Edvo-Kit # 121

Edvo-Kit #121

Detection of Genetically Modified Organisms

(Ready-to-Load™)

Experiment Objective:

In this experiment, students will use agarose gel electrophoresis to explore the molecular methods used by scientists to identify genetically modified organisms.

See page 3 for storage instructions.

Version 121.210628

EDVOTEK®

Table of Contents

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Components (in QuickStrip™ format)		Check (✓)
Store QuickStrip™ samples in the refrigerator upon receipt.		
A	DNA Standard Marker	❑
B	GMO Negative Control	❑
C	GMO Positive Control	❑
D	Corn Sample	❑
E	Wheat Sample	❑
F	Soy Sample	❑

Experiment #121 is designed for 8 groups.

Store QuickStrip™ samples in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

REAGENTS & SUPPLIES

Store the following at room temperature.

- UltraSpec-Agarose™ ❑
- Electrophoresis Buffer (50x) ❑
- Practice Gel Loading Solution ❑
- FlashBlue™ DNA Stain ❑

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipettes with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 mL flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Small plastic trays or large weigh boats (for gel destaining)
- DNA visualization system (white light)
- Distilled or deionized water

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

Background Information

WHAT ARE GENETICALLY MODIFIED ORGANISMS?

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

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

Figure 1A:
Traditional varieties of corn.

Figure 1B:
Cultivated corn

While it used to take years of selective breeding to produce the genomic changes necessary to give rise to such desirable traits, the advent of biotechnology has accelerated this pace. The introduction of genetic engineering now allows scientists to directly manipulate a DNA sequence in order to generate desirable traits. These engineered genes, called transgenes, can be inserted, deleted or mutated in a matter of weeks using recombinant DNA technology. In order to be to be properly expressed *in vivo*, a transgene must include a promoter sequence, which recruits RNA polymerase to the transgene for transcription, and a terminator sequence, which signals RNA polymerase to end transcription (Figure 2). The promoter from the Cauliflower Mosaic Virus (CaMV) and the terminator from the *Agrobacterium tumefaciens* nopaline synthase gene (NOS) are commonly used by genetic engineers because they are recognized by transcriptional machinery of many different types of plants.

Many technologies have been developed to create transgenic plants. One example is the biolistic (or "gene gun") method. Using this approach, the transgene DNA is adhered to gold particles and is shot through the walls of plant cells, where it is incorporated into the host's genome. Another method relies on a natural plant parasite (*A. tumefaciens*) that transfers some

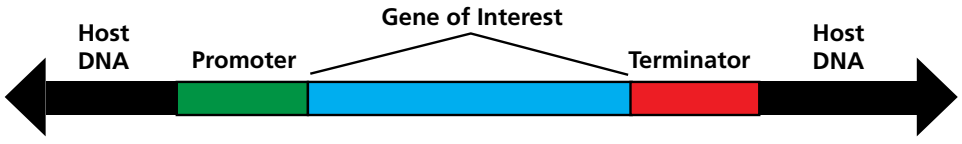


Figure 2: Features of a Transgene

of its own genetic material into plant cells in the form of the Ti-plasmid. Genetic engineers use recombinant DNA technology to replace a bacterial gene with a gene of interest. The bacteria transfer the transgene to the host as part of the Ti-plasmid, thus creating a transgenic plant. This technique works best with plants like tomatoes, apples and soybeans.

Current plant biotechnology promises to increase both the yield and nutritional value of many food crops. For example, the enzyme polygalacturonase (PG) digests pectin in the tomato's cell wall, making the fruit softer and more easily damaged during shipping. The Flavr Savr tomato has been engineered to "turn off" production of the PG enzyme, slowing the softening process. Therefore, the tomatoes are less fragile. "Bt-corn" expresses a naturally occurring pesticide that protects the plant from insects (Figure 3). This technology allows farmers to use less chemical pesticides, some of which are harmful to people and the environment. Another success story is that of "golden rice". Normally, rice, a staple food for much of the world population, does not provide β-carotene or vitamin A. Because vitamin A deficiency is a widespread problem in developing countries, rice has been modified to produce β-carotene, a precursor of vitamin A. Switching to cultivation of "golden rice" and other nutrient-supplemented crops in these areas represents major progress in combating malnutrition.

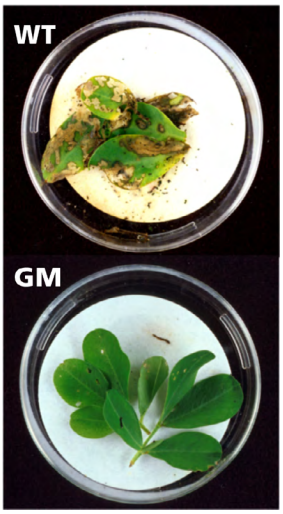


Figure 3:
Insect resistance of GM plants

In addition to boosting crop yields and enhancing nutrition, transgenic technology could be used to create allergy-free peanuts and low protein rice for people with kidney disease. Genetically modified foods may soon allow for the synthesis and delivery of various pharmaceutical products. In "pharming", transgenic plants can be used to make proteins with medicinal value like insulin or growth hormone. "Farmaceuticals" can be produced in many crops, including tobacco, carrots, tomatoes, soybeans and rice. By taking advantage of engineering DNA within the chloroplast, which maintains high protein expression levels and does not distribute its DNA via pollen, scientists may one day be able to generate great amounts of pharmaceuticals with little chance of non-target exposure to the product.

ETHICS OF GENETICALLY MODIFIED ORGANISMS

Tomatoes, soybeans and corn were among the first genetically modified food products approved by U.S. agencies in the 1990s. Since then, the safety, efficacy and benefits of GM foods have been debated at a global level. Many studies of GMOs and related technologies have been published in leading peer-reviewed science journals like Nature and Science. Proponents of GM technology cite studies showing improved quantity and quality of plants, decreasing costs for growers, and benefits for the environment. Critics of GM technology fear the spread of transgenes to other crops, increased allergens, and the creation of unanticipated dangers to people and the environment.

The responsibility of public health and policy concerning GMOs rests on the shoulders of the government and the biotechnology industry. The United States government has carefully monitored GM food production and created strict regulations to protect the health of Americans. There are several federal agencies in the United States that oversee food safety: the Federal Drug Administration (FDA) is responsible for the safety of human and animal food products; the U.S. Department of Agriculture (USDA) oversees the development of new plant varieties and their use in farming; and the Environmental Protection Agency (EPA) monitors pesticide levels in plants and determines what is acceptable for human consumption. To gain acceptance, the plant biotechnology industry must communicate its research and development of new GM food products effectively to these agencies.

USING PCR TO IDENTIFY GMOS

Over the past several years, some food companies have decided to remove GMOs from their foods. In order to determine whether the raw materials (corn, wheat, soy) have been genetically modified, DNA was extracted from the samples and analyzed using the Polymerase Chain Reaction (PCR). PCR has revolutionized biological research because it allows research-

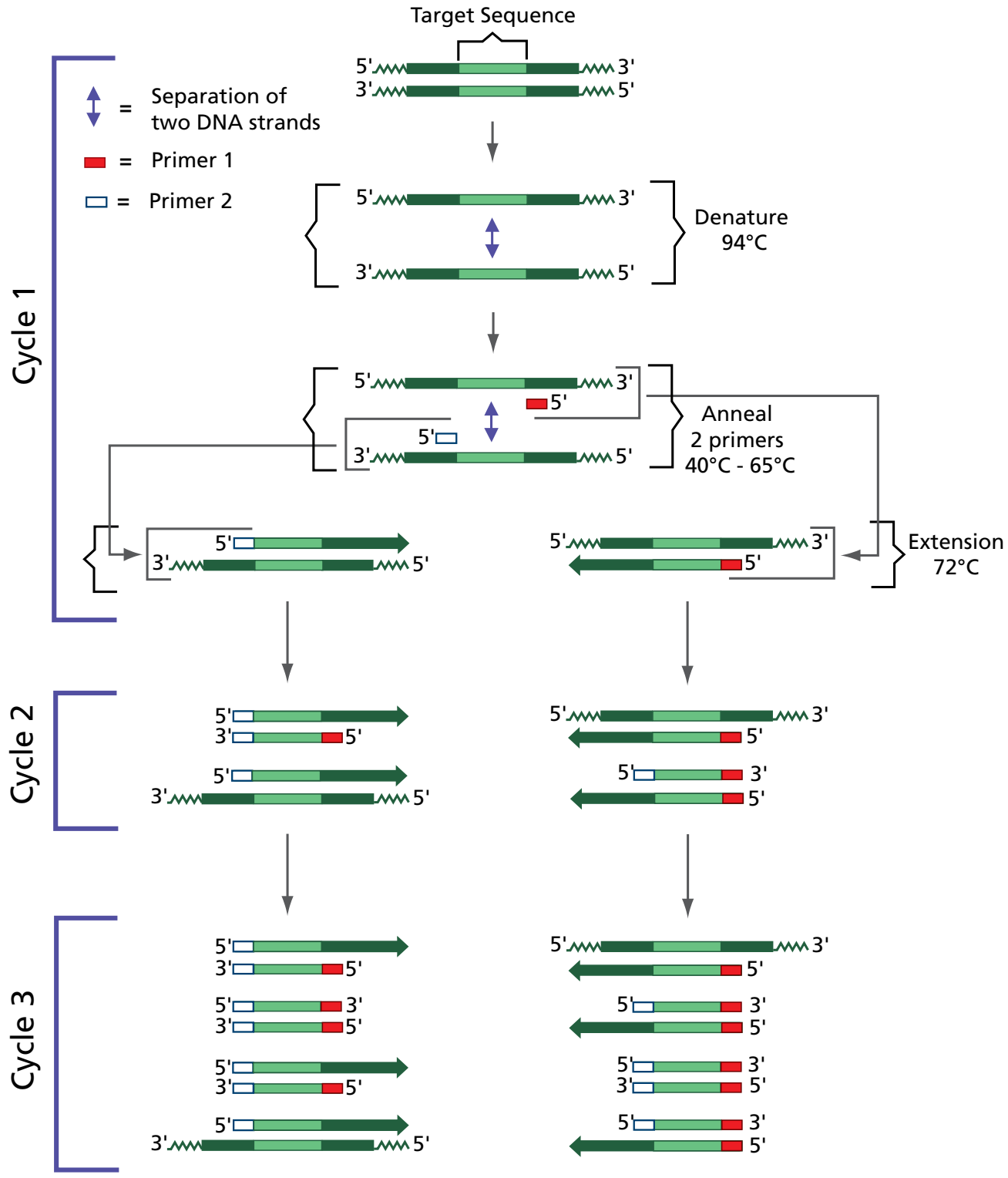
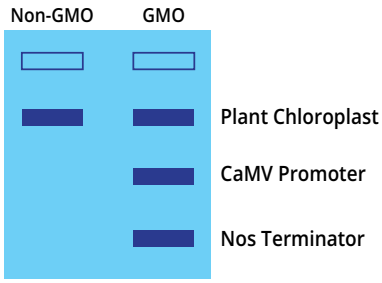


Figure 4:
Three-step PCR

ers to quickly create many copies of a specific region of DNA in vitro using short synthetic DNA molecules that target specific DNA sequences for amplification (primers). In this experiment, the primers were designed to differentiate between wild-type plants and those that have been genetically engineered. PCR can be used to determine whether a plant or food has been genetically modified using primers that target the 35S CaMV promoter and/ or NOS terminator. As a positive control for DNA extraction, the plant chloroplast gene is also amplified.

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



Plant Chloroplast:	4282
CaMV:	2872
Nos:	1282

Figure 5:
PCR Analysis of Non-GMO and GMO corn.

PCR is a simple, fast and reliable method to identify genetic modifications. The primers in this experiment are designed to produce DNA fragments of different sizes depending on whether the plant chloroplast, the CaMV promoter region, and/or the NOS terminator sequences are present in the extracted DNA (Figure 5). In order to analyze the mixture of DNA fragments, scientists use a technique called agarose gel electrophoresis, which separates DNA fragments according to size. The mixture of DNA molecules is added into depressions (or "wells") within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode (Figure 6A).

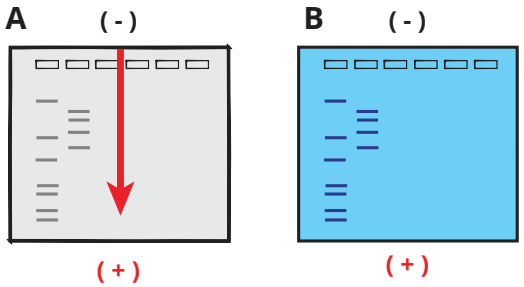


Figure 6:
Overview of Agarose Gel Electrophoresis.

At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA (Figure 6B).

Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, students will use agarose gel electrophoresis to explore the molecular methods used by scientists to identify genetically modified organisms.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

LABORATORY NOTEBOOKS

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

Before starting the Experiment:

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

During the Experiment:

- Record your observations.

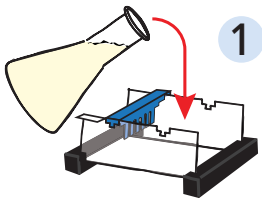
After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

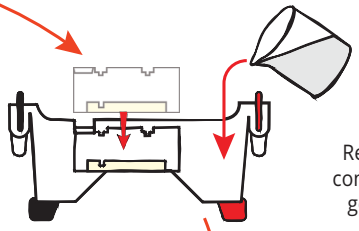
Experiment Overview

MODULE I: Agarose Gel Electrophoresis

Time required: See Table C

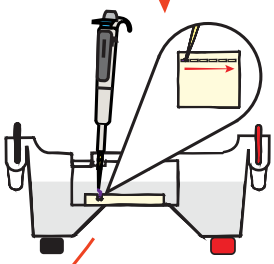


1 Prepare agarose gel in casting tray.

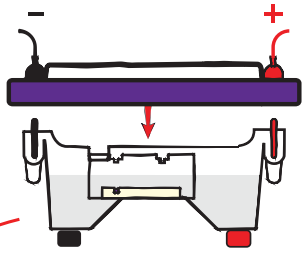


2 Remove end caps & comb, then submerge gel under buffer in electrophoresis chamber.

Quick Reference for EDVO-Kit #121			
Size of gel casting tray	*Groups per gel*	*Placement of comb*	*Wells required per group*
7 x 7 cm	1 group	1st set of notches	6
10 x 7 cm	1 group	1st set of notches	6
14 x 7 cm	2 groups	1st and 3rd sets of notches	6



3 Load each sample in consecutive wells

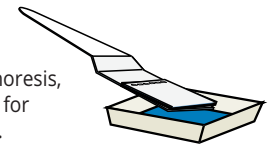


4 Attach safety cover & connect leads to power source to conduct electrophoresis.

MODULE II: Staining Agarose Gels Using FlashBlue™

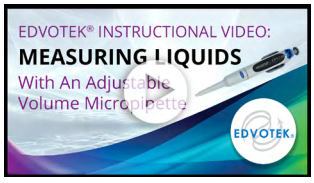
Time required: 30 min.

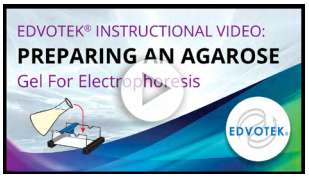
5 After electrophoresis, transfer gel for staining.

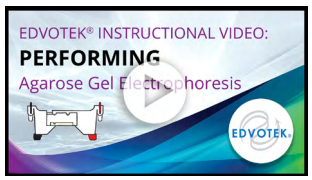


6 Analysis on white light source.

Related EDVOTEK® Instructional Videos

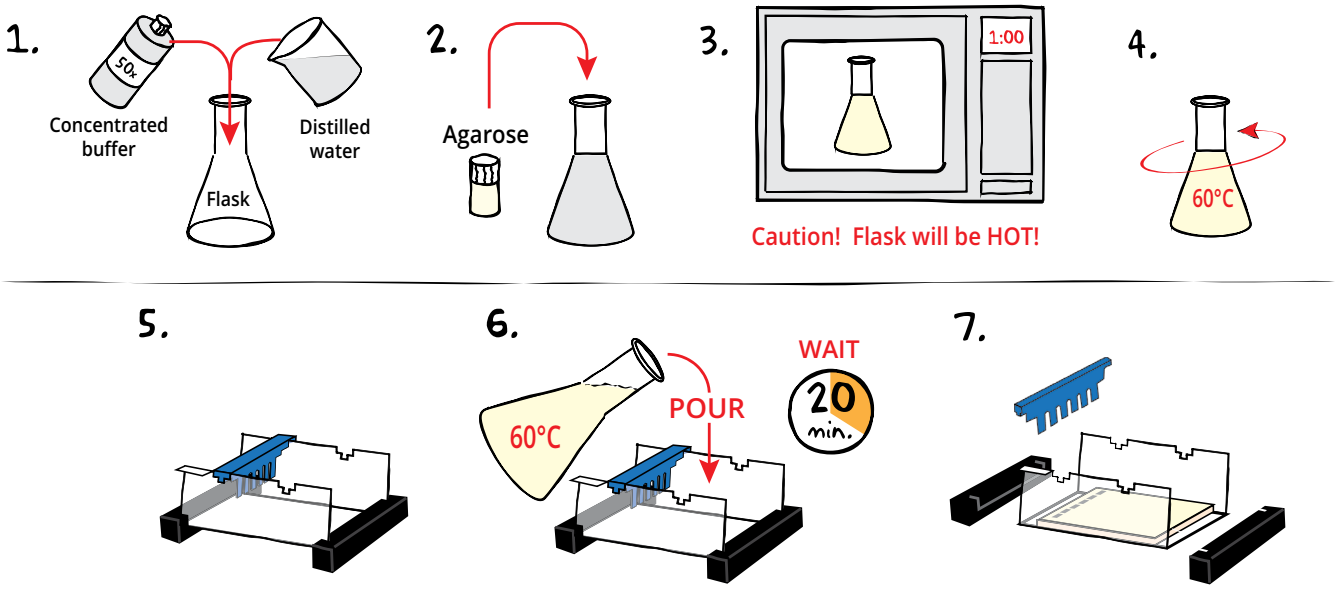






www.youtube.com/edvotekinc

Module I: Agarose Gel Electrophoresis



CASTING THE AGAROSE GEL

1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60 °C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Wear gloves and safety goggles

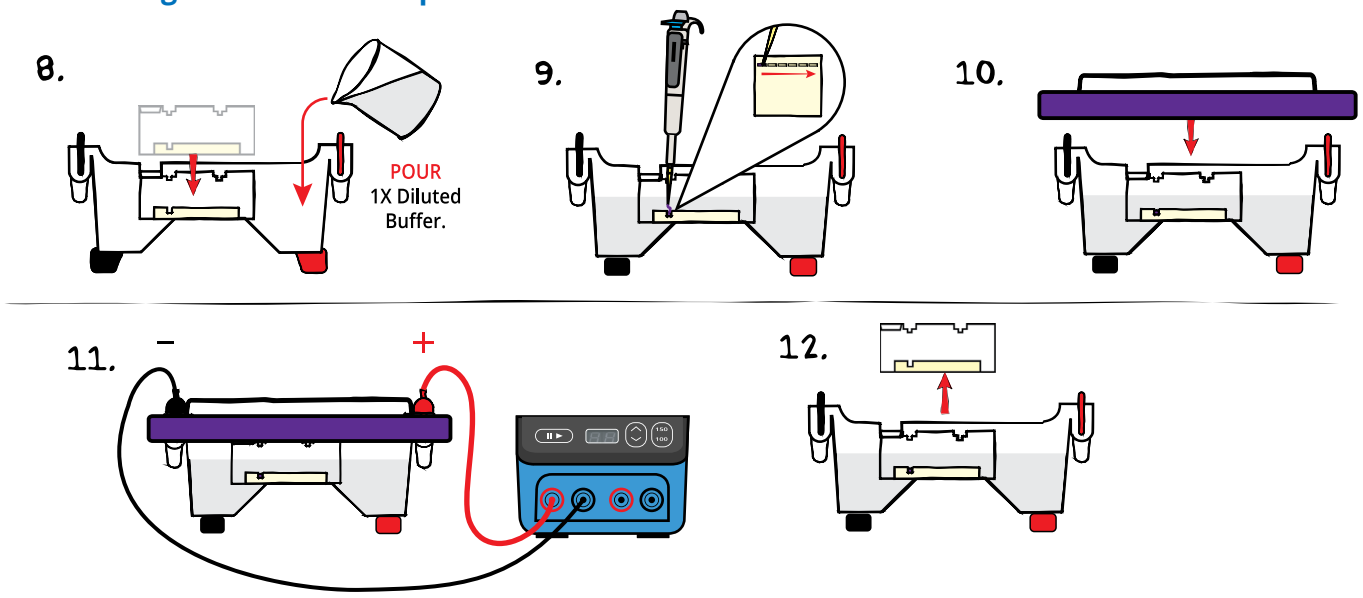
REMINDER:
This experiment requires 0.8% agarose gels cast with 6 wells.

Table A — **Individual 0.8% UltraSpec-Agarose™ Gels**

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 mL	29.4 mL	0.24 g	30 mL
10 x 7 cm*	0.9 mL	44.1 mL	0.36 g	45 mL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL

**Recommended gel volume for the EDGE™ Integrated Electrophoresis System. (Cat. #500).*

Module I: Agarose Gel Electrophoresis



RUNNING THE GEL

8. **PLACE** the gel (still on the tray*) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35 µL) into the well in the order indicated by Table 1, at right.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

PROCEED to Module II: Staining Agarose Gels Using FlashBlue™.

REMINDER:
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

TABLE 1: GEL LOADING

Lane		
1	Tube A	DNA Standard Marker
2	Tube B	GMO Negative Control
3	Tube C	GMO Positive Control
4	Tube D	Corn Sample
5	Tube E	Wheat Sample
6	Tube F	Soy Sample

Table B — **1x Electrophoresis Buffer (Chamber Buffer)**

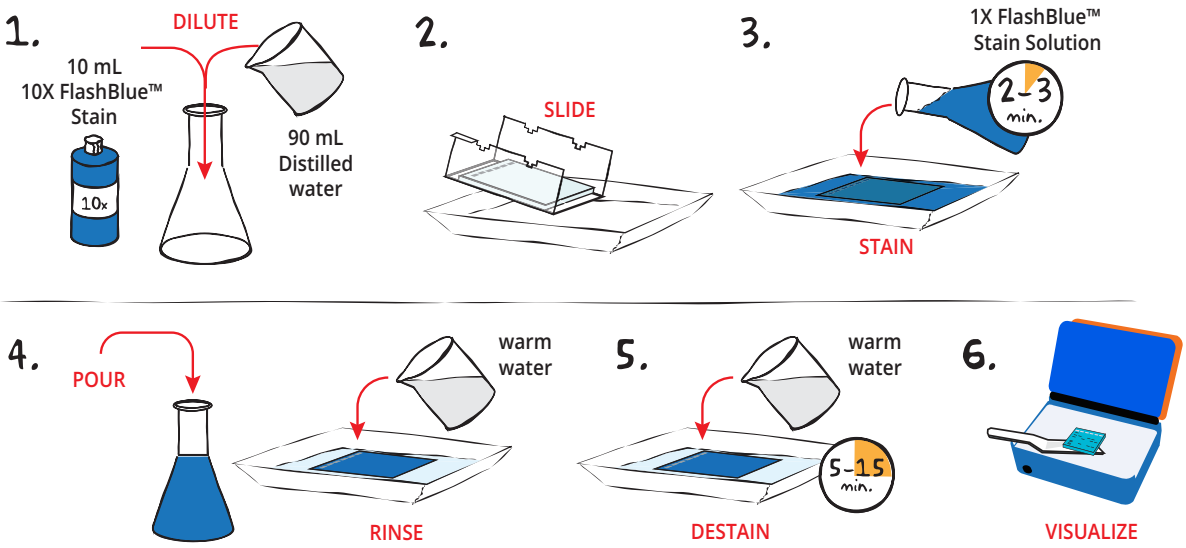
EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

Table C — **Time and Voltage Guidelines (0.8% Agarose Gel)**

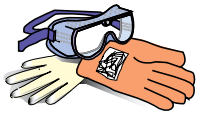
	Electrophoresis Model: EDGE™	M12 & M36
Volts	Min/Max (minutes)	Min/Max (minutes)
150	10/20	20/35
125	N/A	30/45
100	15/25	40/60

*Gels that have previously been removed from their trays should be "anchored" back to the tray with a few drops of molten agarose before placing into the electrophoresis chamber. This will prevent the gels from sliding around in the trays and the chambers.

Module II: Staining Agarose Gels Using FlashBlue™



1. **DILUTE** 10 mL of 10X concentrated FlashBlue™ with 90 mL of distilled water in a flask. **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1X FlashBlue™ stain solution. **STAIN** the gel for 2-3 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 3 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **POUR** the 1X FlashBlue™ back into the flask (the stain can be reused). **COVER** the gel with warm water (40-45 °C). Gently **RINSE** the gel for 20-30 seconds. **POUR** off the water.
5. **COVER** the gel with clean, warm water (40-45 °C). **DESTAIN** for 5-15 minutes with gentle shaking (longer periods will yield better results). DNA bands will start to appear after 5 minutes of destaining. Changing the water frequently will accelerate destaining.
6. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.



Wear gloves and safety goggles

ALTERNATIVE FLASHBLUE™ STAINING PROTOCOL:

1. **DILUTE** 1 mL of 10X FlashBlue™ stain with 149 mL distilled water.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.
4. Carefully **REMOVE** the gel from the staining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Module III: Data Analysis Using a Standard Curve

Agarose gel electrophoresis separates biomolecules into discrete bands, each comprising molecules of the same size. How can these results be used to determine the lengths of different fragments? Remember, as the length of a biomolecule increases, the distance to which the molecule can migrate decreases because large molecules cannot pass through the channels in the gel with ease. Therefore, the migration rate is inversely proportional to the length of the molecules—more specifically, to the log10 of molecule's length. To illustrate this, we ran a sample that contains bands of known lengths called a "standard". We will measure the distance that each of these bands traveled to create a graph, known as a "standard curve", which can then be used to extrapolate the size of unknown molecule(s).

Figure 7:
Measure distance migrated from the lower edge of the well to the lower edge of each band.

1. Measure and Record Migration Distances

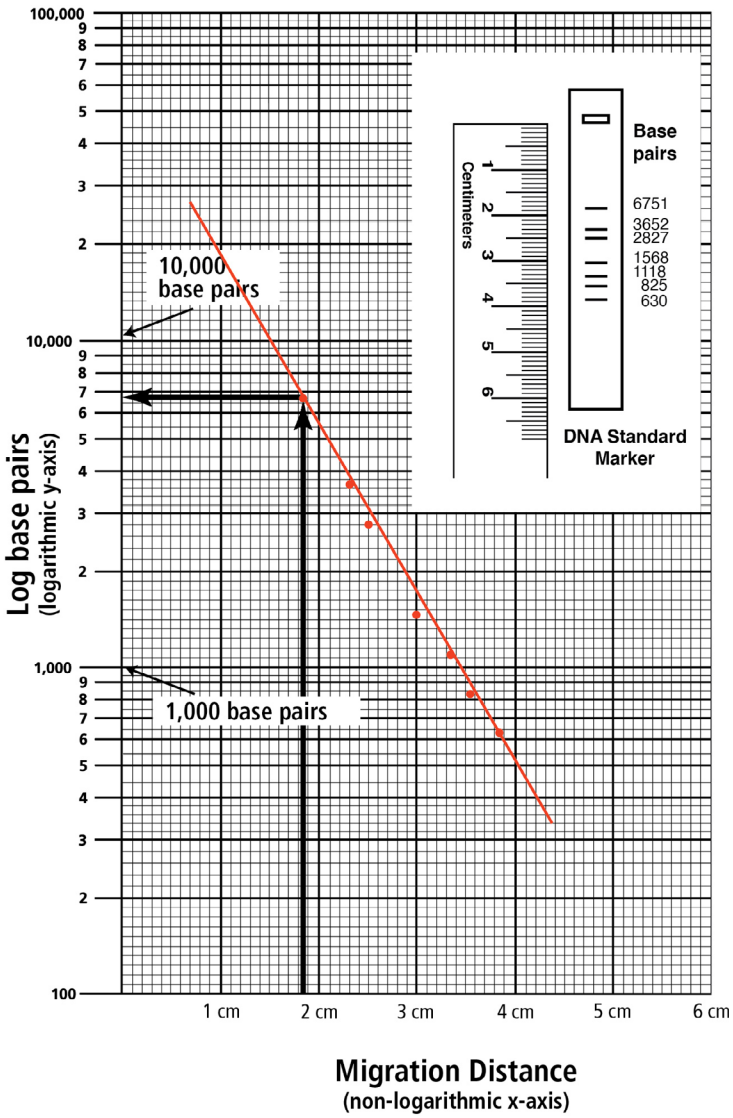
Measure the distance traveled by each Standard DNA Fragment from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters (to the nearest millimeter) in your notebook. Repeat this for each DNA fragment in the standard.

Measure and record the migration distances of each of the fragments in the unknown samples in the same way you measured the standard bands.

2. Generate a Standard Curve

Because migration rate is inversely proportional to the log10 of band length, plotting the data as a semi-log plot will produce a straight line and allow us to analyze an exponential range of fragment sizes. You will notice that the vertical axis of the semi-log plot appears atypical at first; the distance between numbers shrinks as the axis progresses from 1 to 9. This is because the axis represents a logarithmic scale. The first cycle on the y-axis corresponds to lengths from 100-1,000 base pairs, the second cycle measures 1,000-10,000 base pairs, and so on. To create a standard curve on the semi-log paper, plot the distance each Standard DNA fragment migrated on the x-axis (in mm) versus its size on the y-axis (in base pairs). Be sure to label the axes!

Figure 8: Semilog graph example



Module III: Data Analysis Using a Standard Curve

After all the points have been plotted, use a ruler or a straight edge to draw the best straight line possible through the points. The line should have approximately equal numbers of points scattered on each side of the line. It is okay if the line runs through some points (see Figure 8 for an example).

Quick Reference:
DNA Standard fragment sizes - length is expressed in base pairs.

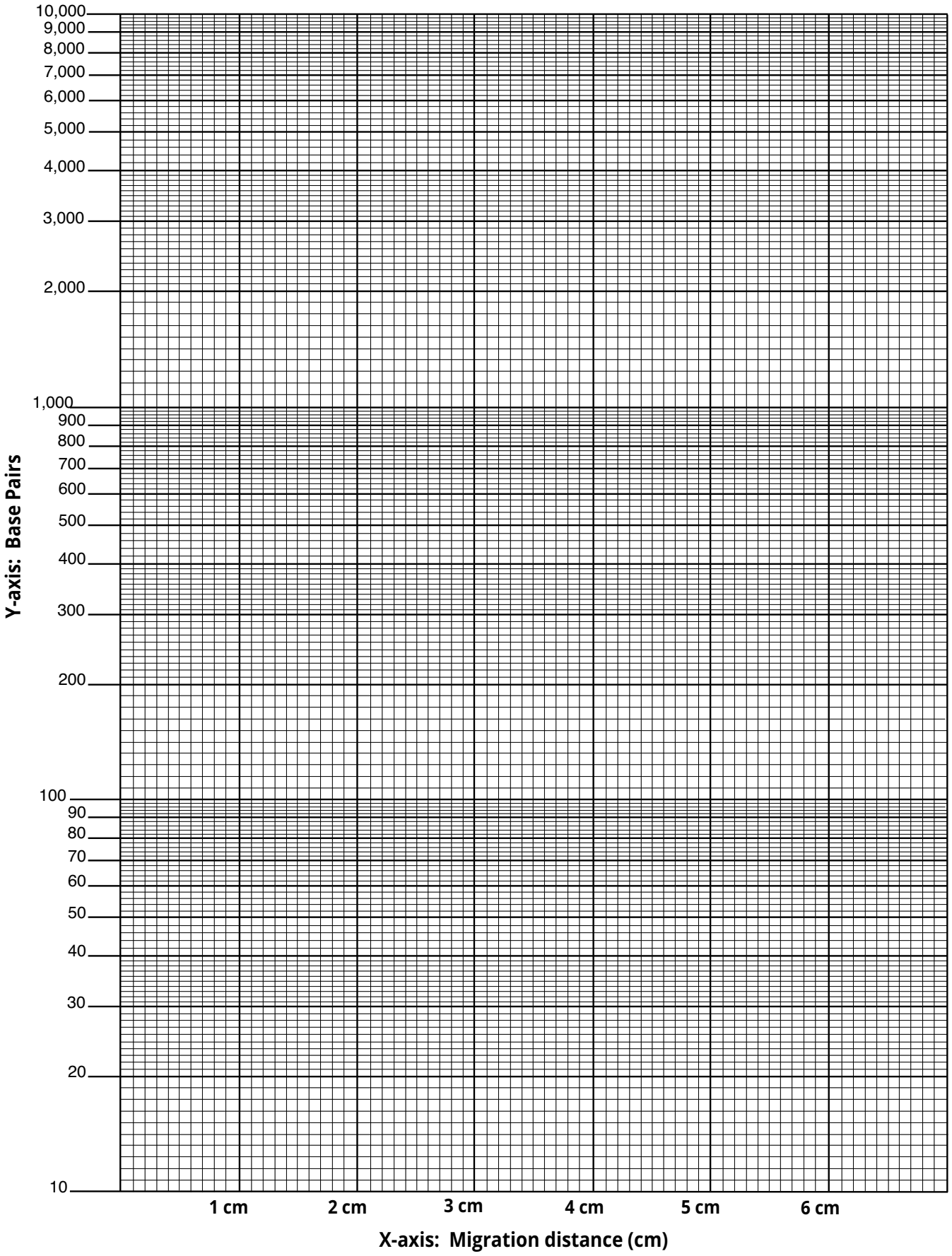
6751, 3652, 2827, 1568, 1118, 825, 630

3. Determine the length of each unknown fragment.

a. Locate the migration distance of the unknown fragment on the x-axis of your semi-log graph. Draw a vertical line extending from that point until it intersects the line of your standard curve.

b. From the point of intersection, draw a second line, this time horizontally, toward the y-axis. The value at which this line intersects the y-axis represents the approximate size of the fragment in base pairs (refer to Figure 8 for an example). Make note of this in your lab notebook.

c. Repeat for each fragment in your unknown sample.



Study Questions

1. What is artificial selection? Describe how farmers use artificial selection to improve crops.

2. What are some benefits of GM plants? What are some common concerns about GM plants?

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

4. What is PCR? How is PCR used to identify Genetically Modified Organisms?

5. Theoretically, you have extracted DNA from three different samples (corn, wheat, and soy) and analyzed them for genetic modifications using PCR. Knowing the results from your electrophoresis experiment, which samples have been genetically modified?

Instructor's Guide

ADVANCE PREPARATION:

PREPARATION FOR:	WHAT TO DO:	WHEN?	TIME REQUIRED:
Module I: Agarose Gel Electrophoresis	Prepare QuickStrips™.	Up to one day before performing the experiment.	45 min.
	Prepare diluted electrophoresis buffer.		
	Prepare molten agarose and pour gels.		
Module II: Staining Agarose Gels Using FlashBlue™	Prepare staining components.	The class period or overnight after the class period.	10 min.

Pre-Lab Preparations: Module I

AGAROSE GEL ELECTROPHORESIS

This experiment requires 0.8% agarose gels. Enough reagents are provided to cast either eight 7 x 7 cm gels, eight 10 x 7 cm gels, or four 14 x 7 cm gels. You can choose whether to prepare the gels in advance or have students prepare their own. Allow approximately 30 minutes for this procedure.

FOR MODULE I
Each group will need:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- QuickStrip™ Samples

Quick Reference for EDVO-Kit #121			
Size of gel casting tray	*Groups per gel*	*Placement of comb*	*Wells required per group*
7 x 7 cm	1 group	1st set of notches	6
10 x 7 cm	1 group	1st set of notches	6
14 x 7 cm	2 groups	1st and 3rd sets of notches	6

NOTE:
This kit is compatible with SYBR® Safe Stain (Cat #608, not included). Instructions for preparing gels and visualizing results can be found in Appendix C.

Individual Gel Preparation:
Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module I in the Student's Experimental Procedure. Students will need 50x concentrated buffer, distilled water and agarose powder.

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

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

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

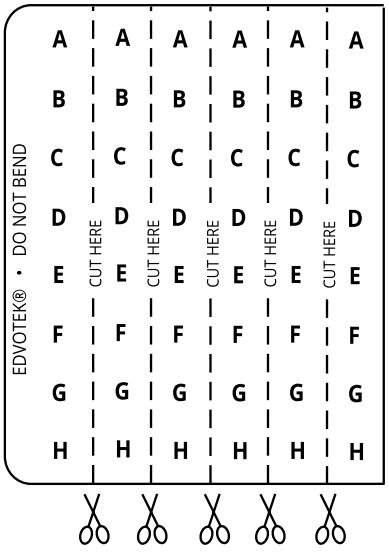
Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

QuickStrip™ tubes consist of a microtiter block covered with a protective foil overlay. Each well contains pre-aliquoted sample.

Using sharp scissors, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the foil overlay while separating the samples.

Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube. Puncture the foil overlay of the QuickStrip™ with a pipet tip to aspirate the sample. ***Do not remove the foil as samples can spill.***



Carefully cut between each set of tubes

Pre-Lab Preparations: Module II

STAINING AGAROSE GELS USING FLASHBLUE™

FOR MODULE II
Each group will need:

- 10 mL 10X concentrated FlashBlue *OR* 100 mL 1x diluted FlashBlue
- Small plastic tray or weight boat
- Distilled or deionized water

FlashBlue™ stain is optimized to shorten the time required for both staining and de-staining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to "equilibrate" in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (**Cat. #552**) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

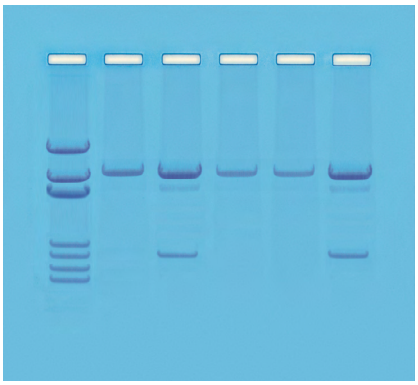
PHOTODOCUMENTATION OF DNA (OPTIONAL)

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

NOTE:
Accurate pipetting is critical for maximizing successful experiment results. EDVOTEK Series 100 experiments are designed for students who have had previous experience with micropipetting techniques and agarose gel electrophoresis.

If students are unfamiliar with using micropipettes, we recommended performing **Cat. #S-44, Micropipetting Basics** or **Cat. #S-43, DNA DuraGel™** prior to conducting this experiment.

Experiment Results and Analysis



In the idealized schematic, the relative positions of DNA fragments are shown but are not depicted to scale.

Lane	Tube	Sample	Result	Molecular Weights (in bp)
1	A	DNA Standard Markers	----------	6751, 3652, 2827, 1568 1118, 825, 630
2	B	GMO Negative Control	GMO Negative	4282
3	C	GMO Positive Control	GMO Positive	4282, 2872, 1282
4	D	Corn Sample	GMO Negative	4282
5	E	Wheat Sample	GMO Negative	4282
6	F	Soy Sample	GMO Positive	4282, 2872, 1282

Plant Chloroplast: 4282
CaMV: 2872
Nos: 1282

Please refer to the kit insert for the Answers to Study Questions

Appendices

A EDVOTEK® Troubleshooting Guide

B Bulk Preparation of Electrophoresis Buffer and Agarose Gels

C Using SYBR® Safe Stain (OPTIONAL)

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
Bands are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	The gel was not stained properly.	Repeat staining protocol.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
After staining the gel, the DNA bands are faint.	The gel was not stained for a sufficient period of time.	Repeat staining protocol.
	The background of gel is too dark after staining with FlashBlue™.	Destain the gel for 5-10 minutes in distilled water.
DNA bands were not resolved.	Tracking dye should migrate at least 3 cm from the wells to ensure adequate separation.	Be sure to run the gel at least 3 cm before staining and visualizing the DNA (approximately 15-20 minutes at 150 V).
DNA bands fade when gels are kept at 4 °C.	DNA stained with FlashBlue™ may fade with time.	Re-stain the gel with FlashBlue™.
There is no separation between DNA bands, even though the tracking dye ran the appropriate distance.	The wrong percent gel was used for electrophoretic separation.	Be sure to prepare the correct percent agarose gel. For reference, the Ready-to-Load™ DNA samples should be analyzed using a 0.8% agarose gel.
There's not enough sample in my QuickStrip™.	The QuickStrip™ has dried out.	Add 40 µL water, gently pipet up and down to mix before loading.

Visit www.edvotek.com for additional troubleshooting suggestions.

Appendix B

Bulk Preparation of Electrophoresis Buffer and Agarose Gels

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

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

Table D

Bulk Preparation of Electrophoresis Buffer			
50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 mL		2,940 mL	3000 mL (3 L)

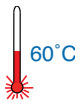
Batch Agarose Gels (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.

Table E

Batch Prep of 0.8% UltraSpec-Agarose™				
Amt of Agarose	+	Concentrated Buffer (50X)	+ Distilled Water	Total Volume
3.0 g		7.5 mL	367.5 mL	375 mL

1. Use a 500 mL flask to prepare the diluted gel buffer.
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60 °C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. Measure 30 mL for a 7 x 7 cm tray, 45 mL for a 10 x 7 cm tray, and 60 mL for a 14 x 7 cm tray. ***For this experiment, 7 x 7 cm gels are recommended.***
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks. Do not freeze gels.

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

PROCEED to Loading and Running the Gel (page 11).

Appendix C
Using SYBR® Safe DNA Stain (OPTIONAL)

If desired, the DNA samples in this experiment can be visualized using **SYBR® Safe DNA stain (Cat #608)**.
We recommend adding diluted SYBR® Safe stain to the liquid agarose gels while casting for easy, reproducible results.
A blue light or UV transilluminator is needed for visualizing SYBR® gels. The TruBlu™ 2 (**Cat. #557**) is highly recommended.

PREPARING SYBR® SAFE STAIN

Instructors:

1. Prepare 1x Electrophoresis Buffer by combining 10 µL of 50X Concentrated Buffer with 490 µL of distilled water.
2. Add 20 µL of the SYBR® Safe to the tube of 1X buffer from Step 1 and mix by tapping the tube several times. The diluted SYBR® Safe Stain is now ready to be used during agarose gel preparation.

AGAROSE GEL PREPARATION

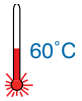
This experiment requires one 0.8% agarose gel for each student group. Instructors can choose whether to prepare the gels in advance (METHOD A) or have the students prepare their own (METHOD B). Allow approximately 30-40 minutes for this procedure.

Table E

Batch Prep of 0.8% UltraSpec-Agarose™			
Amt of Agarose +	Concentrated Buffer (50X) +	Distilled Water	Total Volume
3.0 g	7.5 mL	367.5 mL	375 mL

Instructor Preparation (METHOD A):
For quantity (batch) preparation of agarose gels, see Table E.

1. Use a 500 mL flask to prepare the diluted gel buffer.
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60 °C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Add the entire tube of ***diluted*** **SYBR® Safe** stain to the cooled agarose and mix well.
7. Dispense the required volume of cooled agarose solution for casting each gel. Measure 30 mL for a 7 x 7 cm tray, 45 mL for a 10 x 7 cm tray, and 60 mL for a 14 x 7 cm tray. ***For this experiment, 7 x 7 cm gels are recommended.***
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Solidified gels can be stored in the refrigerator for up to 2 weeks. Place 1-2 mL of electrophoresis buffer in a sealable bag with the gels to prevent them from drying out. Excessive buffer will cause SYBR® Safe to diffuse out of the gels. Do not freeze gels.

PROCEED to Loading and Running the Gel (Steps 8-12 on page 11), followed by the VISUALIZATION procedures on page 27.
NO ADDITIONAL STAINING IS NECESSARY.

Appendix C
Using SYBR® Safe DNA Stain (OPTIONAL)

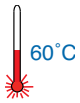
AGAROSE GEL PREPARATION, CONTINUED

Student Preparation (METHOD B):
For student preparation of agarose gels, see Table A.2.

Table A.2 **Individual 0.8% UltraSpec-Agarose™ with SYBR® Stain**

Size of Gel Casting Tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume	Diluted SYBR® (Step 6)
7 x 7 cm	0.6 mL	29.4 mL	0.24 g	30 mL	30 µL
10 x 7 cm*	0.9 mL	44.1 mL	0.36 g	45 mL	45 µL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL	60 µL

** Recommended gel volume for the EDGE™ Integrated Electrophoresis System.*

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A.2).
2. **MIX** agarose powder with 1X buffer in a 250 mL flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60 °C with careful swirling to promote even dissipation of heat. 

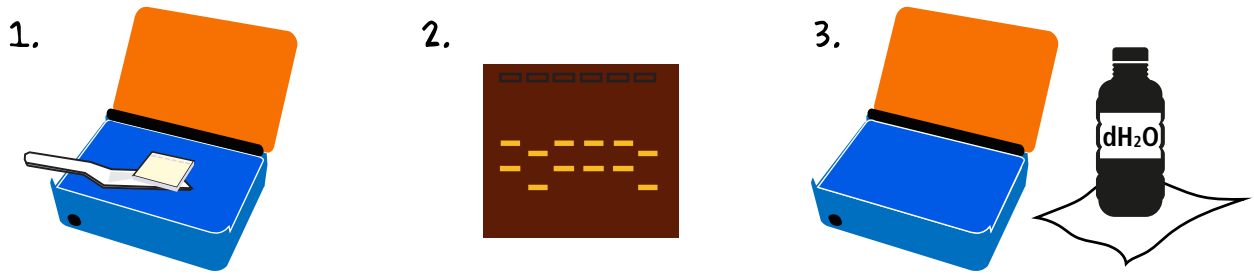
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. Before casting the gel, **ADD** ***diluted SYBR® Safe*** to the cooled agarose and swirl to mix (see Table A.2).
7. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
8. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

PROCEED to Loading and Running the Gel (Steps 8-12 on page 11), followed by the VISUALIZATION procedures on page 27.
NO ADDITIONAL STAINING IS NECESSARY.

Appendix C
Using SYBR® Safe DNA Stain (OPTIONAL)

VISUALIZING THE SYBR® GEL

A blue light or UV transilluminator is needed for visualizing SYBR® gels. The TruBlu™ 2 (Cat. #557) is highly recommended.



1. **SLIDE** gel off the casting tray onto the viewing surface of the transilluminator.
2. Turn the unit **ON**. DNA should appear as bright green bands on a dark background. **PHOTOGRAPH** results.
3. Turn the unit **OFF**. **REMOVE** and **DISPOSE** of the gel. **CLEAN** the transilluminator surfaces with distilled water.

Be sure to wear UV goggles if using a UV transilluminator.