Chapter 5

PCR-Based Detection of Genetically Modified Foods

Diana L. Brandner

Madison Area Technical College
3550 Anderson St.
Madison, WI 53704
dbrandner@madison.tec.wi.us

Diana L. Brandner has been an Instructional Assistant in the Biotechnology Laboratory Technician Program at Madison Area Technical College, since 1990. She received her B.S. in Biology, Chemistry and Secondary Science Education from the University of Wisconsin-Stevens Point.

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Introduction

Synopsis

Students perform DNA isolation on food products (corn or soy / organic and nonorganic) and DNA amplification by polymerase chain reaction (PCR) on food DNA to detect the presence of genetic modification. The students will use genetically modified reference standards as controls and samples will be analyzed using agarose gel electrophoresis.

Objectives

At the end of this lab, students will be able to:

- Discuss a method for detecting genetic modification in food.
- Describe a method for isolating DNA.
- Describe a method of amplifying DNA.
- Describe a method for separating DNA by size using agarose gel electrophoresis.

Level

- High School: Advanced
- Two-year college: Intermediate, Advanced
- Four-year college: Intermediate, Advanced

Length of Lab

A suggested time allotment follows:

- Day 1 Introduction and selection of foods to be tested.
- Day 2 Isolate DNA from food. (If steps 1-5 are prepared ahead of time)
- Day 3 Set up and run polymerase chain reaction.
- Day 4 Load and run agarose gels, stain and photograph
- Day 5 Interpret results

Preparation Time Required

10-15 hours depending if all reagents are on hand.

Concept Information

Introduction

Genetically modified foods are often in the news. While genetic modifications have made improvements in many crops and helped to increase yields, many groups have raised loud protest against "tinkering" with crop plants. In the U.S., however, genetically modified foods have been introduced to the market with little fanfare. For some crops in the US over half of the acres planted are genetically modified varieties (USDA/NASS 2000). Much of the world, in contrast, has experienced strong and increasing resistance to the introduction of any genetically modified foods to the market place. The European Union and other countries require certification that foods entering their countries be Genetically Modified Organism (GMO) free or contain minimal limits.

In the fall of 2000, genetically modified foods caught the attention of the US press when it was revealed by a watchdog group that Taco Bell® brand taco shells contained a type of genetically modified corn that was not approved for human consumption by the USDA. A nationwide recall of corn products was ordered after independent verification of the earlier results. The particular corn modification in these foods was not approved, but the press largely ignored the fact that the USDA, FDA, and EPA already approve many genetically modified foods. With the availability of simple tests like this one to detect genetic modification in food products, public awareness will continue to increase.

Benefits of Genetically Modified Foods

People have been modifying crop plants since the dawn of agriculture. Year after year ancient people selected and saved seeds from plants displaying specific traits. Later, with cross breeding and the development of hybrid plants, traditional plant breeding emerged. Today modern techniques in biotechnology allow plant breeders to introduce very specific traits via particular genes into plants. Inserted genes may come from the same species of plant, from other plant species, or even from animals or bacteria.

Genetic modification of crops can produce four general benefits: 1) agricultural -- increased yield, 2) environmental -- reduced use of pesticides, herbicides, and fuel, 3) nutritional -- improved quality of foods, and 4) disease prevention -- foods that work like edible vaccines.

Agricultural benefits include new methods to improve productivity and profitability while at the same time reducing reliance on pesticides and herbicides (Table 1). Monsanto has several products that contain a gene making plants tolerant of the herbicide glyphosate (trade name Roundup®). Glyphosate kills plants by blocking the pathway for synthesizing essential amino acids (Clark 2000).

Table 1. Selected Genetically Modified Crops Currently Allowed in the US Food Supply, March 2000. (Source: US Food & Drug Administration)

Product	Company	Engineered Trait	Name & Year
Corn	Novartis	Bt toxin to control insect pests	Bt11 1996
Corn	Novartis	Bt toxin to control insect pests	Knock Out™ 1995
Corn	Dow/Mycogen	Bt toxin to control insect pests	NaureGard 1995
Corn	Monsanto/DeKalb	Bt toxin to control insect pests	Bt-Xtra 1997
Canola	Monsanto	Resist glyphosate herbicide to control weeds	Roundup® Ready 1999
Cotton	Monsanto	Bt toxin to control insect pests	Bollgard® 1995
Cotton	Monsanto	Resist glyphosate herbicide to control weeds	Roundup® Ready 1996
Papaya	Cornell Univ./ Univ. Hawaii	Resist papaya ringspot virus	Sunup, Rainbow 1997
Potato	Monsanto	Bt toxin to control insect pests	NewLeaf 1995
Soybean	Monsanto	Resist glyphosate herbicide to control weeds	Roundup® Ready 1995
Sugarbeet	Aventis	Resist glyphosate herbicide to control weeds	Name unknown 2000
Tomato	Monsanto/Calgene	Altered ripening to enhance fresh market value	FlavrSavr™ 1994

Biotech companies have modified crops by introducing a gene that produces *Bt* toxin to control insect pests in lieu of pesticide application. The source of the gene is the bacterium *Bacillus thuringiensis (Bt),* which has long been used for insect control. Organic farmers use the bacterium because it kills plant-eating insects but not beneficial insects like bees. Insects ingest the *Bt* protein, which binds to the epithelial cells of the midgut, killing the insect. Because the *Bt* protein is broken down in an acidic gut, vertebrates are not affected. *Bt* does not contaminate groundwater and is considered nontoxic to humans and livestock (Ramanujan 2000).

Scientists are working to engineer resistance to certain plant pathogens. Researchers at the University of Hawaii and Cornell University have developed two new varieties of papaya which are resistant to the papaya ringspot virus (PRSV). Papaya is Hawaii's second largest fruit crop and an important crop in the tropics everywhere. Worldwide the disease is a serious threat because it is rapidly transmitted and can quickly destroy entire plantations. The modification used in papaya is called 'pathogen derived resistance', where a gene from the pathogen is inserted to fight the pathogen itself (Tennant 1994).

Genetic modification can improve food quality. Consumers benefit by having foods available to them with increased vitamin, mineral, and nutritional content. Golden rice is an important example. The yellow colored grains are produced by rice genetically altered to make beta-carotene, a pigment the body converts to Vitamin A. Vitamin A deficiency leads to blindness and immune system impairment, especially in children. This vitamin deficiency contributes to the death of more than a million children each year in Asia, Africa, and Latin America (Rusting 2000).

Foods that can be engineered to combat human disease offer enormous advances in public health. One day, children may get immunized by eating foods such as bananas, potatoes and tomatoes. The modified plants could be grown locally at low cost eliminating problems of vaccine transport and refrigeration. Edible vaccines would not require syringes or other equipment, which often contribute to infection and disease spread upon reuse (Landridge 2000). Researchers at Loma Linda University School of Medicine have already succeeded in inserting a gene from the cholera

bacterium into potatoes. The modified potatoes produce a nontoxic component of the cholera toxin that triggers the production of antibodies against cholera when eaten (Arakawa 1998).

Finally, many modifications contribute to increasing the amount of food produced worldwide. As the world population continues to increase, it is undeniable that the problem of feeding everyone will require greater food production. Increased crop yields through genetic modification can work toward that end.

Risks of Genetically Modified Foods

Some people argue that along with the benefits of genetically modifying food come risks. Such risks may include: exposure to possible allergens and toxins, harm to the environment, antibiotic resistance, and the spread of introduced genes to non-target plants by out crossing and pollen drift (Obrycki 2001).

In November 2000 the Food and Drug Administration recalled 300 supermarket and restaurant products made with StarLink™ corn. StarLink™, produced by Aventis, Research Triangle Park, N.C., contains the gene Cry9C, which protects the plants against insect pests. The EPA had approved StarLink™ corn in 1998 with the stipulation that it was not for human consumption. Studies had shown that the Cry9C protein produced in the modified corn was heat stable and resistant to stomach acids and enzymes, all characteristics of human allergens, hence the restriction on human use. Aventis failed to keep StarLink™ corn separate from approved and nongenetically modified corn so the unapproved corn entered the market initiating the massive recall (USDA 2000).

A different problem arose for the Terra Prima organic corn chip company in Hudson, Wisconsin, in 1995. Despite strict practices by its organic corn growers, it was discovered that some of Terra Prima's Apache Tortilla chips showed traces of *Bt* corn. Genetic testing revealed that pollen from a crop of Novartis *Bt* corn planted more than a quarter-mile away had contaminated an organic corn field of one of Terra Prima's suppliers. Because of the contamination by pollen drift, Terra Prima recalled and destroyed 90,000 bags of chips, a significant monetary loss to the small company (Ramanujan 2000).

Methods for Genetically Modifying Foods

In order to genetically modify food crops, one needs a reliable means of introducing new genetic material into the host. There are three main methods for introducing foreign DNA: biological vectors (Ti-plasmid from *Agrobacterium*), physical methods (particle gun and electroporation), and chemical methods (polyethyleneglycol and calcium chloride) (Hemmer 1997).

Of the three methods, the biological vector system is used most often. This is a binary vector system. One vector contains the DNA to be transferred (the transgene) and is introduced along with the second vector, the Ti plasmid of *Agrobacterium tumefaciens*, which contains genes encoding the necessary mechanism for the genetic transfer to take place (McBride 1990).

In order for the transgene to work effectively in its new host it needs to be controlled by a promoter sequence and a terminator sequence. This grouping is called a gene cassette (Figure 1). Many potential promoter elements have been identified, but the most commonly used is the CaMV35S promoter derived from the phytopathogenic cauliflower mosaic virus (Spoth 2000). The NOS terminator from the Ti plasmid in *Agrobacterium tumefaciens* is the most common terminator.

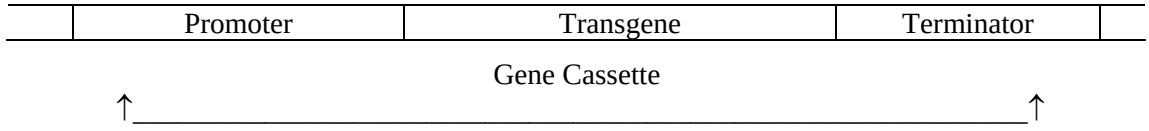

Figure 1. A gene cassette.

Regulation of Genetically Modified Foods

The U.S. government works to ensure new agricultural biotechnology products are safe for animal and human health and safe for the environment (USDA 2000). Three agencies are involved: the Food and Drug Administration (FDA), Environmental Protection Agency (EPA), and United States Department of Agriculture (USDA)/ Animal and Plant Health Inspection Service (APHIS).

Under FDA regulations, companies are legally obliged to ensure that any food product meets the safety standards of the law. This applies equally to both conventional and genetically modified food. The FDA will pull products off the market if they do not meet safety standards (USDA 2000). The EPA approves new herbicides and pesticides (USDA 2000). Companies wishing to field test or move biotechnology-derived plants must obtain APHIS approval under USDA regulations. An APHIS "determination of non-regulated status" must be obtained by companies before a crop can be produced on a wide scale and sold commercially (USDA 2000). Over 5000 field trials have been conducted since 1987 with APHIS approval. About 40 GMO products have met all federal regulatory requirements and are sold commercially (USDA 2000).

In other countries regulations for GMO foods vary. Some countries have adopted labeling regulations. Such regulations necessitate appropriate techniques to identify the presence or absence of genetic modification so food can be labeled properly (Zimmermann 1998). Many countries and communities are developing and standardizing methods for the detection of genetically modified foods.

In the U.S. the USDA Grain Inspection, Packers and Stockyards Administration (GIPSA) is establishing a biotech reference laboratory in Kansas City, MO. The reference lab will evaluate and verify analytical techniques for the detection of genetically enhanced grains and grain products. The lab will also accredit independent analytical laboratories that test for the presence of genetic modification. This new reference lab will alleviate testing problems that occur because currently there is no standardization of reference materials, sampling methods, or extraction procedures for detection tests (USDA/ERS 2000).

Detection of Genetically Modified Foods

Several companies in the United States and Europe test foods for genetic modification. Methods vary for the different companies and countries, but there are two basic means for detecting genetic modification. One method tests food for the product of the transgene, usually a protein. The other method tests for the presence of DNA from the transgene or another portion of the gene cassette.

Proteins are assayed using an ELISA (Enzyme Linked ImmunoSorbent Assay). In the test an enzyme is linked to an antibody bound to the protein, which then reacts with a colored substrate

enabling detection of a specific protein. ELISAs usually cost less than DNA tests, offer quicker results, and can sometimes be done on site. The big drawback is that ELISAs do not work well on processed foods because heat during processing can destroy the protein. In contrast, DNA tests are more expensive, cannot be done on site, and take several hours to complete. Importantly, however, DNA tests are very accurate, work on processed foods, and can be quantified.

About This Lab

This lab has three parts: 1) isolation of DNA from food, 2) PCR, and 3) visualization of the results using agarose gel electrophoresis. The lab uses a polymerase chain reaction (PCR) test to detect foreign DNA in genetically modified food, and it uses primers that are designed to amplify DNA from the CaMV35S promotor component of the gene cassette. Amplification of this promoter yields DNA fragments 195 base pairs (bp) in length.

Since almost all transgenic crops contain the CaMV35S promoter it offers a good target for DNA testing. A positive test for this promoter is not always conclusive, however. Plants in the cabbage family should be treated carefully because these plants may be naturally infected with the *Caulimovirus*, the source of the CaMV35S promoter. In such cases further PCR tests should be run with primers designed to amplify the specific transgene DNA. For plants from other families the risk of infection by the *Caulimovirus* is very small (DG JRC 1998).

The following lab activity has been modified for use in the pre-college or college classroom. The method of DNA isolation and PCR are based on currently validated tests used in the European Union (DG JRC 1998). This lab is designed to be used with corn products and certified reference standard maize powder from the Institute for Reference Materials and Measurements. Efforts have been made to keep costs to a minimum, however, PCR lab activities are generally not considered to be shoestring. This activity assumes the instructor has previous experience and knowledge of PCR and the necessary preparation involved. Instructors should allow several lab periods to complete this lab activity.

Materials

Equipment

- Micropipettors (100-1000 µL, 10-100 µL, and 0.5-10 µL)
- Analytical balance
- Autoclave
- pH meter
- Spatulas
- Thermometer
- 55-60º C incubator
- 70ºC waterbath or dri bath
- Timers
- Vortexer
- Microcentrifuge 14,000 x g
- Minifuge
- Microcentrifuge tube racks
- Vac-Man® Laboratory Vacuum Manifold (Promega A7231)
- Vacuum line or vacuum pump
- Lab markers
- Thermalcycler
- Horizontal gel electrophoresis boxes
- Gel electrophoresis power supplies
- UV transilluminator
- Staining trays
- Camera
- Assorted beakers, graduated cylinders and storage bottles
- Stir bars
- Magnetic stirplate
- Hotplate or microwave oven
- Heat-proof gloves
- Refrigerator
- Freezer
- Goggles or safety glasses

Materials and Consumable Lab Supplies

- Institute for Reference Materials and Measurements (IRMM)-certified reference standards Fluka Biochemika 63194 Maize Powder MZ-Set available from Sigma
- Dried food products containing corn meal or soy flower examples include: corn meal, corn flour, corn chips, soy flour, organic corn meal, or organic soy flour.
- Microcentrifuge tubes 1.5-mL (sterile)
- Sterile sticks (Fisher 01-340)
- Nuclease free water (Promega P1193)
- Weigh boats
- Aerosol resistant pipet tips (100 - 1000 µL, 10- 100 µL, and 0.5 - 10 µL)
- Tris-HCl (Sigma T 5941)
- NaCl (Sigma S 9888)
- EDTA (disodium salt) (Sigma E1644)
- SDS (Sigma l 4509)
- Guanidine-HCl (Sigma G 9284)
- Proteinase K (Promega V3021)
- Labeling tape
- 3-ml syringes (sterile)
- Wizard® minicolumns (Promega A7211)
- Wizard® resin (Promega A7181)
- Isopropanol (Sigma I 9516)
- PCR primers CaMV35S Promoter
- Amersham Pharmacia Ready-To-Go™ PCR beads (27-9555-01)
- Sterile mineral oil (Sigma M 5904)
- 4% precast agarose gels (BioWhittaker Molecular Applications 54926)
- TBE electrophoresis buffer (Sigma T 4415)
- PCR Marker, 50-2000 bp (Novagen 69278-3)
- Blue/Orange Loading Dye, 6X (Promega G1881)
- Deionized or distilled water
- Film
- Ethidium bromide 10 mg/mL (Sigma E 1510)
- Gloves
- Lab coats or aprons

Equipment and Materials for Each Team of Four Students

Equipment

- Micropipettors (100 - 1000 µL, 10 - 100 µL, and 0.5 - 10 µL)
- Timer
- Microcentrifuge tube rack
- Lab marker
- Horizontal gel electrophoresis box
- Staining tray
- Goggles or safety glasses

Materials and Consumable Lab Supplies

- Certified references for the positive and 0% control
- Food products (4)
- Microcentrifuge tubes, 1.5-mL (sterile) several
- Sterile sticks 1/food sample
- 4 mL nuclease free water
- Weigh boats
- Aerosol resistant pipet tips (100 - 1000 µL, 10 - 100 µL, and 0.5 - 10 µL)
- 6 mL extraction buffer
- 700 µL Guanidine-HCl
- 300 µL Proteinase K
- Labeling tape
- 3-mL syringes (6)
- Wizard® minicolumns (6)
- 6 mL Wizard® resin
- 12 mL 80% Isopropanol
- 7 µL of each PCR primer
- Amersham Pharmacia Ready-To-Go™ PCR beads (7)
- TBE electrophoresis buffer

- Agarose
- 6 µL PCR Marker, 50-2000bp
- 40 µL Blue/Orange Loading Dye, 6X
- Gloves, several pair per student
- Lab coat or apron, 1 per student

Notes for Instructor

Directions for Setting Up the Lab

Solution Preparation

- *Extraction buffer*
  10 mM Tris-HCl at pH 7.5
  150 mM NaCl
  2mM EDTA
  1% SDS
  Store at room temperature for a maximum of three months.

- *5M Guanidine-HCl*
  Add 80 mL of distilled water to 47.8 g of guanidine hydrochloride in a flask, and stir until completely dissolved. BTV to 100 mL with distilled water and autoclave. Store at room temperature for a maximum of three months.

- *20mg/mL Proteinase K*
  Add 5 mL nuclease water to 100 mg Proteinase K in a sterile tube or flask. Aliquot and store at -20ºC for a maximum of six months.

- *80% Isopropanol*
  80 mL of 100% isopropanol
  20 mL sterile distilled water
  Makes 100 mL

- *Oligonucleotide Primer Sequences*
  Primers are available from several companies and prices vary.
  Dilute primers to a final concentration of 25 pmol/µL in nuclease free water. Store in freezer for one year.
  35S Promotor
  Sense: 5’ **GCT CCT ACA AAT GCC ATC A** 3’
  Antisense: 5’ **GAT AGT GGG ATT GTG CGT CA** 3’

- *TBE electrophoresis buffer*
  Follow manufacturer directions for 1X solution.

- *PCR Marker*
  Use 6 µL per gel lane. Store in refrigerator.

- *Ethidium Bromide stain 1 µg/mL*

Add 50 µL of 10 mg/mL ethidium bromide to 500 mL of deionized or distilled water. Store in unbreakable opaque bottles at room temperature. Label bottle Caution: Ethidium Bromide

Safety Procedures

- All laboratory procedures should be conducted with gloves, goggles, and aprons.
- Wash hands before and at the conclusion of the lab.
- If the power is on, electrical shock may result from touching the buffer or electrophoresis equipment.
- Never leave the electrophoresis power unit on without supervision. There is a risk of fire if the buffer leaks out or if the buffer should evaporate completely during electrophoresis.
- Never leave stirplates or hotplates on without supervision.
- Be sure that students are familiar with the operating instructions and safety precautions before they use any centrifuge.
- Use caution with hot liquids and glassware. Wear heat-proof gloves to move hot glassware and metals.
- Ethidium bromide is a mutagen and cancer-suspect agent. Wear gloves when handling the stain and stained gels. Designate an ethidium stain work area. Dispose of stain properly (see Micklos and Freyer 1990, pp. 256-257).
- Check MSDS (Material Safety Data Sheets) for all chemicals and reagents in the lab before preparing and running the lab.
- Never look at an unshielded UV light source. Wear a full face UV shield and cover all exposed skin if your UV light source is unshielded.

Teaching Tips

- For help determining how to reconstitute your oligoneucleotide primer sequences. See the following web sites: *www.lifetech.com* TechOnLine, FAQs – Calculations for Custom Oligos or *www.genosys.com* Custom Oligos, FAQ – You And Your Oligos
- If you don't use the Amersham Pharmacia Ready-To-Go™ PCR beads prepare the following PCR master mix. Note that the primer concentrations are different.

	Single PCR (µL)	10 Reactions (µL)
DNA template	5	--
Primer 1 (50 pmol/µL)	1	10
Primer 2 (50 pmol/µL)	1	10
10X Reaction Buffer	10	100
$MgCl_2$ (25 mM Solution)	6	60
PCR Nucleotide Mix (10 mM)	2	20
Taq DNA Polymerase (5 u/µL)	0.5	5
Nuclease Free Water	74.5	745
Total Volume	100	950

- *Thermalcycler Profile for PCR Using CaMV35S*
  Denaturation 3 min/94˚C, amplification 20 sec/94˚C, 40 sec/54˚C, 60 sec/72˚C, for 40 cycles and a final extension of 3 min/72˚C. Forty cycles takes approximately 2.5 hr to complete with the Perkin Elmer Thermalcycler 480.
- Crush food products like corn chips before the lab so they are easier to weigh.
- If you don't have access to a vacuum source. There is an alternative to the Vac-Man® Laboratory Vacuum Manifold. Use 3-mL syringes and plungers instead.
- If you can't use ethidium bromide stain, alternatives include *Carolina*BLU™ DNA Stain, Ward's DNA Stain and Edvotek® DNA InstaStain™. When using a stain with less sensitivity than ethidium bromide, load more of the DNA sample into the gel wells.
- For information on the disposal of ethidium bromide stain consult Micklos (1990) or Horn (1993).
- On day two the instructor may need to prepare the food samples ahead of time for steps 1-5 of the DNA Isolation portion, if class time is limited.
- Use aerosol resistant pipet tips for all pipetting to prevent cross contamination between samples.
- Highly processed foods contain less and poorer quality DNA than less processed foods.
- The Institute for Reference Materials and Measurements (IRMM)-certified reference standards Maize Powder MZ-Set contains at least 1 g of each four control standards: 0%, 0.1%, 0.5% and 2% GMO dry powder.
- Blue/Orange Loading Dye, 6X is a convenient marker dye containing orange G, bromophenol blue and xylene cyanol. The dye is provided in a premixed ready to use form. Store at –20˚C.
- A few minutes after the power supply is turned on and current is applied to the gel, the loading dye should be seen moving toward the positive electrode end of the gel box. The Blue/Orange loading dye will eventually separate into three band colors. The yellowish orange band is orange G. The purplish band is bromophenol blue and the aqua colored band is xylene cyanol. Bromophenol blue dye migrates at approximately the same rate as a DNA fragment of 300 bp and xylene cyanol migrates at approximately the same rate as a DNA fragment of 9000 bp.
- When aliquoting small amounts of liquid for students, each aliquot should contain slightly more than will be required for the lab. To get the contents of the tube in the bottom of the tube, tap the tube on the counter top or centrifuge for a couple seconds.
- The instructor may want to consider dispensing the primers into each student's sample tube due to the very small quantity.
- Prepare negative control samples for the PCR portion of the lab. A negative control is a check on the reagents used in this lab activity. Prepare the sample exactly the same as the others but do not add DNA. Make up the volume difference with water. If a PCR product is present after the amplification, DNA has contaminated the reagents.
- The PCR Marker consists of eight fragments that range in size from 50 – 2000 bp.
- Have students make a chart of all the samples being tested and controls to record the results of the class.

Further Reading

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Student Team Logistics

- Organize the class into teams of 3-4 students.
  - Have each team bring in four food samples for testing. Have each team prepare one 2% positive control sample and one 0% control sample.

Pedagogical Information

The following is a chart of concepts covered in this lab and student misconceptions of the concepts:

Correct Concept	Misconception
No band on the gel may indicate that: • -no genetic modification has taken place. • -the technique used in the genetic modification was not detected by this detection method. • -the amount of the food sample wasn't big enough to detect genetic modification. • -the procedure wasn't working properly.	No band on the gel indicates that the food product has not been genetically modified.
The detection method used in this lab can indicate whether genetic modification utilized the 35S promoter.	The detection method used in this lab can indicate whether genetic modification of any kind has taken place and what gene has been used.

Student Outline

Protocol

DNA Isolation

1. Wearing gloves select your food sample or control sample and weigh out 0.1 g of food and place it into a disposable microcentrifuge tube.
2 Add 200 µL of nuclease free water to the tube.
3. Use a sterile stick to homogenize the food sample to a smooth slurry.
4. Add 860 µL of extraction buffer, 100 µL of 5M Guanidine-HCl and 40 µL of 20 mg/mL Proteinase K to the tube containing the homogenate. Vortex tube.
5. Incubate at 55-60ºC for 3 hours with intermittent mixing.
6. Allow samples to cool at room temperature for 10 minutes.
7. Centrifuge 10 minutes at 14,000 x g in a microcentrifuge.

8. For each sample, attach one labeled 3-mL syringe barrel to the Luer-Lok® extension of a Wizard® minicolumn and attach this minicolumn/syringe barrel assembly to the Vac-Man® Laboratory Vacuum Manifold.
9. Check to ensure all stopcocks are closed before proceeding.
10. Add 1mL of Wizard® resin to each minicolumn/syringe assembly.
11. Carefully remove 300 µL of the cleared supernatant from each sample and transfer it to the barrel of the minicolumn/syringe assembly containing the Wizard® resin.
12. Open the stopcocks and apply a vacuum to pull the resin/supernatant mix into the minicolumn. When the entire sample has passed through the column, close the stopcock and turn off the vacuum. In this step the DNA will stick to the column.
13. Add 2 mL of 80% isopropanol to each minicolumn and reapply the vacuum to draw the solution through the minicolumn. This step washes the column.
14. Remove the syringe barrel and transfer the minicolumn to a 1.5 mL microcentrifuge tube. Centrifuge the minicolumn at 10,000 x g in a microcentrifuge for 2 minutes to remove any residual alcohol.
15. Transfer the minicolumn to a new microcentrifuge tube, add 50 µL of 70ºC nuclease free water to the column and allow it to interact with the resin for 1 minute. This step elutes the DNA from the column.
16. Elute the DNA by centrifugation at 10,000 x g for 1 minute in a microcentrifuge.
17. You may stop here and store the DNA in the refrigerator for about a week. For longer periods, store it in the freezer.

PCR DNA Amplification

1. Wearing gloves obtain one Ready-To-Go™ PCR bead in a 0.5 mL tube for each sample to be tested. Check that the bead in each tube is visible at the bottom of the tube. Label the tube and place it in a rack. See Setting Up Reactions (Table 2).

Table 2. Setting Up Reactions

For each group:

Tube #	Tube w/bead	DNA	Primer mix	H_2O	Mineral Oil	Gel Lane #
Sample #1	✓	5 µL	2 µL	18 µL	1 drop	
Sample #2	✓	5 µL	2 µL	18 µL	1 drop	
Sample #3	✓	5 µL	2 µL	18 µL	1 drop	
Sample #4	✓	5 µL	2 µL	18 µL	1 drop	
0% Standard	✓	5 µL	2 µL	18 µL	1 drop	
2% Standard	✓	5 µL	2 µL	18 µL	1 drop	
Neg. Control = **NO DNA**	✓	0 µL	2 µL	23 µL	1 drop	

2. Add 5 µL of template DNA to the tube. Change pipet tips between samples.
3. Add 2 µL of primer mix to the tube.
4. Add 18 µL of nuclease free water. Cap the tube and gently vortex, then centrifuge briefly to collect the contents at the bottom of the tube. The total volume in the tube should be 25 µL.
5. Overlay the reaction with 1 drop of mineral oil, if it is required for your thermalcycler.
6. Place your tube in the thermal cycler and start the reaction. While you are waiting for the amplification reaction you may begin to prepare your agarose gel for analysis. When the

reaction is complete. You may stop and freeze the reaction tubes or continue with gel electrophoresis.

Agarose Gel Electrophoresis Analysis

1. Wearing gloves obtain one 4% agarose TBE gel with eight wells, a gel box containing TBE 1X buffer, and a power supply.
2. Add 5 µL loading dye to the tubes with your PCR samples, standards and negative control.
3. Load 15 µL of each sample, standard and negative control into separate wells of your gel, avoid mixing mineral oil with the sample. Save a lane for the PCR marker on each gel. Record where each sample is located on the gel.
4. Add 1µL loading dye to your PCR marker. Load the marker into one well of the gel.
5. Attach the gel box to the power supply, turn the power on, and set to 100 -150 volts. Electrophorese for 40 - 60 minutes or until the bromophenol blue band has traveled one-third the length of the gel. Volt settings and time will vary with different equipment.
6. Wearing gloves, carefully remove the gel and put it into a staining tray. Cover the gel with ethidium bromide stain and stain for 5 - 10 minutes.
7. After staining, decant the ethidium bromide stain from the staining tray back into the storage bottle.
8. Rinse the gel with tap water, in the tray, for several minutes to remove background ethidium bromide stain from the gel.
9. View on ultraviolet transilluminator and photograph. Record your results and share with all other groups.

Data Analysis and Interpretation

- Share a copy of your results with all the other groups.
- Are any bands in the 180 -195 bp range?
- How many lanes show PCR bands?
- Does the 0% control standard show a PCR band? Should it? Why?
- Does your negative control show a PCR band? Should it? Why?
- Does your positive control standard show a PCR band? Should it? Why?
- Can you see the PCR Marker ladder? How many bands should there be? Are all the bands visible?
- Do your organic food samples show a PCR band? If they do what does this mean?

Expected Results

No DNA fragments should appear in the negative control lane except bands at or below 50bp in length are artifacts of the primers. Bands of 195bp indicate presence of the CaMV35S promoter. No DNA fragments of 195bp should appear in the 0% control standard lane. A 195bp fragment will appear in the 2% control standard lane. Student samples may or may not show a band at 195bp. Theoretically, organic food products should be free of genetically modified foods. However, organic definitions vary. If a product indicates that it is 100% organic, there should be no band. Non-organic corn products will probably contain detectable levels of genetically modified corn (Figure 2).

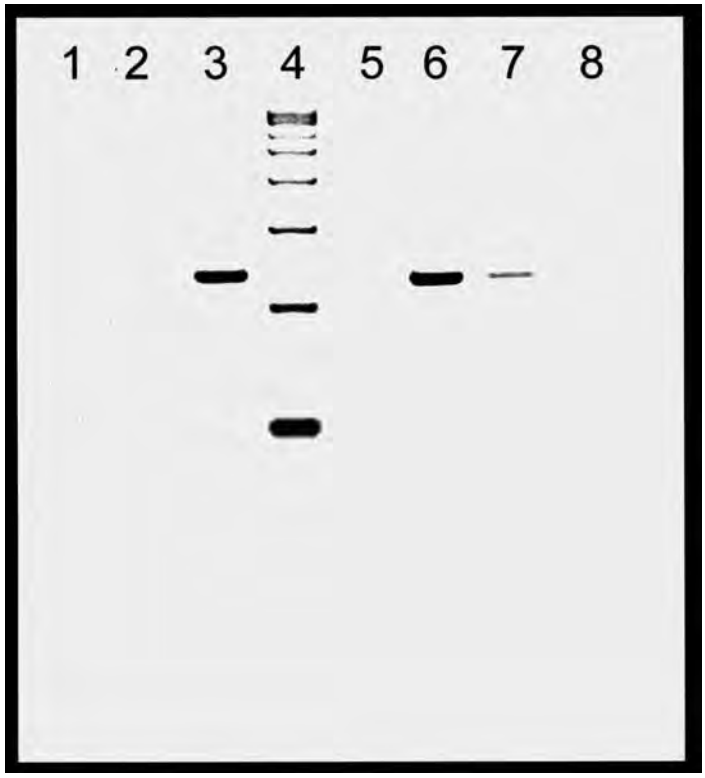

Figure 2. Expected electrophoresis results.

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Appendix A

Reagent and Equipment Vendors

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www.promega.com
www.vwrsp.com
www.lifetech.com
www.novagen.com
www.edvotek.com
www.apbiotech.com
www.fishersci.com
www.nabt.org
www.genosys.com
www.carolina.com
www.wardsci.com