Edvo-Kit #962

Identification of Genetically Modified Foods Using PCR

Experiment Objective:

The objective of this experiment is to utilize PCR to identify genetically modified foods.

See page 3 for storage instructions.

IMPORTANT NOTE:
The PCR cycling conditions and electrophoresis buffer have changed. Please review the literature before performing the experiment.
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Safety Data Sheets can be found on our website: [www.edvotek.com/Safety-Data-Sheets](http://www.edvotek.com/Safety-Data-Sheets)
## Experiment Components

<table>
<thead>
<tr>
<th>Components</th>
<th>Storage</th>
<th>Check (✓)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A PCR EdvoBeads™</td>
<td>Room Temp.</td>
<td></td>
</tr>
<tr>
<td>Each PCR EdvoBead™ contains:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• dNTP Mixture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Taq DNA Polymerase Buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Taq DNA Polymerase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• MgCl₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B GMO Primer mix concentrate</td>
<td>-20° C Freezer</td>
<td></td>
</tr>
<tr>
<td>C 100 base pair ladder</td>
<td>-20° C Freezer</td>
<td></td>
</tr>
<tr>
<td>D GMO Positive PCR Control concentrate</td>
<td>-20° C Freezer</td>
<td></td>
</tr>
<tr>
<td>E DNA Resuspension Buffer</td>
<td>-20° C Freezer</td>
<td></td>
</tr>
<tr>
<td>F NaCl Solution</td>
<td>-20° C Freezer</td>
<td></td>
</tr>
<tr>
<td>G DNA Extraction Buffer</td>
<td>-20° C Freezer</td>
<td></td>
</tr>
<tr>
<td>H Proteinase K</td>
<td>-20° C Freezer, desiccated</td>
<td></td>
</tr>
</tbody>
</table>

### REAGENTS & SUPPLIES

Store all components below at room temperature.

<table>
<thead>
<tr>
<th>Component</th>
<th>Check (✓)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Certified Non-GMO Pine nuts (Negative PCR Control)</td>
<td></td>
</tr>
<tr>
<td>• UltraSpec-Agarose™</td>
<td></td>
</tr>
<tr>
<td>• TBE Electrophoresis Buffer Powder</td>
<td></td>
</tr>
<tr>
<td>• 10x Gel Loading Solution</td>
<td></td>
</tr>
<tr>
<td>• InstaStain® Ethidium Bromide</td>
<td></td>
</tr>
<tr>
<td>• 0.2 ml PCR Tubes</td>
<td></td>
</tr>
<tr>
<td>• Plastic Pestles</td>
<td></td>
</tr>
<tr>
<td>• Transfer Pipets</td>
<td></td>
</tr>
<tr>
<td>• Wax Beads (for thermal cyclers without heated lid)</td>
<td></td>
</tr>
<tr>
<td>• Snap-top Microcentrifuge Tubes</td>
<td></td>
</tr>
</tbody>
</table>

This experiment is designed for 25 reactions.

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Sample volumes are very small. For liquid samples, it is important to quick spin the tube contents in a microcentrifuge to obtain sufficient volume for pipeting. Spin samples for 10-20 seconds at maximum speed.
Requirements

- Recommended foodstuffs that have worked well in the EDVOTEK testing laboratory include: corn bread, corn meal, and papaya. See page 18 for more information.
- Thermal cycler (EDVOTEK Cat. #541 highly recommended)
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- Waterbath 56° C (EDVOTEK Cat. #539 highly recommended)
- UV Transilluminator or UV Photodocumentation system (use for staining with InstaStain® Ethidium Bromide)
- UV safety goggles
- Automatic micropipets (5-50 µl) with tips
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Disposable vinyl or latex laboratory gloves
- Ice buckets and ice
- Distilled or deionized water
- Isopropanol
- 70% Ethanol
Background Information

**WHAT ARE GENETICALLY MODIFIED ORGANISMS?**

Over the past one hundred years, genetic research has expanded our understanding of the genome (the hereditary material of an organism encoded by its DNA) and its role as a blueprint for all processes within an organism. Variations in the DNA sequence, called mutations, can cause changes in the way an organism interacts with its environment. Most mutations result in negative effects for the organism; occasionally, a mutation grants an organism an advantage that promotes survival in its particular environment.

Humans have long recognized and taken advantage of genetic variation through traditional plant and animal husbandry techniques. For centuries, selective breeding and conventional hybridization have been used to increase crops’ yields or give rise to other desirable qualities. For example, the corn we eat today was produced by artificial selection. Ages ago, farmers may have noticed that one plant was producing larger kernels, while another plant was producing more flavorful corn (Figure 1A). By crossing the two plants, these farmers encouraged those traits (observable characteristics) in the next generation. This allowed farmers to grow plants that yielded the best possible product — a plant with many ears of large, delicious kernels of corn (Figure 1B). In this way, over the last fifty years—a period during which the world population more than doubled, but farmland only increased by 10%—selective breeding and new agricultural technologies have allowed food yields to increase 25% per person!

While it used to take years of selective breeding to produce the genomic changes necessary to give rise to such desirable traits, modern biotechnology techniques have accelerated this pace. Genetic engineering now allows scientists to directly manipulate a DNA sequence to generate desirable traits. These engineered genes, called transgenes, can be inserted, deleted or mutated in a matter of weeks using recombinant DNA technology. To be properly expressed in vivo, a transgene must include a promoter sequence, which recruits RNA polymerase to the transgene for transcription, and a terminator sequence, which signals RNA polymerase to end transcription (Figure 2).

Many technologies have been developed to create transgenic plants. One example is the biolistic (or “gene gun”) method. Using this approach, the transgene DNA is adhered to gold particles and then shot through the walls of plant embryonic stem cells. After the treatment, researchers screen the cells for markers that show the transgene has been incorporated into the plant genome. The transgenic stem cells are used to create mature plants. Another method relies on a natural plant parasite (*Agrobacterium tumefaciens*) that transfers some of its own genetic material into plant cells as the Ti-plasmid. Genetic engineers use recombinant DNA technology to replace a bacterial...
gene with a gene of interest. The bacteria transfer the transgene to the host as part of the Ti-plasmid, thus creating a transgenic plant. This technique works best with plants like tomatoes, apples and soybeans.

Some of the first genetically modified plants simplified pest management for farmers. Bacillus thuringiensis, a gram-positive bacterium, had long been used in organic farming for its insecticidal proteins. The bacterial spores needed to be sprayed on the crops several times during the growing season to protect the plants from insect damage. To simplify this treatment, scientists used several of the bacterial genes to create “Bt-corn”, a crop that expresses the insecticidal proteins to protect the plant from pest damage (Figure 3). This technology allows farmers to use less chemical pesticides, some of which are harmful to people and to the environment.

Current plant biotechnology also promises to increase the quality of food crops. One application of biotechnology to food science is antisense RNA technology, which can be used to decrease food spoilage. This technique takes advantage of the natural process of RNA silencing to reduce expression levels of naturally occurring genes. For example, the enzyme polygalacturonase (PG) digests pectin in the tomato’s cell wall, making the fruit softer and more susceptible to fungal infections. The Flavr Savr tomato has been engineered to “turn off” production of the PG enzyme, slowing the softening process and increasing the tomato’s shelf life.

Genetic engineering technology can also enhance food crops by introducing additional nutrients. Rice, a staple food in many developing countries, does not provide the recommended dietary allowance of several key nutrients including vitamin A. In children, vitamin A deficiency (VAD) is the leading cause of preventable blindness. VAD may also increase the risk and frequency of infections. To address this problem, rice has been modified to produce β-carotene, a precursor of vitamin A. Switching to cultivation of “golden rice” and other nutrient-supplemented crops in these areas represents major progress in combating malnutrition.

Besides boosting crop yields and enhancing nutrition, transgenic technology could create allergy-free peanuts and low protein rice for people with kidney disease. Genetically modified foods may soon allow for the synthesis and delivery of various pharmaceutical products. In “pharming”, transgenic plants make proteins with medicinal value like insulin or growth hormones. “Farmaceuticals” can be produced in many crops, including tobacco, carrots, tomatoes, soybeans and rice. Many times, these transgenes are engineered into the chloroplast DNA, which maintains high protein expression levels and is not distributed via pollen. This will generate great amounts of pharmaceuticals with little chance of horizontal gene transfer to surrounding plants.

ETHICS OF GENETICALLY MODIFIED ORGANISMS

Tomatoes, soybeans and corn were among the first genetically modified food products approved by U.S. agencies in the 1990s. Since then, the safety, efficacy and benefits of GM foods have been debated at a global level. Proponents of GM technology cite studies showing improved quantity and quality of plants, decreasing costs for growers, and benefits for the environment. Critics of GM technology fear the spread of transgenes to other crops, increased allergens, and the creation of unanticipated dangers to people and the environment. Many studies of GMOs and related technologies have been published in leading peer-reviewed science journals like Nature and Science. In 2016, the National Academies of Science, Engineering, and Medicine surveyed 20 years of scientific literature to address concerns about GM technology (available online http://nas-sites.org/ge-crops/). While the findings show that current GM technology is safe, it urges caution when introducing any new crop, conventional or GM.
Figure 4: Polymerase Chain Reaction
The responsibility of public health and policy concerning GMOs rests on the shoulders of the government and the biotechnology industry. The United States government has carefully monitored GM food production and created strict regulations to protect the health of Americans. There are three federal agencies in the United States that oversee food safety: the Federal Drug Administration (FDA) monitors the safety of human and animal food products; the U.S. Department of Agriculture (USDA) oversees new plant varieties and their use in farming; and the Environmental Protection Agency (EPA) monitors pesticide levels in plants and determines what is acceptable for human consumption. To gain acceptance, the plant biotechnology industry must communicate the research and development of new GM food products to these agencies.

**USING PCR TO IDENTIFY GMOS**

To determine whether food products have been genetically engineered, DNA is extracted from the samples and analyzed using the Polymerase Chain Reaction (PCR). This technique has revolutionized biological research because it quickly creates many copies of a specific region of DNA *in vitro*. This is accomplished by using short synthetic DNA molecules (primers) to target specific DNA sequences. In this way, PCR can differentiate between wild-type plants and those that have been genetically engineered by amplifying specific DNA sequences common in GM organisms. The first set of primers target the promoter from the Cauliflower Mosaic Virus (CaMV). Genetic engineers commonly use this promoter because it is recognized by transcriptional machinery of many species of plants. A second set of primers target an insecticide gene isolated from *B. thuringiensis* (*cry1F*). This gene is found in corn, cotton, and sometimes soy. As a positive control for DNA extraction, a plant chloroplast gene is also amplified.

To perform PCR, purified double-stranded DNA is mixed with primers, a thermostable DNA polymerase (*Taq*) and nucleotides (Figure 4). First, the mixture is heated to 94° C to denature the DNA duplex (i.e., unzip it into single strands). Next, the sample is cooled to 45° C-60° C, allowing the primers to base pair with the target DNA sequence (called “annealing”). Lastly, the temperature is raised to 72° C, the optimal temperature at which *Taq* polymerase will extend the primer to synthesize a new strand of DNA. Each “PCR cycle” (denaturation, annealing, extension) doubles the amount of the target DNA in less than five minutes (Figure 4). In order to produce enough DNA for analysis, twenty to forty cycles may be required. To simplify this process, a specialized machine, called a “thermal cycler” or a “PCR machine”, was created to heat and cool the samples rapidly.

After PCR is performed, the samples will contain DNA fragments of different lengths depending upon whether the sample contains genetically modified plants. To analyze the mixture of DNA fragments, scientists use a technique called agarose gel electrophoresis, which separates DNA fragments according to size. The mixture of DNA molecules is added into depressions (or “wells”) within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode (Figure 5A).

At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete “bands” within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA (Figure 5B).
EXPERIMENT OBJECTIVE:

The objective of this experiment is to utilize PCR to identify genetically modified foods.

LABORATORY SAFETY:

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

• Wear gloves and goggles while working in the laboratory.
• Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
• Wear protective gloves when working with hot reagents like melted agarose.
• DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
• Always wash hands thoroughly with soap and water after working in the laboratory.
• Be sure to properly dispose of any biological samples according to your institutional guidelines.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you’ll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

• Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
• Predict the results of your experiment.

During the Experiment:

• Record your observations.

After the Experiment:

• Interpret the results – does your data support or contradict your hypothesis?
• If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.
Module I: Isolation of DNA from Food

1. **GATHER** the food sample to be tested. **SMASH** a small amount of food on a clean piece of paper or in a weight boat.
2. **TRANSFER** enough of the smashed food to a microcentrifuge tube to reach the 0.1 mL mark. **LABEL** the tube with your initials or group number.
3. **ADD** 400 µL of DNA extraction buffer to the food sample.
4. Using a micropestle, **GRIND** the food sample until no large pieces remain (approx. 30 seconds).
5. **VORTEX** or flick the tubes for 30 seconds to mix the sample.
6. **INCUBATE** the samples at 56°C for 15 minutes.
7. **ADD** 300 µL of NaCl solution to the tube and mix well by vortexing or flicking for 30 seconds.
8. **CENTRIFUGE** the food sample at full speed for 5 minutes.
9. Carefully **TRANSFER** the supernatant into a fresh, labeled microcentrifuge, tube being careful not to disturb the pellet. **DISCARD** the tubes with pellets.
10. **PRECIPITATE** the DNA from the supernatant by adding an equal volume of room-temperature isopropanol to the tube. **MIX** well.
11. **INCUBATE** the tubes at room temperature for 5 minutes.

**OPTIONAL STOPPING POINT:** Store the tube of precipitated DNA and isopropanol at -20°C if it will not be used immediately.
12. **INSERT** the tube in the rotor so that the hinge is facing towards the outside edge. **CENTRIFUGE** the sample at full speed for 5 minutes.

13. After centrifugation, a very small DNA pellet should be visible at the bottom of the hinge side of the tube. **CIRCLE** the location of the DNA pellet with a marker.

14. Carefully **REMOVE** and **DISCARD** the supernatant, leaving the pelleted DNA at the bottom of the tube.

15. **WASH** the pellet by slowly adding 500 µl of 70% Ethanol to the tube.

16. **CENTRIFUGE** the sample at full speed for 2 minutes.

17. Carefully **REMOVE** and **DISCARD** the supernatant and allow the DNA pellet to dry for at least 5 minutes. The pellet should be completely dry.

18. Completely **RESUSPEND** the pellet in 200 µl of DNA Resuspension Buffer (E) by pipetting up and down several times or by vortexing vigorously.

19. **PLACE** tubes in ice. **PROCEED** to Module II: PCR Amplification.

**OPTIONAL STOPPING POINT:** DNA can be stored at -20° C if it will not be used immediately.
Module II: PCR Amplification

1. **LABEL** a 0.2 ml PCR tube with the sample and your initials.

2. **ADD** 20 µl GMO primer mix, 5 µl extracted DNA (or control DNA) and the PCR EdvoBead™ to the labeled 0.2 ml tube. At least one control reaction should be performed per class to confirm that PCR was successful.

3. **MIX** the PCR sample. Make sure the PCR EdvoBead™ is completely dissolved.

4. **CENTRIFUGE** the sample for a few seconds to collect the sample at the bottom of the tube.

5. **AMPLIFY** DNA using PCR
   **PCR cycling conditions:**
   - Initial denaturation 94°C for 5 minutes
   - 94°C for 60 seconds
   - 58°C for 60 seconds
   - 72°C for 60 seconds
   - Final Extension 72°C for 10 minutes
   - 35 cycles

6. After PCR, **ADD** 5 µl of 10x Gel Loading Solution to the sample. **PLACE** tubes on ice. **PROCEED** to Module III: Separation of PCR Products by Electrophoresis.

**OPTIONAL STOPPING POINT:**
The PCR samples may be stored at -20°C for electrophoresis at a later time.

**NOTES AND REMINDERS:**
This kit includes enough DNA for 25 reactions. At least one positive and one negative control reaction should be performed per class to confirm that PCR was successful.

If your thermal cycler does not have a heated lid, it is necessary to overlay the PCR reaction with wax to prevent evaporation. See Appendix B for guidelines.

**NOTE:**
**PCR Cycling Conditions** have changed. Please review your PCR program before performing the experiment.
Module III: Separation of PCR Products by Electrophoresis

1. **MIX** agarose powder with 1X TBE Electrophoresis Buffer in a 250 ml flask (see Table A).

2. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).

3. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.

4. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.

5. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.

6. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

---

**Table A**

<table>
<thead>
<tr>
<th>Size of Gel Casting Tray</th>
<th>Amount of Agarose (g)</th>
<th>1X TBE Electrophoresis Buffer (ml)</th>
<th>TOTAL Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7 cm</td>
<td>0.5 g</td>
<td>25 ml</td>
<td>25 ml</td>
</tr>
<tr>
<td>7 x 14 cm</td>
<td>1.0 g</td>
<td>50 ml</td>
<td>50 ml</td>
</tr>
</tbody>
</table>
Module III: Separation of PCR Products by Electrophoresis

7. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X TBE Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.

8. **LOAD** the entire sample into the well. **RECORD** the position of the samples in Table 1, below.

9. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.

10. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). For best results, the blue tracking dye should migrate at least 4 cm from the wells.

11. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to **STAINING** the agarose gel.

---

**Table 1: Sample Table**

<table>
<thead>
<tr>
<th>Lane</th>
<th>Recommended</th>
<th>Sample Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 base pair Ladder</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>GMO Negative Control</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>GMO Positive Control</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Soy Beans Extraction</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Cornbread Extraction</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Papaya Extraction</td>
<td></td>
</tr>
</tbody>
</table>

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**Table B**

<table>
<thead>
<tr>
<th>EDVOTEK Model #</th>
<th>Total Volume Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+</td>
<td>300 ml</td>
</tr>
<tr>
<td>M12</td>
<td>400 ml</td>
</tr>
<tr>
<td>M36</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**Table C**

<table>
<thead>
<tr>
<th>Volts</th>
<th>Time &amp; Voltage Guidelines (2.0% Agarose Gels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>Time: 7 x 7 cm gel ~4.0 cm migration</td>
</tr>
<tr>
<td>125</td>
<td>75 min.</td>
</tr>
<tr>
<td>150</td>
<td>40 min.</td>
</tr>
<tr>
<td></td>
<td>30 min.</td>
</tr>
</tbody>
</table>
Module IV: Staining Agarose Gels with InstaStain® Ethidium Bromide

1. Carefully REMOVE the agarose gel and casting tray from the electrophoresis chamber. SLIDE the gel off of the casting tray on to a piece of plastic wrap on a flat surface. **DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.**

2. MOISTEN the gel with a few drops of electrophoresis buffer.

3. Wearing gloves, REMOVE and DISCARD the clear plastic protective sheet from the unprinted side of the InstaStain® card(s). PLACE the unprinted side of the InstaStain® Ethidium Bromide card(s) on the gel. You will need 2 cards to stain a 7 x 14 cm gel.

4. With a gloved hand, REMOVE air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.

5. PLACE the casting tray on top of the gel/card stack. PLACE a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Ethidium Bromide card is in direct contact with the gel surface. STAIN the gel for at least 3-5 minutes. For best results, the gel should be stained for 15 minutes.

6. REMOVE the InstaStain® Ethidium Bromide card(s). VISUALIZE the gel using a mid-range ultraviolet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background.

**BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!**
Study Questions

1. How are gene guns used in plant genetics? Describe how this technique could be used to create Bt-corn.

2. What are common potential concerns about foodstuffs obtained from GM plants?

3. Which Federal agencies are responsible for oversight on GM plants and foods?

4. What genetic modifications were made to the Flavr Savr tomato? How does this modification affect the tomato?

5. List and describe the three steps of PCR. How can PCR be used to detect genetically modified organisms?
Instructor's Guide

OVERVIEW OF INSTRUCTOR’S PRELAB PREPARATION:

This section outlines the recommended prelab preparations and approximate time requirement to complete each prelab activity.

<table>
<thead>
<tr>
<th>Preparation For:</th>
<th>What to do:</th>
<th>When:</th>
<th>Time Required:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Module I: Isolation of DNA from Food</strong></td>
<td>Prepare and aliquot various reagents</td>
<td>Up to one day before performing the experiment. IMPORTANT: Prepare the DNA Extraction Buffer no more than one hour before performing the experiment.</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>Equilibrate waterbath to 56° C.</td>
<td>One hour before performing the experiment.</td>
<td>5 min.</td>
</tr>
<tr>
<td><strong>Module II: PCR Amplification</strong></td>
<td>Prepare and aliquot various reagents (Primer, DNA template, ladder, etc.)</td>
<td>One day to 30 min. before performing the experiment.</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>Program Thermal Cycler</td>
<td>Anytime before performing the experiment.</td>
<td>15 min.</td>
</tr>
<tr>
<td><strong>Module III: Agarose Gel Electrophoresis</strong></td>
<td>Prepare diluted TBE buffer</td>
<td>Up to one day before performing the experiment.</td>
<td>45 min.</td>
</tr>
<tr>
<td></td>
<td>Prepare molten agarose and pour gel</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Module IV: Staining Agarose Gels</strong></td>
<td>Prepare staining components</td>
<td>Anytime before performing the experiment.</td>
<td>5 min.</td>
</tr>
</tbody>
</table>

**NOTE:**
We recommend students work in pairs, with each student extracting DNA from their own sample.

**NOTE:**
The PCR cycling conditions and electrophoresis buffer have changed. Please review the literature before performing the experiment.
Pre-Lab Preparations

MODULE I: ISOLATION OF DNA FROM FOOD

If a precipitate has formed in the DNA extraction buffer, warm at 37° C to redissolve.

NOTE: PREPARE DNA EXTRACTION BUFFER NO MORE THAN ONE HOUR BEFORE PERFORMING THE EXPERIMENT.

1. Add 200 µl of DNA Extraction Buffer (G) to each tube of Proteinase K (H) and allow the pellets to hydrate for a couple of minutes. Add the dissolved Proteinase K back to the remaining DNA Extraction Buffer and mix. Aliquot 1 ml for each pair and keep tubes on ice.

2. Aliquot 800 µl of NaCl solution (F) for each pair.

3. Aliquot 800 µl of DNA Resuspension Buffer (E) for each pair.

4. Save the remaining DNA Resuspension Buffer for preparing the GMO Primer Mix and Control DNA. The isopropanol and 70% ethanol can be placed at a classroom pipetting station for students to share.

<table>
<thead>
<tr>
<th>Recommended Foods</th>
<th>Not Recommended</th>
</tr>
</thead>
</table>
| • Fresh or frozen corn  
• Fresh or frozen papaya  
• Fresh or frozen lime beans  
• Corn bread  
• Pine nuts  
• Corn meal  
• Cake mix  
• Soy beans  
• Soy protein-based foods (veggie burgers, hot dogs, soy flour) | • Oatmeal  
• Potato chips  
• Corn chips  
• Breakfast cereals |

NOTE:
We recommend students work in pairs, with each student extracting DNA from their own sample.

FOR MODULE I
Each Pair should receive:
• 1 ml DNA Extraction Buffer  
• 800 µl NaCl solution  
• 800 µl DNA Resuspension Buffer  
• 2 microcentrifuge tubes with pestles  
• 2 1.5 ml microcentrifuge tubes with snap tops  
• Isopropanol  
• 70% Ethanol
Pre-Lab Preparations

MODULE II: PCR AMPLIFICATION

Preparation of the GMO Primer Mix

1. Thaw the GMO Primer Mix Concentrate (B) on ice.
2. Add 1 ml of DNA Resuspension Buffer (E) to the tube of GMO Primer Mix Concentrate. Cap tube and mix.
3. Aliquot 50 µl of the diluted GMO Primer Mix into labeled microcentrifuge tubes.
4. Distribute one tube of diluted GMO Primer Mix to each student pair.

Preparation of the PCR Controls

1. Thaw the tubes of GMO Positive PCR Control Concentrate (D) on ice.
2. Add 20 µl of DNA Resuspension Buffer (E) to the Control DNA Concentrate tube. Pipet up and down to mix.
3. Dispense 6 µl of the diluted Control DNA for each positive control reaction. Certified non-GMO pine nuts have been included as DNA extraction and PCR negative controls. One pine nut should be used for each negative control DNA extraction. At least one positive control and one negative control should be performed per class to confirm that the PCR was successful.

NOTE:
Negative control samples must be prepared carefully to prevent cross-contamination from positive GMO samples.

Additional Materials:
Each student should receive one PCR EdvoBead™ and one PCR tube. Groups receiving the control DNA should receive an extra PCR tube and PCR EdvoBead™. Alternatively, the instructor can prepare the additional control samples and distribute to students after PCR.

PCR Amplification

The Thermal cycler should be programmed as outlined in Module II in the Student’s Experimental Procedure.

• Accurate temperatures and cycle times are critical. A pre-run for one cycle (takes approximately 3 to 5 min.) is recommended to check that the thermal cycler is properly programmed.

• For thermal cyclers that do not have a heated lid, it is necessary to place a layer of wax above the PCR reactions in the microcentrifuge tubes to prevent evaporation. See Appendix B for instructions.
Pre-Lab Preparations

**MODULE III: SEPARATION OF PCR PRODUCTS BY ELECTROPHORESIS**

**Preparation of Agarose Gels**

This experiment requires one 2.0% gel per 4-5 students, depending upon which control samples are run on the gel. **A 7 x 7 cm gel is recommended.** You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

**Preparation of TBE Electrophoresis Buffer**

For this experiment, we recommend preparing the 1X TBE Electrophoresis Buffer in bulk for sharing by the class. Unused diluted buffer can be used at a later time.

1. Measure 3.7 L of distilled or deionized water and place in a large vessel. **(NOTE: If using purchased water in a gallon jug, remove and discard 80 mL water.)**
2. Add the entire amount of TBE Electrophoresis Buffer powder to the vessel and mix well.
3. Label the vessel as "1X TBE Electrophoresis Buffer".
4. Use within 60 days of preparation.

**Individual Gel Preparation:**

Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module III in the Student’s Experimental Procedure. Students will need 1X TBE Electrophoresis Buffer, distilled water and agarose powder.

**Batch Gel Preparation:**

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. Electrophoresis buffer can also be prepared in bulk. See Appendix C.

**Preparing Gels in Advance:**

Gels may be prepared ahead and stored for later use. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks.

Do not freeze gels at -20°C as freezing will destroy the gels.

Gels that have been removed from their trays for storage should be “anchored” back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

**Additional Materials:**

Each 2.0% gel should be loaded with the 100 base pair ladder and PCR reactions from 3 or 4 foods.

- Aliquot 30 µl of the 100 base pair ladder (C) into labeled microcentrifuge tubes and distribute one tube of EdvoQuick™ DNA ladder per gel.
Pre-Lab Preparations

MODULE IV: STAINING AGAROSE GELS

InstaStain® Ethidium Bromide

InstaStain® Ethidium Bromide provides the sensitivity of ethidium bromide while minimizing the volume of liquid waste generated by staining and destaining a gel. An agarose gel stained with InstaStain® Ethidium Bromide is ready for visualization in as little as 3 minutes! Each InstaStain® card will stain 49 cm² of gel (7 x 7 cm).

Use a mid-range ultraviolet transilluminator (Cat. #558) to visualize gels stained with InstaStain® Ethidium Bromide. BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!

- Standard DNA markers should be visible after staining even if other DNA samples are faint or absent. If bands appear faint, repeat staining with a fresh InstaStain card for an additional 3-5 min. If markers are not visible, troubleshoot for problems with electrophoretic separation.
- Ethidium bromide is a listed mutagen. Wear gloves and protective eyewear when using this product. UV protective eyewear is required for visualization with a UV transilluminator.
- InstaStain® Ethidium Bromide cards and stained gels should be discarded using institutional guidelines for solid chemical waste.

Photodocumentation of DNA (Optional)

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

SYBR® Safe DNA Staining (Optional)

This fast, easy staining protocol incorporates SYBR® Safe into the molten agarose before the gel is poured into the casting tray. This means that the DNA is staining while the electrophoresis experiment is running!

Results can be visualized immediately post electrophoresis with a mid-range ultraviolet transilluminator (Cat. #558) or a TruBlu™ Blue Light Transilluminator (Cat #557).

Find more information on SYBR® Safe DNA stain at www.edvotek.com/608.
Experiment Results and Analysis

In this experiment, results will vary depending upon the type of genetic modification (if any) in the food source chosen by the student(s). Successful genomic DNA purification from foodstuffs can have a significant impact on the PCR amplification and gel electrophoresis results. Poor results and quality of extracted genomic DNA can be caused by an unsuccessful extraction attempt. For optimal DNA preparation, particular attention should be paid to the extraction process as described in the protocol.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 base pair Ladder</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>GMO Negative Control</td>
<td>500 bp plant chloroplast</td>
</tr>
<tr>
<td>3</td>
<td>GMO Positive Control</td>
<td>500 bp plant chloroplast, 200 bp CMV 35s, and 125 bp Cry1F</td>
</tr>
<tr>
<td>4</td>
<td>Soy Beans Extraction</td>
<td>500 bp plant chloroplast and 200 bp CMV 35s</td>
</tr>
<tr>
<td>5</td>
<td>Corn Bread Extraction</td>
<td>500 bp plant chloroplast, 200 bp CMV 35s, and 125 bp Cry1F</td>
</tr>
<tr>
<td>6</td>
<td>Papaya Extraction</td>
<td>500 bp plant chloroplast and 125 bp Cry1F</td>
</tr>
</tbody>
</table>

* Note:
Depending on the PCR conditions used, a diffuse, small-molecular weight band, known as a "primer dimer", may be present below the 100 bp marker. This is a PCR artifact and can be ignored. Other minor bands may also appear due to nonspecific primer binding and the subsequent amplification of these sequences.
Please refer to the kit insert for the Answers to Study Questions
Appendices

A  EDVOTEK® Troubleshooting Guide
B  Preparation and Handling of PCR Samples With Wax
C  Bulk Preparation of Agarose Gels

Safety Data Sheets:
Now available for your convenient download on www.edvotek.com/Safety-Data-Sheets
## DNA EXTRACTION

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor DNA Extraction</td>
<td>Samples not mixed or smashed well enough during extraction</td>
<td>In addition to flicking the tube, vortex or pipet up and down to mix the sample.</td>
</tr>
<tr>
<td></td>
<td>Proteinase K inactive because it was prepared too far in advance.</td>
<td>Prepare Proteinase K within one hour of use.</td>
</tr>
<tr>
<td></td>
<td>Water baths not at proper temperature</td>
<td>Use a thermometer to confirm water bath set point.</td>
</tr>
<tr>
<td></td>
<td>Not enough DNA</td>
<td>Extracting DNA from food products can be challenging. Refer to page 18 for food sample recommendations.</td>
</tr>
<tr>
<td></td>
<td>Too much sample during extraction</td>
<td>Use only enough sample to reach the 0.1 mL mark in the microcentrifuge tube.</td>
</tr>
<tr>
<td>The extracted DNA is very cloudy.</td>
<td>Cellular debris from pellet transferred to tube</td>
<td>Centrifuge sample again and move supernatant to a fresh tube. Take care to avoid pellet.</td>
</tr>
<tr>
<td></td>
<td>Cellular debris not separated from supernatant</td>
<td>Centrifuge sample again. If possible, centrifuge at a higher speed. Move cleared supernatant to a fresh tube.</td>
</tr>
</tbody>
</table>
## Appendix A

### EDVOTEK® Troubleshooting Guides

### PCR AND ELECTROPHORESIS

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>There is very little liquid left in tube after PCR</td>
<td>Sample has evaporated</td>
<td>Make sure the heated lid reaches the appropriate temperature.</td>
</tr>
<tr>
<td></td>
<td>Pipetting error</td>
<td>Make sure students pipet 20 µL primer mix and 5 µL extracted DNA into the 0.2 mL tube.</td>
</tr>
</tbody>
</table>

| The ladder, control DNA, and student PCR products are not visible on the gel. | The gel was not prepared properly. | Ensure that the electrophoresis buffer was correctly diluted. | Gels of higher concentration (> 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of "clumps" and glassy granules before pouring gels. | The proper buffer was not used for gel preparation. Make sure to use 1x Electrophoresis Buffer. |
| | The gel was not stained properly. | Repeat staining. | |
| | Malfunctioning electrophoresis unit or power source. | Contact the manufacturer of the electrophoresis unit or power source. | |

| After staining the gel, the DNA bands are faint. | The gel was not stained for a sufficient period of time. | Repeat staining protocol. |
| | Student DNA sample was not concentrated enough. | Poor DNA extraction. Repeat Module I (Isolation of DNA from food). |
| | Student DNA sample was degraded. | If DNA is not used right after extraction, store sample at -20°C. |
| | Wrong volumes of DNA and primer added to PCR reaction. | Practice using micropipets |

| Some student samples have more/less amplification than others. | Concentration of DNA varies by sample. | There is an inherent variability in the extraction process. |
| | | |

| Low molecular weight band in PCR samples | Primer dimer | Low concentration of extracted DNA in PCR reaction. |

| DNA bands were not resolved. | To ensure adequate separation, make sure the tracking dye migrates at least 3.5 cm on 7 x 7 cm gels and 6 cm on 7 x 14 cm gels. | Be sure to run the gel the appropriate distance before staining and visualizing the DNA. |
Appendix B

Preparation and Handling of PCR Samples with Wax

**ONLY For Thermal Cyclers WITHOUT Heated Lids, or Manual PCR Using Three Waterbaths**

Using a wax overlay on reaction components prevents evaporation during the PCR process.

**How to Prepare a Wax overlay**

1. Add PCR components to the 0.2 ml PCR Tube as outlined in Module II.
2. Centrifuge at full speed for five seconds to collect sample at bottom of the tube.
3. Using clean forceps, add one wax bead to the PCR tube.
4. Place samples in PCR machine and proceed with Module II.

**Preparing PCR Samples for Electrophoresis**

1. After PCR is completed, melt the wax overlay by heating the sample at 94° C for three minutes or until the wax melts.
2. Using a clean pipette, remove as much overlay wax as possible.
3. Allow the remaining wax to solidify.
4. Use a pipette tip to puncture the thin layer of remaining wax. Using a fresh pipette tip, remove the PCR product and transfer to a new tube.
5. Add 5 µl of 10x Gel Loading Buffer to the sample. Proceed to Module III to perform electrophoresis.
Appendix C

Bulk Preparation of Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

BULK ELECTROPHORESIS BUFFER

For this experiment, we recommend preparing the 1X TBE Electrophoresis Buffer in bulk for sharing by the class. Unused diluted buffer can be used at a later time.

1. Measure 3.7 L of distilled or deionized water and place in a large vessel. *(NOTE: If using purchased water in a gallon jug, remove and discard 80 mL water.)*

2. Add the entire amount of TBE Electrophoresis Buffer powder to the vessel and mix well.

3. Label the vessel as "1X TBE Electrophoresis Buffer".

4. Use within 60 days of preparation.

BATCH AGAROSE GELS (2.0%)

Bulk preparation of 2.0% agarose gel is outlined in Table D.

1. Measure 250 ml of 1x TBE Electrophoresis Buffer and pour into a 500 mL flask.

2. Pour 5.0 g of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.

3. With a marking pen, indicate the level of solution volume on the outside of the flask.

4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.

5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.

6. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed.

7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Proceed with electrophoresis (Module IV) or store the gels at 4° C under buffer.

### Table D

<table>
<thead>
<tr>
<th>Amt of Agarose</th>
<th>1x TBE Electrophoresis Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 g</td>
<td>250 ml</td>
</tr>
</tbody>
</table>

**NOTE:**
The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

**NOTE:**
QuickGuide instructions and guidelines for casting various agarose gels can be found on our website. [www.edvotek.com/quick-guides](http://www.edvotek.com/quick-guides)