Genetic Engineering; A Journey into DNA Science

Prepared by LeeAnn Vaughan Omaha North High School April 2004 Lesson title: Introduction to Genetic Engineering

Abstract: The teacher will demonstrate an activity called "Earth - the Apple of our Eyes," which points to the limited resources available to mankind for the production of enough food to feed the world population. Next, students will complete a worksheet while viewing a video presentation that further defines biotechnology. Before a definition of genetic engineering, the teacher will assess prior student knowledge using a KWL chart.

Teacher information/Situations/Setting/Time:

- Time Frame: One 90-minute bock period
- Materials:
- 1) Small apple & knife (see "Earth: The Apple of our Eyes")
- 2) "A Short Course on Biotechnology" self-running video on disk
 - a. Contact Council for Biotechnology & Syngenta Seeds, Inc
 (a) 1-800-478-5428 for a copy of the video
- 3) "Cinna-Apple" posters (Using the "croptechnology" Website as a guide, develop posters to illustrate the 5 steps of GE)
- 4) Student sheets:

Video notes KWL chart GE guided notes

 Teacher Resources: <u>http://citnews.unl.edu/hscroptechnology/html/firstPage.html</u>

Technology requirements/Tools/Materials:

Eiki projector w/computer Computer speakers

Assessment: KWL chart; video questions; discussion

Teacher Instructions/Student Activity/Tasks:

- 1. Follow the "Earth: The Apple of our Eyes" activity instructions to introduce students to the need for science to advance agriculture. It is important that they understand agricultural concerns are not just the business of farmers. We all need food to survive. In addition, most vaccines and medicines originate from plants.
- 2. Transition to the video presentation. Students should be instructed to complete the video questions. *Differentiation options: Allow students 5 minutes to "pairshare" after the video. A word list might also be provided.*
- 3. After the video, refer back to the concept map (from last period.) Discuss the genetic engineering section, explaining that you would like to know what information they already have about genetic engineering.
- 4. Handout the KWL chart and instruct students to make a list of everything they already know about GE in the first column, and a list of what they want to learn about GE in the second column. The third column will be completed at a later

date. Collect the charts and save for future use. *I usually give a grade based on participation.*

- 5. Encouraging student participation, the teacher should record their responses on the classroom marker board, reminding students that any response is acceptable at this point. The last column will later allow us to correct any misconceptions.
- 6. Using the GE posters, walk students through the steps of genetically engineering an apple transformed to include the taste of cinnamon. Students should complete their notes page. Note: I made individual posters, laminated them, and then stuck a magnet on the back of each so that I could toss them onto the marker board as I told the story (hypothetical) of how I created an apple to taste like cinnamon. I really get animated with this story—starting with the question: How many of you had Apple-cinnamon Cheerios for breakfast? I then discuss how much of love the taste of cinnamon with my apple, however, I hate the mess that it makes.

EARTH: THE APPLE OF OUR EYES

(adapted from Teachers' Pet Project)

Consider the earth an apple. Carry out the following sequence:

- 1. Slice an apple into quarters.
 - Set aside 3 of the quarters. **What do these represent?** *The represent the oceans of the world.*

What fraction do you have left? 1/4

Slice the ¼ in half and set on piece aside.
 That piece represents the parts of earth that are inhospitable to people: polar regions, deserts, swamps, very high or rocky mountains

What fraction do you have left? 1/8

The piece that is left is land area where people live, but do not necessarily grow the foods needed for life.

• Slice the 1/8 piece into 4 sections. Set aside 3 of these.

These 3 pieces represent the areas too rocky, too wet, too cold, too steep, or with too poor soil to actually produce food. They also contain the cities, suburban sprawl, highways, shopping centers, schools, parks, factories, parking lots, and other places where people live but do not necessarily grow food.

What fraction do you have left? 1/32

• Carefully peel the 1/32 slice of the earth.

This tiny bit of peeling represents the surface, the very thin skin of the earth's crust upon which mankind depends. It is less than five feet deep and is a quite fixed amount of food-producing land.

Things to consider:

- >3 million acres of the best US farmland is lost every year, in part to urban development.
- Population growth adds 78 million more people to the planet while ~1.9 billion tons of topsoil is lost due to erosion.

WITH A FIXED LAND RESOURCE BASE & AN EVER INCREASING # OF PEOPLE TO FEED FROM THAT BASE, EACH PERSON'S PORTION OF THE APPLE BECOMES SMALLER & SMALLER!

Period_____

Name

KWL CHART (Genetic Engineering)

What I already know about GE	What I would like to learn about GE	What I have learned about GE (end of unit)

Period_____

Name

A Short Course on Biotechnology

(Fill in the blanks as you view the presentation)

Agricultural biotechnology is a science that has evolved over hundreds of years.

 $\underline{1}$ work with peas also led to an explanation of dominant and recessive hereditary traits.

Today, agricultural biotechnology--or genetic-enhancement, as it's often called--is a continuation of the work that Mendel and others began.

Agricultural biotechnology is defined as "a precise scientific process including genetic engineering, used to modify or improve plants, animals, or microorganisms."

How does biotech work?

Example: You want a tall white carnation

Using traditional breeding methods and a lot of time, you *might* chance upon your tall white carnation, but it's a ______2 and inexact process.

And you'd end up with lots of variations. Medium-height white, medium-height red, tall pink, short pink--you get the idea!

But if you used today's genetic-enhancement technology you could very precisely select just the *tall* genetic information from the red carnation and add it to the white carnation.

That's what biotechnology allows us to do with important agricultural products and human health care--to move specific desirable 3 from one organism to another.

Fighting disease

4, used in the treatment of diabetes, is the product of one of the first geneticallyenhanced organisms. Researchers found that they could generate a consistent, reliable, inexpensive source of insulin by inserting a human gene into the genetic code of a bacterium.

Similar biotechnology research has led to the production of interferon for the treatment of ______5, and treatments that can aid people with anemia or heart disease.

Promoting human health

Biotechnology also allows us to boost the <u>6</u> values of foods.

In the not-too-distant future, many grains, fruits, and vegetables may be genetically-enhanced to deliver higher percentages of proteins, vitamins, and minerals than their conventional counterparts.

Combating animal disease

Through recent developments in biotechnology we're able to control "shipping fever," the biggest killer of beef cattle in feed lots.

Increasing yield

In the farm field, herbicide-resistant soybeans improve yield while reducing the need for chemical application, as well as lowering production costs, and reducing the need for soil tillage-which means less soil erosion.

Safer crops

And, insect-protected corn, cotton, and potatoes result in better-quality crops with less reliance on insecticides.

In fact, in 1998, 3.5 million pounds less _____7 were applied to American-grown corn and cotton through the use of insect-protected varieties produced through biotechnology.

Why Biotech Products Are Safe

The facts indicate that biotech products *are* safe, and that the _____8 they provide far outweigh the _____9.

Research & Development

Early on, genetically-enhanced plants are grown in isolation greenhouses. The soil that the hybrids are grown in is sterilized before and after use. And once grown, all plant material is incinerated.

US government agencies that require extensive field and safety tests: FDA (Food & Drug Administration)

The FDA ensures that any foods derived from new plant varieties are safe to eat, holding them to the same high standard of safety as any traditional food product.

Foods derived from biotechnology must be labeled only if they 10 significantly from their conventional counterparts. For example, if the nutritional value or the potential to cause an allergic reaction is altered.

USDA (United States Department of Agriculture)

The USDA is the US government's lead agency regulating the safe field-testing of geneticallyenhanced new plant varieties.

In order to receive permission to test a new biotech plant, an applicant must provide information about the plant, including all new genes and gene products, their origin, the purpose of the test, how it will be conducted, and specific precautions that will be taken to prevent the escape of pollen or plant parts from the field test site.

Impact on the environment, on endangered or threatened species, and on "non-target" species are all considered.

EPA (Environmental Protection Agency)

The EPA has authority over all new pesticides, including genetically-enhanced plants which produce their own protection against pests.

In deciding whether to register a new product, the EPA considers human safety, impact on the <u>11</u>, effectiveness on the targeted pest, and any effects on other "non-target" species, including endangered or threatened species.

Biotech research

From the time research starts on a new genetically-enhanced plant until it receives final clearance (or rejection) for retail sale, often takes $__{12}$ to $__{13}$ years.

Safe to eat

Foods made from current biotech products receive much greater scrutiny by the FDA, the USDA, and the EPA than conventional products.

Thorough testing assures that all biotech products have the same or better nutritional characteristics than conventional varieties.

Safe for the environment

Because of their built-in characteristics, genetically-enhanced crops may require fewer pesticides than are required to grow their conventional counterparts.

Which means less chemistry is being handled by farmers and entering the environment.

Why are biotech products better?

They are very often higher quality, more nutritious, and more plentiful than their conventional counterparts. Because they use fewer resources, they're more friendly toward the environment.

In the 1860s, Louis Pasteur learned that he could improve the wholesomeness of _____14 by gently heating it to kill off harmful bacteria.

When settlers first came to this continent, native Americans taught them to grow ______15.

That maize just barely resembles the $\underline{16}$ that we grow today. But over a long time and through much experimentation and cross-breeding, growers were eventually able to produce stronger, healthier plants with bigger, better-tasting ears of corn.

As you can imagine, early plant breeding involved a lot of chance. It was a hit or miss process!

Benefits to both the grower and the consumer

Growers harvest higher-yielding, higher-quality crops; and consumers get more, better, and healthier choices from their grocery stores' produce departments throughout the year.

Let's look at a few examples. . .

- One of the first major biotech products made available to farmers was Bt _____17.
- **Income spent on food** Farming efficiencies like that--as well as modern food production efficiencies in this country--help keep what North American families spend on food to just 10% of disposable household income versus the 20% spent by European families.
- Another example: Tacos! The shredded cheddar cheese that we put on tacos is a product of biotechnology.

Some years ago however, by isolating the gene that produces rennet, researchers found a way of having common bacteria generate the enzyme. This lets us produce rennet through a much simpler fermentation process.

Today, <u>____0</u>% of all cheeses are made using bioengineered rennet.

- Genetic-enhancement has also created bell ______ that are sweeter and firmer. .
- tomatoes with better flavor, color, and texture; and that stay fresh longer. . .
- cooking oils that are lower in saturated fats. . .
- Frost-resistant (strawberries that resist frost)

Through biotechnology we can develop foods that are higher in protein, vitamins, and minerals; and in sufficient quantities to feed our constantly expanding population.

Healthier animals

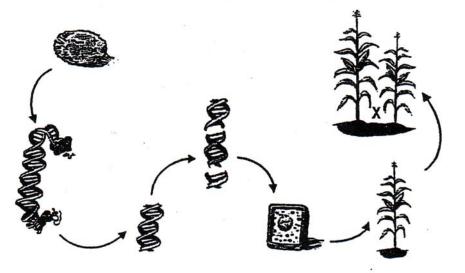
Edible vaccine

Another promising development: scientists are working on a _____25 that will contain an "edible vaccine" to fend off hepatitis, one of the world's most widespread and devastating diseases.

Genetic Engineering Notes

- Genetic engineering is the directed addition of foreign DNA (genes) into an organism.
- Five basic steps in crop genetic engineering:
 - 1. ______ DNA is extracted from an organism known to have the desired trait.
 - 2. _____- The gene of interest is located and copied.
 - Express in a desired way by altering and replacing gene regions.
 - 4. ______ The gene(s) are delivered into tissue culture cells, using one of several methods, where hopefully they will land in the nucleus and insert into a chromosome.
 - 5. _____ Transgenic lines are crossed with elite lines to make high yielding transgenic lines.

Crop Genetic Engineering Process



Lesson title: DNA Extraction

Abstract: Students conceptualize the extraction of DNA from the cell through a handson lab involving bananas. Throughout the lab, students explain how each step contributes to the extraction process. Their explanations and the extraction experience lead students to a better understanding of DNA properties.

Teacher information/Situations/Setting/Time:

- Time Frame: One 90-minute bock period
- Materials: See Extraction Lab for a list of materials Student sheets: Pre-lab worksheet Lab protocol sheet

Technology requirements/Tools/Materials:

Hot water bath Freezer

Assessment: Formative interaction during lab; lab worksheet

Teacher Instructions/Student Activity/Tasks:

- Remind students that the first step in genetic engineering is DNA extraction. Introduce them to the lab through a pre-lab investigation. Students should understand that the DNA they will attempt to extract from the banana will be less pure than DNA extracted through more sophisticated lab methods; however, these procedures will allow us to see clumped strands of DNA.
- 2) Prior to lab procedures, students should complete the pre-lab focus questions. A follow-up class discussion will help the teacher to clarify any misconceptions.
- 3) Students should complete the lab procedures and record their observations. *It is fun to have a contest to see who can "hook" the longest DNA strand!*
- 4) Clean up.

Extension:

Students can go to the following Website to view an animation titled "DNA Extraction." http://croptechnology.unl.edu/download.cgi

Alternative lesson:

The BioRad Company offers a DNA extraction kit called "Genes in a Bottle," which allows students to extract their own cheek cells and save them in a necklace. The kit works really well, and the extracted DNA is clearly a thin strand. While the alternative kit is more sophisticated, it is costly.

Period

Name DNA Extraction Pre-lab Investigation

How can we make DNA visible?

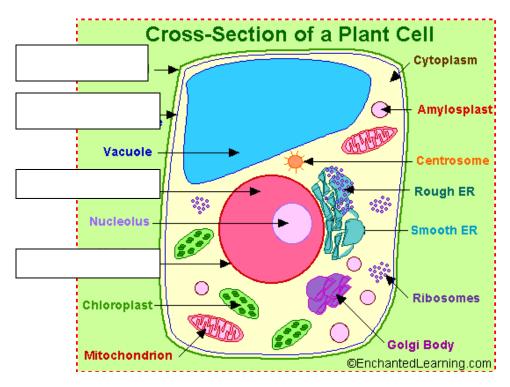
To work with DNA, scientists usually take it out of the cell and gently separate it from the other substances around it. This activity is a procedure for extracting DNA from banana cells. It is imperative that you read the lab protocol prior to the activity. Then, you should answer the questions below to help you process the lab concepts.

Step 1-4: Collect cells

To see the DNA, you will collect banana cells by breaking down tissue through the process of smashing. You will then break the cells open, and condense the DNA from all of the cells together. You can collect thousands of cells from a small sample of banana.

Focus question:

- 1. Below is a schematic image of a plant cell. Label the following compartments:
 - Plasma cell membrane •
 - Cell wall
 - Nucleus
 - Nuclear membrane



2. In which cellular compartment do you expect to find DNA?

Steps 5 & 6: Break open (lyse) the cells

Once you have collected the cells, the cells need to be broken open to release the DNA. Detergent, or shampoo, will dissolve the membranes of your cells, just like dishwashing detergent dissolves fats and proteins from a greasy pan, because cell and nuclear membranes are composed of fats and proteins. Dissolving the membrane results in the release of the DNA. The process of breaking open the cell is called lysis. Once the DNA has been released, it must be dissolved so it can be separated from the substances around it through a filtration process. Another important step is to neutralize the negative ends of the DNA so that it will eventually clump together during the last steps of the lab.

Focus question:

- 3. Do you think your DNA will be visible after you have broken open your cells? Why or why not?
- 4. Explain the purpose of the following steps:
 - a. Smashing the banana
 - b. Adding the shampoo
 - c. Adding the water
 - d. Adding the salt

Steps 7-10: Remove proteins & other substances

DNA is packaged tightly around proteins. Like spools for thread, these proteins keep the DNA tightly wound and organized so that it doesn't get tangled inside the nucleus. For you to see the DNA, it helps to remove the proteins so that the DNA can first loosen and expand, then collect into a mass with the DNA from all the other cells. You will submerge your lysed cells into a hot bath, which breaks down proteins so that they can no longer bind DNA.

Focus question:

5. When washing dishes, what works better, warm or cold water? Which do you think will help the detergent break open the cell, warm or cold temperatures?

Steps 11 & 12: Condense the DNA

Strands of DNA are so thin that it is not possible to see them when they are dissolved in solution. Think of the long, thin strands of DNA as fine white thread. If one long piece of thread were stretched across the room, it would be difficult to see. To make the thread more visible, you could collect it all together and pile it on the floor. In this laboratory experiment, you will use a cold liquid to bring the DNA out of solution, or **precipitate** it. The liquid creates a condition in which DNA doesn't stay in solution, so the DNA clumps together and becomes a solid mass that you can see.

Focus question:

6. What liquid is used to precipitate the DNA? (*Hint: DNA is insoluble in this liquid.*)

What does precipitated DNA look like?

Lab Observations:

1) Use observation terms to describe what you see in the test tube.

2) Use observation terms to describe what the spooled DNA looks like.

3) What pH reading did you get? _____ Is DNA an acid or a base?_____

Extracting DNA From Bananas

Materials (per lab group)

Start with:

- 1/4 ripe banana
- 1 zip-seal freezer bag
- 100 mL beaker
- 100 mL distilled water
- Ice bucket filled with ice
- 1 sealed test tube containing alcohol/student (keep on ice until needed)
- Filter paper (#2 coffee filter)
- pipet
- 1 large "hook"/student (straightened paper clip)

Other materials (use as needed)

- metric measuring spoons
- 1 ml table salt
- 10 mL clarifying shampoo
- pH paper
- Hot bath (shared by entire lab)

Procedures

- 1. Label your freezer bag.
- 2. Peel your portion of banana and break into small pieces, putting them into the freezer bag.
- 3. Seal the bag tightly.
- 4. Smash the banana with your hands.
- 5. Add 100 mL of water to the bag and continue smashing until banana is completely liquefied. Take care not to pop open or tear a hole in the bag!
- 6. To the solution, add 1 mL of salt and 10 mL of shampoo. Mix slowly to avoid foaming!
- 7. Pour the salt/shampoo/water solution into banana bag and mix **SLOWLY** to avoid foaming.
- 8. When banana is thoroughly liquefied and the shampoo, salt and water are uniformly mixed, place bag into 60° Celsius water bath for 10 minutes.
- **9.** Wash out and dry beaker, then place coffee filter about ¹/₄ into it. Fold the edges of filter down around the outside lip of the beaker so that it will stay in place when the beaker is set down.
- **10.** When the hot bath is completed, carefully place the bag into the ice bath for 5 minutes.
- **11.** After the ice bath, slowly pour banana solution through coffee filter and allow it to filter into the beaker until you have approximately 2 cm of filtrate. This may take several minutes.

- **12.** Dispense 2 mL of solution into each of the test tubes containing the alcohol. **Do not shake.**
- **13.** Let the tubes sit for 2 to 3 minutes without disturbing them. Observe and record what you see. You should see the banana DNA precipitates at the interface between the layers in the test tube.
- **14.** Gently lower your hook into the layer of DNA and slowly move the hook in small circles to spool the DNA.
- 15. Observe and record your observations of the spooled DNA.
- **16.** Use pH paper to test the DNA.

AT THE CONCLUSION OF THE LAB, YOU MUST CLEAN UP AND RETURN EVERYTHING TO ITS ORIGINAL CONDITION!

Lesson title: Introduction to Biotechnology

Abstract: An initial engagement activity is intended to illicit "excited" reactions from students based on news of a dangerous chemical (DHMO) that exists in our environment. The activity is designed to promote open-mindedness and to encourage students to become more informed citizens, especially considering the vast media influence in our society. Students then enjoy "DNA smoothies," while a biotech concept map is introduced to help students visualize the scope of biotechnology. The lesson proceeds with a focus on classical biotechnology, including samples from those processes. Students will also be introduced to a current events assignment that encourages them to recognize biotech in the news, and to make connections with what they are learning in the classroom.

Teacher information/Situations/Setting/Time:

- Time frame: 90-minute bock period
- Materials:
 - 1) Smoothie ingredients

Blender

- 1 bag frozen strawberries
- 1 banana
- 1 cup orange juice
- 1/2 cup milk
- 8 oz. yogurt
- 3 Tbs. honey
- small Dixie cups
- napkins
- 2) Biotech-related articles from newspaper, magazine, etc.
 - Products made from classical biotech processes: Bread, soy sauce, yogurt, cheese, aspirin, empty wine bottle, etc.
 - 4) Student sheets: Concept Map
 - Distash in the New
 - Biotech in the News
- Resources: <u>http://www.circus.com/~nodhmo/</u> (Ban Dihydrogen Monoxide) <u>http://www.dhmo.org/facts.html</u> (DHMO facts) <u>http://www.armory.com/~crisper/DHMO/</u> (DHMO Your Friend) <u>http://www.ghg.net/redflame/DHMO.html</u> (Song)

Technology requirements/Tools/Materials:

Eiki projector w/computer

Assessment: Formative interaction with students

Teacher Instructions/Student Activity/Tasks:

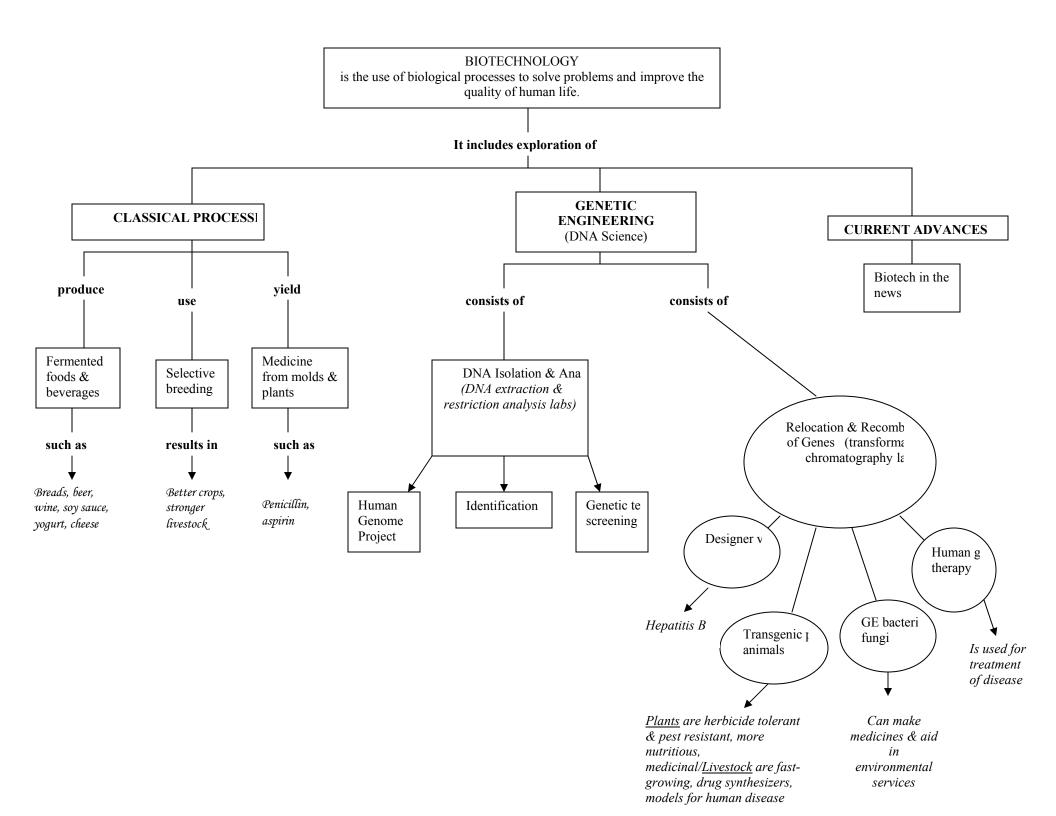
- 1. Begin class with the DHMO manipulation activity. The teacher must be really "pumped up" and animated while engaging students in the exploration of the dangers of DHMO. After explaining to the students that you recently stumbled across an alarming Website, share the sites with them. As you navigate the "Ban" and "Facts" sites, attempt to illicit excited responses from the students. Allow opportunity for student discussion. Point out that it is always important to look for other perspectives when exploring issues. Take them to the "Friends" site. Finally, inform the students that there is even a song about the dangers of DHMO. Encourage students to sing along as you slowly scroll through the verses. As you come to the verse that actually quantifies DHMO as H₂O, be prepared for mayhem!
- 2. Point out that all information presented was true! Press the fact that often the way information is presented influences our thinking. Ask students if they have ever been influenced by negative rumors regarding someone they did not yet know, only to later realize they really liked the person. *This would be a good opportunity to make the connection between misinformation and prejudice.*
- 3. Move to a discussion about how important it is to look at many perspectives, and to become knowledgeable before judging an issue. That is a constant role in science! Scientists must continually search for answers to improve our quality of life, based on a diverse group of interests; on ethics; and on scientific research. Discuss the dangers of automobiles. Ask if our society could easily eliminate cars because of their danger. Of course not! Therefore, scientists and technologists have worked together in order to make automobile travel as safe as possible.
- 4. Inform students that biotechnology is controversial. As young people reach voting maturity, it will be important for them to become knowledgeable and open-minded in order to make decisions on issues. Site the following example of biotech controversy: About six years ago, international news broadcast a protest outside of a European Monsanto plant. In reference to his opposition of genetic engineering, a well-known leader of the protesting group was filmed as he shouted, "I don't care what you say, I will never, ever, eat food that has DNA in it!" Ask the students what was wrong with that statement.
- **5.** Ask students if they would be afraid to eat food with DNA in it. Have some fun with the DNA smoothies! *Extra credit can be assigned to students who come to the next class period with a written explanation of any biotech processes involved in smoothie ingredients.*
- 6. While students enjoy their smoothies, introduce the concept map. Explain that the map is a framework for a course in biotechnology, and point to the areas that will be explored during the current unit.
- 7. Today's lesson should focus on the classical processes. As you discuss this section of the map, display examples of those products developed through biotech processes. Ask students to explain how the products connect to biotech.
- 8. Point to examples of biotech in the news and impress the fact that advances in biotech are popping up everywhere. *I usually show students a brochure that came in my Younkers bill. It advertises bedding products that were made from corn.* Handout and explain the "Biotech in the News" assignment.

Make a DNA Smoothie

To a blender, add the following ingredients and blend until smooth:

- 1 bag frozen strawberries
- 1 banana
- 1 cup orange juice
- 1/2 cup milk
- 8 oz. Yogurt
- 3 Tbs. honey

Serve in small Dixie cups.



"Biotech in the News"

Name	Date	
below. Th	otechnology article <i>that is of interest to you</i> , cut it out (or copy it) and answer the questions he article should be attached to the questions and then handed in by You may find an the newspaper, a magazine, a science journal, Websites, or any other source that highlights plogy.	l
Why did t	this article interest you?	
Read the	biotech article you have chosen and complete the following questions:	
1. Title	2	
Auth	10r	
Sour	Date	
	tify the <u>main</u> ideas of the article. (2-3) Write one or two sentences that rize each main idea.	
A. 1	Main Idea:	
Summ	nary:	
B. 1	Main Idea:	
Sum	nmary:	
C. 1	Main Idea:	
S	Summary:	
-		
-		
-		
-		
-		

3. Identify and define at least 5 terms that are either new to you or are related to biotechnology, or biology topics.

	Term	Definition
A.		
B.		
C.		
D.		
E.		
F		

4. What questions does this article raise? Does the article present differing viewpoints? If so, summarize each point of view.

5. Describe your reaction to the article. Do you accept the findings? Why or why not?

6. How does the information in this article relate to what you have learned in class?______

Lesson title: Welcome to the Wonderful World of Transformation!

Abstract: Completion of this lab allows students to witness genetic transformation as a visible event. After a teacher-led presentation that introduces and explains the process of moving genes from one organism to another, students complete intense pre-lab preparations. Students then explore the mechanisms of gene regulation and genetic selection as they transform bacteria with a gene that codes for GFP, which causes them to glow a brilliant green color; and with a gene that codes for resistance to the antibiotic ampicillin. In addition to providing an excellent example of the central molecular framework of biology in action, this lab allows students an opportunity to practice lab skills and techniques, as well as an opportunity to experience the thought process involved in a lab-based scientific procedure.

Teacher information/Situations/Setting/Time:

- Time Frame: Three 90-minute bock periods
- Materials:

Genetic Transformation PowerPoint Presention w/notes (contact leeann.vaughan@ops.org for a copy)

pGloTM Bacterial Transformation Kit

(contact BioRad @ 1-800-424-6723)

Ice bucket w/crushed ice

Thermometer that reads 42° C

1 L flask

Distilled water

- Bleach
- Student sheets
 - o Power Point handouts (request attachment from Vaughan)
 - o Introduction to Transformation
 - Lab protocol (see BioRad booklet)
 - Focus questions
 - Review questions
 - Data table
 - Analysis & conclusion

Technology requirements/Tools/Materials:

Eiki projector w/computer UV lamp Stopwatch Microwave oven 37⁰ C incubator oven Hot water bath

Assessment: Teacher interaction, discussion, and observations; student sheets

Teacher Instructions/Student Activity/Tasks:

Note: The kit comes with instruction booklets that include the lab procedures. The student sheets included with this lesson have been modified from the BioRad lessons in order to meet the needs of my own students. The teacher preparations should be carefully followed as directed in the BioRad booklet.

Day One

- 1) Students fill in blanks on slide show notes as the teacher presents the Genetic Transformation PowerPoint.
- 2) After slide #16, take a break from the presentation to allow students an opportunity to become more familiar with the lab. Have each small group (3-4 students) collaborate to write the twelve steps of the lab. I instruct the groups to write a one-sentence summary of each step. (*Students will use the lab protocol to complete the lab; however, the condensed summarization activity requires the students to process each step.*)
- 3) Return to the slide show.
- 4) Using their slide show notes, students should then complete the focus questions.

Day Two

- 1) Review purpose and procedures.
- 2) Determine group roles so that each student has a definite responsibility throughout the lab.
- 3) Students should wear lab safety equipment, such as goggles, aprons, and gloves.
- 4) Students should complete the pre-lab investigation, then proceed with the lab procedures.

Day Three

- 1) Teacher should lead students through a prediction discussion. Students should be able to predict what they should see on each of their four plates.
- 2) Students will complete the review questions.
- 3) Next, comes the moment of truth! Have students record their observations on their data tables. When they have finished the "regular" light observations, turn out the lights and have them hold the UV lights over each plate. They should see "green" on one of their plates. This is an EXCITING moment! Make a big deal out of what an incredible task they have just completed.
- 4) Students should finish by completing the analysis and conclusion requirements.

Introduction to pGLO Transformation

In this lab you will perform a procedure known as genetic transformation. Remember that a gene is a piece of DNA, which provides the instructions for making (codes for) a protein. This protein gives an organism a particular trait. Genetic transformation literally means **change caused by genes**, and involves the insertion of a gene into an organism in order to change the organism's trait.

Genetic transformation is used in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest, or spoilage resistance can be genetically transformed into plants. In bioremediation, bacteria can be genetically transformed with genes enabling them to digest oil spills. In medicine, diseases caused by defective genes are beginning to be treated by gene therapy; that is, by genetically transforming a sick person's cells with healthy copies of the defective gene that causes the disease.

You will use a procedure to transform bacteria with a gene that codes for Green Fluorescent Protein (GFP). The real-life source of this gene is the bioluminescent jellyfish *Acquorea Victoria*. Green Fluorescent Protein causes the jellyfish to fluoresce and glow in the dark. Following the transformation procedure, the bacteria express their newly acquired jellyfish gene and produce the fluorescent protein, which causes them to glow a brilliant green color under ultraviolet light.

In this activity, you will learn about the process of moving genes from one organism to another with the aid of a plasmid. In addition to one large chromosome, bacteria naturally contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for one or more traits that may be beneficial to bacterial survival. In nature, bacteria can transfer plasmids back and forth allowing them to share these beneficial genes. This natural mechanism allows bacteria to adapt to new environments. The recent occurrence of bacterial resistance to antibiotics is due to the transmission of plasmids.

The unique pGLO plasmid encodes the gene for GFP <u>and</u> a gene for resistance to the antibiotic ampicillin. PGLO also incorporates a special gene regulation system, which can be used to control expression of the fluorescent protein in transformed cells. The gene for GFP can be switched on in transformed cells by adding the sugar arabinose to the cells' nutrient medium. Selection for cells that have been transformed with pGLO DNA is accomplished by growth on antibiotic plates. Transformed cells will appear white (wild-type phenotype) on plates not containing arabinose, and fluorescent green when arabinose is included in the nutrient agar medium.

You will be provided with the tools and instructions for performing genetic transformation. Your task will be:

- 1) To do the genetic transformation.
- 2) To provide evidence of your understanding through clear explanations of thoughtprovoking questions.

Focus Questions (To be completed prior to lab day)

- 1) What is the host organism?
- 2) What two traits are we attempting to change about the host organism?
- 3) What are the two genes of interest, and what organisms were these genes taken from?
- 4) How many colonies of bacteria are you to transfer into the CaCl microtubules?
- 5) Draw pictures of the four plates you will be using in the lab and label them as shown in step #7 of the lab instructions.

- 6) Why do some of the plates have ampicillin in the agar?
- 7) Why do some of the plates have arabinose in the agar?
- 8) How will you be able to tell if you are transferring the plasmid DNA into the microtubules that contain the E. coli? *(Hint: See step #5.)*

Name____

Pre-lab Investigation: How Can I Tell if Cells Have Been Genetically Transformed?

Recall that the goal of genetic transformation is to change an organism's traits (phenotype). Before any change in the phenotype of an organism can be detected, a thorough examination of its natural (pre-transformation) phenotype must be made. Look at the colonies of *E. coli* on your starter plates.

The following pre-transformation observations of *E. coli* might provide data to make reference to when attempting to determine if any genetic transformation has occurred.

- 1. Describe the colonies on your starter plate:
 - a) Make a sketch of your starter plate.

- b) What color are the colonies?
- 2. Should the bacteria from your starter plate colonies be able to live and reproduce in the presence of an antibiotic such as ampicillin?
- **3.** Do the starter plate colonies glow green when viewed with ultraviolet light?
- 4. Examine the pGLO plasmid solution with the UV lamp. Does it glow green?

Name_____

Period_____

Review Questions

Before collecting data and analyzing your results answer the following questions.

1. On which of the four plates would you expect to find bacteria most like the original non-transformed *E. coli* colonies you initially observed on the starter plates? Explain your predictions.

2. If there are any genetically transformed bacterial cells, on which plate(s) would they most likely be located? Explain your predictions.

3. What is meant by a control plate? What purpose does a control serve?

4. Which plates should be compared to determine if any genetic transformation has occurred? Why?

DATA COLLECTION

lies Absent)	Regular Light	lor UV Light

Analysis of Results

1. What plates should be compared to best prove changes occurred?

- 2. Very often an organism's traits are caused by a combination of its genes and its environment. Think about the green color you saw in the genetically transformed bacteria:
 - a. What two factors must be present in the bacteria's environment for you to see the green color? (Hint: One factor is in the plate, and the other factor is in how you look at the bacteria.)

Conclusion

Refer back to the purpose of this lab and write a paragraph (in complete sentences) that addresses the following: Was the purpose accomplished? If not, what could be some possible reasons for lack of successful transformation? Explain what you learned in this lab. What new questions do you have? What new experiments could this experience lead you to?

Unit Title: Genetic Engineering: A Journey into DNA Science

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Unit Overview: An initial engagement activity is intended to illicit "excited" reactions from students based on news of a dangerous chemical (DHMO) that exists in our environment. The activity is designed to promote open-mindedness and to encourage students to become more informed citizens, especially considering the vast media influence in our society. Students then enjoy "DNA smoothies," while a concept map introduces students to the field of biotechnology. A follow-up lesson allows the teacher to demonstrate an activity called, "Earth: The Apple of our Eyes," which points to the limited resources available to mankind for the production of enough food to feed the world population. Students are then introduced to the field of genetic engineering, as they begin their journey into DNA science. The DNA extraction and genetic transformation labs are designed to promote conceptual understanding of genetic engineering. In addition to a focus on conceptual understanding of DNA science, the guided inquiry-based labs aid students in their understanding of scientific processes, and enrich the knowledge gained through the classroom experiences. The lab activities are also designed to develop laboratory skills that are utilized in scientific research.

Pre-requisites: Students should have a basic understanding of DNA structure, replication, and protein synthesis.

Relevance

- Since DNA is the essence of all life on Earth and is the molecule responsible for the unique characteristics present in all living things, students should have a conceptual understanding of DNA science.
- DNA research and diagnostic information helps to treat and/or cure various diseases that are familiar to most students.
- Genetic engineering has become an important link in advanced agriculture as mankind addresses the problems associated with the inversely proportional relationship between population growth and the loss of farmland.
- Molecular biology has become the forerunner in cutting-edge science, opening the doors of opportunity to those who possess knowledge and skills in the field.
- In addition to understanding the chemical basis of life, students will be better prepared to take informed positions on ethical issues related to scientists' ability to manipulate living organisms.

National Content Standards Science as Inquiry

CONTENT STANDARD A: As a result of activities in grades 9-12, all students should develop

ABILITIES NECESSARY TO DO SCIENTIFIC INQUIRY

 USE TECHNOLOGY AND MATHEMATICS TO IMPROVE INVESTIGATIONS AND COMMUNICATIONS. A variety of technologies, such as hand tools, measuring instruments, and calculators, should be an integral component of scientific investigations. The use of computers for the collection, analysis, and display of data is also a part of this standard. Mathematics plays an essential role in all aspects of an inquiry. For example, measurement is used for posing questions, formulas are used for developing explanations, and charts and graphs are used for communicating results.

Life Science

CONTENT STANDARD C: As a result of their activities in grades 9-12, all students should develop understanding of

THE CELL

- Cells store and use information to guide their functions. The genetic information stored in DNA is used to direct the synthesis of the thousands of proteins that each cell requires.
- Cell functions are regulated. Regulation occurs both through changes in the activity of the functions performed by proteins and through the selective expression of individual genes.

THE MOLECULAR BASIS OF HEREDITY

• Changes in DNA (mutations) occur spontaneously at low rates. Some of these changes make no difference to the organism, whereas others can change cells and organisms.

Science and Technology

CONTENT STANDARD E: As a result of activities in grades 9-12, all students should develop

UNDERSTANDINGS ABOUT SCIENCE AND TECHNOLOGY

 Science often advances with the introduction of new technologies. Solving technological problems often results in new scientific knowledge. New technologies often extend the current levels of scientific understanding and introduce new areas of research.

Components:

- Introduction to Biotechnology: An initial engagement activity is intended to illicit "excited" reactions from students based on news of a dangerous chemical (DHMO) that exists in our environment. The activity is designed to promote open-mindedness and to encourage students to become more informed citizens, especially considering the vast media influence in our society. Students then enjoy "DNA smoothies," while a biotech concept map is introduced to help students visualize the scope of biotechnology. The lesson proceeds with a focus on classical biotechnology, including samples from those processes. Students will also be introduced to a current events assignment that encourages them to recognize biotech in the news, and to make connections with what they are learning in the classroom.
- Introduction to Genetic Engineering: The teacher will demonstrate an activity called "Earth the Apple of our Eyes," which points to the limited resources available to mankind for the production of enough food to feed the world population. Next, students will complete a worksheet while viewing a video presentation that further defines biotechnology. Before a definition of genetic engineering, the teacher will assess prior student knowledge using a KWL chart.
- **DNA Extraction Lab:** Students conceptualize the extraction of DNA from the cell through a hands-on lab involving bananas. Throughout the lab, students explain how each step contributes to the extraction process. Their explanations and the extraction experience lead students to a better understanding of DNA properties.
- **Micropipet Lab:** This laboratory activity introduces micropipets and centrifuge techniques. The initial prep activity serves as an introduction to the instruments, to their functions, and to their purposes. A follow-up lab allows students an opportunity to interact with the mechanics of the instruments. Students practice range determination, dial selection, withdraw and transfer technique, and centrifuge use. Both class sessions include computer exploration. The first serves as a review of protein synthesis. The second walks students through an experiment involving bacteria, which focuses on experimental processes and helps to familiarize students with components that will be used in their transformation lab.
- **Bacterial Explorations:** Students engage in an interactive Internet activity that walks through an entire scientific experiment involving bacteria. The activity serves as a tool to prepare students for their upcoming transformation lab. The site promotes understanding of the scientific method, explains the process of bacteria cultures (including agar preparation,) and offers insight with regard to the nature of bacteria.
- Genetic Transformation Lab: Completion of this lab allows students to witness genetic transformation as a visible event. After a teacher-led presentation that introduces and explains the process of moving genes from one organism to another, students complete intense pre-lab preparations. Students then explore the mechanisms of gene regulation and genetic selection as they transform bacteria with a gene that codes for GFP, which causes them to glow a brilliant green color;

and with a gene that codes for resistance to the antibiotic ampicillin. In addition to providing an excellent example of the central molecular framework of biology in action, this lab allows students an opportunity to practice lab skills and techniques, as well as an opportunity to experience the thought process involved in a lab-based scientific procedure.