

# Biotechnology Explorer™ GMO Investigator™ Kit

Catalog #166-2500EDU

[explorer.bio-rad.com](http://explorer.bio-rad.com)

**Note: Kit contains temperature-sensitive reagents. Open immediately upon arrival and store components at  $-20^{\circ}\text{C}$  or at  $4^{\circ}\text{C}$  as indicated.**

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**BIO-RAD**

## **To the Instructor**

### **Are Your Favorite Foods Genetically Modified?**

Currently, genetically modified (GM) foods do not have to be labeled as such in the US and foods with less than 5% genetically modified content can be labeled "GMO-free". In Europe and Asia, genetically modified foods do require labeling if they contain >1% GM content.

The purpose of this kit is for students to test their favorite store-bought food products (for example corn chips and veggie burgers) for the presence of genetically modified organisms (GMOs). Moreover, students engage in a scientific inquiry experiment where they gather food items from the grocery store, extract DNA from the food, amplify the DNA using the polymerase chain reaction (PCR) and use gel electrophoresis to identify the presence or absence of the amplified GMO sequences.

In this activity students employ state-of-the-art molecular biology techniques to test familiar food items. The kit will work best with students that have some basic understanding of molecular biology and previous experience with some of the techniques involved. The exercise covers a wide variety of subject areas, including: genetic engineering and transformation; DNA transcription and translation; gene regulation; DNA replication and PCR; plant development and physiology; agricultural and environmental science.

### **Teaching Strategy: Guided, Inquiry-Based Investigation**

The GMO Investigator kit allows a guided inquiry approach to this exercise. The students conduct sophisticated scientific procedures that have multiple levels of controls. This allows them to assess the validity of their results. Thus not only is the presence or absence of GMO sequences in their test food determined, but they also ask and answer the questions: did we successfully extract DNA; did our PCR work as expected and do we have contamination?

### **Are GM Crops a Good Thing?**

Many people object to the use of GM crop plants. They argue that there is a potential to create super-weeds through cross-pollination with herbicide-resistant crops or that super-bugs will evolve that are no longer resistant to the toxins in pest-resistant crops. Many are concerned with potential allergic reactions to the novel proteins or antibiotic resistance arising from the selectable markers used to develop the crops or other unforeseen effects on public health. Proponents of genetically modified foods argue these crops are actually better for the environment. Fewer toxic chemicals are put into the environment and thus fewer toxic chemicals can harm the environment and human health. In addition, these crops can preserve arable land by reducing stresses on the land, improve the nutritional value of food in developing countries, and allow crops to be grown on previously unfarmable land. We include a formal debate in Appendix D to aid discussion of these issues.

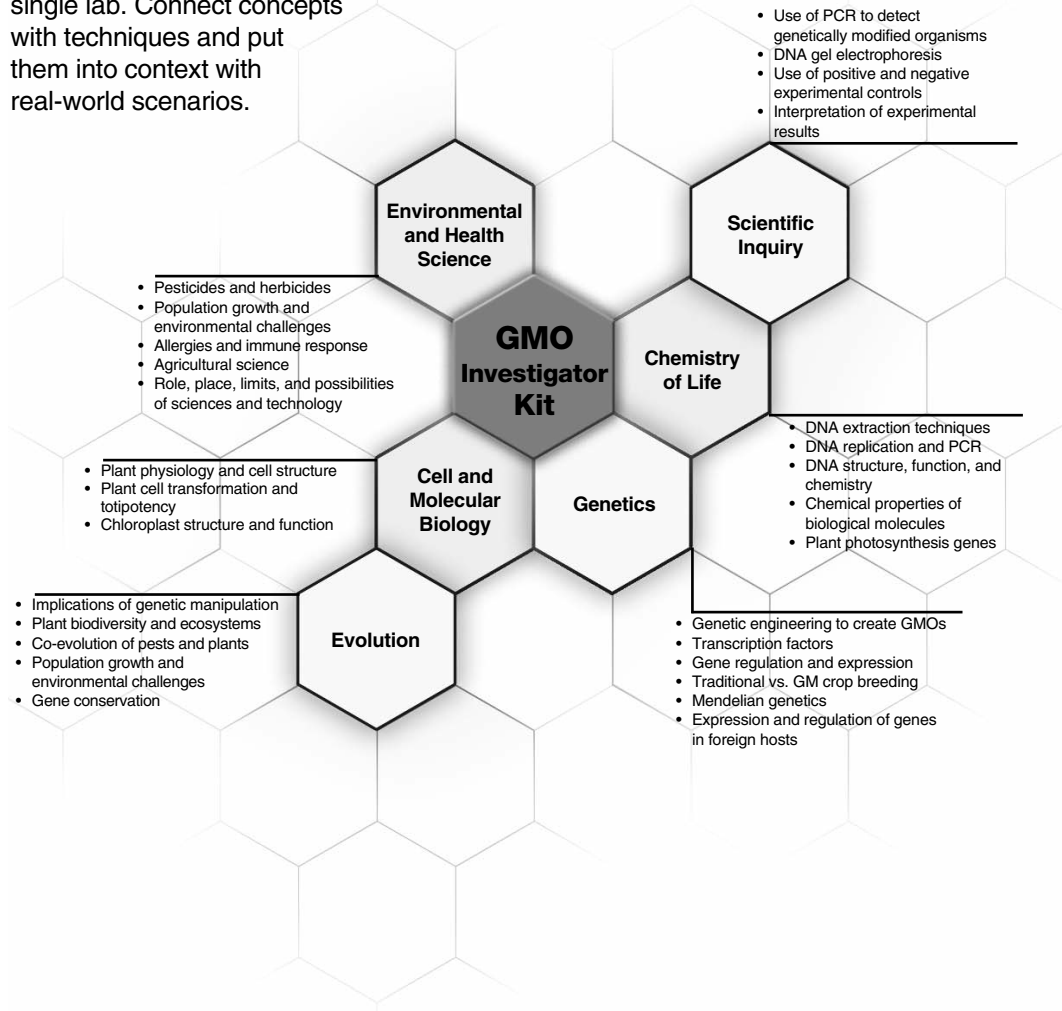
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We strive to continually improve our curricula and products and welcome your stories, ideas and suggestions.

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**Create context. Reinforce learning. Stay current.**

New scientific discoveries and technologies create more content for you to teach, but not more time. Biotechnology Explorer kits help you teach more effectively by integrating multiple core content subjects into a single lab. Connect concepts with techniques and put them into context with real-world scenarios.





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## Kit Summary

The purpose of this kit is to test grocery store food products (e.g., corn chips, vegetarian sausages) for the presence of genetically modified organisms (GMO). This kit contains sufficient materials to extract and amplify DNA from 16 samples and requires a minimum of 4 lab periods:

- Lesson 1: Extraction of DNA from food samples
- Lesson 2: Set up PCR reactions
- Lesson 3: Electrophoresis of PCR products
- Lesson 4: Drying gels and analysis of results

The reagents for gel electrophoresis are available as separate modules; you can choose to perform agarose gel electrophoresis or polyacrylamide gel electrophoresis (PAGE).

The GMO Investigator kit uses PCR to test for the presence of two different GMO-associated sequences: the 35S promoter of the cauliflower mosaic virus (CaMV 35S) and the terminator of the nopaline synthase (NOS) gene of *Agrobacterium tumefaciens*. One or both of these sequences are present in most of the genetically modified crops that are approved for distribution in North America, Asia, and Europe. The GMO Investigator kit allows a guided-inquiry approach to this exercise by providing multiple levels of controls to assess the validity of the results obtained. It mimics the process of research by using multiple procedures to address open-ended questions. The integrity of the plant DNA extracted from food is tested using PCR to identify a third sequence of DNA, the photosystem II chloroplast gene, which is common to most plants. The integrity of the polymerase chain reaction is tested by amplifying the 35S promoter and the photosystem II gene sequences directly from template DNA provided in the kit. Potential contamination of the test samples is identified by extracting DNA from a Bio-Rad certified non-GMO food control provided in the kit and performing PCR to test for the presence of GMO sequences.

## Agarose or Polyacrylamide Gel Electrophoresis?

The DNA fragments amplified from the 35S promoter and NOS terminator are 203 and 225 base pairs (bp) respectively. The PCR product generated from the photosystem II gene is 455 bp. Resolving bands of these sizes requires either a 3% agarose gel or a 10% polyacrylamide gel. Both gel techniques give excellent results. Your choice of gel technique will depend on the equipment that is available to you and the techniques you wish to teach your students. Polyacrylamide gels are much more fragile than 3% agarose gels and thus may be suitable only for more experienced students. However polyacrylamide gels resolve bands to a greater degree, which may allow separation of the similar sized DNA bands generated from a test food that contains both the CaMV 35S promoter and NOS terminator, such as genetically modified papaya. Refer to page 2 for the accessories that you will need depending on whether you choose agarose or polyacrylamide gel electrophoresis.

## Storage Instructions

Place the reagent bag at  $-20^{\circ}\text{C}$  and the InstaGene matrix at  $4^{\circ}\text{C}$  within 1 week of arrival. The other reagents may be stored at room temperature.

## Kit Inventory Checklist

This section lists the components provided in the GMO Investigator kit. It also lists required accessories. Each kit contains sufficient materials for 32 students comprised of 8 student workstations, 4 students per station. As soon as your kit arrives, open it and check off the listed contents to familiarize yourself with the kit. **Immediately place the bag containing the master mix and primers in the freezer (–20°C), and the bottle of InstaGene in the fridge (4°C).** The number of gel boxes and pipets you will need depends on the number of students you will have working at each station.

Kit Components	Number/Kit	(✓)
Bio-Rad Certified Non-GMO food control	1 pack	<input type="checkbox"/>
GMO-positive control DNA, 0.5 ml	1 tube	<input type="checkbox"/>
Master mix, 1.2 ml	1 tube	<input type="checkbox"/>
GMO primers (red), 15 µl	1 tube	<input type="checkbox"/>
Plant PSII primers (green), 15 µl	1 tube	<input type="checkbox"/>
PCR molecular weight ruler, 200 µl	1 tube	<input type="checkbox"/>
Orange G loading dye, 1 ml	1 tube	<input type="checkbox"/>
InstaGene™ matrix, 20 ml	1 bottle	<input type="checkbox"/>
Disposable plastic transfer pipets (DPTPs)	2 packs	<input type="checkbox"/>
Flip top tubes, 1.5 ml	2 packs	<input type="checkbox"/>
Screwcap tubes, 1.5 ml	1 pack	<input type="checkbox"/>
PCR tubes, 0.2 ml	1 pack	<input type="checkbox"/>
Capless PCR tube adaptors, 1.5 ml	1 pack	<input type="checkbox"/>
Manual	1	<input type="checkbox"/>
<b>Required Accessories</b>	<b>Number/Kit</b>	<b>(✓)</b>
2–20 µl adjustable-volume micropipets (cat. #166-0506 EDU) or 10 µl and 20 µl fixed volume pipettes (cat. #166-0512EDU and 166-0513EDU)	8	<input type="checkbox"/>
20–200 µl adjustable-volume micropipet (cat. #166-0506EDU)	1	<input type="checkbox"/>
200–10000 µl adjustable-volume micropipet (cat. #166-0508EDU)	1	<input type="checkbox"/>
2–20 µl pipet tips, aerosol barrier (cat. #211-2006EDU)	8 racks	<input type="checkbox"/>
20–200 µl pipet tips, aerosol barrier (cat. #211-2016EDU)	1 rack	<input type="checkbox"/>
200–1000 µl pipet tips, aerosol barrier (cat. #211-2021EDU)	1 rack	<input type="checkbox"/>
Mortar and pestle	8	<input type="checkbox"/>
Marking pens	8	<input type="checkbox"/>
Test food from grocery store	1–8	<input type="checkbox"/>
Distilled water	3.5 L	<input type="checkbox"/>
Water bath (cat. #166-0504EDU) or dry bath (cat. #166-0562EDU)	1	<input type="checkbox"/>
Microcentrifuge (cat. #166-0602EDU) or mini centrifuge (cat. #166-0603EDU)	1–4	<input type="checkbox"/>
Balance with 0.5–2 g range and weigh boats or paper	1	<input type="checkbox"/>
Thermal cycler (T100™ cat. #186-1096EDU)	1	<input type="checkbox"/>
Power supply (PowerPac™ Basic cat. #164-5050EDU)	2–4	<input type="checkbox"/>



### If using agarose gel electrophoresis:

Required Accessories	Number/Kit	(✓)
Horizontal electrophoresis chambers with gel casting trays and combs (cat. #166-4000EDU)	4–8	<input type="checkbox"/>
Small Fast Blast™ DNA electrophoresis reagent pack (cat. #166-0450EDU) containing 25 g agarose, 100 ml 50x TAE, 100 ml Fast Blast DNA stain	1	<input type="checkbox"/>

### If using polyacrylamide gel electrophoresis:

Required Accessories	Number/Kit	(✓)
Mini-PROTEAN® Tetra cell vertical electrophoresis chambers (cat. #165-8005EDU)	4	<input type="checkbox"/>
10% TBE Mini-PROTEAN precast gels, pkg of 2, (cat. #456-5033EDU)*	8	<input type="checkbox"/>
10x Tris-borate-EDTA buffer (10x TBE) (cat. #161-0733EDU)	1 L	<input type="checkbox"/>
Fast Blast DNA stain (cat. #166-0420EDU)	100 ml	<input type="checkbox"/>
Prot/Elec™ tips (cat. #166-9917EDU)	8 racks	<input type="checkbox"/>

\*Note: Polyacrylamide gels have a shelf life of 3 months, thus order the gels only when the lab is scheduled

Optional Accessories	Number/Kit	(✓)
GelAir™ drying system (cat. #165-1771EDU)	1	<input type="checkbox"/>
Cellophane (if not using GelAir drying system)(cat. #165-1779EDU)	1	<input type="checkbox"/>
Rocking platform (catalog cat. #166-0709EDU)	1	<input type="checkbox"/>
Vortexer (catalog cat. #166-0610EDU)	1	<input type="checkbox"/>
Acetate sheets for tracing gels	8	<input type="checkbox"/>
Floating tube racks (catalog cat. #166-0479EDU)	8 racks	<input type="checkbox"/>
Microcentrifuge racks (catalog cat. #166-0481EDU)	8 racks	<input type="checkbox"/>
PCR tube racks (catalog cat. #TRC-0501EDU)	8 racks	<input type="checkbox"/>

### Refills Available Separately

166-2501EDU	GMO Investigator kit reagent refill pack, includes GMO primers, PSII primers, positive control DNA, PCR molecular weight ruler, Orange G loading dye, 2x master mix containing dNTPs, buffer, DNA polymerase
166-5009EDU	2x Mastermix for PCR
732-6030EDU	InstaGene™ matrix, 20 ml
166-0455EDU	Medium Fast Blast DNA Electrophoresis Reagent Pack, makes 270 1% or 90 3% 7 x 10 cm agarose gels; includes 125 g agarose powder, 100 ml 500x Fast Blast DNA stain, 1 L 50x TAE electrophoresis buffer
166-0460EDU	Large Fast Blast DNA Electrophoresis Reagent Pack, makes 1080 1% or 360 3% 7 x 10 cm agarose gels; includes 500 g agarose powder, 2x 100 ml 500x Fast Blast DNA stain, 5 L 50x TAE electrophoresis buffer
166-0473EDU	Colored 1.5 ml microcentrifuge tubes, 6 colors, 600
224-0110EDU	Conical Tubes, 1.5 ml, with installed O-ring screwcaps, sterile, 500
TWI-0201EDU	0.2 ml Tubes with domed caps, natural, 1,000

## **Curriculum Fit**

In 1996 the US National Academy of Sciences and its working groups, in conjunction with the National Research Council, published the National Science Education Standards. These standards call for a movement away from traditional science teaching, which includes memorizing scientific facts and information, covering many subject areas, and concluding inquiries with the result of an experiment. Instead, teachers are encouraged to engage students in investigations over long periods of time, learning subject matter in the context of inquiry, and applying the results of experiments to scientific arguments and explanations. The Biotechnology Explorer GMO Investigator kit follows this approach. It provides a guided investigation in which students gather common food items, extract DNA from the sample, amplify genetic sequences using PCR, and use gel electrophoresis to identify the presence or absence of the amplified marker sequences. Students are encouraged to analyze their results in the context of the experimental controls to assess whether they can determine if food they commonly consume has been genetically modified (GM). The kit can be used to cover the following content areas.

### **Scientific Inquiry**

- Use of sophisticated techniques to detect GMOs
- Use of multiple positive and negative experimental controls
- Analysis and interpretation of experimental results

### **Chemistry of Life**

- Chemical properties of cell components
- DNA extraction techniques
- DNA replication and PCR
- Gel electrophoresis of DNA

### **Heredity & Molecular Biology**

- Genetic transformation to create GMOs
- Control of gene expression
- DNA profiling techniques
- Crop breeding: traditional vs. GM
- Expression and regulation of genes in foreign hosts

### **Structure & Function of Organisms**

- Plant transformation and regeneration
- Cell structure

### **Evolutionary Biology**

- Implications of genetic manipulation
- Implications of altering plant biodiversity and ecosystems
- Evolutionary race between pests and plants

### **Environmental & Health Sciences**

- Pesticides and herbicides
- Population growth, environmental quality & global challenges
- Role, place, limits & possibilities of science and technology

More specifically, in the US the kit covers the following content standards:

## **Standard**

## **Fit to Standard**

### **Content Standard A**

Students will develop abilities to do scientific inquiry

Students will perform an experiment using sophisticated procedures and multiple controls

Students will develop understanding about scientific inquiry

Students will apply the results of this experiment to scientific arguments

### **Content Standard B**

Students will develop an understanding of the cell

Students will understand how cellular structure affects DNA extraction, and why an understanding of DNA replication is necessary for understanding PCR

Students will develop an understanding of the molecular basis of heredity

Students will understand how genetic engineering supplements traditional methods of plant breeding to generate new traits in crop plants

Students will develop an understanding of biological evolution

Students will think about how changing the genome of an organism can affect its ability to survive in different environments

Students will develop an understanding of the interdependence of organisms

Students will think about how GM crops will interact with other plants and insects in the environment

### **Content Standard F**

Students will develop an understanding of population growth/environmental quality/national and global challenges

Students will learn about how GM food technology is proposed as a solution to the problems of population growth and environmental damage

## Background for Teachers

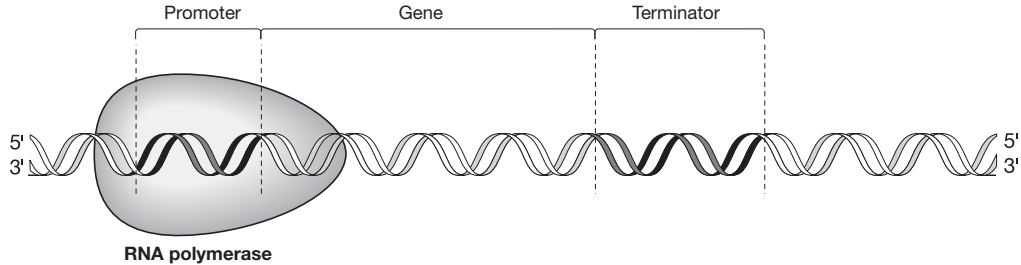
Since the release of the first genetically modified (GM) crop in the US in 1996, scientists have debated the use of these crops because of potential health and environmental risks. GM foods are foods that contain components of GM crops—plants that have been genetically modified by the insertion of foreign genetic material. The foreign genetic material may have come not only from another plant but possibly from a species of another kingdom (e.g., animal, fungal, bacterial). The foreign genetic material is usually a gene that codes for a protein that gives the plant an advantage over similar crop plants. Examples of conferred traits include pest resistance, herbicide tolerance, delayed fruit ripening, improved fruit yield, increased nutrient content, etc.

### How Do You Genetically Modify a Crop?

The first step in the genetic modification process is to identify a protein that has the potential to improve a crop. One popular class of GM crops has a gene from the soil bacterium *Bacillus thuringiensis* (Bt) inserted into their genomes. Bt crops produce a protein called delta-endotoxin that is lethal to European corn borers, a common pest on corn plants. Farmers who plant Bt crops do not have to apply pesticide because the plants produce the toxic protein inside their cells. Bt toxin was first identified on silk farms as a toxin that kills silkworms (which are in the same genus as European corn borers).

The second step is to isolate (clone) the gene that codes for the protein. The entire gene must first be localized within an organism's genome; then it must be copied so that it can be isolated or cloned out of the organism. Although a gene's coding region may just be a few hundred or thousand base pairs long, the gene itself may be tens of thousands of base pairs long, due to its introns (noncoding sequences). The cloning of an entire gene can be very laborious and can take many years.

Genes contain signals that regulate their expression in their host's cells, but these signals are often not understood by another organism's cells. Thus, the third step is to engineer the gene so that the crop plant's cells will read it correctly and manufacture the protein of interest. This is done by streamlining the gene to remove unnecessary introns, and adding or changing sequences that will enable the gene to be expressed within the crop's cells, including a promoter and a terminator (see Figure 1). The promoter serves as a docking site for RNA polymerase and a signal for where it should start transcribing a gene. The terminator is the signal to stop transcription. The native promoters and terminators of unmodified genes interact with other components of a host cell to turn genes on or off depending on cell type and situation, but scientists can engineer the constructs for GMOs so that the foreign gene is continually transcribed and the foreign protein is produced throughout the entire plant. The most common promoter used in GM crops is the 35S promoter from the cauliflower mosaic virus (CaMV 35S). This promoter is chosen because it is already designed by nature to activate transcription in all plant cell types. The most common terminator used in GM crops is the nopaline synthase (NOS) terminator from *Agrobacterium tumefaciens*. The GMO Investigator kit can identify both of these genetic modifications in grocery store food products. One or both of these genetic elements are present in ~85% of all GM crops currently approved around the world.



**Fig. 1. Gene structure.**

Once the gene is engineered with the appropriate promoter and terminator, it needs to be introduced into the plant (see Figure 2). The gene cannot be inserted into all of the cells of an existing plant; instead, individual plant cells are transformed with the engineered gene, and then new plants are grown from those single cells. Cells are first removed from the parent plant, then grown on a special medium that causes them to form a clump of undifferentiated cells referred to as a callus. The engineered gene is then transferred into the cells of the callus by a variety of methods, each of which must get the DNA past the plant's cell wall, plasma membrane, and nuclear membranes. One method is to use a GM version of the soil bacterium *Agrobacterium tumefaciens*. This bacterium causes crown gall disease by inserting some of its DNA to a host plant's genome; this unusual natural property is exploited to transfer the engineered gene into the plant genome. A second method is electroporation, in which an electric current creates pores in cell membranes and allows the entry of the engineered DNA. A third method uses a device called a "gene gun" that physically shoots gold particles coated with the engineered DNA into the plant cells. None of these methods is very efficient, and the few cells that have been transformed need to be identified and selected from among those that were not. To assist this process, selectable markers are inserted into the cells along with the engineered gene. These may be antibiotic resistance markers, or visual markers like the gene for Green Fluorescent Protein. Once the transformed cells have been isolated, they are induced with plant hormones to differentiate and grow into complete plants. The viable insertion of the engineered gene into a plant's genome is called an "event".

The transformation process is very tricky, so the crop strains that have been optimized for transformation are rarely the same crop strains that are used in the field. The fifth and final step in making a GM crop is to back-cross the genetically engineered crop into the most current high-yielding crop strains that are being used in the field. This can take years since only 50% of the high-yield crop's genome is transferred to the genetically modified crop in each cross.

The genetic modification process is very inefficient, costly, and time consuming — there are usually only a handful of successful "events" for each GM crop, and it takes millions of dollars and six to fifteen years to bring each crop to market.

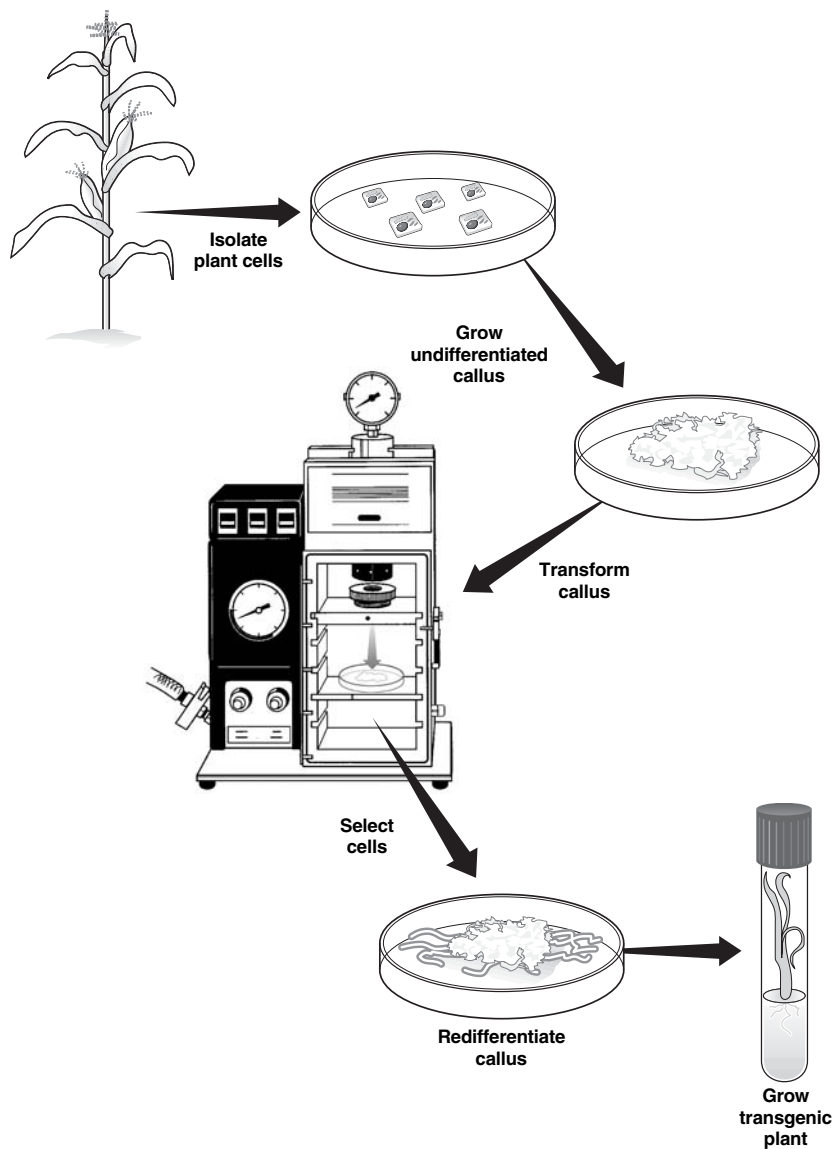


Fig. 2. How to make a GM crop.

## Are GM Crops a Good Thing?

Many people object to the use of GM crop plants. They argue that there is a potential for "superweeds" to arise through cross-pollination of natural weed species with herbicide-resistant crops, or that "superbugs" will evolve that are no longer susceptible to the toxins in pest-resistant crops. Many are concerned about potential allergic reactions to novel proteins, antibiotic resistance arising from the selectable markers used to develop the crops, or other unforeseen effects on public health. Others voice concerns that not enough research has been done to fully understand the implications of altering plant diversity. People also voice concerns on the lack of government requirements for labeling of foods in the US.

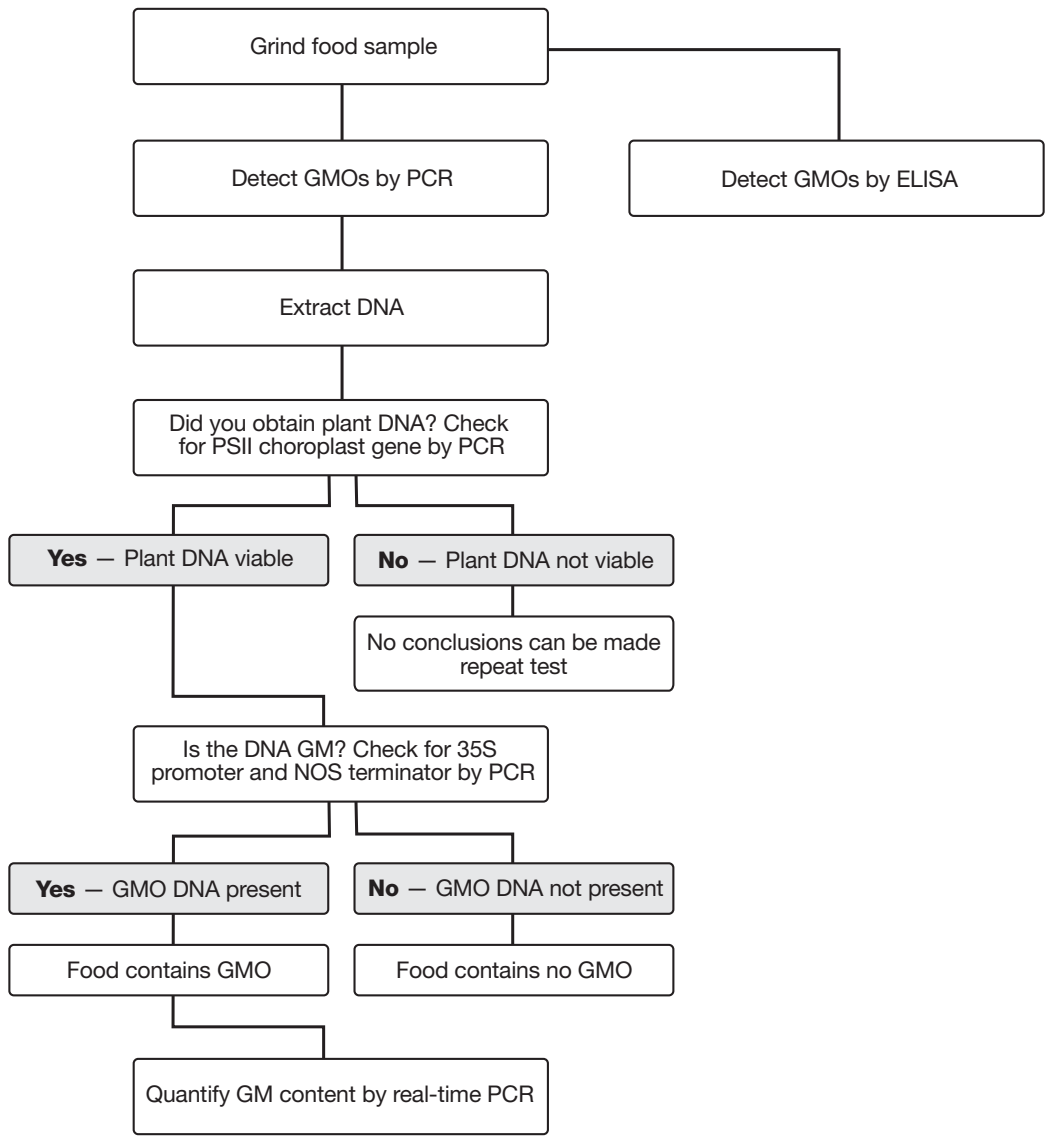
Proponents of GM foods argue that these crops are beneficial for the environment, because they reduce the use of herbicides and pesticides, chemicals that are potentially toxic to the environment and human health. In addition, these crops may preserve arable land by reducing stresses on the land, improve the nutritional value of food in developing

countries, and allow crops to be grown on previously unfarmable land. You may want to organize a debate with your students to address these arguments. A formal debate is included in Appendix D.

## Identifying GM crops

How does one test foods and crops to identify which contain GM genomes (see Figure 3)? Two methods are currently used. One, the enzyme-linked immunosorbent assay (ELISA), identifies proteins. It is an antibody-based test, and it identifies the specific proteins produced by GM plants. ELISA can only test fresh produce, due to protein degradation during food processing. In addition, since ELISA identifies the proteins produced in GMO crops, the tests must be individualized according to the type of crop. For example, a Bt ELISA test can only detect Bt corn, and not herbicide-tolerant GM corn. However, ELISA is inexpensive and accurate, and can be performed in the field with little expertise.

The second test, using the polymerase chain reaction (PCR), identifies sequences of DNA that have been inserted into the GM plant. In contrast to proteins, DNA is a relatively stable molecule, thus DNA fragments can be isolated from highly processed foods and are sufficiently intact to be amplified by PCR. A modified version of PCR, real-time PCR, can also quantitate the percentage of GM material in the food sample. In contrast to an ELISA test that is specific to a single crop, a single PCR test like this one can detect 85% of all GM crops. This is because genetic engineers use only a small number of regulatory sequences (promoter and terminator sequences) to control the expression of the inserted genes, and so these sequences are common to the majority of GM crops. Two of the most common regulatory sequences are the 35S promoter from cauliflower mosaic virus and the nopaline synthase (NOS) terminator from *Agrobacterium tumefaciens*, which are the sequences that are detected by this kit. A review of PCR is included in Appendix A.



**Fig. 3. How to detect GMOs in food.**



## **A Guided Inquiry Experiment**

This kit is an advanced lab because of its use of multiple controls. Your students should be aware that these are the types of controls that are used by scientists in real laboratories and that if the errors occur that these controls identify then the scientists will repeat the test. These controls allow the students to:

### **Check that DNA Extraction Was Successful**

The kit contains one set of primers (colored red) to detect GMO-specific sequences, but also contains a second set of primers (colored green) that identify plant DNA, whether it is GMO-derived or not. The second primer set allows you to tell if a GMO-negative result is due to lack of GMO material or simply an unsuccessful DNA extraction. These primers amplify a 455 bp region of the photosystem II (PSII) chloroplast gene that is common to most plants. Please note that viable DNA is not always extracted from every food. We provide a list on p. 26 of recommended foods that give viable plant DNA. The kit has been optimized to test corn and soy-based foods.

### **Guard Against Contamination**

The kit contains a sample of Bio-Rad certified non-GMO food that should be processed like your chosen test food sample. This sample controls against false positive results. If this sample gives a GMO-positive result, it indicates contamination of the reaction. If your test food also gives a GMO-positive result, you cannot trust this result. Please note that contamination is a very common occurrence in PCR due to its very high sensitivity, and safeguards should be taken to prevent contamination. Refer to Appendix B for a list of precautions to protect against contamination.

### **Ensure That the PCR Reaction Works As Expected**

The kit also contains template DNA that codes for the plant and GMO sequences. This serves as a control against false negatives. If these control sequence are not amplified, there is a problem with the PCR reaction and you cannot trust a GMO-negative result from your test food. This also gives you reference bands for those yielded by the test samples.

### **Test for a Broad Range of GM Foods**

This kit uses "duplex" PCR, which means that two target sequences are simultaneously amplified. The two pairs of primers in the PCR reaction will amplify two DNA sequences, a 203 bp fragment of the CaMV 35S promoter and a 225 bp fragment of the NOS terminator. These primers have been included so that a greater range of GM foods can be detected, since some foods contain just the CaMV 35S promoter, some just the NOS terminator, and some both. By using these two sequences about 85% of all GM foods currently available are detectable with this kit, whereas CaMV 35S primers alone can detect only ~70% of GM foods.

It is not necessary for your students to understand duplex PCR for a full comprehension of the principles of this laboratory, and in the student manual, the text refers to amplification of "GMO sequences", without detailed explanation of these different sequences. However, if a food contains both the CaMV 35S and NOS sequences, such as GM papaya, a doublet band may appear in the GMO lane, where both the 203 and 225 bp PCR products have been generated. This will be especially visible on a polyacrylamide gel.

## **Instructor's Advance Preparation**

This section describes the preparation that needs to be performed by the instructor prior to each laboratory period. If block periods are used, prepare for Lessons 1–2 and Lessons 3–4 at the same time. An estimation of preparation time is included.

### **Timeline**

The entire investigation requires a minimum of four 50-minute laboratory periods or two 90-minute block lessons. Be aware that an additional 4-hour cycling period is needed outside of class time. We also recommend 2–3 days of background review and lectures to prepare your students for the exercise.

### **Prior to Lab**

- Read manual (2 hr)
- Purchase food samples from grocery store (as needed)
- Inventory required accessories (1 hr)
- Perform instructor's advance preparation (30 min–3 hr each lab)
- Set up student workstations (30 min–1 hr each lab)

### **50-minute Lessons**

- Lesson 1: Extract DNA (50 min)
- Lesson 2: Set up PCR reactions (50 min)
- Run PCR reactions (4 hr)—typically overnight
- Lesson 3: Electrophoresis of DNA and staining of gels (50 min)
- Lesson 4: Analysis of results (50 min)

### **90-minute Block Lessons**

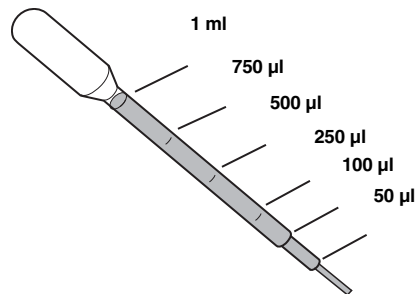
- Lessons 1 – 2: Extract DNA and set up PCR reactions (90 min)
- Run PCR reactions (4 hr)
- Lessons 3 – 4: Electrophoresis of DNA, staining of gels, analysis of results (90 min)

### **Safety Issues**

Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Wearing protective eyewear and gloves is strongly recommended. Students should wash their hands with soap before and after this exercise. If any solution gets into a student's eyes, flush with water for 15 minutes. Although Fast Blast DNA stain is not toxic, latex or vinyl gloves should be worn while handling the stain to keep hands from becoming stained. Lab coats or other protective clothing should be worn to avoid staining clothes.

## Volume Measurements

The instructor's advanced preparation requires a 2–20  $\mu\text{l}$ , a 20–200  $\mu\text{l}$  and a 100–1000  $\mu\text{l}$  adjustable volume pipet and aerosol barrier tips (aerosol barrier tips are necessary to prevent contamination of reagents and your pipets). Sterile graduated disposable plastic transfer pipets (DPTPs) are supplied and can be used for volumes of 50, 100, 250, 500, 750 and 1,000  $\mu\text{l}$ . The illustration shows the marks on the DPTP corresponding to the volumes to be measured. Volumes over 1 ml will require multiple additions. For each step of the laboratory preparation, use a fresh DPTP or a fresh pipet tip.



## Mortars and Pestles

This laboratory requires food to be ground using a mortar and pestle. Please ensure these have been thoroughly washed to remove any residual chemicals that may interfere with the PCR reactions. In addition, rinse the mortars and pestles with 10% bleach, which destroys any residual DNA and then rinse with water to remove the bleach. The student protocol calls for the students to prepare a non-GMO food sample then wash the mortar and pestle with detergent and then to prepare their test food sample. Since the non-GMO food is prepared first there should be no risk of contaminating the test food. It is your decision whether your students use bleach in between samples. However, the mortars and pestles should be rinsed with 10% bleach between different classes.

## Lesson 1 Extraction of DNA From Food Samples

The crux of this lab is the quality and quantity of DNA extracted from your food. The table on p. 26 lists the reliability of different foods with regard to DNA extraction and PCR results; the less reliable foods may produce fainter bands. If you wish your students to find GMO-containing foods, you may want to avoid wheat- and rice-based products, fruits, and fresh vegetables that are almost certainly GMO negative and choose papaya, soy, and corn-based products.

<b>Materials Needed for Advanced Preparation</b>	<b>Quantity</b>
Screwcap tubes	16
Beakers or cups for distilled water	8
InstaGene™ matrix	1 bottle
Disposable plastic transfer pipets (DPTPs)	8–16
Water bath set to 95–100°C	1

### Procedure (Estimated Time: 35 min)

1. Add 500 µl of InstaGene matrix to each of the 16 screwcap tubes using a transfer pipet or 200–1,000 µl adjustable-volume micropipet.

**Note:** The InstaGene matrix needs constant mixing to evenly distribute the microscopic beads. This is easily done by pipetting up and down with the pipet between each aliquot.

2. Put at least 25 ml of distilled water into the clean beakers or cups and label them "DI water".
3. Set the water bath to 95–100°C at least 30 min before the lab.
4. (Optional) Prepare the Bio-Rad certified non-GMO food control. To save time you may want to prepare the non-GMO food control in advance: If you do this, we recommend preparing the sample up to the centrifugation step (see student protocol).
5. Set up the student workstations.
6. Set up the common workstation.

### Student Workstation

<b>Material</b>	<b>Quantity</b>
Screwcap tube with 500 µl InstaGene matrix	2
Beaker of distilled water	1
Transfer pipets	2
Mortar and pestle	1
Test foods*	1–8
Marking pen	1

\* Refer to table on p. 26 for suggestions on foods to use

### Common Workstation

<b>Material</b>	<b>Quantity</b>
Water bath set to 95–100°C	1
Microcentrifuge or mini centrifuges	1 3–4
Balance and weigh boats	1

## Lesson 2 Set Up PCR Reactions

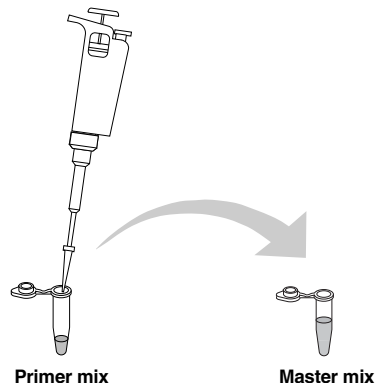
Materials Needed for Advanced Preparation	Quantity
Screwcap tubes	26
PCR tubes	48
PCR tube adaptors	48
Master mix	1 vial
GMO primers (red)	1 vial
Plant PSII primers (green)	1 vial
GMO-positive DNA template	1 vial
Student samples from previous lab	16 tubes
2–20 $\mu$ l adjustable-volume micropipets or 20 $\mu$ l fixed-volume micropipets	8
2–20 $\mu$ l pipet tips, aerosol barrier	8 racks
Beakers with ice or ice baths	8
Foam microtube holders	8
Marking pens	8

### Procedure (Estimated time: 45 min)

**Note:** only add the primers to the master mix and aliquot 30 min before the lesson starts and store prepared master mix on ice.

1. Thaw the GMO-positive DNA template and pulse-spin the tubes in a centrifuge to bring all contents to the bottom. Add 50  $\mu$ l of GMO-positive DNA template to 8 screwcap tubes labeled GMO (+). This can be prepared ahead of time and stored at  $-20^{\circ}\text{C}$  for 1–2 months if necessary.
2. **Perform this step 30 min–1 hr before the lab.** Thaw the master mix & primers and pulse-spin the tubes in a centrifuge to bring all contents to the bottom. **Keep the tubes on ice.**
3. Label the screwcap tubes:
  - a. Label 9 screwcap tubes "PMM" (plant master mix).
  - b. Label 9 screwcap tubes "GMM" (GMO master mix).
4. Add 550  $\mu$ l of master mix to one PMM tube and one GMM tube.

*Before dispensing the primers in steps 5 and 6, pulse-spin the primers tubes again, if necessary, to ensure the contents are not caught in the tube lid.*
5. Add 11  $\mu$ l of green primers to the master mix in the PMM tube, and mix. **Store on ice.**



6. Add 11  $\mu$ l of red primers to the master mix in the GMM tube, and mix. **Store on ice.**
7. Add 65  $\mu$ l of the plant master mix with the newly added primers into each of the remaining 8 tubes labeled PMM.
8. Add 65  $\mu$ l of the GMO master mix with the newly added primers into each of the remaining 8 tubes labeled GMM.
9. Put one PMM tube, one GMM tube, and one GMO (+) tube in an ice bath for each workstation.
10. Set up the student workstations.

### Student Workstations

Material	Quantity
Ice bath containing DNA samples and GMM, PMM, and GMO (+) tubes	1
PCR tubes	6
PCR adaptors	6
Foam microtube holder	1
Marking pen	1
2–20 $\mu$ l adjustable-volume micropipet or fixed-volume 20 $\mu$ l micropipet	1
2–20 $\mu$ l pipet tips, aerosol barrier	1 rack

11. Program the thermal cycler (see Appendix E for detailed instructions).

Step	Function	Number of Temperature	Duration	Cycles
Initial denaturation	Denature	94°C	2 min	1
PCR amplification	Denature	94°C	1 min	40 1 min
	Anneal	59°C		
	Extend	72°C	2 min	
Final extension	Extend	72°C	10 min	1
*Hold	Hold	4°C	Indefinite	1

\* The option to hold temperature at 4°C is not available with some thermal cyclers.

### Lesson 3 Electrophoresis of PCR Products

The DNA fragments amplified from the 35S promoter and NOS terminator are 203 and 225 base pairs (bp) respectively. The PCR product generated from the photosystem II gene is 455 bp. Resolving bands in this size range requires either a 3% agarose gel or a 10% polyacrylamide gel. Both gel techniques give excellent results. Your choice of gel technique will depend on the equipment that is available to you and the techniques you wish to teach your students. Polyacrylamide gels are much more fragile than 3% agarose gels and thus may be suitable only for more experienced students. However, polyacrylamide gels resolve bands to a greater degree, which may allow separation of the similar-sized DNA bands generated from a test food that contains both the CaMV 35S promoter and NOS terminator, such as genetically modified papaya. Separate directions are provided below for each electrophoresis method after the directions common to both.

Materials Needed	Quantity
Orange G loading dye	1 vial
PCR molecular weight ruler	1 vial
Flip-top micro test tubes	16 tubes
20–200 µl adjustable-volume micropipet	1
2–20 µl adjustable-volume micropipets or fixed-volume 20 µl micropipets	8
20–200 µl pipet tips, aerosol barrier or regular	1 rack
2–20 µl pipet tips, aerosol barrier	8 racks
Power supply	2-4
Fast Blast DNA stain	1 bottle
500 ml flask or bottle to store diluted Fast Blast stain	1
Distilled water	3.5 L
Gel staining trays	1–8
Electrophoresis materials and equipment	See below

#### Procedure (Estimated time: 1–3 hr)

1. Thaw the Orange G loading dye and PCR molecular weight ruler, and pulse-spin the tubes in a centrifuge to bring all contents to the bottom.
2. Add 40 µl of Orange G loading dye to the vial of PCR molecular weight ruler. Mix well and pulse-spin.
3. Label the flip-top micro test tubes:
  - Label 8 tubes "LD"
  - Label 8 tube "MWR"
4. Add 70 µl of Orange G loading dye to each of the 8 tubes marked "LD". This can be prepared ahead of time and stored at 4°C for 1–2 months.
5. Add 25 µl of PCR molecular weight ruler to each of the 8 tubes marked "MWR". This can be prepared ahead of time and stored at 4°C for 1–2 months.
6. Prepare the gels, running buffer, and electrophoresis apparatus. Refer to the instructions below for agarose gels or polyacrylamide gels.
7. Prepare Fast Blast DNA stain. Refer to the instructions below for the staining technique you choose.
8. Set up the student workstations.

## Student Workstation

<b>Material</b>	<b>Quantity</b>
Gel (see below)	1
Samples from previous lab	6
Running buffer (see below)	300–350 ml
Orange loading dye	1 vial
PCR molecular weight ruler	1 vial
2–20 µl adjustable-volume pipet or fixed-volume 20 µl micropipet	1
1–20 µl pipet tips, aerosol barrier	1 rack
Gel electrophoresis chamber (may be shared by 2 workstations)	1
Power supply (may be shared by multiple workstations)	1
Fast Blast DNA stain (at common workstation)	1
Gel staining tray	1

## Agarose Gel Electrophoresis

### Preparation of Agarose Gels and TAE Running Buffer

These procedures may be carried out 1–2 days ahead of time by the teacher or done during class by individual student teams. Note: Convenient precast 3% agarose gels (catalog #161-3017EDU) are available from Bio-Rad.

### **Materials (Needed in Addition to Those Indicated for Lesson 3) Quantity**

Agarose	10.5 g
50x TAE	60 ml
Graduated cylinders, 3 L and 500 ml	2
Microwave or magnetic hot plate and stir bar	1
Bottle or Erlenmeyer flask, 1 L	1
Flask, 50 ml (optional)	1
Water bath at 60°C (optional)	1
Gel casting trays	4–8
Gel combs	8
Lab tape (optional)	1 roll
Horizontal electrophoresis chamber	4–8

1. Prepare the electrophoresis buffer. The electrophoresis buffer is provided as a 50x concentrate. 1x TAE buffer is needed to make the agarose gel and is also required for each electrophoresis chamber. Three liters of 1x TAE buffer will be sufficient to run 8 electrophoresis chambers and pour 8 agarose gels. To make 3 L of 1x TAE from 50x TAE concentrate, add 60 ml of 50x concentrate to 2.94 L of distilled water.



2. Make the agarose solution. The recommended gel concentration for this application is 3% agarose. This agarose concentration provides excellent resolution and minimizes run time required for electrophoretic separation of PCR fragments. To make a 3% solution, add 3 g of agarose powder per 100 ml of 1x TAE electrophoresis buffer in a suitable heatproof container that is large enough to accommodate vigorous boiling (e.g., 1,000 ml Erlenmeyer flask, Wheaton bottle, etc.). For 8 gels, you will need approximately 350 ml of molten agarose (10.5 g agarose plus 350 ml 1x TAE buffer). The agarose must be made using electrophoresis buffer, **not** water. Swirl to suspend the agarose powder in the buffer. If using an Erlenmeyer flask, invert a 50 ml Erlenmeyer flask into the open end of the 1,000 ml Erlenmeyer flask containing the agarose. The small flask acts as a reflux chamber, allowing boiling without much loss of buffer volume by evaporation. The agarose can be melted for gel casting on a magnetic hot plate or in a microwave oven.  
**Caution:** Use protective gloves, oven mitts, goggles, and lab coats as appropriate while preparing and casting agarose gels. Contact with boiling molten agarose or the vessels containing hot agarose can cause severe burns.

**Magnetic hot plate method.** Add a stirbar to the flask containing agarose and buffer. Heat the mixture to boiling while stirring on a magnetic hot plate. Bubbles or foam should break before rising to the neck of the flask. Boil the solution until all of the small transparent agarose particles are dissolved. With the small flask still in place, set aside the agarose to cool to 60°C before pouring gels (a water bath set to 60°C is useful for this step).

**Microwave oven method.** Place the flask or bottle containing the agarose solution into the microwave oven. Loosen the bottle cap if present. Use a medium setting and set to 3 minutes. Stop the microwave oven every 30 seconds and swirl the flask to redistribute any undissolved agarose. This technique is the most efficient way to dissolve agarose. Alternate boiling and swirling the solution until all of the small transparent agarose particles are dissolved. With the small flask or bottle cap still in place, set aside to cool to 60°C before pouring (a water bath set to 60°C is useful for this step).

### **Casting Agarose Gels**

Using Bio-Rad's Mini-Sub<sup>®</sup> Cell GT system, gels can be cast directly in the gel box using the casting gates with the gel tray. If casting gates are unavailable, use the taping method for casting gels, as described below. Other methods are detailed in the Bio-Rad Sub-Cell<sup>®</sup> GT instruction manual. 7 x 7 cm gel trays allow a single gel to be cast. 7 x 10 cm gel trays allow casting of a "double" gel, i.e., a gel with two rows of wells that can be loaded with the samples of two student teams. These longer gels do not fit within the casting gates and need to be made by the taping method.

1. Seal the ends of the gel tray securely with strips of standard laboratory tape. Press the tape firmly onto the edges of the gel tray to form a fluid-tight seal and lay the gel tray flat.
2. Prepare an agarose solution of the desired concentration and amount in 1x TAE electrophoresis buffer.
3. Cool the agarose to at least 60°C before pouring (a water bath is useful for this step).
4. While the agarose is cooling, place the comb into the appropriate slots of the gel tray. Gel combs should be placed within ~2 cm of the end of the gel casting tray.
5. Pour 30–50 ml of molten agarose into the tray to a depth of approximately 0.5 cm.
6. Allow the gel to solidify at room temperature for 10 to 20 minutes — it will be translucent when it is ready to use.
7. Carefully remove the comb from the solidified gel. Remove the tape from the edges of the gel tray. Agarose gels can be stored wrapped in plastic wrap, sealed plastic bags or submerged in 1x TAE buffer for up to 2 weeks at 4°C.

### Load and Run Agarose Gels

1. Place the gel in the gel tray onto a leveled DNA electrophoresis chamber so that the sample wells are at the cathode (black) end of the base. DNA samples will migrate towards the anode (red) end of the base during electrophoresis.
2. Fill the electrophoresis chamber with 1x TAE running buffer to about 2 mm above the surface of the gel.
3. Load the gels as directed in the student manual.
4. Run gels at 100 V for 30 min. Greater resolution can be obtained using a longer run time (eg, 45 min), but if double gels are used, only run gels at 100 V for 30 min since the DNA from the upper gel may run into the lower gel. Do not let the orange dye migrate off the gel.
5. Stain the gels in Fast Blast DNA stain — see below.

### Preparation for Staining Agarose Gels

Fast Blast DNA stain is provided as a 500x concentrate that must be diluted prior to use. The stain can be used as a quick stain when diluted to 100x to allow the visualization of DNA within 15–20 minutes, or can be used as an overnight stain when diluted to 1x. Fast Blast DNA stain is a convenient, safe, and nontoxic alternative to ethidium bromide for the detection of DNA. Fast Blast contains a cationic compound that belongs to the thiazin family of dyes. The positively charged dye molecules are attracted to and bind to the negatively charged phosphate groups on DNA. The proprietary dye formula stains DNA deep blue in agarose gels and provides vivid, consistent results. Detailed instructions on using Fast Blast stain are included in the student manual.

### WARNING

**Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes. Dispose of the staining solutions according to protocols at your facility. Use either 10% bleach solution or 70% alcohol solution to remove Fast Blast from most surfaces. Verify that these solutions do not harm the surface prior to use.**

### Preparation for Overnight Staining Protocol (Recommended)

To prepare 1x stain (for overnight staining), dilute 1 ml of 500x Fast Blast with 499 ml of distilled or deionized water in an appropriately sized flask or bottle, and mix. Cover the flask and store at room temperature until ready to use.

### Preparation for Quick Staining Protocol

To prepare 100x stain (for quick staining), dilute 100 ml of 500x Fast Blast with 400 ml of distilled or deionized water in an appropriately sized flask or bottle and mix. Cover the flask and store at room temperature until ready to use.

Destaining requires the use of at least one large-volume container, capable of holding at least 500 ml, at each student workstation. 100x Fast Blast can be reused at least seven times. Please note, in contrast to 1% agarose gels, 3% agarose gels require 5 min staining, prior to destaining in warm water. Due to the high percentage of agarose, gels stained by this quick method may take longer to destain to a satisfactory level than 1% agarose gels. Multiple washes with **warm** tap water will assist the destaining of these gels.

## Polyacrylamide Gel Electrophoresis (PAGE)

### Preparation of Polyacrylamide Gels and TBE Running Buffer

#### Materials needed in addition to those indicated for Lesson 3

	Quantity
10% TBE Mini-PROTEAN precast gels (cat. #456-5033)	8
10x TBE (cat. #161-0733EDU)	300 ml
Graduated cylinder, 3 L	1
Mini-PROTEAN® Tetra cell vertical electrophoresis chamber	4–8
Prot/Elec™ tips	8 racks
Sharp knife or razor	1

#### Ready Gel 10% TBE Precast Polyacrylamide Gels

Polyacrylamide gels should be stored in a refrigerator until the time of use. Order gels 2–3 weeks before the lab for optimal results. **Do not freeze them.** To set up the gels for the laboratory, cut the gel packages open over a sink or container, drain out the excess buffer, and throw away the filter paper and plastic wrap. Remove the comb from between the plates by pushing it upward gently with your fingertips. Peel off the strip of plastic covering the bottom of the gel, as indicated on the gel cassette. Make sure the entire section of tape is removed completely, to allow the full length of the bottom of the gel to be exposed to electric current. For best results, use a transfer pipet and 1x TBE running buffer to rinse any debris out of the wells. **Note:** The Mini-PROTEAN TBE gels used to electrophorese DNA for this laboratory are different from the 15% SDS-containing gels used to run proteins for SDS-PAGE and the two types should not be substituted for each other.

**Note:** Instructors may choose to assemble the gel boxes up to 1 hour prior to the laboratory.

#### Prepare Mini-PROTEAN Tetra Cell Electrophoresis Chambers

1. **1x TBE running buffer.** One Mini-PROTEAN Tetra cell with two gels requires 700 ml of 1x TBE running buffer. One Mini-PROTEAN Tetra cell using the companion running module to run four gels requires 1.1 L of 1x TBE running buffer. To make 3 L of 1x TBE running buffer, mix 300 ml of 10x TBE with 2,700 ml of distilled water. Store at room temperature.

Tip: You may want to prepare 1–2 L of extra 1x TBE buffer in case your gel boxes leak after assembly. If you do have a leak, the outer chamber of the gel box can be filled to above the inner small plates to equalize the buffer levels in both reservoirs. This requires approximately 1,200 ml of 1x TBE buffer per gel box and is a more convenient fix than reassembling the apparatus mid-lesson.

2. Follow Appendix G for detailed instructions in assembling the apparatus.

### **Load and Run Polyacrylamide Gels**

1. If available, place a sample loading guide on the top of the electrode assembly. The guide will direct the pipet tip to the correct position for loading each sample in a well.
2. Use Prot/Elec tips to load the samples into the wells. These very narrow tips can fit between the two gel plates and deliver the samples directly into the wells. If Prot/Elec or similar tips are not available, hold the tip directly above the well and between the two gel plates, and allow the sample to gently fall into the well.
3. After loading, run the polyacrylamide gels at 200 V for 30 min. It is acceptable for the orange dye front to migrate out but stop the electrophoresis if the red dye gets to 2 cm from the bottom of the gel.
4. When the gels are finished running, turn off the power supply and disconnect the leads. Remove the lid and lift out the electrode assembly and clamping frame.
5. Pour out the running buffer from the electrode assembly. Open the clamps and remove the gel cassettes.
6. To keep the gel free of contamination from your fingertips, wear gloves to handle the gels from this point on. Lay a gel cassette flat on the bench with the short plate facing up. Carefully pry apart the gel plates using the gel opening key supplied with the gels. The gel will usually adhere to one of the plates. Transfer the plate with the gel adhering to it to a tray containing 1x Fast Blast stain (see below), allowing the liquid to detach the gel from the plate. The gel may also be lifted directly (very gently!) from the plate and placed into the stain.

### **Preparation for Staining Acrylamide Gels**

Fast Blast DNA stain is provided as a 500x concentrate that must be diluted to 1x prior to use and stains DNA in polyacrylamide in around 30 minutes. It is a convenient, safe, and nontoxic alternative to ethidium bromide for the detection of DNA. Fast Blast contains a cationic compound that belongs to the thiazin family of dyes. The positively charged dye molecules are attracted to and bind to the negatively charged phosphate groups on DNA. The proprietary dye formula stains DNA deep blue in acrylamide gels and provides vivid, consistent results. Detailed instructions on using Fast Blast are included in the student manual.

#### **WARNING**

**Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes. Dispose of the staining solutions according to protocols at your facility. Use either 10% bleach solution or 70% alcohol solution to remove Fast Blast from most surfaces. Verify that these solutions do not harm the surface prior to use.**

#### **Preparation for Staining Protocol**

To prepare 1x stain, dilute 1 ml of 500x Fast Blast with 499 ml of distilled or deionized water in an appropriately sized flask or bottle, and mix. Cover the flask and store at room temperature until ready to use.

### **Lesson 4: Drying Gels and Analysis of Results**

For a permanent record of the experiment, gels can be dried between cellophane sheets and incorporated into lab notebooks; see below and student manual for protocols on these two drying methods.

To document the wet gels, they can be scanned, photocopied (a yellow backing provides optimal contrast), or traced onto acetate film. Note: 3% agarose gels do not adhere well to agarose gel support film.

**GelAir™ drying method:**

**Materials Needed for Drying 8 Gels**

<b>Using Gel Drying System</b> (cat. #165-1771EDU)	<b>Quantity</b>
GelAir cellophane (cat. #165-1779EDU)	4 sheets
GelAir assembly table (cat. #165-1776EDU)	1
GelAir drying frames (cat. #165-1775EDU)	2
GelAir clamps (cat. #165-1780EDU)	16
GelAir drying oven (optional) (cat. #165-1777EDU)	1
Distilled water	500 ml

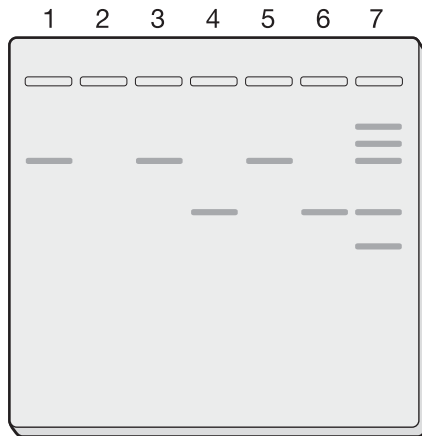
**Alternatively, you may use the cellophane sandwich and plastic container method:**

<b>Materials Needed for Drying 8 Gels Using Plastic Containers</b>	<b>Quantity</b>
GelAir cellophane (cat. #165-1779EDU)	16 sheets
Plastic container	8
Rubber bands	16
Distilled water	500 ml

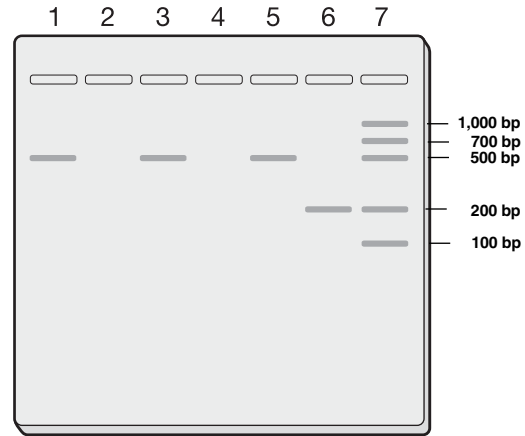
**Procedure**

1. Prewet 2 sheets of cellophane in a container of water for 15–20 seconds.
2. Place one sheet of cellophane over a plastic container. Pull the cellophane taut so that it makes a flat surface over the top of the container, and use a rubber band to hold the sheet in place.
3. Place a gel onto the cellophane. Flooding the surface of the cellophane around the gel with water will aid in the removal of bubbles.
4. Place the second sheet of wetted cellophane over the gel. Because of their thickness you cannot avoid bubbles at the edges of agarose gels, but avoid bubbles between the cellophane and the face of the gel. Secure the second sheet of cellophane to the box with a second rubber band.
5. Allow the gel to dry for several days in a well-ventilated area.
6. Contrast on agarose gels can be improved by peeling off the cellophane once the agarose gels have dried. This is not possible with polyacrylamide gels.

## Typical Classroom Results



**Result for GMO-positive food**



**Result for Non-GMO food**

The presence or absence of a 200 bp band in lane 5 indicates whether or not the test food contains GMOs. However, the validity of this result depends on the results from the other PCR reactions. The plant primers determine whether plant DNA was successfully extracted from the sample. The non-GMO food control is an indicator of false positive results, should they occur. If the non-GMO food control comes out as GMO-positive (showing a band in lane 2) it means that the PCR was contaminated at some point during processing. If your test food is also GMO-positive, you cannot trust this result. The GMO-positive template control is an indicator of false negatives. If the GMO-positive template control does not amplify, there is a problem with the PCR reaction and you cannot trust a GMO-negative result from your test food. The flow chart on the next page shows how to evaluate these controls in a step-by-step manner.

PCR Sample	Band Size
Lane 1: Non-GMO food with plant primers	455 bp
Lane 2: Non-GMO food with GMO primers	No band
Lane 3: Test food with plant primers	455 bp
Lane 4: Test food with GMO primers	200 bp or no band
Lane 5: GMO-positive template with plant primers	455 bp
Lane 6: GMO-positive template with GMO primers	200 bp
Lane 7: PCR molecular weight ruler	1,000, 700, 500, 200, 100 bp

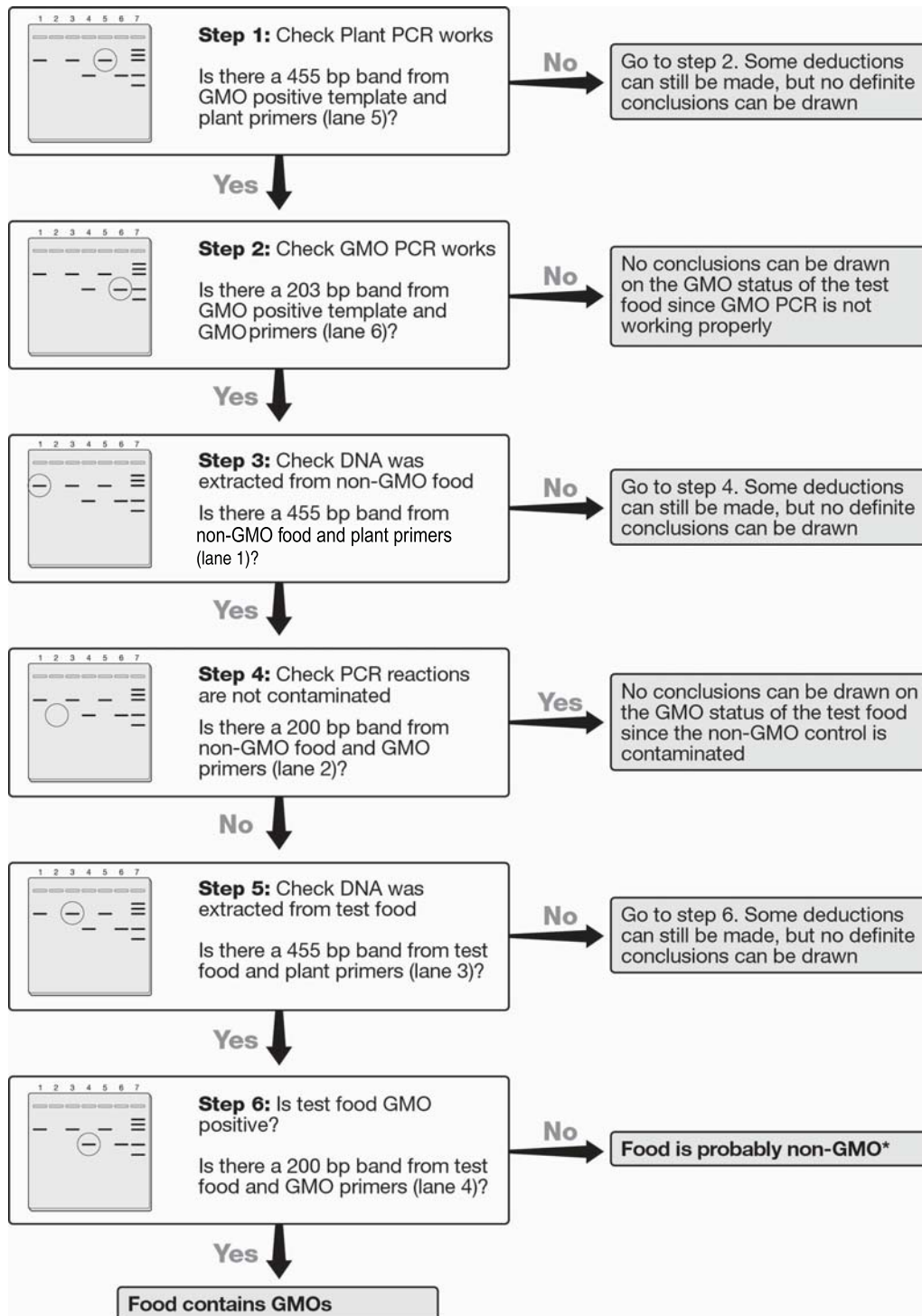


Fig. 4. Step-by-step guide to analysis of results.

\* Note: this kit detects approximately 85% of GM food. Thus you cannot definitively conclude that a food is non-GMO.

## Tips and Frequently Asked Questions

### Lesson 1 Extraction of DNA From Food Samples

#### Bio-Rad Certified Non-GMO Food Control:

- Grinding whole grains takes a while, but total grinding is not necessary. You will find that the water will help to soften the grains and facilitate grinding.
- It is important to process the Bio-Rad certified non-GMO sample first, since PCR is very sensitive and any GMO-positive DNA may contaminate your equipment.
- To reduce the risk of contamination or to save time, you may want to prepare this sample ahead of time and have your students prepare only their test samples.

#### What Foods Should I Choose for the Lab?

Although each group can investigate the genetic modification of a different food, it is advisable to have multiple student teams test each sample to validate each others' results. The extraction method used in this kit may not successfully generate template DNA from all foods. Furthermore, different foods will amplify differently. The table below summarizes foods that generate template DNA that can be amplified with plant primers. **Note:** This is not a list of foods that contain genetic modifications.

The purpose of the activity is to stimulate student interest in the role biotechnology plays in their everyday lives. Although this activity does not require the test food to be genetically modified, here are some tips to help find GM-positive foods in the grocery store:

- Avoid using fresh corn or soy — in the U.S., it is rare to find fresh corn or soy that is genetically modified
- Test nonorganic foods — organic foods are generally not genetically modified
- Use processed food such as cheese-flavored puffed corn snacks, which frequently use GM corn
- Test inexpensive meat products, which often use soy as a filler and are frequently good candidates for GM soy protein

**Note:** Since Europe and Japan have much stricter GM regulations than the U.S., it is very difficult to find GM-positive foods in these regions.

#### Table of foods that reliably generate template DNA that can be amplified with plant primers.

Very Reliable	Reliable	Less Reliable	Very Difficult/Not Possible
Fresh corn	Veggie sausages	Veggie burgers	Oil
Fresh papaya	Tortilla chips	Fried corn snacks	Salad dressing
Corn bread/cake mix	Flavored tortilla chips	Popcorn	Cereal (eg, cornflakes)
Corn meal	Puffed corn snacks	Fries	Wheat flour
Soy flour	Meatballs and burgers containing soy protein	Potato chips	
	Soy-based protein drinks/powders		

#### Prevent Contamination

Part of this lab involves looking for a negative result (i.e., that DNA extracted from your non-GMO food control is not amplified with GMO primers). If this sample gets contaminated with any GMO-positive DNA, yielding a band on the gel, the results of the entire lab will be inconclusive because all of the samples could have been contaminated as well and you cannot trust a GMO-positive result for your test food samples. Therefore, it is imperative that you and your students take proper steps to safeguard against contamination.



Remember that DNA can aerosolize, get itself into pipet barrels, and float about in the air. Keeping tubes capped except during immediate use, using aerosol barrier pipet tips at all stages of the lab, wiping down work areas and equipment, and rinsing out pipet barrels with 10% bleach (to destroy DNA) will assist in reducing contamination risk. Detailed guidelines are given in Appendix B.

### **InstaGene™ matrix: What Function Does It Perform?**

InstaGene matrix consists of a suspension of negatively charged, microscopic beads that bind divalent cations such as magnesium ( $Mg^{2+}$ ). It is important to remove divalent cations from the extracted DNA samples because the cations assist enzymes that degrade the DNA template. When cheek cells are lysed and boiled in the presence of InstaGene matrix, the divalent cations released from the cells bind to the beads, and the heat inactivates the DNA-degrading enzymes. The beads are pelleted by centrifugation, and the supernatant, which contains clean, intact genomic DNA, can be used as template in PCR reactions.

The beads in the InstaGene matrix quickly settle out of the suspension. It is therefore extremely important that the vial of matrix be thoroughly mixed before pipetting aliquots for each student workstation, so that the aliquots contain equivalent amounts of beads.

If the DNA samples are not going to be amplified within 24 hours, they can be stored in the refrigerator in the InstaGene matrix for up to 1 week. For longer storage, place samples in the freezer to prevent DNA degradation. Before the samples are used in PCR, the beads should be repelleted by centrifugation just prior to making up the PCR reactions.

## **Lesson 2 Set Up PCR Reactions**

### **Contamination**

Again, the students should be reminded to guard against contamination, to use fresh aerosol-filtered tips at each step, and to keep tubes capped unless they are immediately adding a reagent to them.

### **Do I Have to Remove the InstaGene Matrix Before PCR?**

It is extremely important to pellet the InstaGene beads completely before any of the lysate is removed for the PCR reaction. The beads bind and remove divalent cations such as  $Mg^{2+}$ , which is essential to the function of *Taq* polymerase. Thus, if any beads are carried over into the PCR reaction, the reaction could be inhibited. The InstaGene matrix can be effectively pelleted by centrifugation (6,000 x g for 5 min). When transferring the DNA samples from the InstaGene samples, carefully remove 20  $\mu$ l of the supernatant above the beads (which contains the genomic DNA).

### **Master Mix: What Is It?**

The master mix contains a mixture of nucleotides, or dNTPs (dATP, dTTP, dCTP, and dGTP), buffer, and *Taq* DNA polymerase. Complete master mix is prepared by adding primers to the master mix just prior to the laboratory period. When 20  $\mu$ l of the DNA template is added to 20  $\mu$ l of complete master mix, all of the necessary components for a 40  $\mu$ l PCR reaction are present.

**Note:** Once the master mix and primers are mixed, the complete mix should be kept on ice and used within 30 minutes to 1 hr. These reagents are extremely sensitive.

### **Why Are the Primers Red and Green?**

The primer mixes contain PCR-compatible dyes that allow students to easily visualize and distinguish the different master mixes. The dyes also migrate in the gel giving a visual demonstration of electrophoresis.

## PCR in a Thermal Cycler

The PCR amplification takes place in a thermal cycler that performs cycles of alternating heating and cooling steps. This lab utilizes a three-step cycle: the DNA undergoes denaturation at 94°C for 1 minute, annealing at 59°C for 1 minute, and extension at 72°C for 2 minutes. This cycle is repeated 40 times during the course of PCR amplification. During the denaturation, the two strands of the DNA template are melted apart to provide access for the PCR primers. During the annealing step, the PCR primers recognize and bind to the DNA template. Once the primers are bound, *Taq* DNA polymerase extends the primers to replicate the segment of DNA during the extension step. The PCR reaction will take approximately 3.5 hours to complete.

The PCR tubes are very small and require care when handling. It is important to carefully and completely cap the tubes before placing them into the thermal cycler. If the tubes are not closed completely, substantial evaporation can take place, and PCR amplification will be inhibited. Bio-Rad's thermal cyclers were developed for oil-free operation. Oil is not needed in the thermal block wells or in the sample tubes. The sample wells are shaped to provide uniform contact with most standard 200 µl thin-wall PCR tubes. **Do not use 500 µl thin-wall micro test tubes with these thermal cyclers.** The heated sample block cover maintains a higher temperature than the sample block at all times during a thermal cycling program. This keeps water vapor from condensing under the cap of the sample tube, thereby reducing sample evaporation and eliminating the need for oil overlays in the tubes.

### How Stable Are Newly Set Up PCR Reactions?

Extended incubation of master mix and genomic DNA decreases amplification efficiency. Thus if you wish to put two classes into one PCR machine or if you have more PCR reactions than you have space in your thermal cycler we suggest incubating the reactions on ice for no more than one hour prior to cycling.

### Manual PCR

It is possible to perform PCR manually without an automated thermal cycler, although the PCR will not be as efficient. For manual PCR amplification, reactions should be performed in screwcap tubes and topped off with a drop of mineral oil to prevent evaporation. The tubes are placed in a heat block or water bath set at 95°C for 1 minute, then manually transferred to a heat block or water bath set at 59°C for 1 minute, and finally transferred to a heat block or water bath set at 72°C for 2 minutes. Forty cycles of manual PCR should take ~3 hours. It is tedious but it works. Good luck!

## Lesson 3 Electrophoresis of PCR products

### Agarose or Polyacrylamide Gel Electrophoresis?

The DNA fragments amplified from the 35S promoter and NOS terminator are 203 and 225 base pairs (bp) respectively. The PCR product generated from the photosystem II gene is 455 bp. Resolving bands in this size range requires either a 3% agarose gel or a 10% polyacrylamide gel. Both gel techniques give excellent results. Your choice of gel technique will depend on the equipment that is available to you and the techniques you wish to teach your students. Polyacrylamide gels are much more fragile than 3% agarose gels and thus may be suitable only for more experienced students. However polyacrylamide gels resolve bands to a greater degree, which may allow for separation of the similar-sized DNA bands generated from a test food that contains both the CaMV 35S promoter (203 bp) and NOS terminator (225 bp), such as genetically modified papaya. Refer to page 3 for the accessories that you will need depending on whether you choose agarose or polyacrylamide gel electrophoresis.

### **Orange G Loading Dye**

Before the amplified samples are electrophoresed, students need to add 10 µl of 5x orange G loading dye to each of their PCR tubes. The loading dye contains glycerol, which increases the density of the sample and ensures that it sinks into the well of the agarose gel. In addition, the loading dye contains a dye called Orange G that comigrates with DNA of ~50 bp in a 3% agarose gel or with ~20 bp in a 10% acrylamide gel.

### **Dye Migration**

**Agarose Gels** — The orange dye from the loading dye should not be allowed to migrate off an agarose gel, otherwise some samples may be lost.

**Polyacrylamide Gels** — The orange dye front may migrate off the polyacrylamide gel. The red dye front from the GMO primer dye should not be allowed to migrate off polyacrylamide gels.

As a side point, the different dyes used to color the primers migrate at different rates due to charge differences, and they provide a useful visible demonstration of electrophoresis.

### **Can I Use Ethidium Bromide to Stain My Gels?**

This lab has been optimized for use with Fast Blast DNA stain, a nontoxic, safe DNA stain. Ethidium bromide (EtBr) is the traditional stain used to visualize DNA and is more sensitive than Fast Blast, and it will work well to stain gels for this lab. However, EtBr is a known mutagen and suspected carcinogen and requires the use of UV light to visualize DNA. One disadvantage of using EtBr is that, due to its higher sensitivity, primer-dimer bands may be more visible with EtBr than with Fast Blast and may confuse interpretation of results with less experienced students. If EtBr is used as a stain for agarose gels, the gels should contain 0.05 µg/ml EtBr in the agarose. This concentration produces maximum contrast of the amplified bands. Note: Fast Blast DNA stain quenches EtBr staining, so visualize with EtBr before Fast Blast stain. Polyacrylamide gels must be stained after electrophoresis. Stain the gels in 0.05 µg/ml EtBr and destain in water at least 2 times for 20 min.

## **Lesson 4 Analysis of Results**

### **Why Do Foods Labeled As "Non-GMO" or "Organic" Come up As GMO-Positive?**

First, check your controls. Does your non-GMO food control test negative for GMO? If the answer is yes, you may still have contamination in just that one sample, rather than in all of the reactions, so the best way to confirm your result is to repeat the test. However, there may well be GMOs in food labeled as "non-GMO". Different countries have different regulations for food labeling. Most countries allow food to be labeled as "non-GMO" (or alternatively, **not** labeled as "GMO") when the percentage of GMO-derived material in the food is below a legislated level (usually 1–5%). The PCR test is sensitive enough to detect these low levels. Quantitative tests for detecting the percentage of GMOs in food can be performed by a GMO testing laboratory using real-time PCR.

### **Why Are My Non-GMO Controls GMO-Positive?**

Somewhere in the process the samples were contaminated with GMO-positive DNA. Refer to Appendix B for ways to safeguard against contamination.

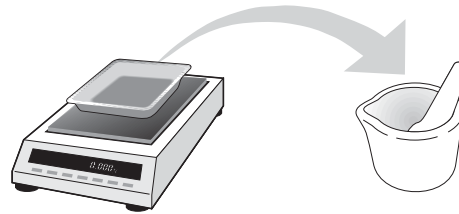
### **Why Did I Not Get Viable Plant DNA?**

Mistakes may have been made during DNA extraction, which can be verified by repeating the test. However, some foods do not yield PCR amplifiable plant DNA. This kit was optimized to test corn, soy, and papaya based foods. Refer to the table on p. 26 for recommended reliable foods.

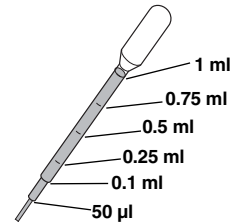
## Quick Guide

### Day One: Extraction of DNA From Food Samples

1. Find your screwcap tubes and label one “non-GMO” and one “test”.
2. Weigh out 0.5–2 g of certified non-GMO food and put it into the mortar.



3. Add 5 ml of distilled water for every gram of food. To calculate the volumes of water you need, multiply the mass in grams of the food weighed out by 5 and add that many milliliters.

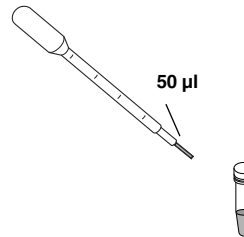


Mass of food = \_\_\_\_ g x 5 = \_\_\_\_ ml

4. Grind with pestle for at least 2 min to form a slurry.
5. Add another 5 ml of distilled water for every gram of food. Mix or grind further with the pestle until the slurry is smooth enough to pipet.



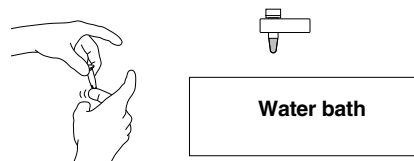
6. Pipet 50 µl of ground slurry to the screwcap tube containing 500 µl of InstaGene labeled “non-GMO” using the 50 µl mark on a graduated pipet. Recap tube.



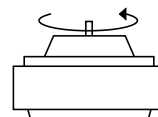
7. Repeat steps 2–5 to prepare the test food sample.

8. Pipet 50 µl of ground test food slurry to the screwcap tube labeled “test”. Recap tube.

9. Shake or flick the non-GMO food and test food InstaGene tubes and place tubes in 95°C water bath for 5 min.



10. Place tubes in a centrifuge in a balanced conformation and centrifuge for 5 min at max speed.



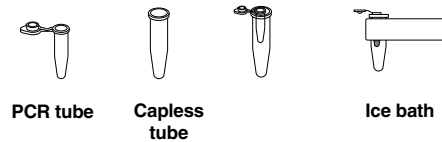
11. Store tubes in a refrigerator until next lesson.

## Day 2: Set Up PCR Reactions

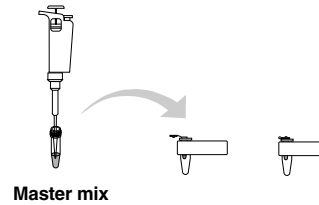
- Number PCR tubes 1–6 and initial them. The numbers should correspond to the following tube contents:

Tube number	Master Mix	DNA
1	20 $\mu$ l Plant MM (green)	20 $\mu$ l Non-GMO food control DNA
2	20 $\mu$ l GMO MM (red)	20 $\mu$ l Non-GMO food control DNA
3	20 $\mu$ l Plant MM (green)	20 $\mu$ l Test food DNA
4	20 $\mu$ l GMO MM (red)	20 $\mu$ l Test food DNA
5	20 $\mu$ l Plant MM (green)	20 $\mu$ l GMO positive control DNA
6	20 $\mu$ l GMO MM (red)	20 $\mu$ l GMO positive control DNA

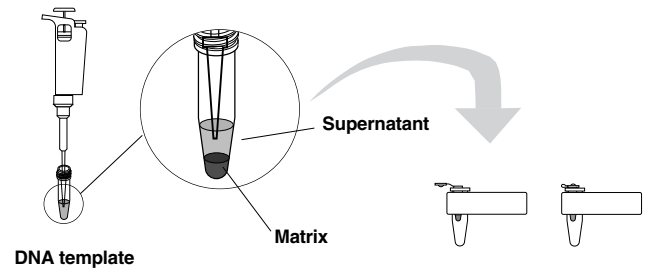
- Place each tube in a capless microtube adaptor and place in the foam float on ice.



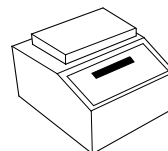
- Referring to the table and using a fresh tip for each addition, add 20  $\mu$ l of the indicated master mix to each PCR tube, cap tubes.



- Referring to the table and using a fresh tip for each tube, add 20  $\mu$ l of the indicated DNA to each PCR tube, being sure to avoid the InstaGene pellet at the bottom of the tubes. Mix by pipetting gently up and down; recap tubes.



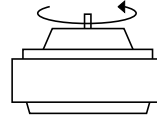
- When instructed, place PCR tubes in thermal cycler.



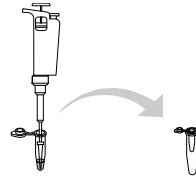
### Day 3: Electrophoresis of PCR products

1. Set up your gel electrophoresis apparatus as instructed.

2. Obtain your PCR tube from the thermal cycler and place in the capless microtube adaptor. Pulse-spin the tube for ~3 seconds.

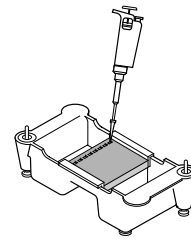


3. Using a fresh tip each time, add 10  $\mu$ l of Orange G loading dye (LD) to each sample and mix well

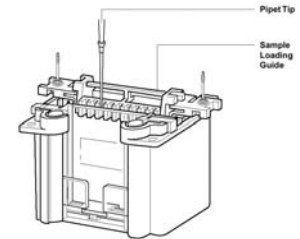


4. Load 20  $\mu$ l of the molecular weight ruler and 20  $\mu$ l each sample into your gel in the order indicated below:

Lane	Sample	Load volume
1	Sample 1: Non-GMO food control	
	with plant primers	20 $\mu$ l
2	Sample 2: Non-GMO food control	
	with GMO primers	20 $\mu$ l
3	Sample 3: Test food with plant primers	20 $\mu$ l
4	Sample 4: Test food with GMO primers	20 $\mu$ l
5	Sample 5: GMO positive DNA	
	with plant primers	20 $\mu$ l
6	Sample 6: GMO positive DNA	
	with GMO primers	20 $\mu$ l

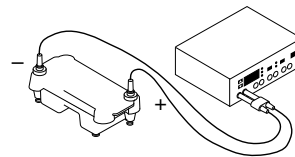


Agarose Gel

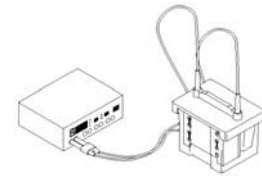


Polyacrylamide Gel

5. The run time and voltage will depend on the type of gel you are running. Run an agarose gel for 30 min at 100 V and run a polyacrylamide gel at 200 V for 20 min.

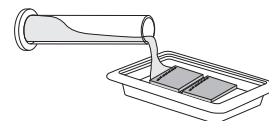


Agarose Gel Electrophoresis



Polyacrylamide Gel Electrophoresis

6. Stain in Fast Blast DNA stain. Refer to specific instructions depending on gel type.



# Student Manual

## Background

With the world population exploding and farmable land disappearing, agricultural specialists are concerned about the world's ability to produce enough food to feed the growing population. Environmentalists are concerned about the overuse of pesticides and herbicides and the long-term effects of these chemicals on the environment and human health. Might there be a solution to both of these problems? The biotechnology industry thinks so. Its proponents believe genetically modified organisms (GMOs), particularly genetically modified (GM) crop plants, can solve both problems. This proposed solution, however, has met with great opposition throughout the world. Dubbed "frankenfoods" by opponents and restricted in most European countries, GMOs are widely produced and sold in the United States. Currently in the US, foods that contain GMOs do not have to be labeled as such.

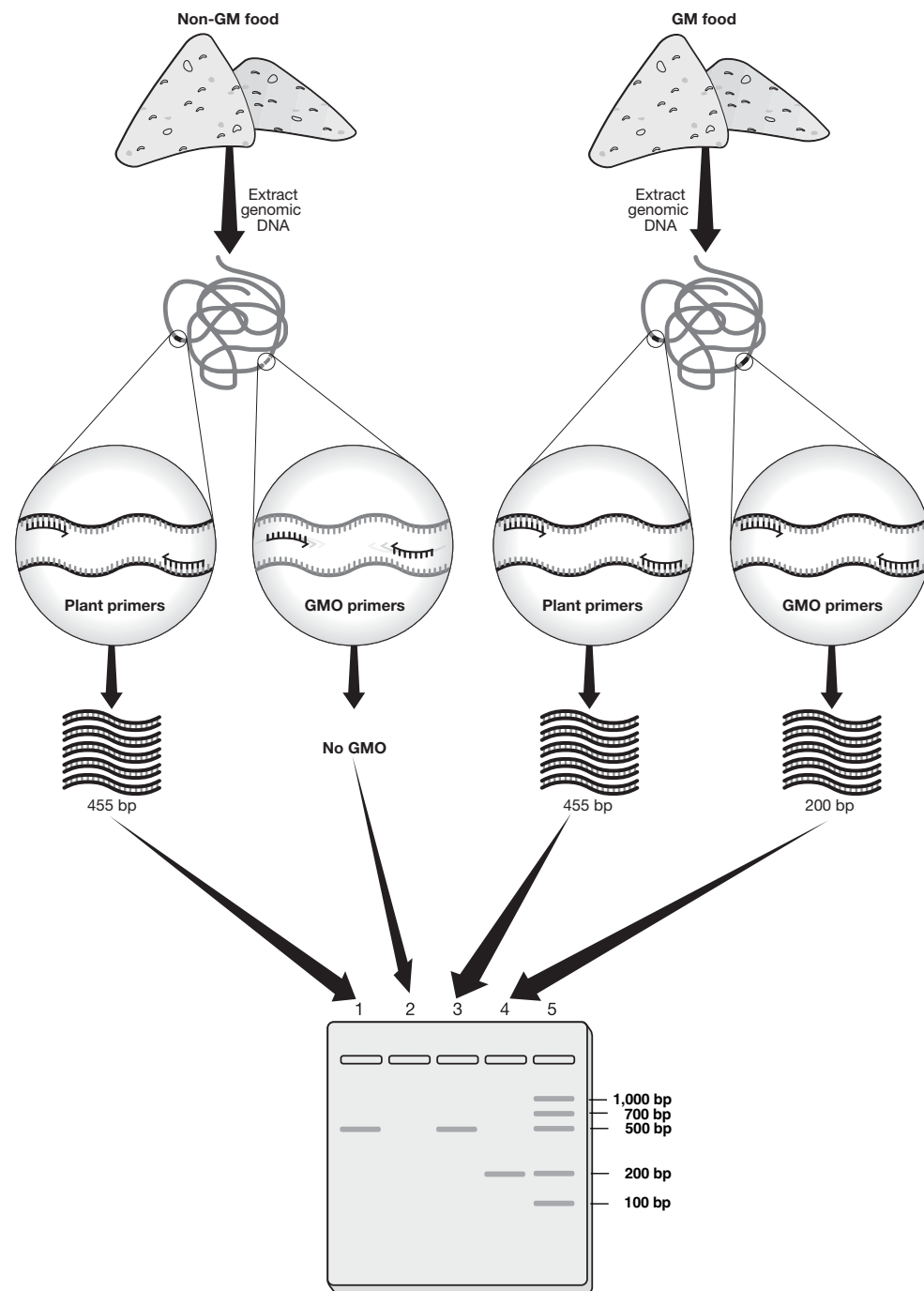
Genetic manipulation of crop plants is not new. Farmers have been genetically modifying crops for centuries and crop breeding to encourage specific traits, such as high yield, is still an important part of agriculture today. However, there is now the option to place genes for selected traits directly into crop plants. These genes do not have to originate from the same plant species—in fact, they do not have to come from plants at all. One popular class of GM crops has a gene from the soil bacterium *Bacillus thuringiensis* (Bt) inserted into their genomes. Bt crops produce a protein called delta-endotoxin that is lethal to European corn borers, a common pest on corn plants. Farmers who plant Bt crops do not have to apply pesticide because the plants produce the toxic protein inside their cells. When the corn borers feed on the genetically modified plant, they die. Other GMOs include those that are herbicide-resistant, delayed for fruit ripening, are resistant to fungi or drought, have increased crop yield, or bear improved fruits.

Many people object to the use of GM crop plants. They argue that there is a potential to create super-weeds through cross-pollination with herbicide-resistant crops or that super-bugs will evolve that are no longer resistant to the toxins in pest-resistant crops. Many are concerned with potential allergic reactions to the novel proteins or antibiotic resistance arising from the selectable markers used to develop the crops or other unforeseen effects on public health. Proponents of GM foods argue these crops are actually better for the environment. Fewer toxic chemicals are put into the environment and thus fewer toxic chemicals can harm the environment and human health. In addition, these crops can preserve arable land by reducing stresses on the land, improve the nutritional value of food in developing countries, and allow crops to be grown on previously unfarmable land.

Whatever position one takes in the GMO debate, it would be beneficial to be able to test foods found in the grocery store for the presence of GMO-derived products. This can be done in several ways. One would be to use an antibody-based test such as the enzyme-linked immunosorbent assay (ELISA), which can detect the proteins that are produced specifically by GM crops. However, the ELISA is not useful for testing foods that have been highly processed, because the proteins have most likely been destroyed and different GM foods produce different proteins. Another method is to use the polymerase chain reaction (PCR) to look for a DNA sequence common to GM foods. DNA is more resistant than proteins to processing and can be extracted from even highly processed foods. It is these GMO DNA sequences that we will be testing for in this laboratory.

In the first lesson you will extract genomic DNA from food samples, in the second lab you will run PCR reactions to amplify GMO and natural plant sequences from the DNA, and in the third lab you will electrophorese the amplified samples to visualize the DNA.

Let's see if your favorite food contains GMOs!



**Fig. 1. Detecting GM foods by PCR.** Genomic DNA is extracted from test foods (Lesson 1) and then two PCR reactions are performed on each test food genomic DNA sample (Lesson 2). One PCR reaction uses primers specific to a common plant gene (plant primers) to verify that viable DNA was successfully extracted from the food. No matter whether the food is GM or not, this PCR reaction should always amplify DNA (See lanes 1 and 3 of the gel above). The other PCR reaction uses primers specific to sequences commonly found in GM crops (GMO primers). This PCR reaction will only amplify DNA if the test food is GM (See lane 4). If the test food is non-GM, then the GMO primers will not be complementary to any sequence within the test food genomic DNA and will not anneal, so no DNA will be amplified (see lane 2). To find out whether DNA has been amplified or not, the PCR products are electrophoresed on a gel and stained to visualize DNA as bands (Lesson 3). A molecular weight ruler (lane 5) is electrophoresed with the samples to allow the sizes of the DNA bands to be determined.



## Lesson 1 Extraction of DNA From Food Samples

In this lesson you will extract DNA from a control non-GMO food and a grocery store food item that you will test for the presence of GMOs. The most commonly modified foods are corn and soy-based, and so the test food could be fresh corn or soybeans, or a prepared or processed food such as cornmeal, cheese puffs, veggie sausage, etc. You will process the non-GMO control first.

You will first weigh your food sample, then grind it with water to make a slurry. You will then add a tiny amount of the slurry to a screwcap tube containing InstaGene matrix and boil it for 5 minutes.

The cellular contents you are releasing from the ground-up sample contain enzymes (DNases) that can degrade the DNA you are attempting to extract. The InstaGene matrix is made of negatively charged microscopic beads that “chelate” or grab metal ions out of solution. It chelates metal ions such as  $Mg^{2+}$ , which is required as a cofactor in enzymatic reactions. When DNA is released from your sample in the presence of the InstaGene matrix, the charged beads grab the  $Mg^{2+}$  and make it unavailable to the enzymes that would degrade the DNA you are trying to extract. This allows you to extract DNA without degradation. Boiling the samples destroys these enzymes.

After you centrifuge the samples to remove the InstaGene matrix and debris, the supernatant will contain intact extracted DNA. This extracted DNA will be used in the next laboratory as your target DNA.

## **Lesson 1 Extraction of DNA From Food Samples**

### **Focus Questions**

1. How can you test a food to find out if it contains material derived from a genetically modified organism (GMO)?
2. In what organelles is plant DNA located?
3. Many foods containing GM crops are highly processed. Can you suggest how DNA from whole plants may differ from that extracted from processed foods, e.g., corn chips, cornmeal, etc.?
4. What molecules are present in the cell that might interfere with DNA extraction?
5. Why do you also perform analysis on food that is known to be a non-GMO food control?
6. Why was the non-GMO food control prepared prior to your test food sample?

## Student Protocol – Lesson One

Materials and supplies required at the workstation prior to beginning this exercise are listed below.

### Student Workstation

Material	Quantity
Screwcap tube with 500 $\mu$ l InstaGene matrix	2
Beaker of distilled water	1
Food samples	1 or 2
Disposable plastic transfer pipets (DPTP)	2
2–20 $\mu$ l micropipet (if preparing non-GMO food control)	1
2–20 $\mu$ l pipet tips, aerosol barrier	1 rack
Mortar and pestle	1
Marking pen	1

### Common Workstation

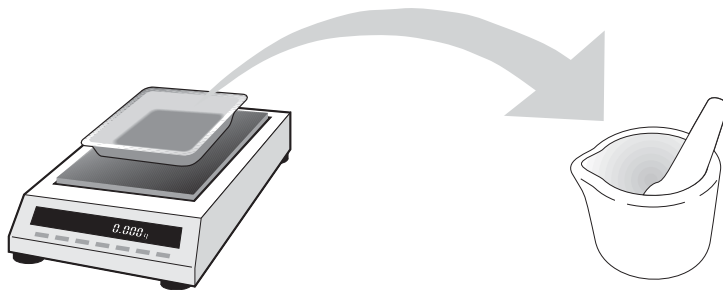
Material	Quantity
Water bath set to 95–100°C	1
Microcentrifuge or mini centrifuges	3–4
Balance and weigh boats	1

### Protocol

**Note:** ALWAYS process the non-GMO control before the test sample to reduce the risk of contamination.

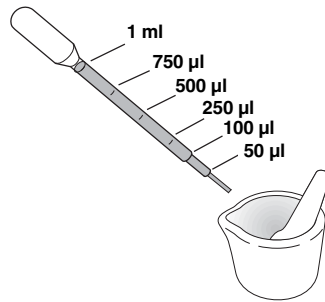
**Grind non-GMO food control** (your instructor may perform this step for you)

1. Find your screwcap tubes containing 500  $\mu$ l of InstaGene matrix and label one “non-GMO” and one “test”.
2. Weigh out 0.5–2 g of the certified non-GMO food control and place in mortar.

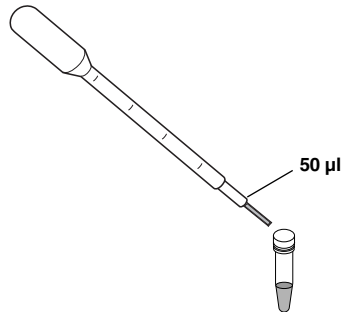


- Using the transfer pipet, add 5 ml of distilled water for every gram of food using the graduations on the transfer pipet. To calculate the volume of water you need, multiply the mass in grams of the food weighed out by 5 and add that many millimeters.

Mass of Food = \_\_\_\_\_ g x 5 = \_\_\_\_\_ ml



- Grind with pestle for at least 2 min until a slurry is formed.
- Add another 5 ml of distilled water for every gram of food. Mix or grind further with the pestle until the slurry is smooth enough to pipet.
- Add **50 µl** of ground slurry to the screwcap tube containing 500 µl of InstaGene matrix labeled “non-GMO” using a transfer pipet.



- Recap tube and shake well.



- Wash mortar with detergent and dry.

### Grind Test Food

- Weigh out 0.5–2 g of test food and place in mortar.**
- Using the transfer pipet, add 5 ml of distilled water for every gram of food using the graduations on the transfer pipet. To calculate the volume of water you need, multiply the mass in grams of the food weighed out by 5 and add that many millimeters.

Mass of food = \_\_\_\_\_ g x 5 = \_\_\_\_\_ ml

- Grind with pestle for at least 2 min until a slurry is formed.

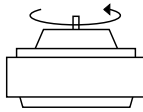
4. Add another 5 ml of distilled water for every gram of food and mix or grind further with pestle until the slurry is smooth enough to pipet.
5. Add **50  $\mu$ l** of ground slurry to the screwcap tube labeled "Test" using the 50  $\mu$ l mark on a transfer pipet.
6. Recap tube and shake well.

#### **Process Samples to Extract DNA**

1. Place non-GMO food control and test food sample tubes in 95°C water bath for 5 min.



2. Place tubes in a centrifuge in a balanced conformation and spin for 5 min at max speed.



3. Store tubes in refrigerator until the next lesson.

## Lesson 2 Set Up PCR Reactions

In the last laboratory, you extracted DNA from a certified non-GMO food sample and a test food sample that you are analyzing for the presence of GMO DNA sequences. In this lab you will prepare those two samples and a positive control (GMO-positive template DNA) for the polymerase chain reaction (PCR).

PCR is DNA replication in a test tube. PCR allows you to amplify specific sections of DNA and make millions of copies of the target sequence. Your experiment is to determine whether or not the DNA you extracted from food in Lesson 1 contains or does not contain the target sequences of interest typically found in genetically modified (GM) foods.

### PCR Review

PCR is such a powerful tool because of its simplicity and specificity. All that is required are minute quantities of the DNA template you want to amplify, DNA polymerase, two DNA primers, four DNA base pair subunits (deoxyribonucleotide triphosphates of adenine, guanine, thymine, and cytosine) and buffers.

Because PCR identifies a specific sequence of DNA and makes millions of copies of (or amplifies) that sequence, it is one of the most useful tools of molecular biology. Scientists use PCR to obtain the large amounts of a specific sequence of DNA that are necessary for such techniques as gene cloning, where DNA is physically moved from one genome to another. You are using the property of PCR that allows identification of a specific sequence, namely, the ability of PCR to search out a single sequence of a few hundred base pairs in a background of billions of base pairs. For example, the corn genome has 2.5 billion base pairs of DNA. This sequence is then amplified so that there are millions of copies of it so that it stands out from the few copies of the original template DNA.

PCR locates specific DNA sequences using primers that are complementary to the DNA template. Primers are short strands of DNA (usually between 6 and 30 base pairs long) called oligonucleotides. Two primers are needed to amplify a sequence of DNA, a forward primer and a reverse primer. The two primers are designed and synthesized in the laboratory with a specific sequence of nucleotides such that they can anneal (bind) at opposite ends of the target DNA sequence on the complementary strands of the target DNA template. The target DNA sequence is copied by the DNA polymerase reading the complementary strand of template DNA and adding nucleotides to the 3' ends of the primers (see fig 2). Primers give the specificity to the PCR, but they are also necessary because DNA polymerase can only add nucleotides to double-stranded DNA.

During PCR, double-stranded DNA template is separated by heating it, then each primer binds (anneals) to its complementary sequence on each of the separated DNA strands, and DNA polymerase elongates each primer by adding nucleotides to make a new double-stranded DNA (see fig 2).

The DNA polymerase used in PCR must be a thermally stable enzyme because the PCR reaction is heated to 94°C, which would destroy the biological activity of most enzymes. The most commonly used thermostable DNA polymerase is *Taq* DNA polymerase. This was isolated from a thermophilic bacterium, *Thermus aquaticus*, which lives in high-temperature steam vents such as those in Yellowstone National Park.

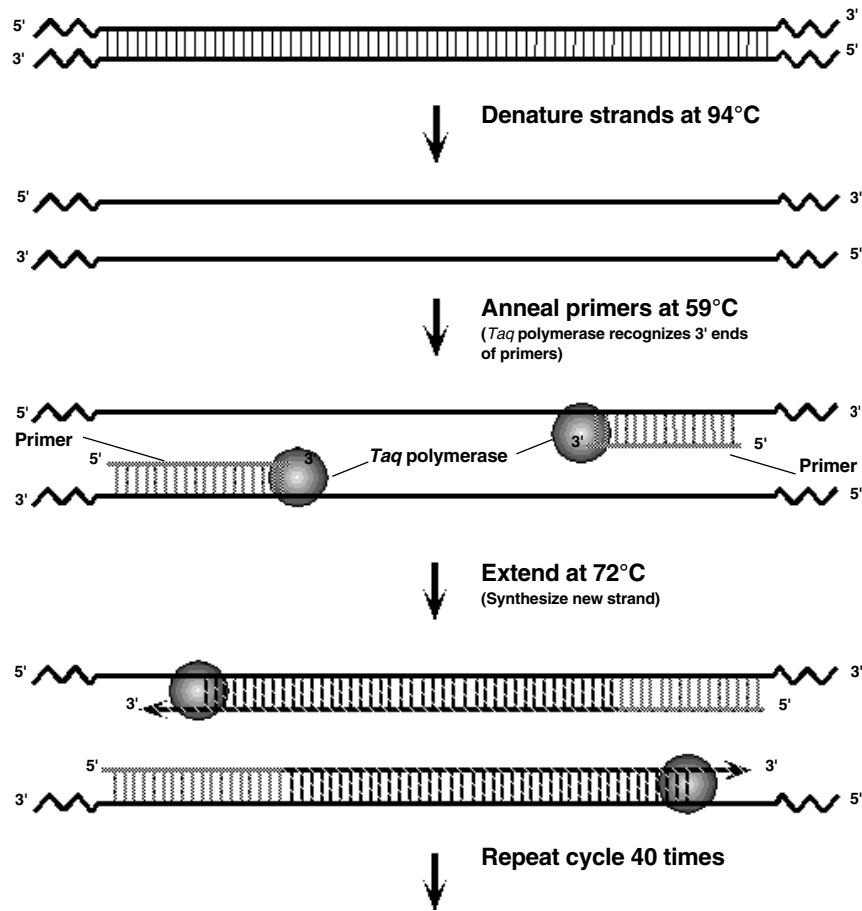


Fig. 2. A complete cycle of PCR.

### PCR Step by Step

PCR has three steps, a denaturing step, an annealing step, and an elongation step. During the denaturing step, the DNA template is heated to 94°C to separate (or denature) the double-stranded DNA molecule into two single strands. The DNA is then cooled to 59°C to allow the primers to locate and anneal (bind) to the DNA. Because the primers are so much shorter than the template DNA, they will anneal much more quickly than the long template DNA strands at this temperature. The final step is to increase the temperature of the PCR reaction to 72°C, which is the optimal temperature for the DNA polymerase to function. In this step the DNA polymerase adds nucleotides (A, T, G, or a C) one at a time at the 3' end of the primer to create a complementary copy of the original DNA template. These three steps form one cycle of PCR. A complete PCR amplification undergoes multiple cycles of PCR, in this case 40 cycles.

The entire 40 cycle reaction is carried out in a test tube that has been placed in a thermal cycler or PCR machine. This is a machine that contains an aluminum block that can be rapidly heated and cooled. The rapid heating and cooling of this thermal block is known as thermal cycling.

Two new template strands are created from the original double-stranded template during each complete cycle of PCR. This causes exponential growth of the number of target DNA molecules, i.e., the number of target DNA molecules doubles at each cycle; this is why it is called a chain reaction. Therefore, after 40 cycles there will be  $2^{40}$ , or over 1,100,000,000,000 times more copies than at the beginning. Once the target DNA sequences of interest have been sufficiently amplified, they can be visualized using gel electrophoresis. This allows researchers to determine the presence or absence of the PCR products of interest.

### **Your Task for This Lesson**

For this experiment you will set up two PCR reactions for each DNA sample, which makes 6 PCR reactions in total. One PCR reaction, using the plant master mix (PMM), is a control to determine whether or not you have successfully extracted plant DNA from your test food. This is done by identifying a DNA sequence that is common to all plants by using primers (colored green in the kit) that specifically amplify a section of a chloroplast gene used in the light reaction (photosystem II). Why is this necessary? What if you do not amplify DNA using the GMO primers? Can you conclude that your test food is not GM or might it just be that your DNA extraction was unsuccessful? If you amplify DNA using the plant primers, you can conclude that you successfully amplified DNA, therefore whether or not you amplify DNA with your GMO primers, you will have more confidence in the validity of your result.

The second PCR reaction you carry out will determine whether or not your DNA sample contains GM DNA sequences. This is done by identifying DNA sequences that are common to most (~85%) of all GM plants using primers specific to these sequences. These primers are colored red and are in the GMO master mix (GMM).

Why do you have to set up a PCR reaction with DNA from certified non-GMO food? What if some GMO-positive DNA got into the InstaGene or master mix from a dirty pipet tip or a previous class? This DNA could be amplified in your test food PCR reaction and give you a false result. By having a known non-GMO control that you know should not amplify the GMO target sequences, you can tell if your PCR reactions have been contaminated by GMO-positive DNA.





Remembering that DNA polymerases can only add nucleotides to the 3' end of DNA, design a forward primer and a reverse primer, each 10 bases long, to amplify a target sequence of the DNA that is at least 100 bp long. Write the sequence of the primers below, with their 3' and 5' ends indicated. Also indicate on the sequence above which strand they are complementary to (will anneal to).

**Forward primer sequence:**

**Reverse primer sequence:**

4. Why are you performing two PCR reactions on each DNA sample?

5. What is the purpose of the GMO-positive control DNA?

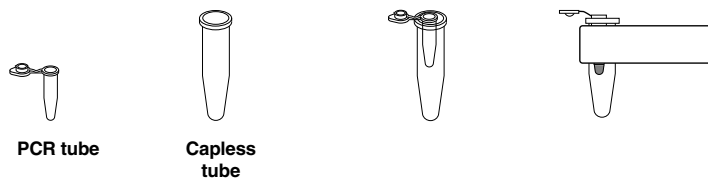
## Student Protocol – Lesson Two Set Up PCR Reactions

### Student Workstations

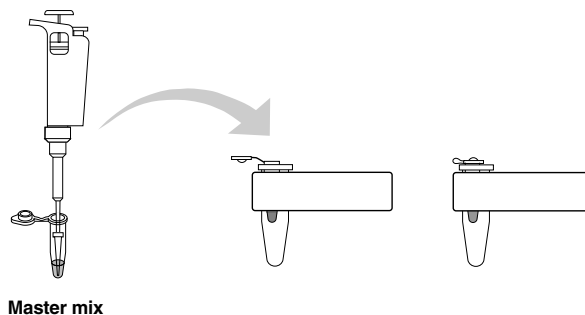
Material	Quantity
Ice bath containing 3 tubes	1
GMO master mix (red) (on ice)	1
Plant master mix (green) (on ice)	1
GMO positive control DNA (on ice)	1
Test food DNA (from previous lab)	1
Non-GMO food control DNA (from previous lab)	1
PCR tubes	6
PCR adaptors	6
Foam microtube holder	1
Marking pen	1
2–20 µl adjustable-volume micropipet or fixed-volume 20 µl micropipet	1
2–20 µl pipet tips, aerosol barrier	1 rack

1. Number six PCR tubes 1–6 and label them with your initials. The numbers correspond to the following tube contents:

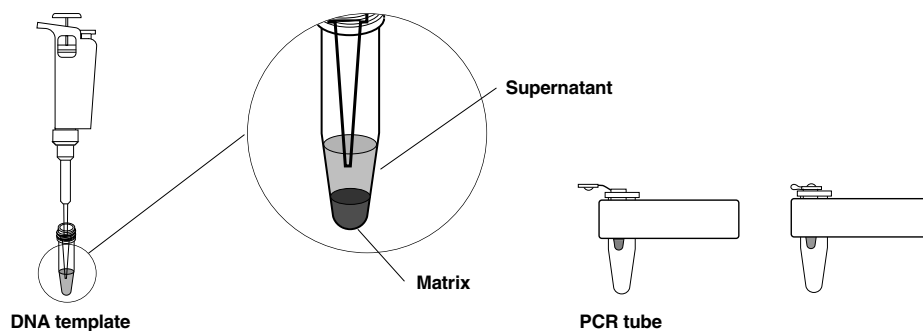
Tube Number	DNA	Master Mix
1	20 µl Non-GMO food control DNA	20 µl Plant master mix (green)
2	20 µl Non-GMO food control DNA	20 µl GMO master mix (red)
3	20 µl Test food DNA	20 µl Plant master mix (green)
4	20 µl Test food DNA	20 µl GMO master mix (red)
5	20 µl GMO positive control DNA	20 µl Plant master mix (green)
6	20 µl GMO positive control DNA	20 µl GMO master mix (red)



2. Keep the tubes on ice for the remaining steps.
3. Using a fresh tip each time, add 20 µl of the indicated master mix to each tube. I.E. add 20 µl of green plant master mix (PMM) to tubes 1, 3, and 5. Then add 20 µl of red GMO master mix (GMM) to tubes 2, 4, and 6. Cap each tube.



- Using a fresh pipet tip for each tube, add 20  $\mu$ l of the DNA to each tube as indicated in the table above. Take care not to transfer any of the InstaGene beads to your PCR reaction. If the beads are disrupted, recentrifuge your DNA samples to pellet the beads. Add 20  $\mu$ l of non-GMO food control DNA to tube 1 and pipet up and down to mix. Discard your tip. Use a fresh tip to add 20  $\mu$ l of non-GMO food control DNA to tube 2 and mix. Discard your tip. Similarly add 20  $\mu$ l of test food DNA to tubes 3 & 4, and add 20  $\mu$ l of GMO positive control DNA to tubes 5 & 6, changing your tip for every tube. Recap tubes.



- When instructed to, place the PCR tubes in the thermal cycler.

### **Lesson 3 Electrophoresis of PCR Products**

You have completed your PCR amplification. You cannot, however, at this point determine whether or not you have PCR products. To do this, you must visualize your products. You will do this using gel electrophoresis.

Your PCR product bands are very small compared to those in other DNA experiments you may have done. For example, fragments produced from a *Hind*III digest of lambda DNA are 23,130, 9,416, 6,557, 4,361, 2,322, 2,027, and 500 base pairs (bp). The product band sizes in this lab are 455 bp for the plant primers and 200 bp for the GMO primers, and a 1% gel would not separate these bands. Instead, a tighter gel matrix is needed to impede the movement of these bands so that they are separated more on the gel and can be seen. Therefore, if you are using agarose electrophoresis, you will use a 3% agarose gel. Alternatively, your teacher may elect to use a polyacrylamide gel, which has smaller pores, to separate your products. Polyacrylamide gel electrophoresis (PAGE) is used to separate smaller molecules for visualization.

Regardless of the gel type, you will load a molecular weight ruler (DNA standard) so that you have a reference to determine your product bands' sizes. The gel will then be stained with Fast Blast stain to make the bands visible.

### Lesson 3

#### Focus Questions

1. Why did you resolve your PCR products by electrophoresis?
2. Explain why DNA fragments separate according to size in an electrophoresis gel.
3. Why do you need a molecular weight ruler alongside your samples?
4. What results do you expect in each lane? Fill in the chart below.

<b>Lane</b>	<b>Sample</b>	<b>Expect band (Yes, No, Don't know)?</b>
1	Sample 1: Non-GMO food control with plant primers	
2	Sample 2: Non-GMO food control with GMO primers	
3	Sample 3: Test food with plant primers	
4	Sample 4: Test food with GMO primers	
5	Sample 5: GMO positive control DNA with plant primers	
6	Sample 6: GMO positive control DNA with GMO primers	

## Lesson 3

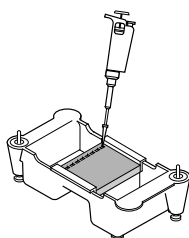
### Student Workstation

Material	Quantity
Gel (3% agarose or 10% polyacrylamide)	1
Samples from previous lab period	6
Running buffer (1x TAE for agarose gels or 1x TBE for polyacrylamide gels)	300–350 ml
Orange G loading dye	1 vial
PCR molecular weight ruler	1 vial
2–20 $\mu$ l adjustable-volume pipet or fixed-volume 20 $\mu$ l micropipet	1
1–20 $\mu$ l pipet tips, aerosol barrier	1 rack
Gel electrophoresis chamber (may be shared by 2 workstations)	1
Power supply (may be shared by multiple workstations)	1
Fast Blast DNA stain, 1x or 100x depending on protocol (at common workstation)	1
Gel staining tray	1

### Protocol

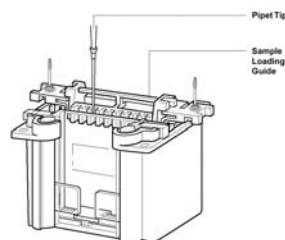
- Set up your gel electrophoresis apparatus as instructed.  
*Details on setting up electrophoresis equipment can be found in the Instructor's guide.*
- Using a fresh tip each time, add 10  $\mu$ l of Orange G loading dye to each sample and mix well.
- Load 20  $\mu$ l of the PCR molecular mass ruler and 20  $\mu$ l of each sample onto your gel in the order indicated below.

Lane	Sample	Load volume
1	Sample 1: Non-GMO food control with plant primers	20 $\mu$ l
2	Sample 2: Non-GMO food control with GMO primers	20 $\mu$ l
3	Sample 3: Test food with plant primers	20 $\mu$ l
4	Sample 4: Test food with GMO primers	20 $\mu$ l
5	Sample 5: GMO positive DNA with plant primers	20 $\mu$ l
6	Sample 6: GMO positive DNA with GMO primers	20 $\mu$ l
7	PCR molecular weight ruler	20 $\mu$ l
8	Leave empty	



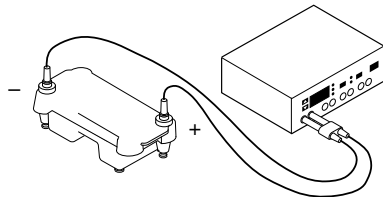
Agarose gel

or



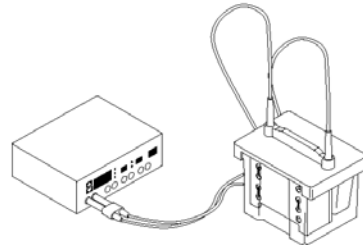
Polyacrylamide gel

4. The run time and voltage will depend on the type of gel you are running.
  - Run an agarose gel at 100 V for 30 minutes. Do not let the orange dye front migrate out of the agarose gel.
  - Run a polyacrylamide gel at 200 V for 30 minutes and do not let the red GMO primer dye front run out of the gel.



Agarose Gel Electrophoresis

or

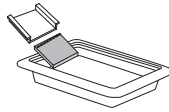


Polyacrylamide Gel Electrophoresis

5. Stain the gel in Fast Blast DNA stain. Refer to specific instructions below for your gel type.

### Staining of Agarose Gels

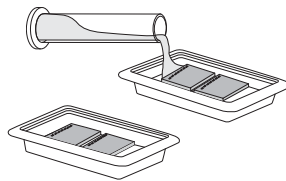
1. When electrophoresis is complete, turn off the power and remove the lid from the gel box.
2. Carefully remove the gel tray and the gel from the gel box. Be careful, the gel is very slippery. Nudge the gel off the gel tray with your thumb and carefully slide it into your plastic staining tray.



3. There are two protocols for staining your gel. Your instructor will inform you which one you will use.

### Protocol One: Overnight Staining

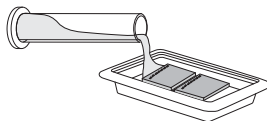
- a. Immerse your gel in 1x Fast Blast.
- b. Let the gels stain overnight, with gentle shaking for best results. No destaining is required.



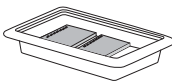


**Protocol Two: Quick Staining** (requires 20 minutes)—This method will allow you to see bands quickly (within 15 min) but may require extensive destaining to obtain optimal band-to-background intensity. Note: it is important to use **warm** water for destaining steps of this protocol.

- a. Immerse your gel in 100x Fast Blast.



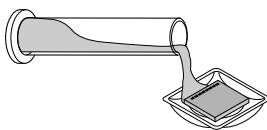
- b. Stain the gel for 5 minutes with gentle agitation. Save the used stain for future use.
- c. Transfer the gels into a large washing container and rinse with **warm** (40–55°C) tap water approximately 10 seconds.



- d. Destain by washing three times in **warm** tap water for 5 minutes each with gentle shaking for best results. You should be able to visualize bands after 10 min if you view the gel with light coming through the bottom of the staining tray. If necessary continue destaining in **warm** water until the desired contrast is reached.

### Staining of Polyacrylamide Gels

1. When gels are finished running, turn off the power supply and disconnect the leads. Remove the lid and lift out the electrode assembly and clamping frame.
2. Pour out the running buffer from the electrode assembly. Open the cams and remove the gel cassettes.
3. To keep the gel free of contamination from your fingertips, wear gloves to handle the gels from this point on. Lay a gel cassette flat on the bench with the short plate facing up. Cut the tape along the sides of the gel cassette. Carefully pry apart the gel plates, using a spatula or your fingertips. The gel will usually adhere to one of the plates. Transfer the plate with the gel adhering to it to a tray containing 1x Fast Blast stain (see below), allowing the liquid to detach the gel from the plate. The gel may also be lifted directly (and gently!) from the plate and placed into the stain.



4. Bands will start to appear after 10 minutes and staining will be complete in 1 hour. However, gels can be left in stain overnight. No destaining is required.

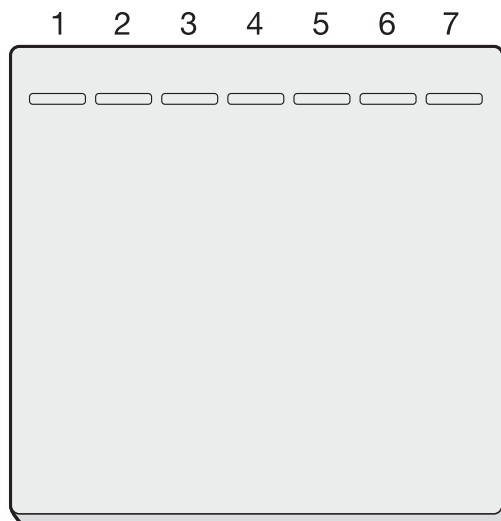


### Procedure

1. Prewet 2 sheets of cellophane in a container of water for 15–20 seconds.
2. Place one sheet of cellophane over a plastic container. Pull the cellophane taut so that it makes a flat surface over the top of the container, and use a rubber band to hold the sheet in place.
3. Place a gel onto the cellophane. Flooding the surface of the cellophane around the gel with water will aid in the removal of bubbles.
4. Place the second sheet of wetted cellophane over the gel. Because of their thickness, you cannot avoid bubbles at the edges of agarose gels, but avoid bubbles between the cellophane and the face of the gel. Secure the second sheet of cellophane to the box with a second rubber band.
5. Allow the gel to dry for several days in a well-ventilated area.

### Analysis of Results

Lane	Sample	Bands?	Band Sizes (bp)
1	Sample 1: Non-GMO food control with plant primers		
2	Sample 2: Non-GMO food control with GMO primers		
3	Sample 3: Test food with plant primers		
4	Sample 4: Test food with GMO primers		
5	Sample 5: GMO positive control DNA with plant primers		
6	Sample 6: GMO positive control DNA with GMO primers		
7	PCR molecular weight ruler		



## **Lesson 4**

### **Focus Questions**

1. What was your test food?
2. Did your test food generate a 200 bp band with GMO primer (lane 4)?
3. What does this tell you about the GMO status of your food?
4. What other information do you need to confirm the GMO status of your sample?
5. How do the results of your other five PCR reactions help support or undermine your result for your test food?
6. If you were to repeat the procedure what laboratory practice might yield better results?

# Appendix A

## Introduction to PCR

In 1983, Kary Mullis at Cetus Corporation developed the molecular biology technique that has since revolutionized genetic research, earning him the Nobel Prize in 1993. This technique, termed the **polymerase chain reaction (PCR)**, transformed molecular biology into a multidisciplinary research tool. Many molecular biology techniques used before PCR were labor intensive, time consuming, and required a high level of technical expertise. Additionally, working with only trace amounts of DNA made it difficult for researchers in other biological fields (pathology, botany, zoology, pharmacy, etc.) to incorporate molecular biology into their research schemes.

PCR had an impact on four main areas of biotechnology: gene mapping, cloning, DNA sequencing, and gene detection. PCR is now used as a medical diagnostic tool to detect specific mutations that may cause genetic disease, in criminal investigations and courts of law to identify suspects on a molecular level, and in the sequencing of the human genome. Prior to PCR, the use of molecular biology techniques for therapeutic, forensic, pharmaceutical, or medical diagnostic purposes was not practical or cost-effective. The development of PCR technology changed these aspects of molecular biology from a difficult science to one of the most accessible and widely used tools in genetic and medical research.

## PCR and Biotechnology - What Is It and Why Did It Revolutionize an Entire Research Community?

PCR produces exponentially large amounts of a specific piece of DNA from trace amounts of starting material (template). The template can be any form of double-stranded DNA, such as genomic DNA. A researcher can take trace amounts of DNA from a drop of blood, a single hair follicle, or a corn chip and use PCR to generate millions of copies of a desired DNA fragment. In theory, only a single molecule of double-stranded template DNA is needed to generate millions of copies. Prior to the development of the PCR technique, it would have been impossible to do forensic or genetic studies with a minute sample containing only a few molecules of source DNA. The ability to amplify a precise sequence of DNA to a sufficient quantity that a researcher can analyze and manipulate is the true power of PCR.

PCR amplification requires the presence of at least one DNA template strand. In this kit, plant DNA isolated from grocery store foods provides the template strands. One of the main reasons PCR is such a powerful tool is its simplicity and specificity. All that is required are inexpensive buffers, four DNA subunits (deoxynucleotide triphosphates of adenine, guanine, thymine, and cytosine), a DNA polymerase, two DNA primers, and minute quantities of the template sequence that one wants to amplify. Specificity comes from the ability to target one specific segment of DNA (or gene) out of a complete genome through the use of sequence-specific primers.

## PCR Makes Use of Two Basic Processes in Molecular Genetics

1. Complementary DNA strand hybridization
2. DNA strand synthesis via DNA polymerase

In the case of PCR, complementary strand hybridization takes place when two different oligonucleotide primers anneal to each of their respective complementary base pair sequences on the template. The two primers are designed and synthesized in the laboratory with a specific sequence of nucleotides such that they can anneal at the opposite ends and on the opposite strands of the stretch of double-stranded DNA (template) to be amplified.

Before a region of DNA can be amplified, one must identify and determine the sequence of an area of DNA upstream and downstream of the region of interest. These areas are then used to determine the sequences of oligonucleotide primers that will be synthesized and used as starting points for DNA replication. Primers are needed because DNA polymerases can only add nucleotides to the end of a preexisting chain. The DNA polymerase used in PCR must be a thermally stable polymerase because the polymerase chain reaction cycles between 59°C–94°C. The thermostable DNA polymerase (*Taq*) used in PCR was isolated from a thermophilic bacterium, *Thermus aquaticus*, which lives in high-temperature steam vents such as those found in Yellowstone National Park.

Two new template strands are created from the original double-stranded template during each complete cycle of the strand synthesis reaction. This causes exponential growth of the number of template molecules, i.e., the number of DNA strands doubles at each cycle. Therefore, after 30 cycles there will be  $2^{30}$ , or over  $10^9$ , times more copies than at the beginning. Once the DNA of interest has been sufficiently amplified, it can be visualized using gel electrophoresis. This allows researchers to determine the presence or absence of the desired PCR products.

### PCR Step by Step

PCR involves a repetitive series of cycles, each of which consists of template denaturation, primer annealing, and extension of the annealed primer by *Taq* DNA polymerase. Before beginning DNA amplification, genomic DNA is prepared from samples—in this lab, from plant-derived food items.

Following sample preparation, the template DNA, oligonucleotide primers, thermostable DNA polymerase (*Taq*), the four deoxynucleotides (A, T, G, C), and reaction buffer are mixed in a single micro test tube. The tube is placed into the MyCycler™ thermal cycler. These thermal cyclers contain an aluminum block that holds the samples and can be rapidly heated and cooled across wide temperature differences. The rapid heating and cooling of this thermal block is called **temperature cycling** or **thermal cycling**.

The first step of the PCR temperature cycling procedure involves heating the sample to 94°C. At this high temperature, the template strands separate (denature). This is called the **denaturation step**.

The thermal cycler then rapidly cools to 59°C to allow the primers to anneal to the separated template strands. This is called the **annealing step**. The two original template strands may reanneal to each other, or compete with the primers for the primers' complementary binding sites. However, the primers are added in excess such that the primers actually outcompete the original DNA strands for the primers' complementary binding sites.

Lastly, the thermal cycler heats the sample to 72°C for *Taq* DNA polymerase to extend the primers and make complete copies of each DNA strand. This is called the **extension step**. *Taq* polymerase works most efficiently at this temperature. Two new copies of each complementary strand are created. There are now two sets of double-stranded DNA (dsDNA). These two sets of dsDNA can now be used for another cycle and subsequent strand synthesis.

At this stage, a complete temperature cycle (thermal cycle) has been completed.

**Temperature cycle = denaturation step + annealing step + extension step**

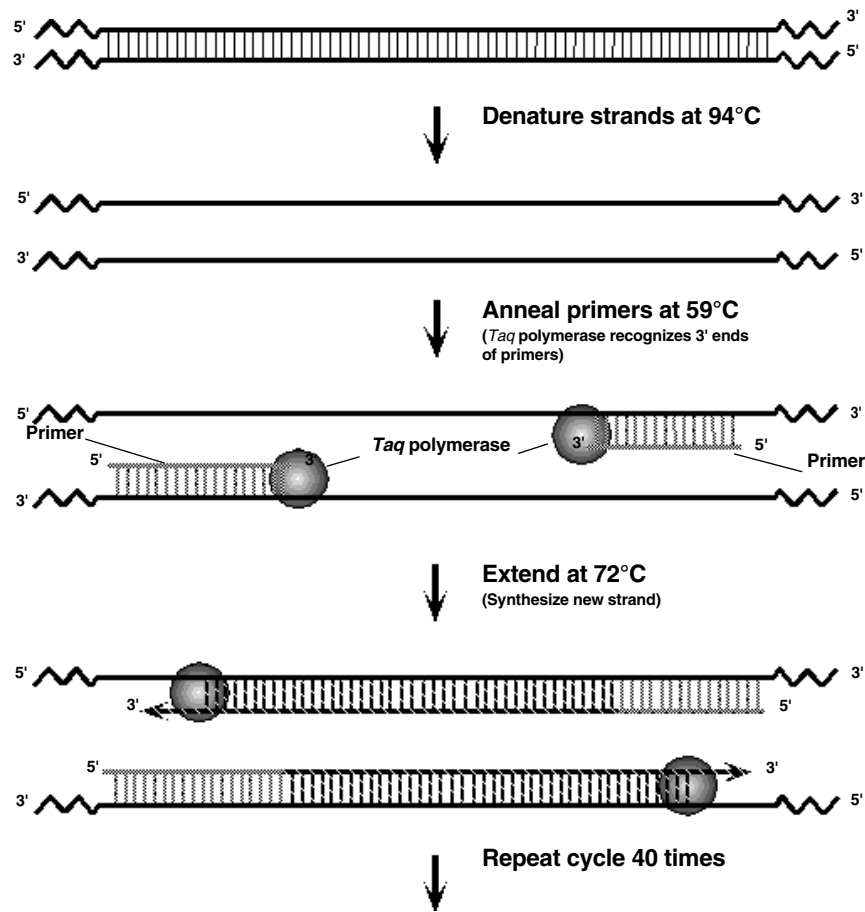


Figure A1. A complete cycle of PCR.

Usually, thermal cycling continues for about 40 cycles. After each thermal cycle, the number of template strands doubles, resulting in an exponential increase in the number of template DNA strands. After 40 cycles there will be  $1.1 \times 10^{12}$  more copies of the original number of template DNA molecules.

PCR generates DNA of a precise length and sequence. On the first cycle, the two primers anneal to the original genomic template DNA strands at opposite ends and on opposite strands. After the first complete temperature cycle, two new strands are generated that are shorter than the original template strands but still longer than the length of the DNA that the researcher wants to amplify. It isn't until the third thermal cycle that fragments of the precise length are generated.

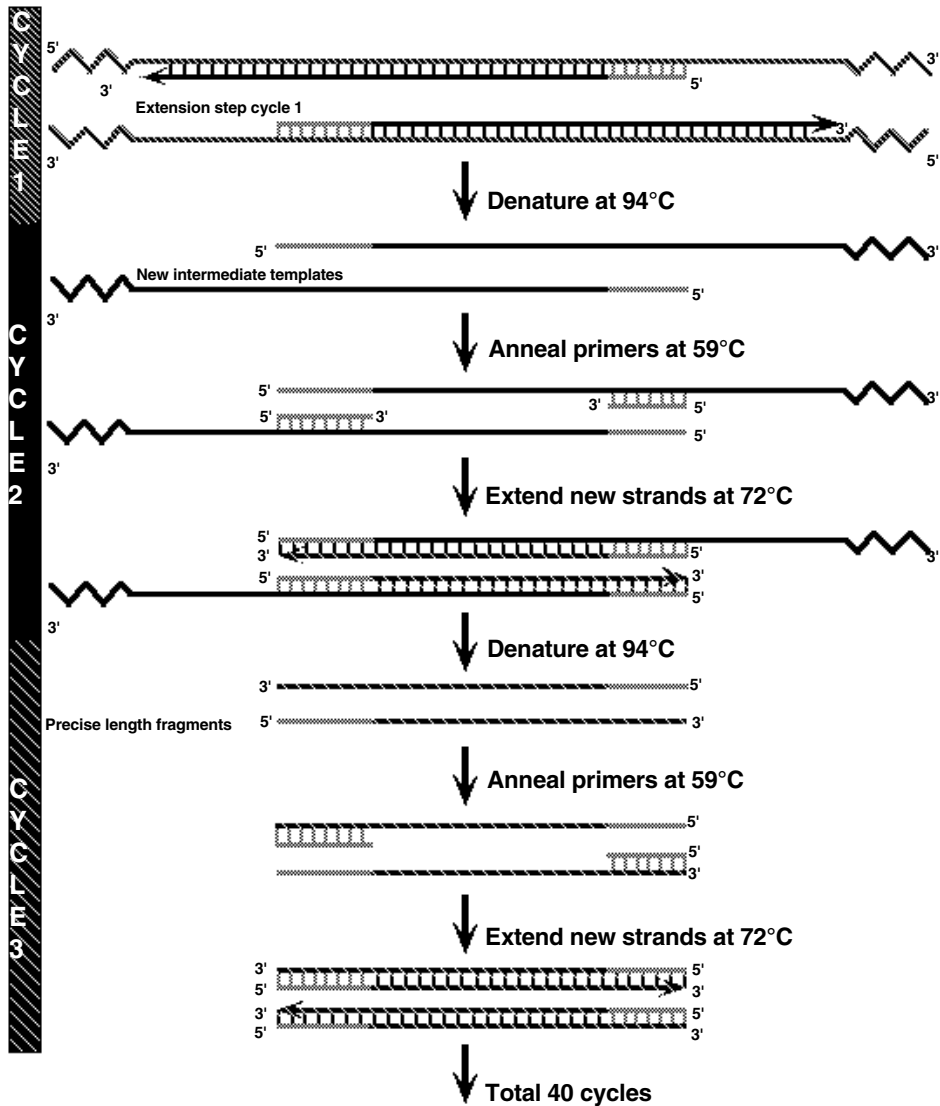


Figure A2. Generation of precise-length fragments.

It is the template strands of the precise length that are amplified exponentially ( $X^n$ , where  $X$  = the number of original template strands and  $n$  = the number of cycles). There is always one set of original long-template DNA molecules that are never fully duplicated. After each thermal cycle, two intermediate-length strands are produced, but because they can only be generated from the original template strands, the intermediate strands are not exponentially amplified. It is the precise-length strands generated from the intermediate strands that are amplified exponentially at each cycle. Therefore, if 20 thermal cycles were conducted from one double-stranded DNA molecule, there would be 1 set of original genomic template DNA strands, 20 sets of intermediate template strands, and 1,048,576 sets of precise-length template strands. After 40 cycles, there would be 1 set of original genomic template DNA strands, 40 sets of intermediate template strands, and  $1.1 \times 10^{12}$  sets of precise-length template strands.



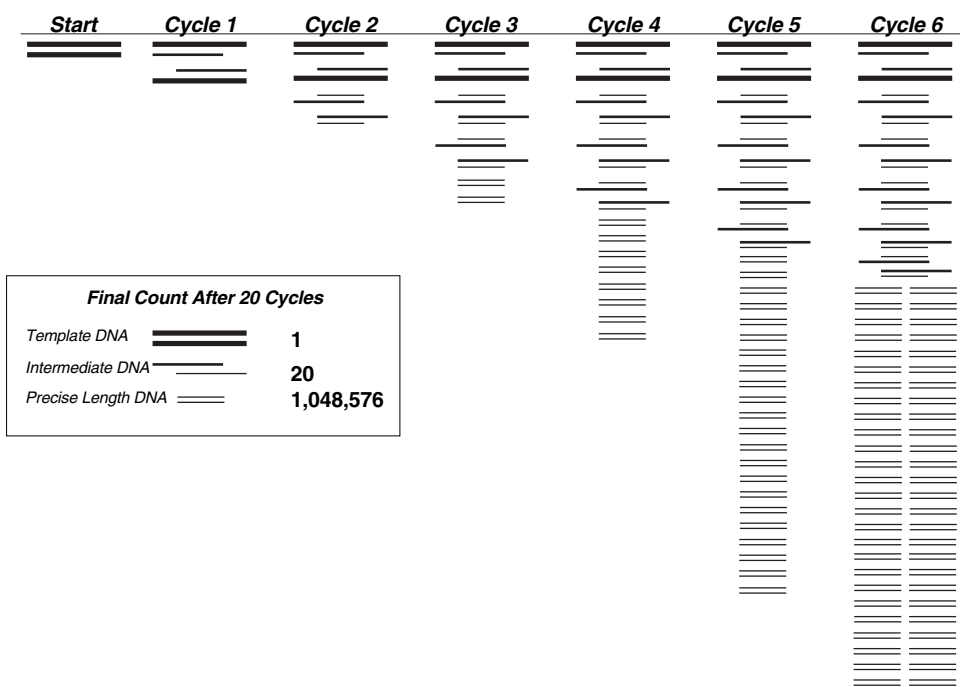


Figure A3. Schematic of PCR amplification of DNA fragments.

## **Appendix B**

### **PCR Amplification and Sterile Technique**

PCR is a powerful and sensitive technique that enables researchers to produce large quantities of specific DNA from very small amounts of starting material. Because of this sensitivity, contamination of PCR reactions with unwanted DNA is always a possible problem. Therefore, utmost care must be taken to prevent cross-contamination of samples. Steps to be taken to prevent contamination and failed experiments include:

1. Filter-type pipet tips. The end of the barrels of micropipets can easily become contaminated with aerosolized DNA molecules. Pipet tips that contain a filter at the end can prevent aerosol contamination from micropipets. DNA molecules that are found within the micropipet cannot pass through the filter and contaminate PCR reactions. Xcluda® aerosol barrier pipet tips (catalog #211- 2006EDU and 211-2016EDU) are ideal pipet tips to use in PCR reactions.
2. Aliquot reagents. Sharing of reagents and multiple pipetting into the same reagent tube can easily introduce contaminants into your PCR reactions. When at all possible, divide reagents into small aliquots for each team, or if possible, for each student. If only one aliquot of a reagent does become contaminated, then only a minimal number of PCR reactions will become contaminated and fail.
3. Change pipet tips. Always use a new pipet tip when entering a reagent tube for the first time. If a pipet tip is used repeatedly, contaminating DNA molecules on the outside of the tip will be transferred to other solutions, resulting in contaminated PCR reactions. If you are at all unsure if your pipet tip is clean, err on the safe side and discard the tip and get a new one. The price of a few extra tips is a lot smaller than the price of failed reactions.
4. Use good sterile technique. When opening tubes or pipetting reagents, leave the tubes open for as little time as possible. Tubes that are open and exposed to the air can easily become contaminated by aerosolized DNA molecules. Go into reagent tubes efficiently, and close them as soon as you are finished pipetting. Also, try not to pick tubes up by the rim or cap, as you can easily introduce contaminants from your fingertips.
5. Bleach at a concentration of 10% destroys DNA, so wiping down surfaces and rinsing plastic pipet barrels, mortars, and pestles with 10% bleach can get rid of any surface DNA contamination that may arise.

## Appendix C

### Glossary of Terms

**Aliquot** – The division of a quantity of material into smaller, equal parts.

**Annealing** – Binding of single-stranded DNA to complementary sequences. Oligonucleotide primers anneal to denatured (single-stranded) DNA strands.

**Bt** – *Bacillus thuringiensis* – In the context of GM crops Bt refers to a specific modification in which a gene for a member of the Cry family of proteins from the soil bacterium *Bacillus thuringiensis* is inserted into the crop. The gene confers resistance to the European corn borer.

**Back-cross** – In the context of GMO crops, the method by which a newly made genetically engineered crop is repeatedly bred into a commercially viable crop to transfer the genetic modification into a high yield or commercially viable background.

**Callus** – An undifferentiated mass of plant cells.

**Chelate** – To bind metal ions in solution. An example of a common chelating agent is EDTA (ethylene diamine tetraacetic acid).

**Cofactor** – Ion or other small molecule needed by an enzyme to function properly. For example, *Taq* DNA polymerase needs  $Mg^{2+}$  in order to function properly.  $Mg^{2+}$  is considered a cofactor.

**Denaturation** – The process of melting apart two complementary DNA strands. In vivo denaturation is accomplished by enzymes; in the (in vitro) PCR reaction, denaturation is accomplished by heat.

**DNase** – Enzyme that degrades DNA.

**dNTPs** – Commonly used abbreviation for all four deoxynucleotide triphosphates (dATP, dTTP, dGTP, dCTP) used in synthesizing DNA.

**Ethidium bromide** – A fluorescent dye that is used to detect DNA. It intercalates between DNA base pairs and fluoresces when exposed to ultraviolet light.

**Exons** – The coding regions of a transcribed messenger RNA that get spliced together and leave the nucleus for translation into protein sequence.

**Extension** – Elongation of a primer by addition of dNTPs (deoxynucleotide triphosphates — dATP, dTTP, dCTP, or dGTP) by a DNA polymerase. Extension follows the base pairing rule and proceeds in the 5' to 3' direction.

**Genomic DNA** – The sum total of the DNA that is found within a cell.

**Genetic engineering** – The process by which scientists change the genetic makeup of an organism.

**GM** – Genetically modified

**GMO** – Genetically modified organism

**InstaGene™ matrix** – Microscopic beads that bind divalent cations in solution. The binding of divalent cations to these beads prevents their availability to enzymes that can degrade DNA.

**Intron** – Region of a transcribed messenger RNA that is spliced out of and is not translated into protein sequence.

**Lysis** – The process of rupturing a cell to release its constituents. In this laboratory, plant cells are lysed to release genomic DNA for the PCR reactions.

**Master mix** – A premixed reagent solution designed for PCR reactions, containing all of the necessary components (dNTPs, primer, buffer, salts, polymerase, Mg<sup>2+</sup>) of the reaction except the template DNA.

**Nucleotide** – A fundamental unit of DNA or RNA. Consists of a sugar (deoxyribose or ribose), phosphate, and nitrogenous base (adenine, cytosine, guanine, thymine, or uracil).

**PCR** – Polymerase chain reaction. A process used to amplify (synthesize large quantities from a small starting sample) DNA within a test tube.

**Primer** – A small chain of nucleotides (usually 16–24 bases in length) that provides a free end for DNA polymerase to extend from. Primers for PCR are designed (synthesized in a laboratory) to be complementary to specific sequences near the target DNA sequence, so that they will "anchor" to the template and provide a starting point for the DNA polymerase to copy the region of interest.

**Taq DNA polymerase** – Heat-stable DNA polymerase that was isolated from the heat-stable bacterium *Thermus aquaticus*. This DNA polymerase is commonly used in PCR reactions.

**Template** – The DNA that contains the sequence to be copied (into a complementary sequence) in a DNA-synthesizing reaction. Double-stranded DNA serves as a template for replication of copies of itself, because each strand's sequence serves as a template for the replication of the other strand's sequence. A single-stranded DNA, on the other hand, can only serve as template for copies of its complementary sequence, and not for copies of itself.

## Appendix D

### Post-Lab Debate Activity

Many people object to the use of GM crop plants. They argue that there is a potential to create super weeds through cross-pollination with herbicide resistant crops or that super bugs will evolve that are no longer resistant to the toxins in pest resistant crops. Many are concerned with potential allergic reactions to the novel proteins or antibiotic resistance arising from the selectable markers used to develop the crops or other unforeseen effects on public health. Proponents of genetically modified foods argue these crops are actually better for the environment. Less toxic chemicals are put into the environment and thus less toxic chemicals can harm the environment and human health. In addition, these crops can preserve arable land, by reducing stresses on the land, improve the nutritional value of food in developing countries and allow crops to be grown on previously un-farmable land. Here we include a debate activity to facilitate discussion of these issues.

#### Day One — Set the Stage

Randomly divide the class into two groups and randomly assign one group to support the development and use of GM crops and the other to oppose the use and development of GM crops. Explain the format of the debate and have each team pick a captain.

#### Days Two–Five — Student Research

- Students conduct research on the development and use of GM crops using the pro/con data sheet on next page (optional — assign for homework).
- Teams compile research from all members.
- Teams write 4 minute opening statements and assign spokespersons.

#### Day Six–The Debate

##### Debate Format

**Opening Statement:** Proponents of GMO use present an opening statement outlining the benefits of GMO crops (4 minutes).

**Break:** Opponents assemble a list of questions they believe shows holes in the proponents' argument (2 minutes).

**Questions:** Opponents present questions (2 minutes).

**Opening Statement:** Opponents of GMO use present an opening statement outlining the reasons why GMO crops should not be allowed (4 minutes).

**Break:** Proponents assemble a list of questions they believe shows holes in the opposition's argument (2 minutes).

**Questions:** Proponents present questions (2 minutes).

**Rebuttal:** Proponents present answers to opponents' questions (2 minutes).

**Rebuttal:** Opponents present answers to proponents' questions (2 minutes).

**Closing arguments:** opposing view (3 minutes).

**Closing arguments:** supporting view (3 minutes).

## **Grading Ruberic**

### **Opening Statements**

- 4 Eloquent, very well organized, researched, and presented.
- 3 Well organized, researched, and presented.
- 2 Somewhat organized, researched, and presented.
- 1 Lacking organization, partially correct research, not well presented.

### **Questions**

- 4 Questions were thoughtful, raised legitimate concerns, were research based and were well presented.
- 3 Questions were somewhat thoughtful, raised some concerns, and were well presented.
- 2 Questions were not research based, did not raise legitimate concerns, or not well presented.
- 1 Questions were unrelated to the subject, did not raise legitimate concerns, or not well presented.

### **Rebuttal**

- 4 Students used research to directly refute the questions.
- 3 Students used research to partially refute the questions.
- 2 Students improperly used research to attempt to refute the questions.
- 1 Students did not refute the questions.

### **Closing statements**

- 4 Closing statement was eloquent, very well organized, presented.
- 3 Closing statement was well organized, researched, and presented.
- 2 Closing statement was somewhat organized, researched, and presented.
- 1 Closing statement lacked organization, used partially correct research, and was not well presented.

### **Working as a team member** (as ranked by other team members)

- 4 Fully participated and contributed to the team.
- 3 Participated and contributed to the team.
- 2 Partially participated, somewhat helpful.
- 1 Little participation, little help.

## **Pro/Con Data Sheet**

Make a list of why we should use GM crops (include references).

Make a list of why we should not use GM crops (include references).

If you are pro, find research to refute the con. If you are con, find research to refute the pro. Include these in your opening or closing statements.

## **Appendix E**

### **Programming Instructions for T100 or MyCycler Thermal Cycler**

Abbreviated instructions for programming your T100 or MyCycler for the proper amplification cycles and temperatures used in this lab are provided below. Refer to the T100 or MyCycler instruction manual for more detailed instructions and troubleshooting.

#### **T-100 Thermal Cycler**

**Program the T100** (only necessary the first time you perform the lab)

Turn on the T100 by flipping the power switch at the rear of the machine

Select "New Protocol"

Press "50 µl" on top right of the screen

Enter 40

Press "OK"

#### **Program the Initial Denaturation**

Press "95°C" in column 1

Enter 94

Press "OK"

Press "3:00" in column 1

Enter 200

Press "OK"

#### **Program the 40 PCR cycles**

Press "95°C" in column 2

Enter 94

Press "OK"

Press "0:30" in column 2

Enter 100

Press "OK"

Press "55°C" in column 3

Enter 59

Press "OK"

Press "0:30" in column 3

Enter 100

Press "OK"

Press "1:00" in column 4

Enter 200

Press "OK"

Press "34X" in column 5

Enter 40

Press "OK"

#### **Program the Final Extension**

Press "5:00" in column 6

Enter 1000

Press "OK"

#### **Save the Protocol**

Press "Save" on the lower menu bar of the screen

Enter "GMO"

Press "Save"

Press "Home" on the lower menu bar of the screen

### **Run the GMO Program**

Press "Saved Protocols"

Press "Main" in the Folders column

Press "GMO" in the Files column

Press "Run" on the lower menu bar of the screen

Press "OK"

### **MyCycler Thermal Cycler**

**Program the MyCycler** (only necessary the first time you perform the lab)

Select "Standby" to turn the machine on

Select "Create"

Scroll down to "Standard-3"

Press "Enter"

#### **Program the Initial Denaturation**

Enter 94.0

Press the down arrow

Enter 2.00

Press the down arrow

Enter 1.00

Press the right arrow

#### **Program the 40 PCR Cycles**

Enter 94.0

Press the down arrow

Enter 1.00

Press the right arrow

Press the up arrow

Enter 59.0

Press the down arrow

Enter 1.00

Press the right arrow

Press the up arrow

Enter 72.0

Press the down arrow

Enter 2.00

Press the down arrow

Enter 40X cycles

Press Enter

#### **Program the Final Extension**

Press the right arrow

Enter 72.0

Press the down arrow

Enter 10.00

Press the down arrow

Enter 1X cycle

Press the right arrow

#### **Program the Final Chill Hold**

Enter 1X cycle

Press "Done"

#### **Save the Protocol**

Press "Save Protocol As"



Press "Enter"  
Enter "GMO" using the alphanumeric keypad  
Press "Enter"

### **Run the GMO Program**

Select "Protocol Library"  
Select "GMO"  
Press "Enter"  
Press "Enter" to run protocol  
Enter "Algorithmic Measurement"  
Enter 40  $\mu$ l volume  
Select "No Hot Start"  
Select "Begin Run"  
The MyCycler should now begin running

# Appendix F

## Teacher Answer Guide

### Lesson 1 Extraction of DNA from food samples

1. How can you test a food to find out if it contains material derived from a genetically modified organism (GMO)?

*There are two methods to test for foods containing GMOs. The ELISA test is used to see if particular proteins are in a sample. PCR is used to amplify regions of GMO genomes.*

2. In what organelles is plant DNA located?

*Plant DNA is not only found in the nucleus, it is also found in the mitochondria and chloroplasts. Plants and other autotrophic organisms are the only organisms with chloroplasts. Plant DNA is more difficult to obtain intact because the cell wall must be destroyed.*

3. Many foods containing GM crops are highly processed. Can you suggest how DNA from whole plants may differ from that extracted from processed foods, e.g., corn chips, cornmeal, etc.?

*High temperatures or physical manipulation of the plant tissue during processing may destroy or fragment DNA.*

4. What molecules are present in the cell that might interfere with DNA extraction?

*Enzymes, such as DNases, may degrade DNA. Metal ions act as cofactors and coenzymes for enzymes that degrade DNA. Cellulose plant cell walls may act as a barrier to DNA extraction.*

5. Why do you also perform analysis on food that is known to be a non-GMO control?

*To make sure samples have not been contaminated. It is also used as a comparison to show how a non-GMO banding pattern should look.*

6. Why was the non-GMO food control prepared prior to your test food sample?

*In the grinding process, airborne particles can travel through the air and contaminate samples of non-GMO foods. Also a mortar and pestle that is not properly washed can transfer minute sample. PCR only needs ONE molecule of DNA to make amplified product.*

### Lesson 2 Set Up PCR Reactions

1. What chemicals and molecules are needed for PCR, and what is the function of each component?

- *Taq DNA polymerase – a polymerase that is not sensitive to heat. It links the deoxynucleotide triphosphates to make a DNA strand that is complementary to the template*
- *Deoxynucleotide triphosphates (dATP, dCTP, dGTP, dTTP) – the basic units that are connected to make the complementary strand*
- *Primers – short sequences of DNA that serve as beginnings of newly synthesized DNA.*

- *Buffers and cofactors needed to make the reaction take place at an optimal rate.*3. Examine the 150 base sequence below.

5'TAGAAAAGGA AGGTGGCTCC TACAAATGCC ATCATTGCGA TAAAGGAAAG  
3'ATCTTTTCTTCCACCGAGGATGTTTACGG ATCAAACGCT ATTTCTTTTC  
**forward 5'AGGAAGGTGG3'**

GCTATCATT C AAGATGCCTC TGCCGACAGT GGTC CCAAAG ATGGACCCCC  
CGATAGTAAG T TCTACGGAG ACGGCTGTCA CCAGGGTTTC TACC TGGGGG

**3'CTTCT GCAAG5' Reverse primer**

ACCCACGAGG AGC ATCGTGG AAAAAGAAGA CGTTCCAACC ACGTCTTCAA3'  
TGGGTGCTCC TCGTAGCACC TTTTCTTCT GCAAGGTTGG TGCAGAAGTT5'

Write in the sequence of the complementary strand and mark the 3' and 5' ends of the complementary strand (see italics)

Remembering that DNA polymerases can only add nucleotides to the 3' end of DNA, design a forward primer and a reverse primer, each 10 bases long, to amplify a target sequence of the DNA that is at least 100 bp long. Write the sequence of the primers below, with their 3' and 5' ends indicated. Also indicate on the sequence above which strand they are complementary to (will anneal to).

*These primers can be any sequence so long as their orientation and complementarity matches these examples in bold.*

**Forward primer 5'AGGAAGGTGG3'**

**Reverse primer 3'CTTCTGCAAG5'**

*If you are teaching about primer design in more depth, you may want to give more criteria for their primer design such as % GC content and primer dimer formation.*

4. Why are you performing two PCR reactions on each DNA sample?

*One reaction is a control to show we extracted plant DNA using primers to a universal plant DNA sequence. The second reaction is to identify the GMO target sequence.*

5. What is the purpose of the GMO positive control DNA?

*We want to make sure our PCR reaction worked; if the positive control produces a positive result but I do not get a band in my test sample, the test is most likely non-GMO. If I do not get the 200 base pair band in the positive control, I can assume the PCR reaction did not work.*

### **Lesson 3 Electrophoresis of PCR products**

1. Why did you resolve your PCR products by electrophoresis?

*Gel electrophoresis separates DNA molecules based on charge and size. After the bands are separated the gel is stained to visualize the band pattern. We can calculate the size of the DNA molecules, in base pairs, in each band.*

2. Explain why DNA fragments separate according to size in an electrophoresis gel.

*DNA is negatively charged and is repelled by the negative electrode (cathode) and attracted by the positive electrode (anode) when an electric current is applied across the gel. It separates because different lengths of DNA move through the gel matrix at different rates. Longer fragments move more slowly than shorter fragments.*

- Why do you need a molecular mass ruler alongside your samples?

*We need a molecular mass ruler to calculate the size of each of our bands. We know exactly how many bands are in the ruler and the size of each of those bands. We can graph the size of the bands against the distance they moved in the gel to create a standard curve. We can then measure the distance our PCR product bands moved in the gel and use our standard curve to calculate the sizes of the product bands.*

- What results do you expect in each lane? Fill in the chart below.

Lane	Sample	Expect Band?
1	Sample 1: Non-GMO food control with plant primers	Yes
2	Sample 2: Non-GMO food control with GMO primers	No
3	Sample 3: Test food with plant primers	Yes
4	Sample 4: Test food with GMO primers	Don't know
5	Sample 5: GMO positive control DNA with plant primers	Yes
6	Sample 6: GMO positive control DNA with GMO primers	Yes

#### **Lesson 4 Drying Gels and Analysis of Results**

- What was your test food?

*Answer to be determined by instructor.*

- Did your test food generate a 200 bp band with GMO primer (lane 4)?

*Yes or no.*

- What does this tell you about the GMO status of your food?

*A band indicates that the food may be GMO-positive, the absence of a band indicates the food may be GMO-negative.*

- What other information do you need to confirm the GMO status of your sample?

*If there was a band in lane 4, we need to determine that there was not contamination of the samples to ensure the result is not a false positive.*

*If there was no band in lane 4, we need to confirm that DNA was extracted from the sample and that the PCR reaction was functioning properly to ensure the result is not a false negative.*

- How do the results of your other five PCR reactions help support or undermine your result for your test food?

*Refer to the flow chart on next page.*

- If you were to repeat the procedure what laboratory practice might yield better results?

*Accept all reasonable answers.*

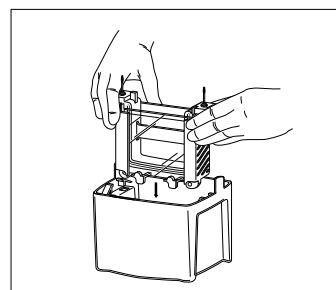
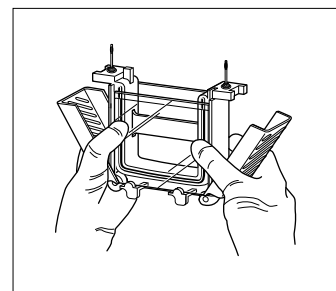
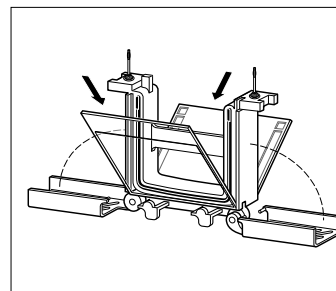
## Appendix G

### Mini-PROTEAN® Tetra Cell Electrophoresis Module Assembly

1. Set up Mini-PROTEAN Tetra gel box.
2. Prepare a Mini-PROTEAN TBE gel by peeling away the plastic strip from the bottom of the gel.
3. Remove the comb from the gel.
4. Place the gel cassette into the electrode assembly that has the banana plugs with the short plate facing inward. Place a buffer dam or another gel cassette on the opposite side of the electrode assembly, with notch on buffer dam facing inward.
5. Push both gels (or gel and buffer dam) toward each other, making sure that they are against the green gaskets that are built into the clamping frame; make certain that the short plates sit just below the notch at the top of the green gasket. Slide the green arms of the clamping frame over the gels, locking them into place.
6. Lower the electrode assembly with the gels in it into the mini tank on the side of the tank with the plastic tabs. Make sure that the red banana plug goes on the side of the tank with the red oval.

**CAUTION: When running 1 or 2 gels only, DO NOT place the Companion Running Module in the tank. Doing so will cause excessive heat generation and prevent electrophoretic separation.**

7. Completely fill the inner chamber with 1x TBE electrophoresis buffer, making sure the buffer covers the short plate (~150 ml).
8. Fill mini tank with approximately 700 ml of 1x TBE electrophoresis buffer until the buffer reaches the 2 gels line on the tank.
9. If using, place sample loading guide on top of the electrode assembly.



# Mini-PROTEAN Tetra Cell Assembly

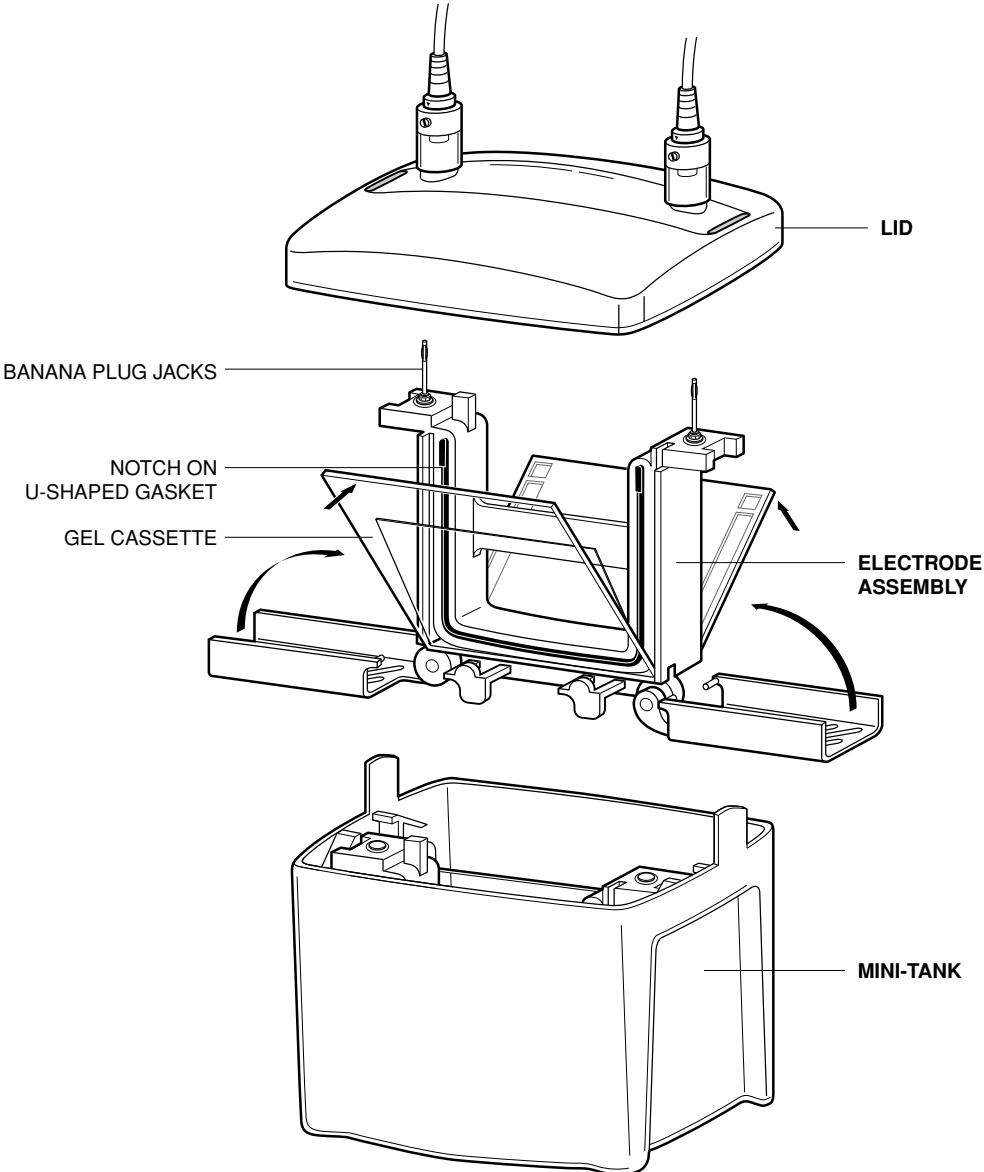


Fig. G1. Assembling the Mini-PROTEAN Tetra cell.

## **Appendix H**

### **Recommended GMO-Based Websites and References**

University of Nebraska online lessons (<http://croptechnology.unl.edu/>).

Center for Environmental Risk Assessment database of GMO crops, how they were made and when they were approved (<http://www.cera-gmc.org>).

U.S. Dept of Agriculture Economic Research Service data on how much of the US is growing GMO crops (<http://www.ers.usda.gov/Data/biotechcrops/>).

European Commission, Joint Research Centre, Review of GMO Detection and Quantification Techniques, 2002-07-23, Bonfini Laura, Heinze Petra, Kay Simon, Van den Eede Guy (<http://mbg.jrc.ec.europa.eu/home/documents/EUR20384Review.pdf>).

Pro-GMO web site with educational links (<http://www.monsanto.com>).

Anti-GMO web site (<http://www.greenpeace.org>).

## **Appendix I**

### **Run Agarose DNA Gels in Under 20 Minutes**

Bio-Rad's BioEducation R&D team has developed a new electrophoresis buffer formula. Using a reduced concentration of running buffer (0.25x TAE), and higher voltage (200 V), any agarose gel can be run 33% faster. Advantages of this new formula include:

- Excellent gel resolution
- Minimal run time
- Fast separation of DNA in gels of any agarose gel concentration (0.8–4.0%)
- Compatibility with all Bio-Rad Biotechnology Explorer program kits

TAE buffer is provided as a 50x concentrate that can be mixed with distilled water to yield the necessary concentrations for making agarose gels and electrophoresis running buffer.

#### **Use 1x TAE to make agarose gels:**

350 ml of 1x TAE is sufficient to pour eight 7 x 10 cm agarose gels. To make 350 ml of 1x TAE from a 50x TAE concentrate, add 7 ml of concentrate to 343 ml of distilled water. Detailed instructions for making agarose gels can be found in individual kit instruction manuals.

- Use 1x TAE to make 3% agarose gels for the GMO Investigator™ kit
  - With the small DNA electrophoresis pack, dissolve 10.5 g of agarose in 350 ml of 1x TAE buffer, boil, and pour 40 ml per gel to make 8 handcast 3% agarose gels. Gels can be stored submerged in buffer for several weeks at 4°C
  - For added convenience, precast 3% agarose gels made with 1x TAE are available from Bio-Rad (catalog #161-3017EDU)

#### **Use 0.25x TAE to make electrophoresis running buffer:**

A 2.5 L volume of 0.25x TAE buffer is required to run eight 7 x 10 cm agarose gels. To make 2.5 L of 0.25x TAE from a 50x TAE concentrate, add 12.5 ml of concentrate to 2.49 L of distilled water. To make 2.5 L of 0.25x TAE from a 1x TAE solution, add 625 ml of 1x TAE to 1,875 ml of distilled water.

**Note:** Do not use 0.25x TAE to make agarose gels; doing so can lead to a loss of DNA resolution.

#### **To run gels:**

Place the gel in an electrophoresis chamber and cover it with 0.25x TAE; ensure the gel is submerged. Run gels at 200 V for no more than 20 min. Monitor gel loading dye progress to get a relative idea of electrophoresis progress.



## **Legal Notices**

Notice regarding Bio-Rad thermal cyclers and real-time systems: Purchase of this instrument conveys a limited, non-transferable immunity from suit for the purchaser's own internal research and development and for use in applied fields other than Human In Vitro Diagnostics under one or more of U.S. Patents Nos. 5,656,493, 5,333,675, 5,475,610 (claims 1, 44, 158, 160–163, and 167 only), and 6,703,236 (claims 1–7 only), or corresponding claims in their non-U.S. counterparts, owned by Applera Corporation. No right is conveyed expressly, by implication, or by estoppel under any other patent claim, such as claims to apparatus, reagents, kits, or methods such as 5' nuclease methods. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA. Bio-Rad's real-time thermal cyclers are licensed real-time thermal cyclers under Applera's United States Patent No. 6,814,934 B1 for use in research and for all other fields except the fields of human diagnostics and veterinary diagnostics. Purchase of iTaq™ DNA polymerase includes an immunity from suit under patents specified in the product insert to use only the amount purchased for the purchaser's own internal research. No other patent rights (such as 5' Nuclease Process patent rights) are conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA

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