HudsonAlpha Institute for Biotechnology

Middle School GPS — navigating genetics content in your classroom

Activity: Dairy Identical Cheese

Overview

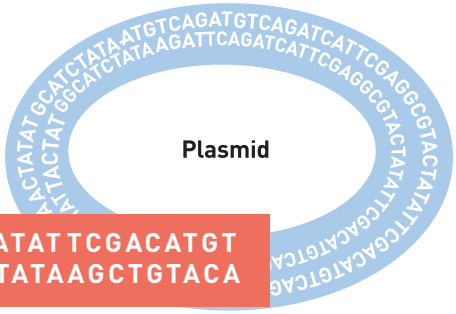
In this activity, students will take on the role of a molecular biologist/genetic engineer to create a genetically modified plasmid involved in the production of cow casein protein. Students will create paper models of the plasmid and the casein gene (CSN2). Students will simulate the activity of restriction enzymes, using scissors to cut DNA at specific sites. Following the restriction enzyme cuts, tape will be used to connect the sticky ends of the plasmid to the gene. This simulates ligation and the creation of a genetically modified plasmid that could be used to produce casein. The casein could then be used to produce dairy identical products.

Learning Targets

- I can define genetic engineering.
- I can identify and describe the functions of the tools that are required in the process of genetic engineering.
- I can explain how genetic engineering is used in the process of producing dairy-identical cheese.
- I can create a model demonstrating the basic principles of genetic engineering.

Materials *(per group of 2 students)*

1 Plasmid sequence (copy on any color of paper other than white)
1 bovine (cow) casein gene sequence (copy on white paper)
Scissors
Tape



GTCAGATCATTCGAGGCTACTATATTCGACATGT
CAGTCTAGTAAGCTCCGATGATATAAGCTGTACA

1 bovine (cow) casein gene sequence

Timeframe

This activity can be completed in 45-60 minutes.

Instructor Background

What is biotechnology?

In its purest form, "biotechnology" refers to using living organisms or their products to alter human health and the environment. Biotechnology has been around since prehistoric times to grow better crops and breed animals. As humans learned that fruit juices could be fermented to make wine, or that milk could be made into cheese or yogurt, biotechnology was born. When bakers made soft, spongy bread instead of a hard, thin cracker, or when beer was made by fermenting malt and hops, biotechnology was in use. Today, biotechnology often involves the manipulation of DNA to produce those new or enhanced products.

What is genetic engineering?

The field of biotechnology expanded once scientists learned to directly manipulate DNA, rather than using traditional breeding or cross-fertilization approaches. Today, scientists are able to isolate, manipulate and reintroduce DNA into cells or model organisms with the intent of introducing new characteristics.

Genetic engineering is most often defined as the process of modifying or altering the genetic material of an organism through a method that does not occur in nature. The organisms that result from this process are often referred to as Genetically Modified Organisms (GMOs). There is a new field in genetic engineering referred to as synthetic biology, which is the use of genetic engineering to design and create biological systems that carry out specified functions.

Genetic engineering is often focused on introducing a new or altered characteristic to the modified organism. These organisms may be used in agriculture (genetically modified crops such as Round Up Ready® Cotton), in drug development (producing human insulin - Humulin®) or in the quest for bio-fuels or pollution indicators.

What tools are used in this process?

Genetic engineers utilize a set of basic molecular tools, many of which were isolated from living cells. Commonly used biotechnology tools include:

- **Plasmid** — This is a small, circular, extra-chromosomal piece of bacterial DNA. It is used as the vector, or molecule that contains the DNA with the new characteristic to be introduced.
- **Restriction enzymes** — Proteins that are often thought of as molecular scissors that cut DNA nucleotide sequences at specific sites.
- **Ligase** — An enzyme that acts as molecular glue to stick the backbone of DNA together.

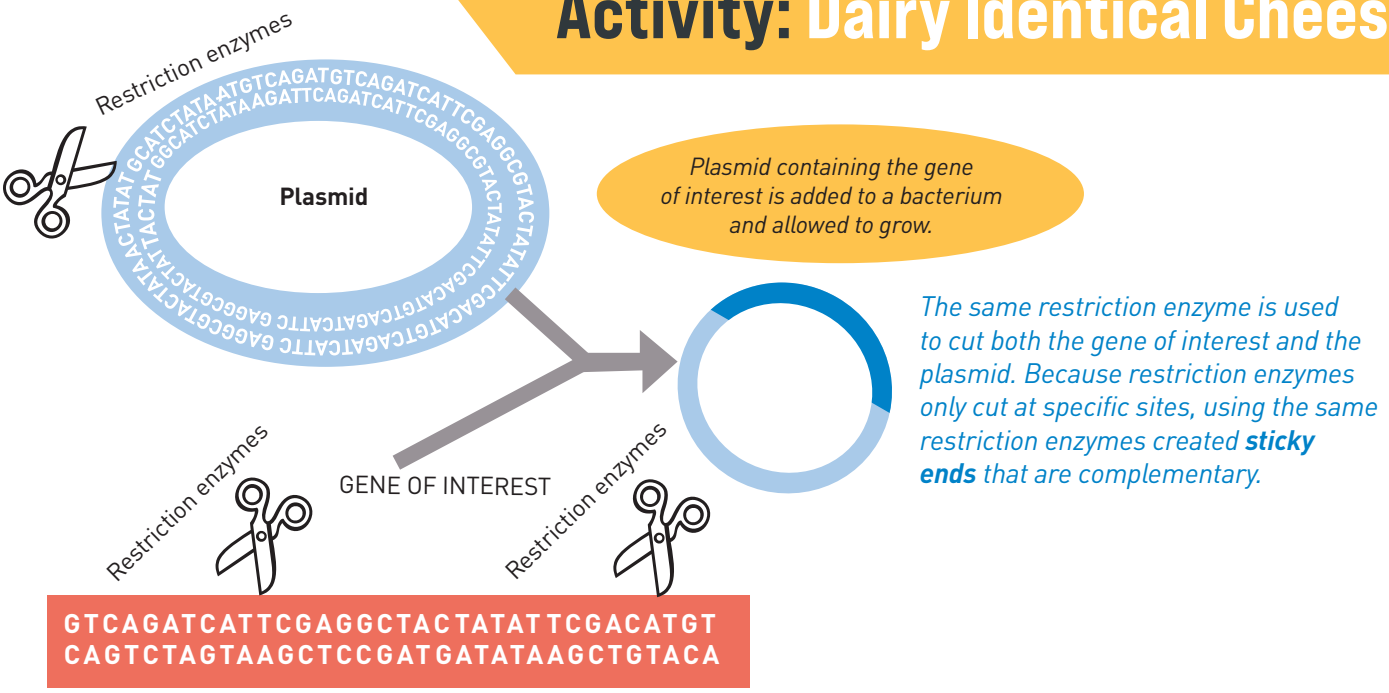
How are these tools used to make genetically engineered organisms?

Let's look at how bacteria or yeast have been engineered to produce casein, the protein found in cows that allows for the production of dairy products. The gene for casein has been identified and located within the cow genome. In order to isolate this gene, scientists use a specific kind of tool called a restriction enzyme. Restriction enzymes cut the DNA on either side of the casein gene, leaving the gene free from most of the surrounding DNA. This creates what scientists refer to as sticky ends flanking both sides of the gene. Scientists then cut the plasmid with the same restriction enzyme used to cut the gene. The restriction enzyme cuts the circular plasmid into a linear DNA fragment with sticky ends on both sides.

See animations found at the following link: https://www.hudsonalpha.org/plasmid-engineering/

The DNA fragment containing the gene is then ligated (fused) into the plasmid (See image on next page). The plasmid carries the gene of interest into the host cells. In the case of casein, the host may be yeast or bacteria. The process of introducing foreign genetic material into another cell is known as transformation. Once the gene is introduced into the bacteria or yeast, the process of fermentation is initiated by feeding the host organisms plant sugars, such as glucose and galactose. The addition of sugars promotes enhanced growth of the yeast or bacteria. As they grow and divide, they will transcribe and translate the genetic code for casein, producing the casein protein. Casein proteins are discharged into the growth solution and are harvested by isolating them from the cells, sugars and other components of the growth solution. Once purified, the protein can be used to produce dairy-identical products.

Activity: Dairy Identical Cheese



*The same restriction enzyme is used to cut both the gene of interest and the plasmid. Because restriction enzymes only cut at specific sites, using the same restriction enzymes created **sticky ends** that are complementary.*

Instructor Protocol

Preparation

1. Copy the casein gene sequence for CSN2 on white paper. Each pair of students will need a copy of the partial gene sequence.
2. Copy the Plasmid sequence onto paper that is any color except white.
3. Download the animations showing restriction enzyme digestion and ligation to be used to illustrate these processes to students. The animations are housed on the HudsonAlpha Educator Resource Hub: https://www.hudsonalpha.org/plasmid-engineering/

Procedure

1. **Direct students to read the Student Background information.**
   Students are introduced to the need for the production of dairy-identical products. They will explore how genetically modified bacteria or yeast may be used to produce dairy-identical products in the absence of livestock to meet the emerging dietary needs within society. Additional information is provided: What is Biotechnology? What is genetic engineering? What tools are used in genetic engineering and How are these tools used?
2. **Facilitate a discussion with students about applying biotechnology to existing problems.**
   Remind students that biotechnology has been in use for thousands of years as humans have attempted to improve crops and select animals with desirable traits. The process has been used to produce a host of products including wine, cheese, yogurt and bread.
3. **Direct students to cut out the Gene Sequence strips (white paper) and tape them together in numerical order. This will make one long strip that is approximately 120 nucleotides long.**
   This sequence represents the bovine casein gene. It is only a portion of the gene that is more than 1200 nucleotides long. This activity uses a shorter section of the casein gene that will represent the entire gene. This gene contains the directions for building casein, one of the proteins found in cow milk. This protein will be needed for the production of dairy identical cheese.

4. **Direct students to cut out the Plasmid Sequence strips (printed on paper that is not white) and tape them in numerical order. Tape the ends of the plasmid strips to form a circle.**
Plasmids are used to carry genetic information. They are circular, extra-chromosomal DNA found in bacteria. The plasmid will be taped in a circle, while the gene strips will remain linear.

5. **Describe restriction enzymes and explain their function as a tool in the field of biotechnology.**
In addition to plasmids, scientists turn to bacteria for another tool, an enzyme. Specifically, this is a restriction enzyme that recognizes and cuts a unique sequence of DNA. These enzymes are used to cut DNA in a specific way that allows them to fit together like pieces of a puzzle.

6. **Show the HudsonAlpha video clips, "Plasmid Uncut" and "Plasmid Cut." These illustrate how two different restriction enzymes respond to a potential cut site. Use this link to access the video clips:**
https://www.hudsonalpha.org/plasmid-engineering/
Highlight the formation of 'sticky ends' in the cut video clip. These are short single-stranded regions of the DNA. Ask students to describe the relationship between the sticky ends. Students should recognize that the ends are complementary to each other and have the potential to re-bond to seal the cut in the plasmid.

7. **Direct students to observe the Plasmid Sequence strip. Ask them to find the dashed line between the base pairs of the sequence GAATTC. Cut along the dashed line. This will form the sticky ends seen in the "Plasmid Cut" video clip.**
The dashed line indicates where a specific enzyme, called ECORI will cut. Watch carefully as students cut along the dotted line to verify that they do not create a blunt cut. Showing the "Plasmid cut" clip again may reinforce the shape of the cut.

8. **Direct students to observe the Gene Sequence strip. Focus on the shaded region. This represents a portion of the gene that encodes for the casein protein.**
The casein gene is actually over 1200 nucleotides long. The sequence is a small portion of the gene.

9. **Direct students to locate their Gene Sequence strip (printed on white paper) and notice the two areas that are marked with dashed lines between the base pairs of the sequence GAATTC. One of these is located before the gene region and the other is after the gene region.**

10. **Cut along the dashed lines forming two sticky ends on the gene sequence.**
Ask students why some sequence outside the shaded area is included before and after the gene region. Responses should include that in order for the gene to function properly, the entire gene sequence must be included. The additional sequence is an area that a particular restriction enzyme can recognize and cut while keeping the entire gene sequence intact.

11. **Carefully observe the sticky ends of the gene and the sticky ends of the plasmid.**
Students should notice that the segments of DNA are complementary on the sticky ends. An "A" on one sticky end of the plasmid will pair with a "T" on the sticky end of the gene. The goal for the scientist is to incorporate the cow casein gene into the bacterial plasmid. These complementary sticky ends allow the two pieces of DNA from different sources to be put together.

12. **Show the "Ligation" clip. The video can be found on the HudsonAlpha Educator Resource Hub website:** https://www.hudsonalpha.org/plasmid-engineering/
Emphasize the complementary base pairing during ligation. Tell students that multiple enzymes will be involved in a ligation reaction. The animation includes a second enzyme whose action is largely off-screen. Reiterate to students that the ligase enzyme would not be able to connect DNA strands if the base pairs were not complementary.

13. **Match the sticky ends from the gene to the sticky ends of the plasmid and tape them together. This creates one large circular paper plasmid.**
This plasmid contains the cow casein gene. Other components present in the plasmid will allow it to be copied and ultimately for the inserted gene to be expressed.

14. **Facilitate a discussion regarding the casein protein that will be produced to be used in the production of dairy identical cheese.**
This process of inserting a gene of interest into a plasmid allows researchers to produce casein protein that is essential for producing dairy products that typically rely on production from a cow. Once the gene has been inserted into a bacterium or yeast, they are fed glucose or galactose which stimulates growth. As the modified organisms produce casein, they secrete the protein into the growth solution. The harvested proteins are isolated from other parts of the growth solution and additional components are added to produce dairy-free products.

15. **Facilitate a summary discussion to check for understanding. During this discussion, focus on the learning targets:**
- **I can define genetic engineering.**
- **I can identify and describe the functions of the tools that are required in the process of genetic engineering.**
- **I can explain how genetic engineering is used in the process of producing dairy-identical cheese.**
- **I can create a model demonstrating the basic principles of genetic engineering.**

******Note: The tools necessary for genetic engineering include: plasmids, restriction enzymes and ligase as opposed to some more common tools, such as pipettes or thermal cyclers used for PCR.***

Consider the following questions in formatively assessing student understanding:

What is genetic engineering?

What might genetic engineering be used for?

What are the tools that are needed for genetic engineering?

What is the function of each of these tools?

Use the paper model to describe how dairy-identical cheese is produced.

Student Protocol

Student Background

Activity: Dairy Identical Cheese

Introduction

For many years, humans have used selective breeding to create organisms that have desired characteristics. One of the drawbacks of this process is that it does not allow traits to be transferred from one species to another. Research over the course of the past 100 years or so has revealed a process for completing a transfer between different species. The scientific inquiries exposed the link between traits and the genetic sequence that codes for them. Recombinant DNA is the name given to a tool that resulted from this work. It allows scientists to add genetic information from one species to another.

In recent years, some people have been shifting their diets. This has created a need for more plant-based products along with dairy-identical products. In order to produce dairy-identical products, scientists found the genes that make casein and whey. These proteins are made in cows and are crucial for the production of dairy products. Knowledge of the code for the production of these proteins allowed scientists to produce dairy products, without cows. In this activity, you will explore genetic engineering. You will learn how this valuable process has been used to produce dairy-identical products.

What is biotechnology?

Biotechnology refers to using living organisms or their products to alter human health and the environment. It has been around since prehistoric times when it was used to grow better crops and breed animals. Humans learned that fruit juices could be fermented to make wine and that milk could be made into cheese or yogurt. Today, biotechnology often involves changing DNA to produce new or enhanced products.

What is genetic engineering?

The field of biotechnology grew once scientists learned to directly alter DNA. Today, scientists are able to isolate, change and insert DNA into cells or model organisms. This allows them to introduce new characteristics.

Genetic engineering is most often defined as the process of modifying the genetic material of an organism through a method that does not occur in nature. The organisms that result from this process are often referred to as Genetically Modified Organisms (GMOs). Synthetic biology is a new field that uses this technology to design and create biological systems that carry out distinct functions.

This technology is often focused on introducing a new or altered aspect to the modified organism. It may be used in agriculture to make genetically modified crops such as Round Up Ready® Cotton. In addition, it may be used to develop drugs, such as producing human insulin or Humulin® or in the quest for bio-fuels or pollution indicators.

What tools are used in this process?

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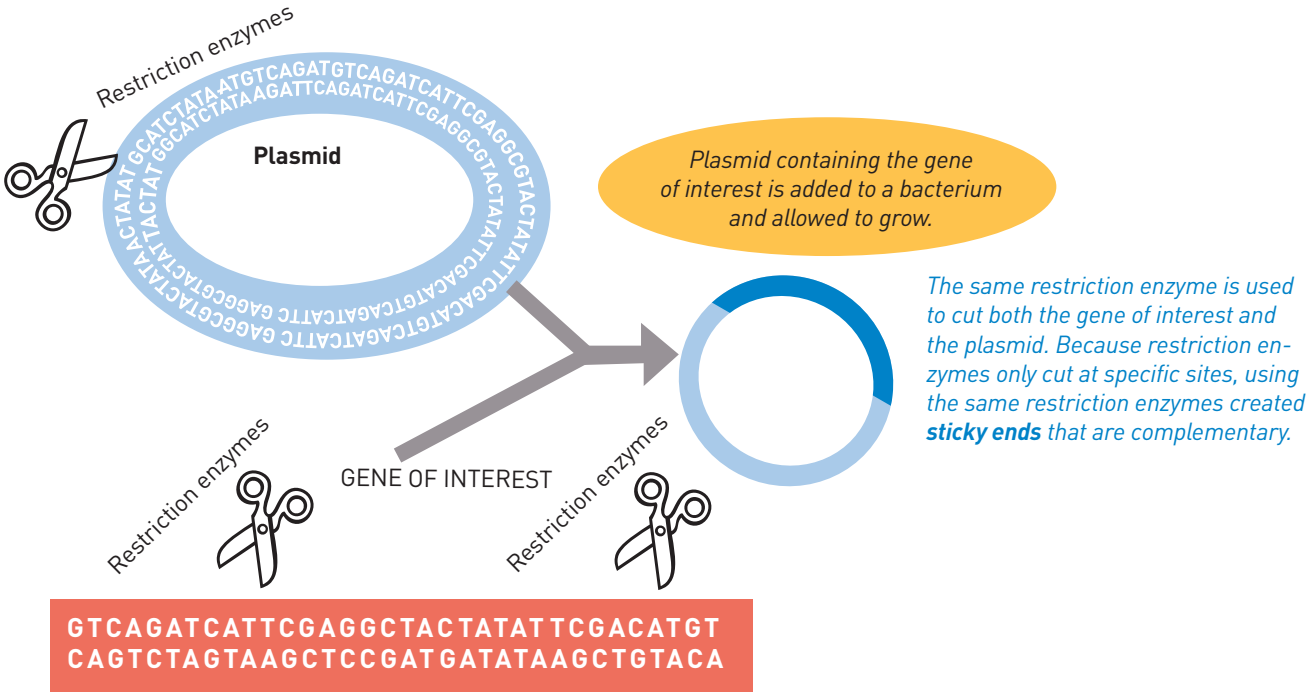
LIT FOR LEARNING SCIENCE

Activity: Dairy Identical Cheese

How are these tools used to make genetically engineered organisms?

Let's look at how bacteria or yeast have been engineered to produce casein. Remember casein is the protein found in cows that allows for the production of dairy products. The gene for casein has been identified and located within the cow genome. Scientists use a unique tool called a restriction enzyme to isolate the gene. These enzymes cut the DNA on either side of the casein gene, leaving the gene free from most of the surrounding DNA. This creates what scientists refer to as sticky ends flanking both sides of the gene. Scientists then cut the plasmid with the same restriction enzyme used to cut the gene. The restriction enzyme cuts the circular plasmid into a linear DNA fragment with sticky ends on both sides. The DNA fragment containing the gene is then fused into the plasmid (*See image below*). The plasmid carries the gene of interest into the host cells. In the case of casein, the host may be yeast or bacteria. The process of including foreign genetic material into another cell is known as transformation. Once the gene is introduced, the process of fermentation begins. Plant sugars are added to promote the growth of yeast or bacteria. As they grow and divide, they will transcribe and translate the genetic code for casein, making the needed protein. Casein proteins are released into the growth solution. The proteins are withdrawn by freeing them from the other parts of the growth solution. Once purified, the protein can be used to produce dairy-identical products.



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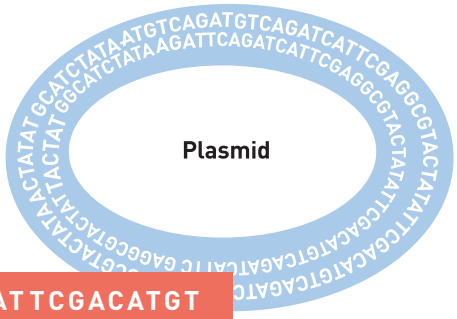

GTCAGATCATTCGAGGCTACTATATTCGACATGT
CAGTCTAGTAAGCTCCGATGATATAAGCTGTACA

Procedure

1. Read the Student Background information and be prepared to discuss the content in a whole-class discussion.

2. Cut out the CSN2 Gene Sequence strip printed on white paper and tape the strips together in numerical order in one long sequence.

3. Cut out the Plasmid Sequence strips printed on colorful paper. Use tape to attach them in numerical order. Tape the ends of the plasmid strips to form a circle.

4. Observe the Plasmid Sequence strip printed on colorful paper. Find the dashed line between the base pairs of the sequence **GAATTC**. Cut along the dashed line. This cut will form what scientists refer to as sticky ends.

5. Now focus on the CSN2 Gene Sequence strip. Notice the gray shaded region. This portion of the sequence indicates the CSN2 gene and represents the gene for cow casein.

6. Continue observing the Gene Sequence strip and notice the two areas marked with a dashed line between the base pairs of the sequence **GAATTC**. One area occurs before the gene region and the other appears after the gene.

7. Cut exactly along the dashed lines forming two sticky ends of the gene region. Notice that the segments of DNA are complementary on the sticky ends of the plasmid and the gene region.

8. Match the complementary base pairs on the sticky end of the gene region to the sticky ends of the plasmid and use the tape to attach them. This action will create one large circular paper plasmid.

9. The newly formed plasmid contains the cow casein (CSN2) gene and plasmid DNA from bacteria or yeast. Consider what steps will be necessary to produce casein for the production of dairy-identical products.

10. Complete the **Check for Understanding** questions.

Check for Understanding

Define biotechnology in your own words and provide two examples of how biotechnology is being currently used or may be used in the future. Do not use the dairy-identical products example modeled in this activity.

Biotechnology _____

_____

1. _____

_____

2. _____

_____

3. List and describe in detail the functions of the tools that are used in biotechnology.

_____

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4. Thinking about the model you built today, what did you model? Demonstrate your understanding through the use of a diagram, figure, cartoon or any other illustration that will allow you to show your understanding of the components of the model and what these components are responsible for in this process. Be creative.

5. What is the relationship between the model that you built and the production of dairy identical cheese? Utilize the following terms in your description: plasmid, restriction enzyme, CSN2 gene, plasmid, ligase, gene of interest.

_____

_____

_____

_____

_____

Gene Sequence - CSN2 - part of the bovine casein beta gene

Plasmid Sequence

1

A T C G G C T T T A C G G C T A A A C C T A G C

T A G C C G A A A T G C C G A T T T G G A T C G

2

T T A T C G C C A A A A C A T A C G A T T A

A A T A G C G G T T T T G T A T G C T A A T

3

T T A G G G C C C C A A C T T A A G G C T A T C

A A T C C C G G G G T T G A A T T C C G A T A G

4

T A C C G T A C C T A G T A C G T C A A C G T A

A T G G C A T G G A T C A T G C A G T T G C A T