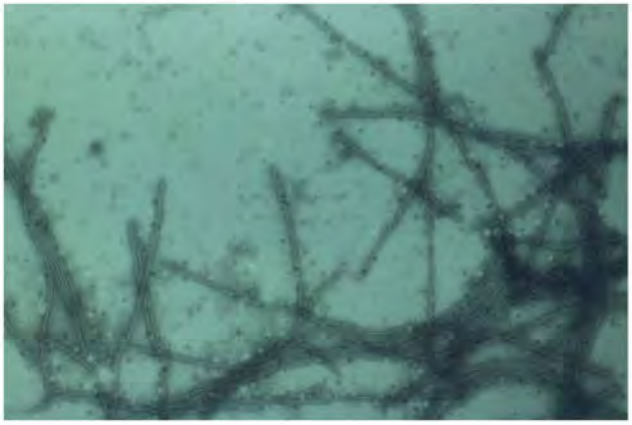

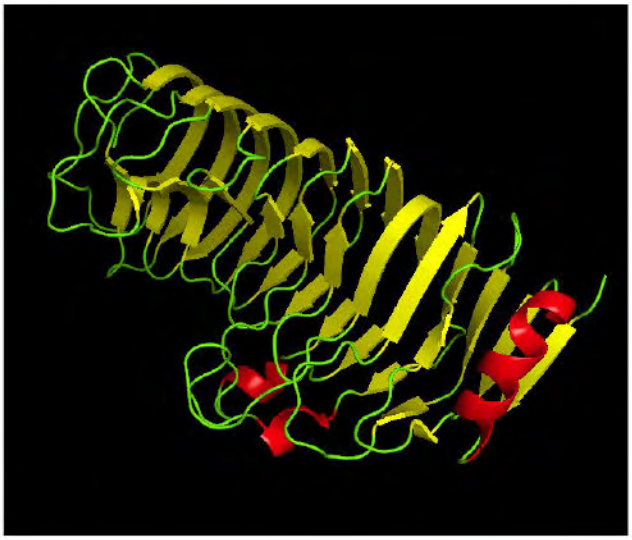

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Zoarces americanus
Ocean Pout

Key genes
Antifreeze glycoproteins or AFGP gene

Properties of gene products
Permit survival in subzero environments

GM use
The promoter for the antifreeze protein gene is used in conjunction with the growth hormone taken from a Chinook salmon, which leads to a higher concentration of the growth hormone in the blood, causing the genetically modified salmon to grow much more rapidly than it would naturally.

Coat protein (CP) of Papaya Ringspot Virus (PRSV)

Key genes
PRSV HA 5-1

Properties of gene products
Provide resistance to PRSV

GM use
Confer resistance to PRSV

DONOR

Name
Agrobacterium tumefaciens

Key genes
CaMV 35S

Properties of gene products
Code for the coat protein (CP) encoding sequences from zucchini yellow mosaic virus (ZYMV) and the watermelon mosaic virus (WMV2).

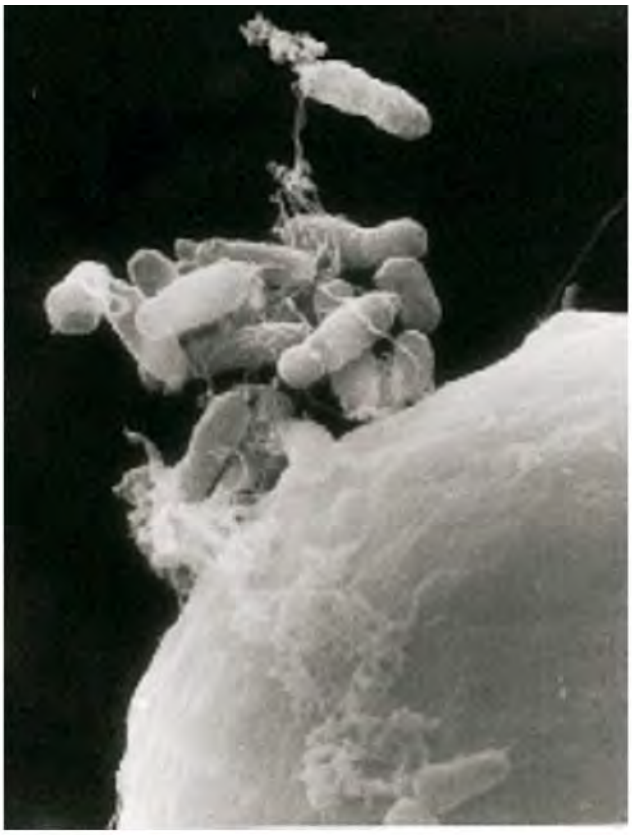
GM use
Provides protection against these viruses.

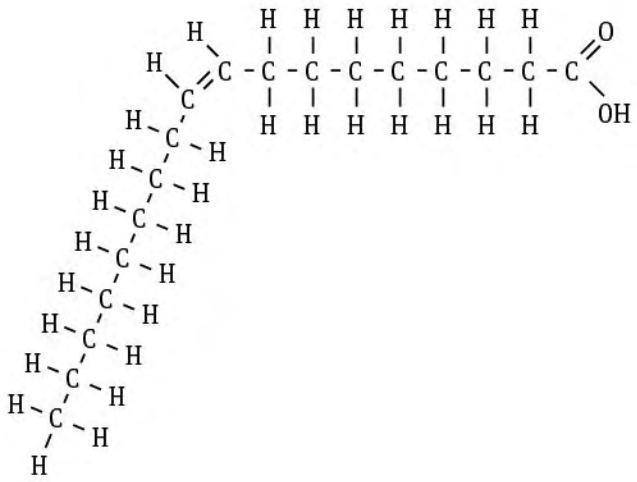
DONOR

Name
Polygalacturonase (PG)

Properties of gene products
Antisense DNA Keeps Polygalacturonase (PG), the major cell wall degrading enzyme of tomato fruit, from forming.

GM use
By inhibiting the development of PG, the fruit should stay fresher longer.





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Delta-12 oleate desaturase

Key genes
gm-fad2-1; FAD2-1

Properties of gene products
An antisense RNA strand is created to silence the formation of the enzyme that converts oleic acid into linoleic acid using the omega-6 desaturase encoding gene.

GM use
Inhibits conversion of oleic acid to linoleic acid, keeps oleic acid levels high for healthier oil.

Agrobacterium tumefaciens

Key genes
CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) encoding gene.

GM use
Inhibits action of glyphosate, the key ingredient in RoundUp (Monsanto).

RECIPIENT

Name
Gossypium hirsutum
Cotton

Suitability as a GM recipient
Important crop for textile fibres but many insect pests attack it and the crop must be kept free of weeds.

RECIPIENT

Name
Zea mays
Maize or Sweetcorn

Suitability as a GM recipient
Major food source for animals and humans and as a source of starch and sugars for processed food. Many insects attack the crop, its yield falls in drought conditions and the crop must be kept free of weeds.

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Solanum tuberosum
Potato

Suitability as a GM recipient
Major carbohydrate food source in Europe and America. Potatoes are easy to grow and can give high yields but suffer from many diseases such as blight, which lower yields. They can be engineered to make vaccines but these must be grown under cover to prevent gene flow to other potatoes and to stop antigenic potatoes accidentally entering the human food chain.

Glycine max
Soybean

Suitability as a GM recipient
Major food source for animals and for humans as a source of protein in processed food. Many insects attack the crop and the crop must be kept free of weeds.

RECIPIENT

Name
Oryza sativa
Rice

Suitability as a GM recipient
Major food source in Asia and a suitable vehicle for therapies like treating children with diarrhea (rice enhanced with human lactoferrin) and preventing vitamin A deficiency (genes from maize or daffodil and a soil bacterium).

RECIPIENT

Name
Daucus carota
Carrot

Suitability as a GM recipient
Field-grown crops generally have been found to be unsafe to use as vehicles for production of pharmaceutical drugs, but carrot cells grown in culture in bioreactors are a new 'expression platform' for human proteins that can be used as medical drugs.

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Ovis aries
Sheep

Suitability as a GM recipient
Female sheep produce plenty of milk.
A gene for a pharmaceutical protein is linked to a promoter to switch the gene on in the mammary glands, so that the protein appears in the milk. Sheep have been used to make factor IX to treat sufferers of haemophilia B.

Capra aegagrus hircus
Goat

Suitability as a GM recipient
Female goats produce plenty of milk.
A gene is linked to a promoter to switch the gene on in the mammary glands, so that the protein product appears in the milk. So-called 'spider-goats' produce silk in their milk for medical and military applications. Other GM goats produce a drug, human anti-thrombin III, used as an anticoagulant in surgery.

RECIPIENT

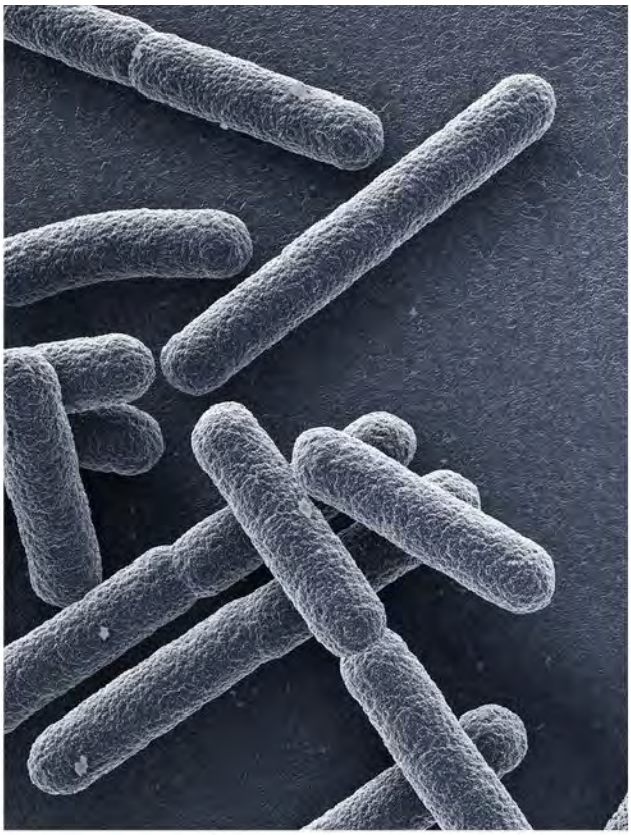
Name
Homo sapiens
Human

Suitability as a GM recipient
People suffering from genetic diseases caused by two recessive non-functional alleles can be treated with gene therapy. The dominant functional allele is inserted into affected somatic cells. Trials have included treatment of cystic fibrosis and Leber's congenital amaurosis. The limitation on treating a human with another human allele is whether the cells that need the foreign DNA are accessible (e.g. lung epithelium) and stable (not replaced every few days).

RECIPIENT

Name
Mus musculus
Mouse

Suitability as a GM recipient
It is a genetic model organism with a well-known, fully-sequenced genome. As a mammal its genome is very similar to that of humans. Mice are small so are cheap to feed and house. Many GM techniques applicable to humans or farm mammals are first tried on mice. Fluorescent GM NeonMice are sold as pets in the USA.



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Escherichia coli

Suitability as a GM recipient
GM bacteria divide rapidly in a fermenter to produce proteins like human insulin and bovine chymosin for cheese-making.
E. coli is a genetic model organism with a well- known, fully-sequenced genome. Its plasmids are widely used as vectors. However, some strains of *E. coli* are pathogenic and the GM process may involve inserting antibiotic resistance genes into the bacteria.

Rerio danio
Zebrafish

Suitability as a GM recipient
It is a genetic model organism with a well-known, fully-sequenced genome. It is a useful, simple vertebrate for research.
GM zecubrafish expressing genes for fluorescent proteins are on sale in the pet trade in the USA marketed as Glo-Fish™.

RECIPIENT

Name
Salmo salar
Salmon

Suitability as a GM recipient
Wild salmon disappeared from many rivers during the twentieth century due to overfishing and habitat change.

RECIPIENT

Name
Carica papaya
Papaya

Suitability as a GM recipient
The papaya is cultivated in most tropical countries. However, it is susceptible to the Papaya Ringspot Virus (PRSV). Since 1992, PRSV has destroyed nearly all non-GMO papaya in Hawaii.

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Solanum lycopersicum
Tomato

Suitability as a GM recipient
Tomatoes are picked as green fruits and artificially ripened by ethylene treatment, which gives a ripe tomato color but not the full vine-ripened tomato flavor.

Fragaria × ananassa
Strawberry

Suitability as a GM recipient
Strawberries grow in temperate climate regions which are capable of having low temperatures and frost.
Spring frosts cause damage to the flowers of the plant leading to poor yields and erratic fruiting. Frost on average causes millions of dollars in damages and drives up the price of the fruit for the consumer.

RECIPIENT

Name
Glycine max
Soybean

Suitability as a GM recipient
Soybean oil is hydrogenated as a preservative to extend shelf life. High oleic oil does not need to be hydrogenated.

RECIPIENT

Name
Cucurbita pepo
Summer squash

Suitability as a GM recipient
Viral diseases are a limiting factor to squash production, particularly during summer and fall months. Mosaic viruses include the cucumber mosaic cucumovirus (CMV), zucchini yellow mosaic potyvirus (ZYMV) and watermelon mosaic potyvirus (WMV2).

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Glycine max
Soybean

Suitability as a GM recipient
Soybeans chief rivals in the field are weeds. If the plant can resist herbicide spraying, the control of weeds is much easier.

Bacillus thuringensis	Maize	insect resistant crop
Agrobacterium sp. C4		herbicide resistant crop
Bacillus subtilis		drought resistant crop
Bacillus thuringensis	Cotton	insect resistant crop
Agrobacterium sp. C4		herbicide resistant crop
Bacillus thuringensis	Soybean	insect resistant crop
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Giant leaf frog	Potato	disease resistant crop
Hepatitis B virus		vaccine production
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