BIOTECHNOLOGY (HS)

Moving genes

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 HS-LS1-1	**Classroom connection:** Students sequence the steps of creating a genetically modified organism, using cards showing the steps.

Science and engineering practices

Constructing Explanations and Designing Solutions	**Classroom connection:** Students will use the cards to construct an explanation for how organisms are genetically modified.

Disciplinary core ideas

LS1.A: Structure and Function	**Classroom connection:** Students will see the tools utilized in genetic modification and explain how they are used in sequence.

Cross-cutting concepts

Structure and Function	**Classroom connection:** Various tools are used and students can explain how each tool is important to the process of genetic modification.

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

Teacher preparation

Copy *Steps in Bacterial Transformation cards* onto card stock, laminate, and cut.

Procedure

1. Pass out to students so that the cards are in random order.
2. Ask students to place the cards into an order that "makes sense." Students may work individually, in pairs, or in small groups.
3. Ask questions as you circulate around the room.
4. 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.
5. Review the correct order with them.
6. 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.

Differentiation

Other ways to connect with students with various needs:

- **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, and 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: **msutoday.msu.edu/feature/2018/gmos-101/**
- **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.)
- **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
  hhmi.org/biointeractive/crispr-cas-9-mechanism-applications

Assessments

Rubric for assessment

Skill	Developing	Satisfactory	Exemplary
Structure and Function: Construct an explanation that uses structure and function as evidence.	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	Unsure
My group was able to properly sequence the steps in moving genes.			
I can explain the steps in moving genes.			

Steps in bacterial transformation

Student instructions

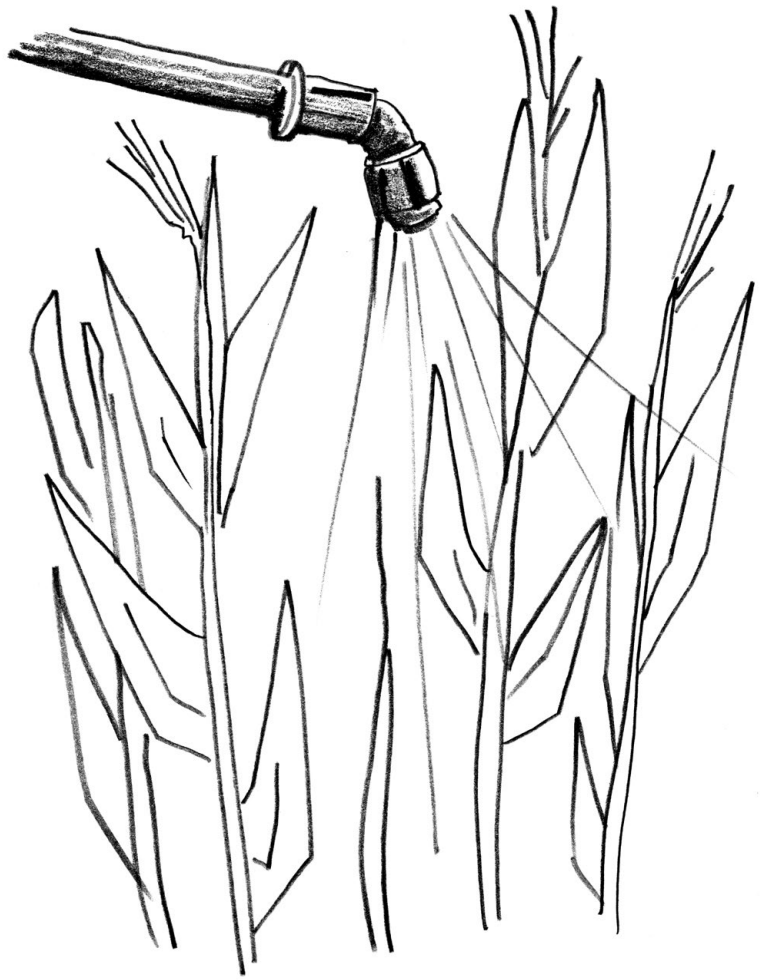
These cards contain the basic steps in using plasmid vectors to transform bacteria. Sort them into the correct order.
Be sure to learn and understand the meanings of any words that are unfamiliar to you.

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.

B

Identify a phenotype of interest



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

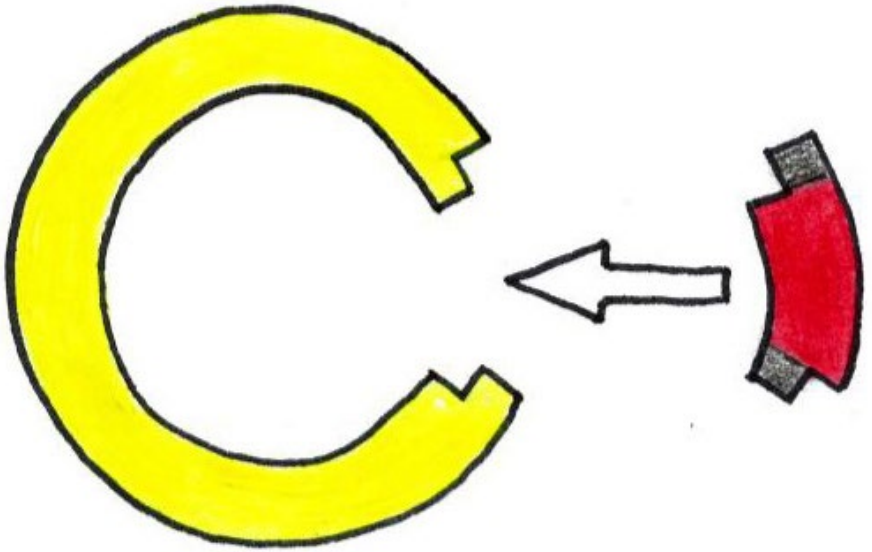
Identify a gene that codes for the desired phenotype

D

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

Gene of interest is engineered into a plasmid vector

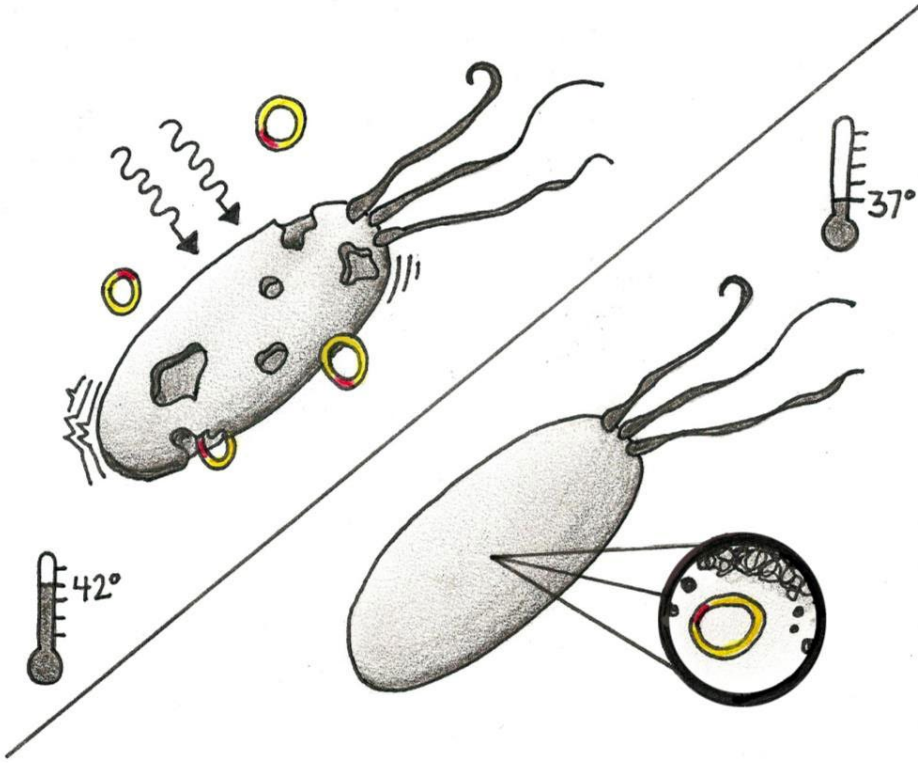
C



- 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

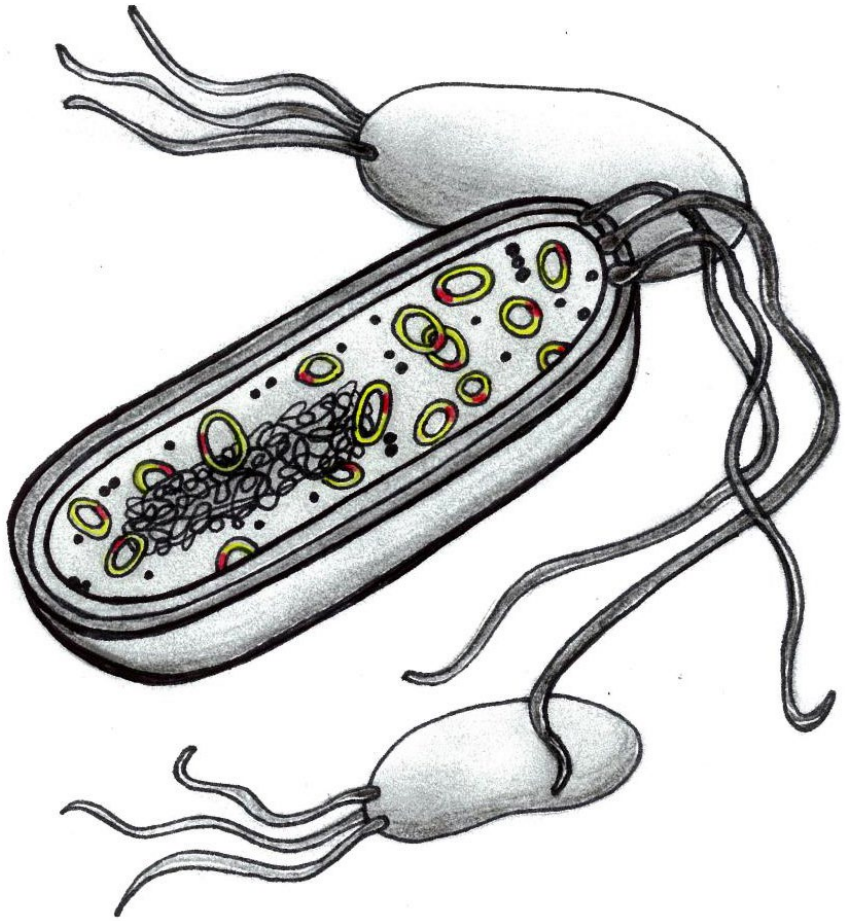
E



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.