Genetically Modified Organisms (GMO) PCR

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Innovative Elements in our Food!

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Detecting Genetically Modified Foods and Plants by PCR

Student Version

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Objectives - student should be able to:

1. Describe the relationship between genes, proteins, and traits.
2. Define GMO and explain how a genetically modified plant differs from an organic one.
3. Explain how transgenes are used to introduce new qualities in a genetically modified plant.
4. Debate the pros and cons of these added modifications applicable in today's agricultural and ecological revolution.

Introduction

Recently, news articles have reported that several counties in Northern California have passed measures to ban growth and cultivation of genetically modified organisms (GMOs). Whether you are part of the pro- or anti-GMO groups, have you wondered if these genetically modified (GM) foods are actually part of your daily food consumption? A quick look at the variety of food products available in your local supermarkets may reveal more sources of GMOs than you would have expected. Today, an estimated 60%-70% of fresh and processed food products found in your local supermarket contains elements of genetically modified crops —and the prevalence of GM crops continues to grow each year. According to the U.S. Department of Agriculture, in 2005 alone, genetically modified varieties of soybeans accounted for 87% of U.S. acreage while the percentage of modified corn varieties was a little over half of all corn acreage[1]. As 2005 marked the 10th anniversary of the commercialization of GM or transgenic crops (generally referred as biotech crops), the number of acres of biotech crops are on the rise as farmers are more willing to adopt these new varieties with more enthusiasm. Soybean is the leading GM crop (60% globally), followed by corn (24%), cotton (11%) and canola (5%) [2].

So, why has there been such a boom in genetically modified crops? By introducing genes that code for a variety of positive attributes from insect resistance to drought tolerance into common crops, farmers are experiencing increased yield, better pest control, lower environmental impact, and decreased use of harmful pesticides. Many people see GM crops as a step towards increased productivity, which is especially important in a world with limited land for cultivation and continuous population growth. However, even as GM crops are becoming more accepted, scientific experts, lawmakers, and the common consumer groups are still concerned by a host of issues that arise when dealing with creating, planting, regulating, and consuming GM crops. As the debate over GM crops rages on, it is critical that the average consumer has an informed and accurate understanding of the scientific process of creating genetically modified foods, as well as the potential benefits and risks associated with them – for both consumers and environment.

Regulation of Agricultural Biotechnology

For the past decade or so, introduction of genetically modified organisms in the U.S. has gone smoothly and without much impediment. For some major U. S. crops, more than half of the acreage planted today is the modified varieties. Much of the rest of the world, by contrast, has experienced strong and increasing resistance to the preamble of biotech products. In fact, the EU (European Union) and other countries require certification of all foods entering their countries to be GMO free or have minimal levels. The U.S. government works to ensure that new agricultural biotechnology products are safe for human and animal health and are relatively safe for the

[1] http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bba/acrg0605.pdf
[2] http://www.isaaa.org/

environment. The three agencies involved are: the EPA, the FDA and USDA/Animal and Plant Health Inspection service (APHIS).

The **EPA** (Environmental Protection Agency) creates and regulates laws protecting human health and the environment. Under the FIFRA (Federal, Insecticide, Fungicide, and Rodenticide Act) stature, it also oversees all genetically engineered microbes, used as pesticides and biodegradation tools, and certain GM crops. Pesticides such as Bt (*Bacillus thuringiensis*) proteins, used to keep insects and caterpillars from eating corn, thus fall under EPA's jurisdiction. EPA also regulates all herbicides, non-pesticidal and non-food microbial products under the Toxic Substances Control Act (TSCA).

The Food and Drug Administration (**FDA**) is responsible for protecting public health by assuring the safety, efficacy and security of all human and veterinary drugs, medical devices, biological products, food supplies for human and animals (whether GM or not), cosmetics and products that emit radiation. All biotech and medical related companies are legally obligated to ensure that their medical, food or cosmetics products meet the safety standards of the law. FDA has specific policies that govern the introduction of all new GM crops. However, at the moment, it does not regulate any food made from these GM crops.

The US Department of Agriculture (**USDA**) focuses on regulating the production of all food, agriculture, natural resources, and related issues such as development of new vaccines for animals and poultry and livestock safety and inspections. Companies wishing to field-test or transport biotechnology-derived plants must obtain **APHIS** approval under the USDA regulations. For companies looking to commercialize their new plant breeds, APHIS will need to classify their products under a "non-regulated status" for them to continue to wide scale production.

Within the folds of USDA, National Organic Program (**NOP**) regulates the standards for any farm (plants, marine organisms and /or livestock), wild crop harvesting, or handling operations producing organic food products. Under the **Organic Foods Production Act of 1990**, any agricultural product that fall in this category shall:

(1) Be produced and handled without the use of synthetic chemicals;
(2) Except for livestock, not be grown on land with any prohibited substances, including synthetic chemicals;
(3) Be produced and handled in compliance with the organic agreements settled between the producer and the certifying agent;
(4) Need an area designated specifically for farming that also includes some buffer zones for handling and harvesting the produce;
(5) Maintain a 3 year history of farm management showing that there were no prohibited substances or chemicals used;
(6) Have records and documents available for inspection. Facilities and products are subject to regular testing by certifying agents.

Labeling and processing of organic products has drawn a huge interest from consumers. Many outside regulatory agencies like NOSB (National Organic Standards Board) work with USDA to adopt new policies into the organic certification program. According to the Organic Foods Production Act of 1990, GMOs are now considered an "excluded method" of production. This means, that in order for a food to be considered organic, it cannot be created using any of the following methods: " …cell fusion, microencapsulation and macroencapsulation, and recombinant DNA technology (including gene deletion, gene doubling, introducing a foreign gene, and changing the position of genes when achieved by recombinant DNA technology)". Such methods do not include the use of traditional breeding, conjugation, fermentation, hybridization, *in vitro* fertilization, or tissue culture."[3]

A Short History of Genetic Modification

For the past 10,000 years, humans have interacted with and manipulated plant and animal species in the environment to make them better suited for their own use. As human society became largely dependent on agriculture, farmers discovered that some plants in each harvest possessed exceptional traits—such as larger fruit or frost resistance. Seeds from these plants were then saved and planted the following year in hopes that the next harvest would yield more plants with the same characteristics. Plants with the special trait were continuously selected for over successive generations. Most people might be surprised to find out that after thousands of generations of this **selective breeding**, what we identify as a stalk of corn today barely resembles its original ancestor. In fact, all plants we currently eat have in some way been intentionally genetically modified by human efforts. **Hybrids** can also be created, whereby two plants of the same species, each with a different quality, are crossbred combining the preferred traits from the two parent plants. In addition to the traits of interest, all other

[3] National Organic Program Overview, Subpart A - Definitions

genetic material is also exchanged between the two plants during this process. These plants often exhibit traits of better quality than either parent, referred to as hybrid vigor. Additionally, almost all fruits (apples, pears, grapes, nectarines, etc.) are produced through a technique called **grafting**, where branches of one type of plant are physically attached to the trunk and roots of another type.

For centuries, farmers practiced these techniques successfully without a clear understanding of how traits were inherited by subsequent generations. However, for the past 50 years, developments with **recombinant DNA technology** have become responsible for the "second green revolution". What used to take many years of cultivating plants to develop desired trait(s) using traditional plant breeding methods can now be done much more quickly and precisely through genetic engineering. The fact that these advancements in research have exciting possibilities is generally not disputed; however, whether we *should* genetically engineer organisms or even pursue the extent of some cross-species modifications is still a topic of strong of debate among scientists and the general public.

DNA and Transgenes

To understand how scientists have managed to engineer plants with properties from bacteria or viruses, you need to start with some basic molecular biology. At a first glance, the wide spectrum of living organisms do not seem to closely resemble each other—your pet cat, your favorite flower, or the bacteria living on your skin all have different shapes, sizes, and behaviors. However, closer inspection at the molecular level reveals that all living things are made up of small living units called cells, and in these cells, they all share a common molecule -- **deoxyribonucleic acid** (**DNA**).

The basic building block of DNA in all living things is the **nucleotide**. A nucleotide is composed of a deoxyribose sugar, a phosphate, and one of the four bases A (adenine), C (cytosine), G (guanine), or T (thymine). In the DNA molecule, nucleotides are linked together in a chain. DNA is a **double helix**; two chains of nucleotides are wound around each other to form a spiral structure. Hydrogen bonds between the bases on the opposing strands hold the double helix together. The A's on one strand hydrogen bond with the T's on the other strand. The G's on one strand interact with the C's on the other. Therefore, A's and T's are said to be **complementary** as are G's and C's. Complementary bases, when hydrogen bound in the double helix, are called **base pairs** (**bp**).

Organisms can be single-celled or multicellular, and amazingly, each cell in an organism (except for sex cells) contains the exact same copy of DNA. In complex organisms in which a broad range of functions needs to be efficiently executed and coordinated, each individual cell is specialized to perform its own particular function. However, regardless of what that cell does, its biochemical reactions are all driven by the presence of this common molecule, DNA.

Proteins are the workforce of a cell, performing most of a cell's daily activities. Specific proteins called **enzymes** catalyze biochemical reactions while other proteins transport molecules within the cell or allow the cell to interact with the outside environment. One of the most prevalent enzymes on the planet is found in plants. Ribulose-1,5-bisphosphate carboxylase oxygenase, commonly known by the abbreviation **RuBisCO**, is located in the chloroplast and is involved in carbon fixation, the process that converts carbon dioxide from the air into sugars and carbohydrates. Still other proteins provide the integral shape and cytoskeletal structure of the cell. For example, in plants, a particular protein, cellulase, is responsible for digesting the tough fibrous cell wall matrix. In most eukaryotes, another protein, actin, is involved in facilitating cell shape changes and cellular movement. The instructions for creating these and other proteins are spelled out in specific sequences of the four bases of the DNA blueprint. These coding regions, that determine the construction of a particular protein, are called **genes**.

When a cell needs to make a specific protein, complex interactions occur between the DNA and various other enzymes and signaling proteins. In a eukaryotic cell, protein synthesis starts in the nucleus. The enzyme RNA polymerase first binds to the **promoter** site, a sequence of DNA bases upstream of a particular gene. During the process of **transcription**, RNA polymerase moves along the DNA template and produces a complementary strand of RNA. This RNA transcript is then modified and processed before finally exiting the nucleus to the cytoplasm as **messenger RNA** (mRNA). In the cytoplasm, the mRNA attaches to a **ribosome**, and the second stage of protein synthesis, **translation**, begins. In the ribosome, three bases (also called **codons**) on the mRNA transcript are read at a time. Each codon corresponds to an amino acid, the building block of proteins. As the mRNA is processed through a ribosome, **transfer RNA** (tRNA) molecules bring the matching amino acids to

each codon. As the amino acids line up on the ribosome platform, peptide bonds are formed between adjacent amino acids resulting in the formation of a polypeptide chain. After further modification by other proteins, the polypeptide chain folds into its characteristic 3-D conformation. This unique shape gives the protein its structural and functional identity necessary to perform its specialized function in the cell.

Amazingly, and critical in the development of genetic engineering, nucleotides and amino acids that make up DNA and proteins, respectively, are chemically conserved across all living organisms. In the 1930s, scientists honed in on a useful technology called **transformation,** which allowed small pieces of DNA to transfer from one organism to another. This new piece of foreign DNA gave the recipient organism new properties or characteristics. It wasn't until the 1960s, however, that another breakthrough occurred with the discovery of restriction enzymes. **Restriction enzymes** are molecular scissors that cut DNA at specific sequences. These enzymes allowed scientists to remove specific genes or gene segments from one organism and insert them into another organism in the process called **recombinant DNA technology**. To add to scientists' delight, a transformed cell was unable to differentiate its own DNA from the foreign gene, so it would synthesize a functional foreign protein based on the newly inserted gene. One common example is the use of genetically modified *E. coli* bacteria to produce human insulin for people afflicted with diabetes mellitus. The mere presence of foreign DNA in another organism makes it a genetically modified organism - **GMO**. Although, most man-made genetic modifications are developed in labs, let's not forget that in nature, pieces of DNA containing one or more traits are also passed on to another, especially within microorganisms.

Scientists have used this innovative technique to insert genes from a variety of unlikely sources, including bacteria and jellyfish, into plant cells thereby conferring upon them new properties. Most of these modifications are done on agriculturally important plants where inserted genes introduce traits such as resistance to insects, herbicides, or viral diseases, or to improve a plant's nitrogen fixing ability, nutritional value, photosynthetic efficiency, or the ability to grow under adverse conditions. These genetic modifications are intended to increase harvest yields of crops, reduce the massive use of chemicals, improve nutritional quality of foods, reduce the spread of diseases, and conserve valuable resources like water and land.

There are two classic examples of gene transfers resulting in improved crop species. The first, ***Bt* gene** from the soil bacterium *Bacillus thuringiensis*, allows plants to produce a toxin that is deadly to harmful insect species like the European corn borer and bollworm. Introduction of the *Bt* gene into corn has resulted in insect resistant crops, which has reduced crop devastation and increased the amount of usable crop that can be harvested each season. Another common transgene, the **glyphosate resistance gene** from the bacterium *Agrobacterium*, protects plants from the widely used herbicide Roundup®. Plants that have been engineered with this gene are therefore "RoundUp® Ready" and can resist the herbicide RoundUp that effectively kills non-engineered, invasive weeds in the same field.

Modification Technologies

Genetic modification in a plant involves the transfer of a desired trait in the form of foreign DNA into a plant of interest. This process is also referred to as **transformation**. To produce a transgenic crop, one would need to have a reliable and efficient technique to transfer DNA. There are generally three classic methods used to transfer foreign DNA into a host plant: bacterial/viral vectors (Ti plasmid from *Agrobacterium tumefaciens*), physical methods (microinjection, biolistics and electroporation), and chemical-induced methods (PEG – polyethyleneglycol and calcium chloride). Due to higher efficiency rates of the **biolistics (**gene gun) and the **bacterial vector** (Agrobacterium) methods, they are most commonly used in producing transgenic monocot and dicot plants.

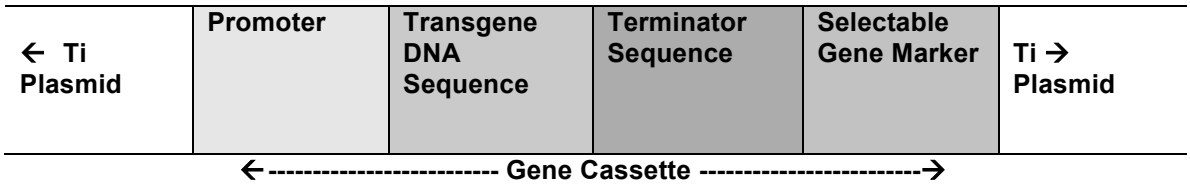
With the biolistics method, the desired gene DNA construct is coated onto fine gold/tungsten particles and then the particles are shot into the callus tissue (a mass of non-specific cells) of a plant of choice. While it is not entirely understood how cells acquire the DNA, it is thought that as the cells repair their injuries, they integrate the foreign DNA into their genome, thus allowing the host to transcribe and translate desired protein(s).

Agrobacterium-mediated (**bacterial vector**) method was only useful with dicots at first, but new discoveries and tinkering of experimental conditions have made this an effective method with monocots such as corn and rice. The greatest advantage with this method over the others is that the Ti (tumor-inducing) vector, integral in helping with the transfer, does not limit the size of the foreign DNA. In nature, when Agrobacterium infects plants, the Ti plasmid is injected into the plant cell and gets incorporated into the cell's chromosomal DNA.

Normally, the expression of the inserted Ti genes triggers the formation of crown galls or tumor in the host plant cell. However, the tumor causing elements can be replaced with foreign DNA or a man-made gene cassette . As long as the left and the right borders are maintained in the engineered Ti plasmid, transfer of DNA from the Agrobacterium to the host cell can still occur. This discovery allowed scientists to insert new genes into plants using the Ti plasmid thus creating GM (genetically modified) crops.

The man-made gene cassette is a group of genes that will be inserted into the Ti vector to be introduced into the new organism. The cassette is usually composed of a promoter, transgene, terminator and a selectable gene marker (a trait that is used to distinguish which cells have acquired the new DNA). In order to ensure successful and efficient expression of the transgene in a plant, a powerful promoter is often inserted upstream of the foreign gene. The most commonly used promoter for plants is from the Cauliflower Mosaic Virus – CaMV35S promoter. In the majority of genetically modified plants, the **35S promoter** sequence drives continuous production of the foreign DNA's protein product in all of the plant cells. The terminator sequence is derived from the Ti plasmid – called NOS. The selectable marker is a gene that confers resistance to either an herbicide or antibiotic. The purpose of this marker is to allow the scientist to quickly screen for transformants; only plants that have received the gene cassette will be able to survive the screening treatment.

Illustration of a Gene Cassette:



Methods Used in the Detection of GMO Foods

Several companies in the US and around the world provide assays and kits for detecting genetically modified foods for commercial purposes. Many of the government agencies, plant biotech companies and others use these methods to inspect, test and validate their protocols for GM products in the agricultural and food industries.

Two most common methods in use today for detecting genetic modifications are:

1. **ELISA** (Enzyme Linked Immuno Sorbent Assay) - whereby the product of the transgene (usually a protein) is used for the test. Here, the enzyme-linked assays are designed so they are very specific for each transgene protein. An antibody to the protein of interest is usually tagged with a color-changing substrate. Once the enzyme binds to its specific protein, a chemical substrate attached to the enzyme undergoes a color change indicating the presence of that particular protein. The ELISA assays are quick, relatively inexpensive and can be portable onsite if needed. The biggest disadvantage of this assay is that it requires intact protein. Since, most processed foods are heated during processing, most of the proteins in these foods are denatured. This makes it difficult to rely on ELISA assays for accuracy in processed materials.

2. **PCR** (**P**olymerase **C**hain **R**eaction) – detects the presence of DNA of a specific portion of the gene cassette, generally targeting the promoter or the transgene elements of modifications. Detecting any piece of DNA using PCR can be costly and time consuming. However, this method allows more reliable and quantifiable results compared to ELISA. This method of testing is usually not portable to the field site, but only very minuscule amounts of DNA isolated from processed foods are needed to detect the presence or absence of any genetic modification(s) Benefits and Risks

3.

As with any new technology, there are perceived benefits and risks. Genetic modification of plants and animals is a particularly controversial topic *because* of the long list of both benefits and risks associated with it. Furthermore, what may be considered a positive aspect of the technology to one person may be a reason to oppose it for another! Therefore, determining which aspects of genetic modification belong in the "pro" and "con" lists can be rather confusing and complicated. Below are just some of the potential benefits and risks of genetic modification of organisms:

Potential Benefits

- Breeders are not restricted to the same species for improved traits.
- Increased crop yield in the same area of land.
- Improved nutritional quality of staple crops.
- Reduced amount of chemicals in the environment.
- Detoxification of soils by specialized plants.
- Decreased fuel use, since farms do not have to fertilize/spray their fields as often.

Potential Risks

- Pesticide resistant insects.
- Antibiotic resistant bacteria.
- Herbicide resistant weeds.
- Exposure to new allergens/toxins in familiar foods.
- Decreased diversity in crop plants.
- Reliance on agribusiness to supply seeds (farmers can't save seed for next year).
- Integrity of human food supply.
- Contamination and loss of wild type plant genomes.
- Unexpected or unpredictable environmental and organismal consequences.

Since the birth of agriculture, we have tinkered naturally, selecting and breeding traits of importance, in plants. With the intervention of modern techniques we have taken this DNA transfer to a new level. New genes need not come from the same plant species; they can vary widely – from other plants, animals or even bacteria. GM technology is intended to increase the productivity and profitability of many companies vested in this agricultural revolution but, can they and government agencies, guarantee the safety of our food supply? Are we making a real difference, being equitable globally? As an informed consumer, it is important to do your research to identify your own "pros" and "cons" of genetic engineering

Things to Keep in Mind When Selecting a Food Product to Test

- Familiarize yourself with the table below that lists the types of genetically engineered crops grown globally **as of the year 2012**. These are crops that have received regulatory approval to be grown and marketed to consumers. You should not get a positive result for any food items that are not listed on this table. If you do, it will likely be due to a false positive PCR result or a new crop being introduced (which is unlikely).

- An estimated 60%-70% of all processed U.S. foods likely contain some GE material. That is largely because two such crops (corn and soybeans, where farmers have widely adopted GE varieties) are used in many different processed foods.

- Globally, the US ranks first in global area of biotech crops. **There are a total of eight crops that have federal regulatory approval to be commercially marketed in the US: corn, soybean, cotton, canola, sugarbeet, alfalfa, papaya, and squash.**

- **There are only two federally approved and commercially marketed crops grown in the US that contain the Bt transgene: Corn and cotton.** If your PCR results show the presence of Bt in anything other than corn or cotton, it is a false positive result. The only food products that contain the Bt transgene will be corn-based.

- Hawaiian papaya was almost entirely decimated by the ringspot virus in the 1990's. Papaya from Hawaii contains the 35S promoter and a gene that confers resistance to the virus. The fact that papaya can still grow in Hawaii is one of the success stories of biotech crops.

Table of Globally Commercialized Biotech Crops, 2012

Rank	Country	Area*	Crop
1	USA	69	Maize, Soybean, Cotton, Canola, Sugarbeet, Alfalfa, Papaya, Squash
2	Brazil	30.3	Soybean, Maize, Cotton
3	Argentina	23.7	Soybean, Maize, Cotton
4	India	10.6	Cotton
5	Canada	10.4	Canola, Maize, Soybean, Sugarbeet
6	China	3.9	Cotton, Papaya, Poplar, Tomato, Sweet Pepper
7	Paraguay	2.8	Soybean
8	Pakistan	2.6	Cotton
9	South Africa	2.3	Maize, Soybean, Cotton
10	Uruguay	1.3	Soybean, Maize
11	Bolivia	0.9	Soybean
12	Australia	0.7	Cotton, Canola
13	Philippines	0.6	Maize
14	Myanmar	0.3	Cotton
15	Burkina Faso	0.3	Cotton
16	Mexico	0.2	Cotton, Soybean
17	Spain	0.1	Maize
18	Colombia	<0.1	Cotton
19	Chile	<0.1	Maize, Soybean, Canola
20	Honduras	<0.1	Maize
21	Portugal	<0.1	Maize
22	Czech Republic	<0.1	Maize
23	Poland	<0.1	Maize
24	Egypt	<0.1	Maize
25	Slovakia	<0.1	Maize
26	Romania	<0.1	Maize
27	Sweden	<0.1	Potato
28	Costa Rica	<0.1	Cotton, Soybean
29	Germany	<0.1	Potato

** Area measured in million hectares*

Sources:
Agricultural Biotechnology: Background and Recent Issues. *Congressional Research Service*. Tadlock Cowan. 2011.
Global Status of Commercialized Biotech/GM Crops: 2011. Clive James. *International Service for the Acquisition of Agri-Biotech Applications* (ISAAA). 2011.

Laboratory Exercise: DNA Extraction

In the following laboratory exercise, you will use several techniques to determine whether your food sample has been genetically modified. You will first isolate the template plant DNA from your samples. The plant materials used for this lab will be ground and then boiled in a detergent/buffer mixture to extract the DNA from the cells. A short precipitation/drying step with isopropanol will follow to purify the DNA further before use.

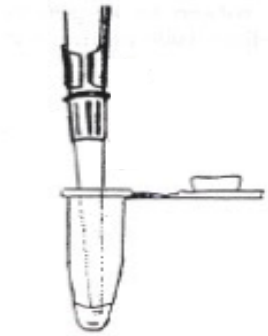
You will then use a process called Polymerase Chain Reaction (PCR) to examine two regions of DNA: a non-GM region and a genetically modified region (if present). These PCR amplification targets are the **rbcL** gene (non-GM) and the **transgene** element (the 35S promoter region or the *Bt* gene).

The gene for rbcL is located in the chloroplast, the location of photosynthesis in all plants. The protein product of this gene is thought to be the most abundant protein on earth. Ribulose-1,5-bisphosphate carboxylase oxygenase, commonly known by the abbreviation **RuBisCO**, helps convert carbon dioxide from the air into sugars and carbohydrates. rbcL refers to the large subunit (L) of this enzyme, and it is the gene that you will be amplifying with PCR.

Since the rbcL gene is present in all plant cells regardless of any modifications, the amplification of this gene region acts as an internal control for the PCR reaction. Therefore, with each successful DNA isolation, you should expect to observe the rbcL gene PCR products in both GM and non-GM PCR reactions. Finally, a process called gel electrophoresis will be used to visualize the amplified products. The presence or absence of the amplified transgene element on your gels will provide evidence as to whether the plant DNA you tested has been genetically modified.

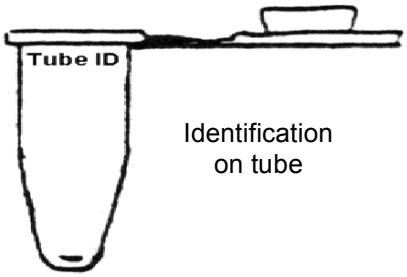
Objectives - student should be able to:

1. Successfully isolate DNA from plant tissue and/or food products.
2. Set up PCR amplification reactions for the rbcL and transgene loci.
3. Cast an agarose gel to run your PCR products. After electrophoresis of your sample, you will then analyze the photo results of the gel, comparing it with data from the rest of the class.
4. Explain your results, as well as why you used the positive and negative controls for this lab.

<table>
<tr><th colspan="2">Important Laboratory Practices</th></tr>
<tr><td>a. Add reagents to the bottom of the tubs, not to its side
b. Add each additional reagent directly into the previously added reagent
c. Do not pipet up and down, as this introduces error. This should only be done only when resuspending the cell pellet and not to mix reagents.
d. Make sure contents are all settled into the bottom of the tube and not on the side or cap of tube. A quick spin may be needed to bring contents down.
  </td><td>a. Pipet slowly to prevent contaminating the pipette barrel.
b. Change pipette tips between each delivery.
c. Change the tip even if it is the same reagent being delivered between tubes. Change tip every time the pipette is used!
  </td></tr>
<tr><td>Keep Reagents on Ice.
  </td><td>Check the box next to each step as you complete it.
  </td></tr>
</table>

** Part 1: Preparation & Lysis of Food Sample **

1. Obtain a clean 1.5 mL microfuge tube. Label each tube with your material and your identification number.



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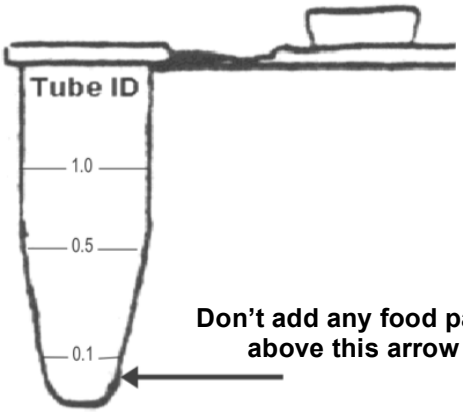
2. **a) If you are isolating DNA from plant tissue:** Use the back of a P-1000 pipette tip (like a cookie cutter) to punch out one disk of plant tissue from your leaf. Place the disk in your labeled tube.

b) If you are isolating DNA from food products:

1. Crush a small amount of dry food product in between two pieces of clean paper or paper towel to produce fine powder like particles.
2. Add the crushed food product to your labeled tube about halfway to the 0.1 mL mark. **Do not add any food above this point, as too much food will inhibit the DNA extraction.**



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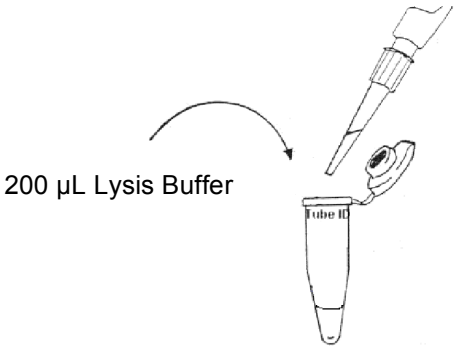
3. Add **200 µL of Lysis Buffer** to your tube containing the plant tissue or ground food product.

Note: The Lysis Buffer breaks the cell and releases the DNA into solution. DO NOT contaminate the stock Lysis Buffer solution!



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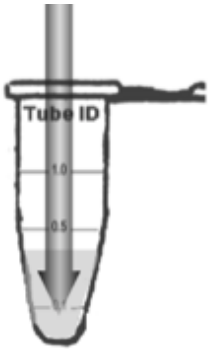
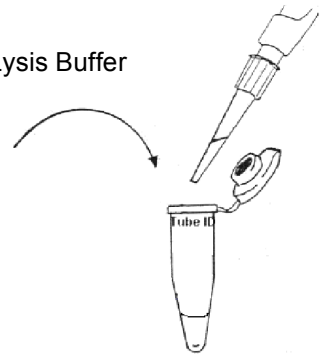
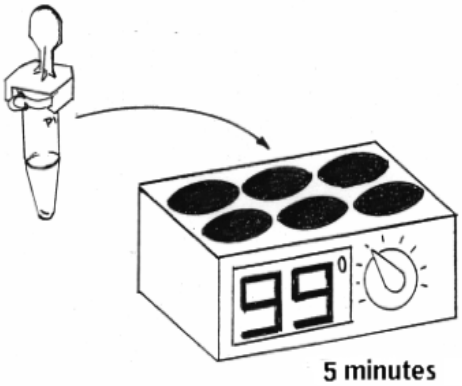
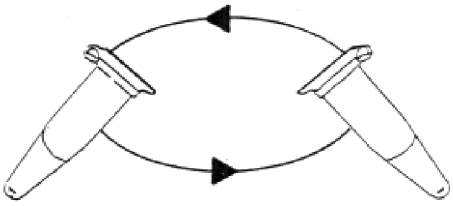
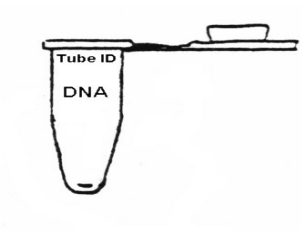
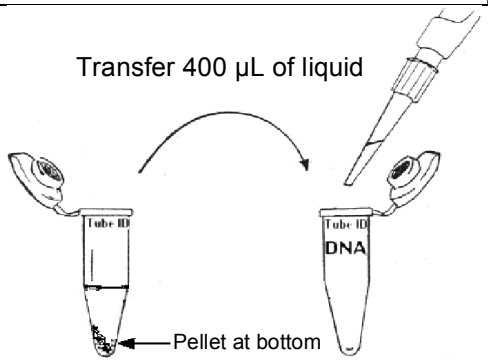
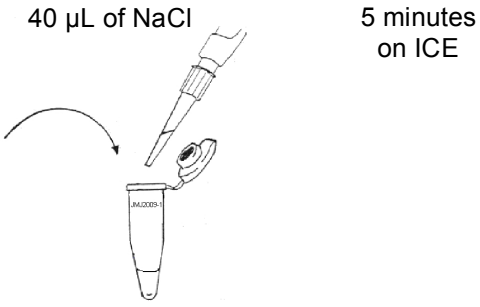
1. Macerate the food product by twisting the plastic micropestle for at least **1 minute** or until only small bits remain. You must use considerable downward and rotating force to adequately macerate the tissue. **If the food material gets stuck at the bottom of the tube, use a clean pipette tip to dislodge it.** **Note**: Some food products will absorb all the liquid, compacting the sample material at the bottom of your tube. If this happens, quickly rack or vortex your tube. **Use a pipette tip to maneuver and resuspend the food product before you resume grinding.**	 ☐
4. Once the food product is sufficiently macerated, add 8**00 µL Lysis Buffer.** Make sure the contents of the tube are thoroughly mixed by vortexing or by "racking" the tube. How much Lysis Buffer should you have in the tube at this point? _____µL	800 µL Lysis Buffer  ☐
5. Slide a cap lock onto your tube and place it in the 99°C heat block or water bath for **5 minutes**. Keep track of where your tube is in the heat block or water bath. The cap locks prevent tubes from popping open due to vapor pressure. **Note:** This step denatures proteins, including DNA-digesting enzymes.	 ☐
6. After heating, open tube briefly to release pressure. Then vortex or shake tube to mix, and place in a balanced centrifuge. Spin the tube for **5 minutes** to pellet cell and food debris. **Centrifuge speed should be set to 10,000 x g (≈10,000 rpm).** **Note:** This step pellets insoluble material at the bottom of the tube leaving DNA in the supernatant.	 5 minutes at 10,000 x g ☐

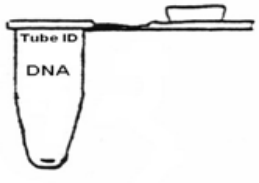
** Part 2: Removing Impurities **

During these steps, the DNA is located in the upper liquid portion, not the bottom pellet.

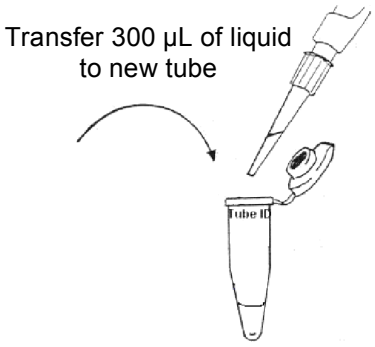
7. Obtain a second clean 1.5 mL microfuge tube. Label the tube with your material and your identification number.	 ☐
8. Retrieve your tube from the centrifuge. You will see food debris at the bottom of the tube. Without disturbing it (the pellet), withdraw **400 µL of the liquid (supernatant)** from the centrifuged tube and transfer to the newly labeled DNA tube. **Discard the old tube containing the pellet.** **Note:** You may notice an oily layer above the supernatant, which is composed of residual fat from the food. If so, draw the liquid from below the oily layer. Be careful not to disturb the pellet or any other solid debris in the tube.	 ☐
9. Add 40µl of 5M NaCl to the tube containing the liquid that you removed. Shake the tube a few times to mix. Incubate on ice for 5-10 minutes. Solution may become cloudy. **Note:** The NaCl binds to detergents in the Lysis Buffer that we need to remove in order to have a clean DNA sample.	 ☐
10. Place tube with NaCl into centrifuge and spin again as described in step 6 above.	5 minutes at 10,000 x g ☐

** Part 3: Isolating the DNA **

During these steps, the DNA is located in the pellet at the bottom of the tube.

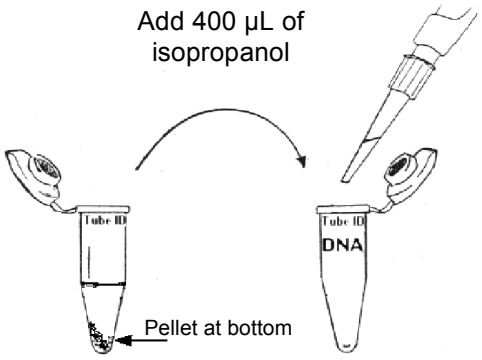
11. Obtain a third clean 1.5 mL microfuge tube. Label the tube with your material and your identification number.	 ☐

12. Retrieve your tube from the centrifuge. There may be a noticeable pellet at the bottom of the tube. Repeat what you did in step 9, but this time take **300µl of the liquid from the top of the tube.** Be careful not to take anything from the pellet at the bottom of the tube.



13. Add **400 µL of isopropanol** to your new tube of the transferred liquid. Mix contents by inverting your tube several times.

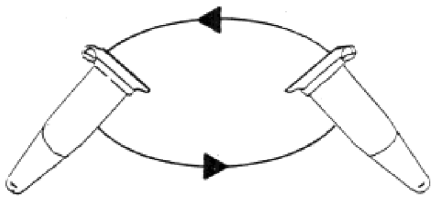
Note: The isopropanol precipitates DNA and makes it settle to the bottom of the tube.



14. Place the tube with the isopropanol mixture into a balanced centrifuge. **Centrifuge speed should be set to 10,000 x g (≈10,000 rpm).**

    VERY IMPORTANT: Orient the hinge of the tube to point outward and away from the middle of the centrifuge. Spin at top speed for **5 minutes**.

Note: Nucleic acids (DNA) will pellet at the bottom-side of the tube under the hinge during centrifugation.

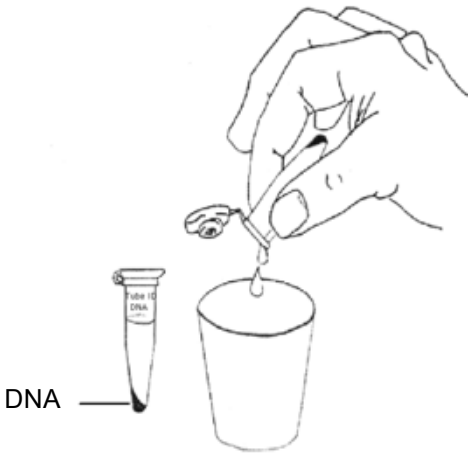


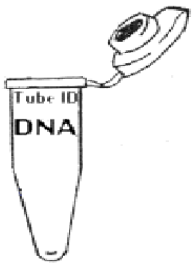
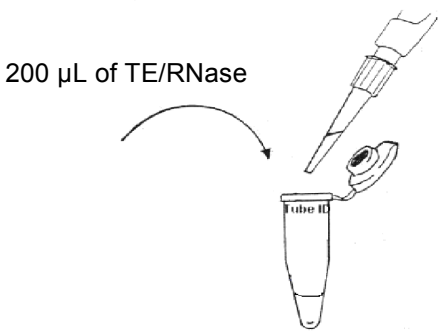
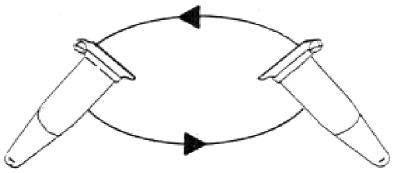
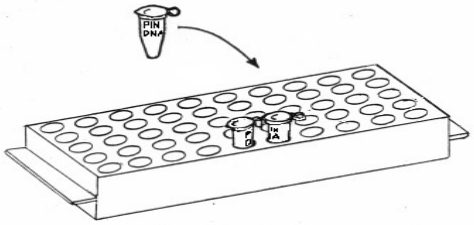
5 minutes

15. Retrieve your tube from the centrifuge. Carefully pour the liquid out of the tube. Tap the mouth of the tube lightly onto a clean paper towel to remove the liquid on the lip of the tube. Spin quickly again in the centrifuge to pool the rest of the liquid and **use a pipette to completely remove all of the supernatant**. Aim the tip **away** from the pellet to remove the liquid. DO NOT disturb the pellet.

Note: The DNA is now in the bottom of the tube. The DNA pellet **may** leave a teardrop-shaped mark or may appear as minute speckles on the hinge-side of the tube. **Do not worry if there is no visible pellet.**

With live plant tissue samples, a greenish pellet containing cellular debris will form at this step. Try not to disturb this pellet when using the pipette to remove excess liquid.



16. Air-dry the DNA pellet for **about 5-10 minutes** to evaporate any remaining isopropanol. Keep the cap open. **Note:** To speed up the evaporation process, place tubes on a heat block set at about 50-70°C. Keep caps open and monitor for evaporation. If most of the liquid has been removed from the tubes beforehand, this should take less than 5 minutes.	 ☐
17. **IMPORTANT: Use filter tips for this step to avoid contamination of the pipette barrel.** Add **200 µL of TE/RNase buffer** to your tube. Scrape the side of the tube where the pellet is (or should be) with the tip to facilitate resuspension. Pipette up and down several times to collect DNA accumulated on the area underneath the hinge. **Note:** TE buffer stabilizes the DNA. RNase destroys any isolated RNA that might interfere with the PCR reaction.	 ☐
18. Observe your DNA. If it is clear, proceed to the next step. If it is cloudy or if there is a lot of debris, add another 200 µl TE/RNase.	☐
19. After the resuspension, place your tube in a centrifuge. Balance and spin the tube for **1 minute** to pellet any particulates that did not dissolve in solution. **Note:** This is your isolated plant DNA! It may contain nucleases that can degrade the DNA at room temperature. Keep the DNA tubes on ICE to limit any leftover nuclease activity.	 1 minute ☐
20. Place your DNA tube in the class rack. Your teacher will **FREEZE your isolated DNA** until you are ready to prepare your PCR amplification. **Note:** Your plant DNA extract may contain nucleases that can degrade the DNA at room temperature. Keep the DNA tubes on ICE to limit any leftover nuclease activity.	 ☐
21. Be sure to clean microtube pestles for reuse and soak in ethanol or isopropanol to sterilize. This will reduce cross-contamination when they are used by the next class.	☐

Laboratory Exercise: Polymerase Chain Reaction

Objectives - student should be able to:

1. Explain the importance of each component of PCR and compare it to *in vivo* DNA replication.
2. Associate the temperature changes with the cycling steps of PCR.
3. Understand the concept of a duplex PCR reaction.
4. Explain the purpose of the two different sets of primers used in this duplex PCR.

The **polymerase chain reaction** (PCR) is a technique used by scientists to rapidly multiply specific segments of DNA in a small tube. Essentially, PCR reproduces some of the mechanisms of cellular DNA replication and thus has the capacity to churn out millions of copies of a targeted DNA segment. This PCR amplification is used in many aspects of science including forensics, human identification, genetic disease diagnostics, and in the cloning of rare genes. One of the reasons PCR has become such a powerful technique is that it does not require large amounts of DNA; a drop of blood at a crime scene, tiny quantities of food matter, a small leaf of a plant, or the back of a licked postage stamp usually has sufficient DNA for PCR amplification.

There are some essential reaction components and conditions needed to amplify DNA by PCR. First, it is necessary to have a sample of DNA containing the segment you wish to amplify. This DNA is called the **template** because it provides the base sequence to be duplicated during the PCR process. Along with template DNA, PCR requires two short single-stranded pieces of DNA called **primers**. These are usually about 20 bases in length and are complementary to the opposite strand of the template. Each primer is designed to sit down at one end of the target DNA segment being amplified. Primers attach (**anneal**) to their complementary sites on the template and are used as initiation sites for synthesis of new DNA strands. **Deoxynucleoside triphosphates** (dNTPs) containing the bases A, C, G, and T are also added to the reaction. The enzyme **DNA polymerase** binds to one end of each annealed primer and strings the dNTPs together to form a new DNA chain complementary to the template. The DNA polymerase enzyme requires the metal ion magnesium ($\mathbf{Mg^{++}}$) for its activity. It is supplied to the reaction in the form of $MgCl_2$ salt. A **buffer** is used to maintain an optimal active pH level for the DNA polymerase.

PCR is accomplished by cycling a reaction through several temperature steps. In the first step, the two strands of the template DNA molecule are separated, or **denatured**, by exposure to a high temperature (usually 94° to 96°C). Once in a single-stranded form, the bases of the template DNA are exposed and are free to interact with the primers. In the second step of PCR, called **annealing**, the reaction is lowered to a temperature usually between 37˚ to 65˚C. At this lower temperature, stable hydrogen bonds can form between the complementary bases of the primers and the template. Although human genomic DNA is billions of base pairs in length, the primers require only seconds to locate and anneal to their complementary sites. In the third step of PCR, called **extension**, the reaction temperature is raised to an intermediate level (65˚ to 72˚C). During this step, the DNA polymerase starts adding nucleotides to the ends of the annealed primers. These three phases are repeated over and over again, doubling the number of DNA molecules with each cycle. After 25 to 40 cycles, millions of copies of desired DNA are produced. The PCR process taken through four cycles is illustrated on the following page (Figure 1).

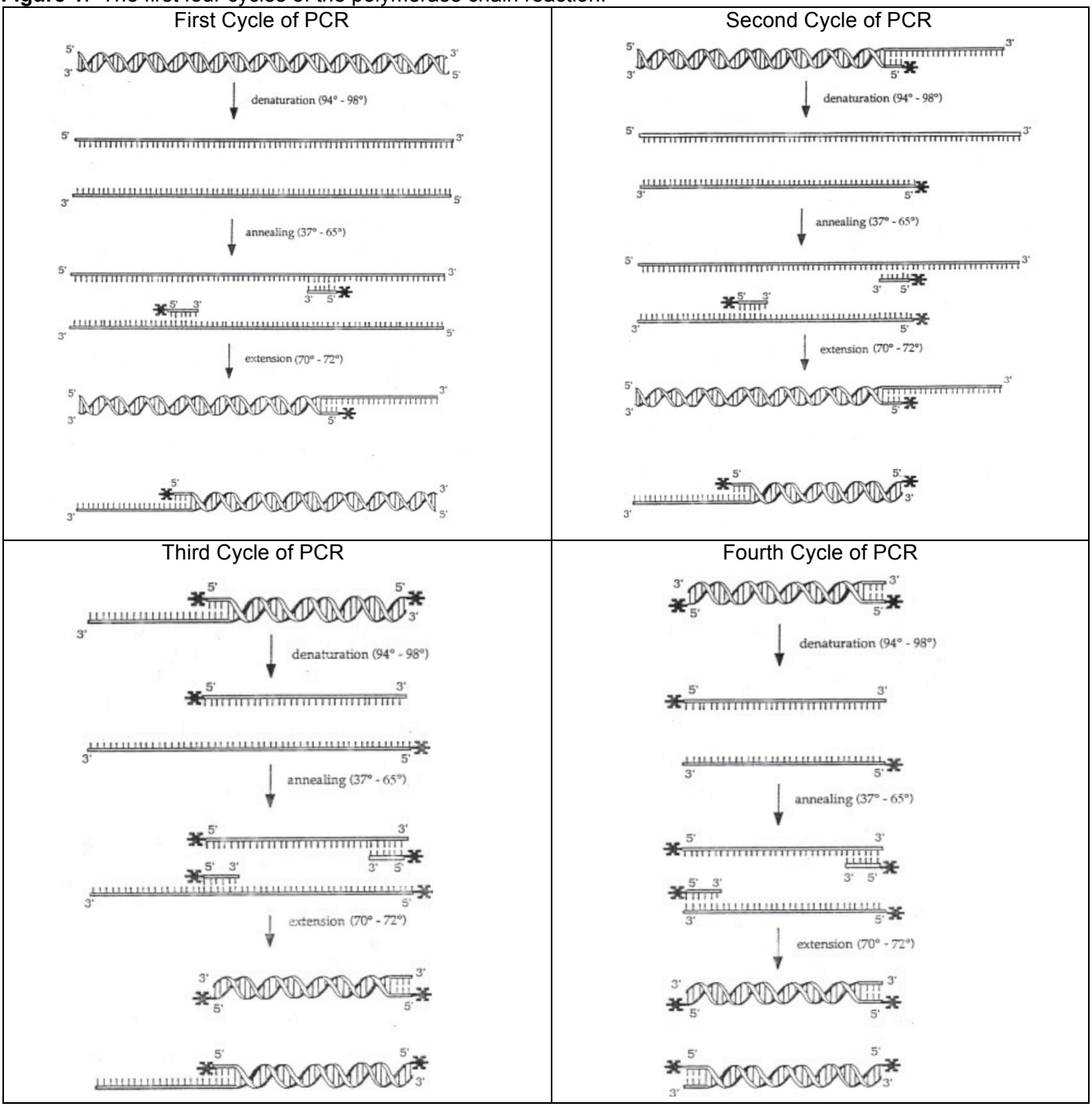
In this experiment, we will be performing a **duplex PCR.** A duplex PCR is one where there are two sets of primer pairs and two independent PCR reactions occur within the same tube. The tube contains two sets of primers for the purpose of amplifying two different gene regions. In our duplex PCR reaction, one primer set will amplify a control gene and the other set will amplify a gene that gives us information about whether the item has been genetically engineered

We will be performing two duplex PCR reactions. One will test for the presence of the control gene rbcL and the 35S promoter region (rbcL/35S). The second will test for the presence of the control gene rbcL and the Bt gene (rbcl/Bt). Although primers for the control gene rbcL will be present in both reactions, you will amplify a different GMO gene in each reaction. rbcL serves to assure us that if your sample does not contain the transgene, it was not due to a poor DNA extraction or a failed PCR reaction.

A **PCR product** is the name for the amplified section of the gene produced by the PCR reaction. The PCR product for rbcL will be 599 base pairs long and it is the largest gene that we will amplify. The 35S PCR product is 221 base pairs in length, and the Bt gene is 421 base pairs.

Illustration of the Polymerase Chain Reaction

Figure 1. The first four cycles of the polymerase chain reaction.



PCR images from Life Technologies, formerly Applied Biosystems, Inc.

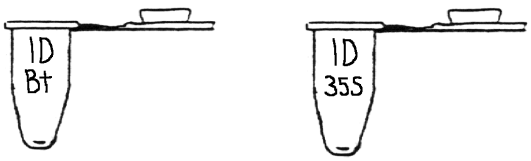
An excellent animated tutorial showing the steps of PCR is available at the DNA Learning Center web site.
http://www.dnalc.org/ddnalc/resources/pcr.htmL

Note: You will need Macromedia Flash plug-in to view this on-line and to download the animation files to you computer.

Polymerase Chain Reaction

1. Two PCR reactions will be set up for each DNA sample. Obtain two 0.2mL PCR tubes for your team. Label them with your group ID number and either Bt or 35S, just under the lip of the tube.

Note: Keep ALL tubes, samples, and reagents ON ICE until you are ready to transfer the tube to the PCR machine.



Label tubes with your ID and Bt or 35S

☐

2. Pipette **20 µL of Master Mix** into both PCR tubes.

20 µL of GMO Master Mix

☐

3. Change your pipette tip and add **20 µL of Actin/ Bt primer mix** to the Bt tube. Change tips again and add **20 µL of Actin/ 35S primer mix** to the 35S tube.

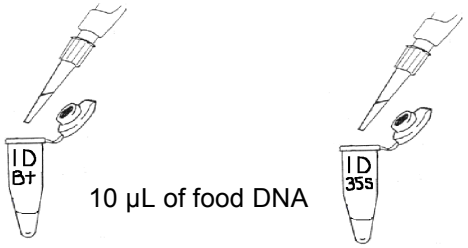
20 µL of GMO Primer Mix

☐

4. With a new tip each time add **10 µL of isolated food DNA** into both PCR tubes.

Note: Make sure that all the liquids are settled into the bottom of the tube and not on the side of the tube or in the cap. If not, you can give the tube a quick spin in the centrifuge. Do not pipette up and down, it introduces error.

What is the total volume in your PCR tube?
_____ µL



10 µL of food DNA

☐

5. **Setting up the controls:**

a) Two students will be asked to set up the positive control reactions for the class. They will use the positive control DNA provided in the kit. There should be enough PCR sample for one lane on each gel.

b) Another two students will set up negative control reactions for the whole class. They will use sterile water. There should be enough PCR sample for one lane on each gel.

Note: *The positive and negative control reactions will be for both primer sets.*

Control	Master Mix	rbcL / 35S Primer Mix	rbcL / Bt Primer Mix	Template
+	20 µL	20 µL	--	10 µL +C DNA
+	20 µL	--	20 µL	10 µL +C DNA
-	20 µL	20 µL	--	10 µL sterile H_2O
-	20 µL	--	20 µL	10 µL sterile H_2O

6. Check the volume of your PCR tubes by comparing them to a reference tube with 50 µL in it. This should be near the thermal cycler, set by your teacher.

Note: If the volume of your tube does not match, see your instructor to troubleshoot. You may need to set up a new reaction.

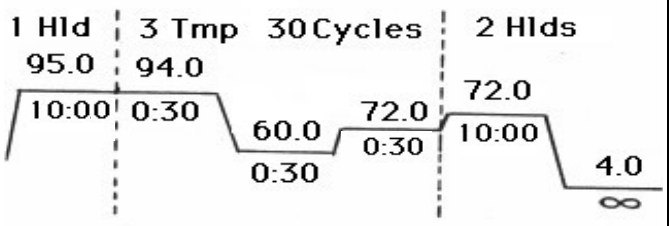
Your PCR tube Reference

☐

7. The cycling protocol for amplification is:

95°C—10 minutes
94°C—30 seconds
60°C—30 seconds
72°C—30 seconds
} 30 cycles
72°C—10 minutes
4°C—hold, ∞ infinity

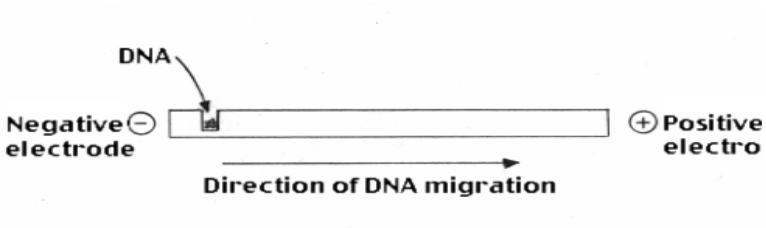
Thermal cycler Instrument displaying program parameters



Agarose Gel Electrophoresis

To determine whether or not your food item contains genetically modified elements, you will need to visualize the products of your amplification. This will be done using a process called **gel electrophoresis** in which electric current forces the migration of DNA fragments through a special gel material. Since DNA is negatively charged, it will migrate in an electric field towards the positive electrode (Figure 2). When electrophoresed through a gel, shorter fragments of DNA move at a faster rate than longer ones.

Figure 2. Side view of an agarose gel showing DNA loaded into a well and the direction of DNA fragment migration during electrophoresis.



The gel material to be used for this experiment is called **agarose,** a gelatinous substance derived from a polysaccharide in red algae. When agarose granules are placed in a buffer solution and heated to boiling temperatures, they dissolve and the solution becomes clear. A comb is placed in the casting tray to provide a mold for the gel. The agarose is allowed to cool slightly and is then poured into the casting tray. Within about 15 minutes, the agarose solidifies into an opaque gel having the look and feel of coconut Jell-O™. The gel, in its casting tray, is placed in a buffer chamber connected to a power supply and running buffer is poured into the chamber until the gel is completely submerged. The comb can then be withdrawn to form the wells into which your PCR sample will be loaded.

Loading dye is a colored, viscous liquid containing dyes (making it easy to see) and sucrose, Ficoll, or glycerol (making it dense). To a small volume of your total PCR reaction, you will add loading dye, mix and then pipet an aliquot of the mixture into one of the wells of your agarose gel. When all wells have been loaded with sample, you will switch on the power supply. The samples should be allowed to electrophorese until the dye front (either yellow or blue, depending on the dye used) is 1 to 2 cm from the bottom of the gel. The gel can then be moved, stained and photographed.

Calculations for Preparing 2% Agarose Gel

You will need a 2%, mass/volume agarose gel for electrophoresis of your PCR products. If your agarose gel casting trays holds 50 mL, then how much agarose and buffer would you need? The definition of m/v % in biology is grams (mass) / 100 mL (volume). Therefore, for 2% agarose, it will be 2 g /100 mL buffer.

Step 1: Calculate the mass of agarose needed for 50 mL total volume of agarose solution.

$$\frac{2\text{ g}}{100\text{ ml}} = \frac{X\text{ g}}{50\text{ ml}} \qquad X = 1\text{ gram}$$

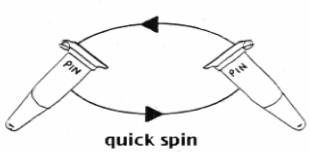
Step 2: Calculate the amount of buffer needed to bring the agarose solution to 50 mL. By standard definition, 1 gram of H_2O = 1 mL of H_2O. The amount of buffer for the 2% agarose solution will be 49 mL (50 mL – 1 mL (1 gram of agarose)).

Note: If using an Invitrogen E-Gel® (pre-cast agarose gel) and E-Gel® PowerBase™, please follow supplementary instructions located on page 21.

Electrophoresis of Amplified DNA

1. Retrieve your PCR tube and place it in a balanced configuration in a microcentrifuge. Spin it briefly (10 seconds) to bring the liquid to the bottom of the reaction tube.

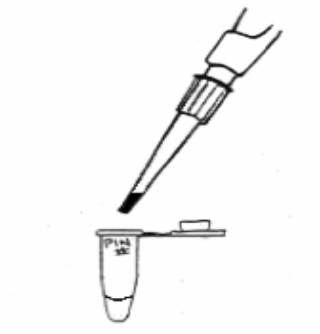
Note: Make sure the centrifuge adapters are in place before putting the tiny PCR tube into the centrifuge rotor.



2. If you are NOT performing DNA sequencing:
   Add **5 µL** of loading dye to your PCR tube.
   If you plan to sequence your DNA:
   Remove **20 µL** of your PCR sample and dispense into a new tube. Add **2 µL** of loading dye to it.

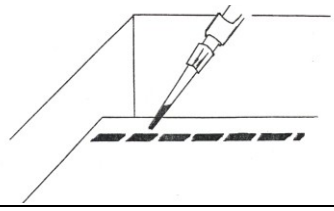
Note: your PCR sample can't contain loading dye for sequencing.

Note to Teachers:
Make sure that the loading dye mixture contains dyes that do not migrate at the same rate as your PCR amplicons, otherwise they may overlap with your bands of interest and they will be more difficult to see.



3. Carefully load **15 to 20 µL** of the DNA/loading dye mixture into a well in your gel. Make sure you keep track of what sample is being loaded into each well.

Note: Avoid poking the pipette tip through the bottom of the gel or spilling sample over the sides of the well. Use a new tip for each sample.

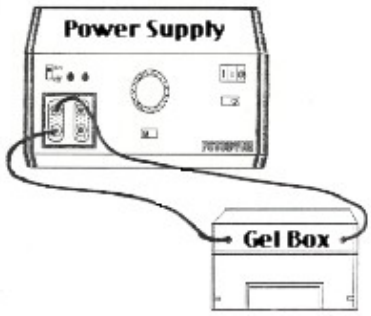


4. One student (or the instructor) should load **5-10 µL** of 100 bp ladder (molecular weight marker) into one of the wells of **each gel**.

Note to Teachers:
If you are running double-welled gels, then make sure that you add 100 bp ladders in a well in each row.

5. When all samples are loaded, attach the electrodes from the gel box to the power supply. Have your teacher check your connections and then electrophorese your samples at **150 Volts** for **25–40 minutes**.

Note to Teachers:
Keep an eye on the gels as they may heat up and begin to melt if they are run at too high a voltage. For double-combed gels, about 25 minutes is sufficient. Single-combed gels can be run longer for better resolution. Running time may vary according to gel length and is given in relation to BABEC equipment. Check manufacturer suggestions for your own equipment.



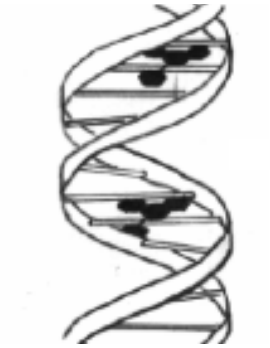
6. After electrophoresis, the gels will be ready to stain and photograph.

Note to Teachers:
If gels cannot be immediately photographed or if gel trays are needed for another class, gels can be stored in a tray covered with some buffer and bagged to prevent drying. However, the longer the gels are in buffer, the more diffuse the bands will be.

Staining and Photographing Agarose Gels

The PCR products separated on your agarose gel are invisible to the naked eye. If you look at your gel in normal room light, you will not be able to see the amplified products of your reaction. In order to "see" them, we must stain the gel with a fluorescent dye called **ethidium bromide (EtBr)**. Molecules of ethidium bromide are flat and can **intercalate,** or insert, between adjacent base pairs of double stranded DNA (Figure 3). When this interaction occurs, they take on a more ordered and regular configuration causing them to fluoresce under ultraviolet light (UV). Exposing the gel to UV light after staining, allows you to see bright, pinkish-orange bands where there is DNA (figure 4).

Figure 3. Ethidium bromide molecules intercalated between DNA base pairs.



Your teacher may stain your agarose gel and take a photograph for you so that you may analyze your GMO PCR results.

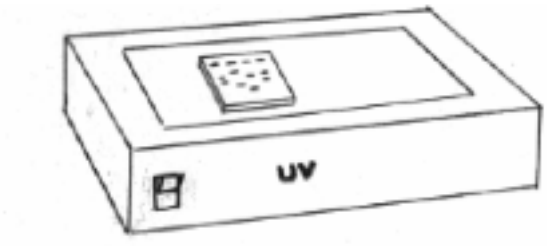
Gel staining is done as follows:

1. Place the agarose gel in a staining tray.
2. Pour enough ethidium bromide (0.5µg/ mL) to cover the gel.
3. Wait **20** minutes.
4. Pour the ethidium bromide solution back into its storage bottle.
5. Pour enough water into the staining tray to cover the gel and wait 5 minutes.
6. Pour the water out of the staining tray into a hazardous waste container and place the stained gel on a UV light box.
7. Place the camera over the gel and take a photograph.
8. Check with your district on how to dispose of hazardous waste liquid and solids.

CAUTION: Ethidium bromide is considered a carcinogen and neurotoxin. Always wear gloves and appropriate PPE (personal protective equipment) like safety glasses when handling. Students should NEVER handle EtBr.

CAUTION: Ultraviolet light can damage your eyes and skin. Always wear protective clothing and UV safety glasses when using a UV light box.

Figure 4. After staining an agarose gel with ethidium bromide, DNA bands are visible upon exposure to UV light.

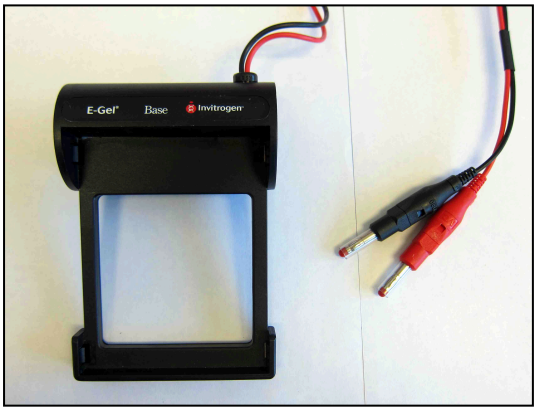


Instructions for Using Invitrogen E-Gel® and E-Gel® PowerBase™

Before getting started, determine which kind of power base you have. There are two options:

Option 1: A red E-Gel base, which is connected to a single power cable with an adaptor. It can be plugged directly into an electrical outlet.

Option 2: A black E-Gel base, which has two power cables: a red one that leads to the anode (+), and a black one that leads to the cathode (-). It needs to be plugged into a power supply.

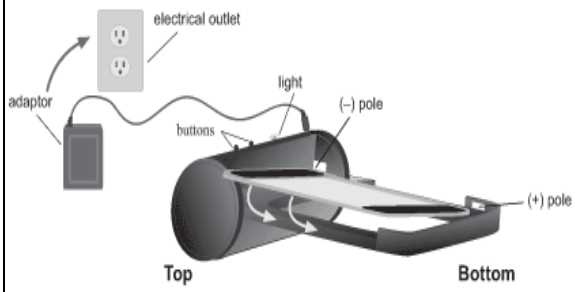


Directions for Option 1: Red E-Gel base

1. Remove E-Gel from the package and insert it with the comb in place into the base, right edge first. The Invitrogen logo should be located at the bottom of the base. Press firmly at the top and bottom to seat the E-Gel into the base.

Plug the base into an electrical outlet using the adaptor plug on the base. A steady, red light illuminates if the gel is correctly inserted. Wash hands.

Note: Wear gloves when assembling the E-Gel, which contains ethidium bromide. Small amounts of buffer may emerge from the wells during assembly.



2. Remove combs from the E-Gel cassette and dispose of as EtBr waste.

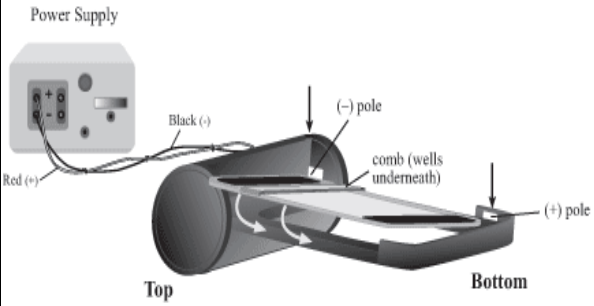
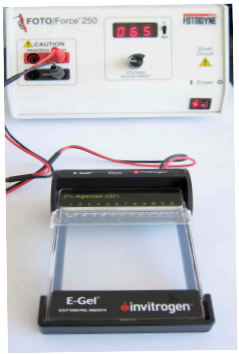
Load 20µl of DNA ladder.
Load 20µl of prepared sample into each well.
Load 20µl of water into any remaining empty wells.

Press the 30-minute button to start the run.
Light will turn green while run is in progress.
E-Gel automatically stops but will continue to beep until unit is turned off.

Note: Leaving empty wells will cause the E-Gel to run unevenly. **Remember to add water to empty wells.**

3. The run can be interrupted at any time. 20-30 minutes will be sufficient. Loading dye should move at least halfway through the length of the gel, as shown. Run progress can be checked on a transilluminator. If band separation is not complete, simply return E-Gel to base and run longer. Alternatively, entire unit can be placed under the transilluminator to check progress. The E-Gel already contains ethidium bromide. Gel staining is not necessary. At the end of the run, proceed directly to imaging the E-Gel.	

Directions for Option 2: Black E-Gel base

1. Remove E-Gel from the package and insert it with the comb in place into the base right edge first. The Invitrogen logo should be located at the bottom of the base. Press firmly at the top and bottom to seat the E-Gel into the base. Plug the two cables located on the black base into an electrophoresis power supply. The red (+) cable connects to the red slot and the black (-) cable connects into the black slot. Wash hands. Note: Wear gloves when assembling the E-Gel, which contains ethidium bromide. Small amounts of buffer may emerge from the wells during assembly.	
3. Remove combs from the E-Gel cassette. Make sure power supply is turned off. Load 20µl of DNA ladder. Load 20µl of prepared sample into each well. Load 20µl of water into any remaining empty wells. Turn on power supply and run E-Gel at 60-70 volts. **Note:** Leaving empty wells will cause the E-Gel to run unevenly.	
4. The run can be interrupted at any time. 20-30 minutes will be sufficient. Loading dye should move at least halfway through the length of the E-Gel. Run progress can be checked on a transilluminator. If band separation is not complete, simply return E-Gel to base and run longer. Alternatively, entire unit can be placed under the transilluminator to check progress. The E-Gel already contains ethidium bromide. Gel staining is not necessary. At the end of the run, proceed directly to imaging the E-Gel.	

Results

By examining the photograph of your agarose gel, you will determine whether your plant tissue or food product has been genetically modified. The primer mix you used when setting up your PCR contained two sets of primers. One set amplified the **rbcL** gene, which is present in all plants. Any sample that contains DNA should therefore produce an rbcL gene fragment of **599 bp.** The other set of primers was specific to a transgene that shows evidence of genetic modification. If your plant tissue or food product has been genetically modified in any way, it will most likely contain the **35S promoter region**. PCR amplification of this 35S site generates a **221 bp** gene fragment. The 35S region by itself does not confer a plant with any new properties, but scientists have engineered the majority of genetically modified plants with the 35S promoter region followed by a particular gene of interest. The promoter region controls the expression of the following gene. Some corn and cotton plants are specifically engineered with the *Bt* gene, which provides them with resistance to certain harmful insects. If your plant tissue or food product contains the ***Bt* gene**, your PCR reaction will reveal the presence of a Bt gene fragment at **421 bp**. Figure 5 shows the structure of three possible plant chromosomes: one which has not been genetically modified, one which contains the 35S promoter region followed by an unknown gene of interest, and one which has been genetically modified with the 35S promoter region followed by the *Bt* gene.

Figure 5. All plant cells (whether fresh tissue or in processed foods) contain the rbcL gene, so all successful DNA extractions should produce the ~400 bp band. This band is used here as an internal control. Most biotech plants contain the 35S promoter region. Regardless of the modification, samples amplified with the 35S primers will give a band that is ~162 bps. For corn that has Bt modification, a ~157 bp band will be produced in the reactions. Plants that have the Bt gene insert are resistant to certain insects.

Illustration of the amplification of the 35S fragment

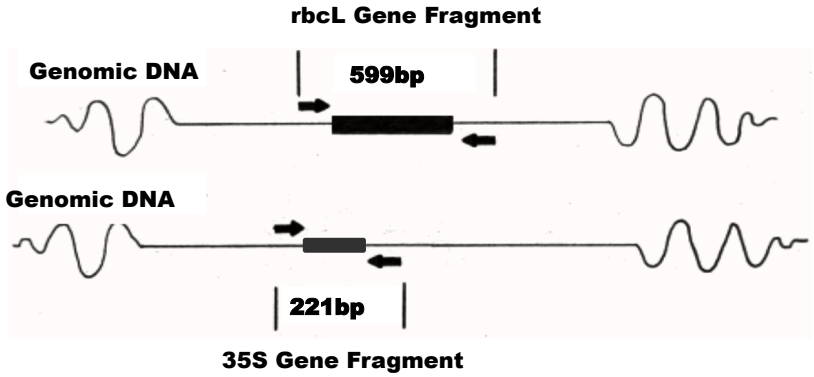
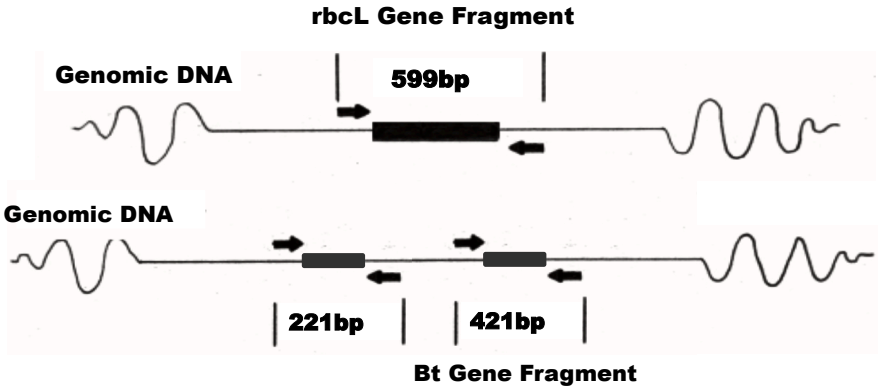


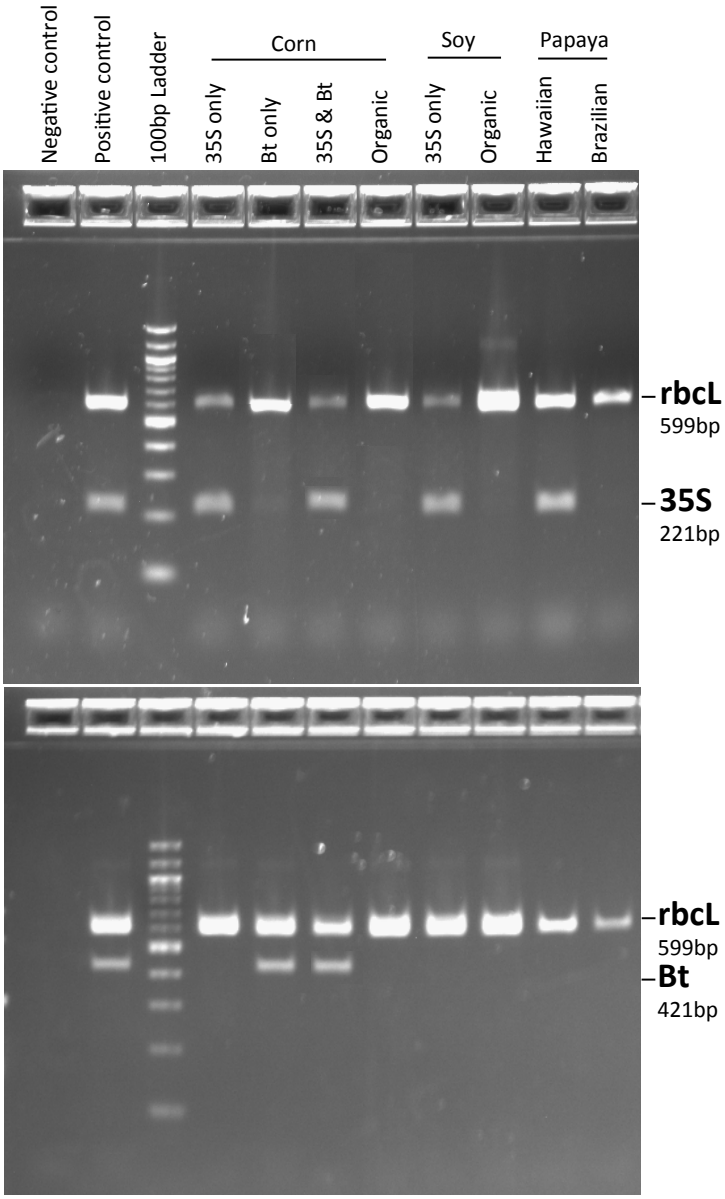
Illustration of the amplification of the Bt fragment



Results

The gel image below shows a representation of a possible experimental outcome.

- All isolated DNA samples were subjected to two separate PCR reactions: one with rbcL + 35S primers (top gel) and another with rbcL + Bt primers (bottom gel).
- rbcL (RuBisCO) is present in all plants and is used as an internal positive control for these PCR reactions. Therefore, all successful DNA isolations followed by good PCR reactions should provide rbcL bands in all samples *regardless of any genetic modification.*
- A 100 base pair ladder is loaded in the third lane and used as a size marker.
- GMO and non-GMO corn samples are tested in the next 4 lanes. All samples show the presence of rbcL. Note that when a transgene is present, the intensity of the rbcL band may be decreased. This is because there is competition for PCR reagents in a duplex PCR reaction when more than one gene is amplified.
- Lanes 8 and 9 contain GMO and non-GMO soy. Organic Soy does not contain 35S.
- The last 2 lanes are GMO and non-GMO papaya. Hawaiian papaya contains the 35S gene because the ringspot virus decimated the crops and Hawaiian papayas now contain a gene for virus resistance. The virus may not affect papaya from other regions of the world.



Things to remember when looking at your gel:

- ✓ The only crops that contain Bt are corn and cotton. If you see a Bt band for any food other than one containing corn, it is most likely a false positive result.
- ✓ Keep in mind the types of crops that have been approved for commercialization.
- ✓ Most foods contain a mixture of non-GM and GM ingredients, so it is common for the shorter transgene (Bt or 35S) band to appear less intense than the longer rbcL band. This is because there is a lower ratio of GM targets available in comparison to rbcL targets in the isolated DNA.

Genetically Modified Food Internet Resources

USDA Agricultural Research Service
http://www.ars.usda.gov/main/main.htm

Teach Food Science: food science and nutrition education curricula from the FDA
http://teachfoodscience.com/curriculum.cfm

U.S. Food and Drug Administration (FDA): food biotechnology page
http://www.fda.gov/Food/Biotechnology/default.htm

Biotechnology Industry Organization (BIO)
http://www.bio.org/

World Health Organization: 20 questions on genetically modified foods
http://www.who.int/foodsafety/publications/biotech/20questions/en/

Center for Food Safety
http://truefoodnow.org

Council for Biotechnology Information: created by leading biotechnology companies
http://whybiotech.com/

GMO Compass: information on GMOs from the EU
http://www.gmo-compass.org/

ISAAA: a nonprofit international organization that shares the benefits of crop biotechnology to resource-poor farmers in developing countries
http://www.isaaa.org/

Issues in Agricultural Biotechnology, USDA & University of Delaware
http://ag.udel.edu/agbiotech/

PBS-Harvest of Fear
http://www.pbs.org/wgbh/harvest

The Pew Initiative on Food and Biotechnology
http://pewagbiotech.org/

Union of Concerned Scientists
http://www.ucsusa.org/

Get Biotech Smart: curricula from the United Soybean Board
http://www.getbiotechsmart.com

USDA Economic Research Service: statistics about GE adoption in the US
http://www.ers.usda.gov/Data/BiotechCrops/

Selected Internet Resources on GMOs, from the University of North Texas Libraries
http://www.istl.org/10-winter/internet2.html

Information Systems for Biotechnology, USDA and Virginia Tech
http://www.nbiap.vt.edu/Default.aspx

Name_____

Date _____ Period_____

Internet Exploration – All About GMOs

The following questions are designed to help you gain an in-depth understanding of the basic principles involved in understanding genetically modified foods. Use with the web page resources to help you find the answers.

I. The What, How and Why of Genetic Modification

1. What is a GMO?

2. How is plant biotechnology SIMILAR to traditional plant breeding?

3. How is plant biotechnology DIFFERENT from traditional plant breeding?

4. Below are the three main parts of a transgene construct. What is the purpose of each part?
   a. Promoter Sequence

   b. Transgene

   c. Selectable Marker Gene

5. Describe the two main methods used to transform plants:
   a.

   b.

6. How do we know a plant cell has been transformed?

7. Once we have a transformed cell, how to we get an entire plant?

8. Describe the two most common traits used in GM crops:
   a.

   b.

II. Food of the World

1. Use the chart below to compare the percentage of GM crop acreage in the US in 2000 and 2005, and in the world:

Transgenic Crop	Percent of US acres in 2000	Percent of US acres in 2005	Percent of world acres in 2005
Biotech Soy			
Biotech Cotton			
Biotech Corn			

2. List three leading countries planting GM crops:

3. Is the increase in GMO acreage faster in developing or industrialized nations?

4. How much of our food in the US contains at least a small quantity of some crop that has been genetically engineered?

5. Use the chart below to show the four major GM crops and the foods in which you will likely find that crop:

Crop	Foods likely found in:

6. Use The True Food Network's "True Food Shopping Guide" to look up some of your favorite foods to see if they are on the GMO list. List three of them.

III. Should we or shouldn't we?

1. Describe three ways that GM crops may help to protect the environment and three ways they may harm the environment:

2. Describe three ways that GM crops may help human health and nutrition and three ways they may harm it:

3. Describe three ways that GM crops may help to feed a growing world population.

4. Describe at least three possible future transgenic crops and how they could benefit us.

5. Is it possible for antibiotic resistance genes used as markers in transgenic crops to transfer to pathogenic bacteria? Why or why not?

6. Pollen contains the genetic material of the host plant, including transgenes, if present. Why is this a concern?

7. Describe two ways to prevent the spread of transgenes via pollen.

8. In developing countries, farmers save seed from one harvest to plant for the next season. Discuss the implications of worldwide adoption of transgenic crops on these farmers.

9. Of the above benefits that you researched, which is (are) the most convincing argument(s) for GM crops?

10. In the US, we have a system for regulating transgenic crops at every stage, from research planning to field-test. Briefly describe the role of each of the following regulators:
    a. Animal and Plant Health Inspection Service (APHIS) of USDA

    b. Food and Drug Administration (FDA)Environmental Protection Agency (EPA)

    c. Environmental Protection Agency (EPA)

11. What are the requirements for labeling GM foods in the US?

12. Many countries, particularly those in Europe, oppose growing and importing genetically modified crops. Why do you think these countries' perception of GMOs is so different from the US's?

13. Describe your ethical stance on GMOs.

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