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.
Table of Contents

| Experiment Components                      | 3 |
| Experiment Requirements                    | 3 |
| Background Information                     | 4 |
| Experiment Procedures                      | 8 |
| - Experiment Overview                      | 10 |
| - Module I: Agarose Gel Electrophoresis    | 10 |
| - Module II: Staining Agarose Gels         | 12 |
| - Study Questions                          | 14 |
| Instructor’s Guidelines                    | 15 |
| - Pre-Lab Preparations                     | 16 |
| - Experiment Results and Analysis          | 18 |
| - Study Questions and Answers              | 19 |
| Appendices                                 | 20 |

Safety Data Sheets can be found on our website: [www.edvotek.com/safety-data-sheets](http://www.edvotek.com/safety-data-sheets)
Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS
Store QuickStrip™ samples in the refrigerator immediately upon receipt.
All other components can be stored at room temperature.

Components (in QuickStrip™ Format) Check (√)
A  DNA Standard Marker
B  GMO Negative Control
C  GMO Positive Control
D  Corn Sample
E  Wheat Sample
F  Soy Sample

REAGENTS & SUPPLIES
• UltraSpec-Agarose™
• Electrophoresis Buffer (50x)
• FlashBlue™ DNA Stain
• InstaStain® Blue cards
• 1 ml pipet
• Microtipped Transfer Pipets

Requirements
• Horizontal gel electrophoresis apparatus
• D.C. power supply
• Automatic micropipets 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

Experiment #121 is designed for 8 gels if stained with FlashBlue™ or InstaStain® Blue (both included) or 16 gels if stained with SYBR® Safe or InstaStain® Ethidium Bromide (not included).

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

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.
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!

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 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 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.

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.
Figure 4:
Three-step PCR
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 researchers 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.

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).

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 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.

**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

1. Prepare agarose gel in casting tray
2. Remove end blocks & comb, then submerge gel under buffer in electrophoresis chamber
3. Load each sample in consecutive wells
4. Attach safety cover, connect leads to power source and conduct electrophoresis
5. After electrophoresis, transfer gel for staining
6. Analysis on white light source.

Gel pattern will vary depending upon experiment.
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.

**Table A**

<table>
<thead>
<tr>
<th>Size of Gel Casting tray</th>
<th>Concentrated Buffer (50x)</th>
<th>Distilled Water</th>
<th>Amt of Agarose</th>
<th>TOTAL Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7 cm</td>
<td>0.6 ml</td>
<td>29.4 ml</td>
<td>0.23 g</td>
<td>30 ml</td>
</tr>
<tr>
<td>7 x 10 cm</td>
<td>1.0 ml</td>
<td>49.0 ml</td>
<td>0.39 g</td>
<td>50 ml</td>
</tr>
<tr>
<td>7 x 14 cm</td>
<td>1.2 ml</td>
<td>58.8 ml</td>
<td>0.46 g</td>
<td>60 ml</td>
</tr>
</tbody>
</table>
Module I: Agarose Gel Electrophoresis

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 3.5 cm from the wells.

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

---

### Table 1: Gel Loading

<table>
<thead>
<tr>
<th>Lane</th>
<th>Tube</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tube A</td>
<td>Standard DNA Marker</td>
</tr>
<tr>
<td>2</td>
<td>Tube B</td>
<td>GMO Negative Control</td>
</tr>
<tr>
<td>3</td>
<td>Tube C</td>
<td>GMO Positive Control</td>
</tr>
<tr>
<td>4</td>
<td>Tube D</td>
<td>Corn Sample</td>
</tr>
<tr>
<td>5</td>
<td>Tube E</td>
<td>Wheat Sample</td>
</tr>
<tr>
<td>6</td>
<td>Tube F</td>
<td>Soy Sample</td>
</tr>
</tbody>
</table>

---

### Table B: 1X Electrophoresis Buffer (Chamber Buffer)

<table>
<thead>
<tr>
<th>EDVOTEK Model #:</th>
<th>Total Volume</th>
<th>Dilution 50x Conc. Buffer + Distilled Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+ &amp; M12 (new)</td>
<td>300 ml</td>
<td>6 ml + 294 ml</td>
</tr>
<tr>
<td>M12 (classic)</td>
<td>400 ml</td>
<td>8 ml + 392 ml</td>
</tr>
<tr>
<td>M36</td>
<td>1000 ml</td>
<td>20 ml + 980 ml</td>
</tr>
</tbody>
</table>

---

### Table C: Time & Voltage Guidelines (0.8% Agarose Gel)

<table>
<thead>
<tr>
<th>Electrophoresis Model</th>
<th>Min. / Max.</th>
<th>Min. / Max.</th>
<th>Min. / Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+</td>
<td>150 15/20 min.</td>
<td>20/30 min.</td>
<td>25 / 35 min.</td>
</tr>
<tr>
<td>M12 (new)</td>
<td>125 20/30 min.</td>
<td>30/35 min.</td>
<td>35 / 45 min.</td>
</tr>
<tr>
<td>M12 (classic) &amp; M36</td>
<td>75 35 / 45 min.</td>
<td>55/70 min.</td>
<td>60 / 90 min.</td>
</tr>
</tbody>
</table>
Module II-A: Staining Agarose Gels Using FlashBlue™

1. **DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 ml of water in a flask and **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.
5. 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.

**ALTERNATIVE PROTOCOL:**

1. **DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH₂O.
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.

**NOTE:** DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.
Module II-B: Staining Agarose Gels Using InstaStain® Blue

1. Carefully REMOVE the agarose gel and casting tray from the electrophoresis chamber. SLIDE the gel off of the casting tray on to a piece of plastic wrap on a flat surface.

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

3. Wearing gloves, PLACE the blue side of the InstaStain® Blue card on the gel.

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

5. PLACE the casting tray on top of the gel/card stack. PLACE a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Blue card is in direct contact with the gel surface. STAIN the gel for 10 minutes.

6. REMOVE the InstaStain® Blue card. If the color of the gel appears very light, reapply the InstaStain® Blue card to the gel for an additional five minutes.

7. TRANSFER the gel to a small, clean gel-staining tray. COVER the gel with about 75 mL of distilled water and DESTAIN for at least 20 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. To accelerate destaining, warm the distilled water to 37°C and change it frequently.

8. 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.

ALTERNATIVE PROTOCOL:

1. Carefully SLIDE the agarose gel from its casting tray into a small, clean tray containing about 75 mL of distilled/deionized water or used electrophoresis buffer. The gel should be completely submerged.

2. Gently FLOAT the InstaStain® Blue card(s) on top of the liquid with the stain (blue side) facing toward the gel. Each InstaStain® Blue card will stain 49 cm² of gel (7 x 7 cm).

3. COVER the tray with plastic wrap to prevent evaporation. SOAK the gel in the staining liquid for at least 3 hours. The gel can remain in the liquid overnight if necessary.

4. Carefully REMOVE the gel from the staining tray. VISUALIZE results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.
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?
## OVERVIEW OF INSTRUCTOR’S PRELAB PREPARATION:

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

<table>
<thead>
<tr>
<th>Preparation for:</th>
<th>What to do:</th>
<th>When?</th>
<th>Time Required:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Module I: Agarose Gel Electrophoresis</td>
<td>Prepare QuickStrips™</td>
<td>Up to one day before performing the experiment</td>
<td>45 min.</td>
</tr>
<tr>
<td></td>
<td>Prepare diluted electrophoresis buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prepare molten agarose and pour gels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Module II: Staining Agarose Gels</td>
<td>Prepare staining components</td>
<td>The class period or overnight after the class period</td>
<td>10 min.</td>
</tr>
</tbody>
</table>

---

**EDVO-TECH Service**

**1.800.EDVOTEK**

Mon. - Fri. 8am-5:30pm EST

Please Have the Following Info:
- Product Number & Description
- Lot Number on Box
- Order/Purchase Order #

Fax: 202.370.1501 • info@edvotek.com • www.edvotek.com
Pre-Lab Preparations

AGAROSE GEL ELECTROPHORESIS

This experiment requires a 0.8% agarose gel per student group. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

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 overlay. Each well contains pre-aliquoted DNA.

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 protective 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.

If using SYBR® Safe or InstaStain® Ethidium Bromide for DNA visualization, each QuickStrip™ is shared by two groups. 18 μl of the DNA sample will be loaded into each well. Proceed to visualize the results as specified by the DNA stain literature.
Pre-Lab Preparations

MODULE II-A: STAINING WITH INSTASTAIN® BLUE

The easiest and most convenient DNA stain available is InstaStain® Blue. InstaStain® Blue does not require the formulation, storage and disposal of large volumes of liquid stain. Each InstaStain® Blue card contains a small amount of blue DNA stain. When the card is placed in water, the DNA stain is released. This solution simultaneously stains and destains the gel, providing uniform gel staining with minimal liquid waste and mess.

You can use a White Light Visualization System (Cat. #552) to visualize gels stained with InstaStain® Blue.

MODULE II-B: STAINING WITH FLASHBLUE™

FlashBlue™ stain is optimized to shorten the time required for both staining and destaining 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.

MODULE II: 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.

FOR MODULE II-A
Each Student Group should receive:
• 1 InstaStain® card per 7 x 7 cm gel

FOR MODULE II-B
Each Student Group should receive:
• 10 ml 10X concentrated FlashBlue OR 100 mL 1x diluted FlashBlue
• Small plastic tray or weight boat
• Distilled or deionized water

Wear gloves and safety goggles
Experiment Results and Analysis

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

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample</th>
<th>Result</th>
<th>Molecular Weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard DNA Marker</td>
<td>........</td>
<td>........</td>
</tr>
<tr>
<td>2</td>
<td>GMO Negative Control</td>
<td>GMO Negative</td>
<td>4282</td>
</tr>
<tr>
<td>3</td>
<td>GMO Positive Control</td>
<td>GMO Positive</td>
<td>4282 2872 1282</td>
</tr>
<tr>
<td>4</td>
<td>Corn Sample</td>
<td>GMO Negative</td>
<td>4282</td>
</tr>
<tr>
<td>5</td>
<td>Wheat Sample</td>
<td>GMO Negative</td>
<td>4282</td>
</tr>
<tr>
<td>6</td>
<td>Soy Sample</td>
<td>GMO Positive</td>
<td>4282 2872 1282</td>
</tr>
</tbody>
</table>

Plant Chloroplast: 4282  
CaMV: 2872  
Nos: 1282

Includes EDVOTEK’s All-NEW DNA Standard Marker

- Better separation
- Easier band measurements
- No unused bands

NEW DNA Standard ladder sizes: 6751, 3652, 2827, 1568, 1118, 825, 630
Please refer to the kit insert for the Answers to Study Questions
Appendices

A EDVOTEK® Troubleshooting Guide
B Bulk Preparation of Agarose Gels
C Data Analysis Using a Standard Curve

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

**EDVOTEK® Troubleshooting Guides**

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bands are not visible on the gel.</td>
<td>The gel was not prepared properly.</td>
<td>Ensure that the electrophoresis buffer was correctly diluted.</td>
</tr>
<tr>
<td></td>
<td>The gel was not stained properly.</td>
<td>Repeat staining.</td>
</tr>
<tr>
<td></td>
<td>Malfunctioning electrophoresis unit or power source.</td>
<td>Contact the manufacturer of the electrophoresis unit or power source.</td>
</tr>
<tr>
<td>After staining the gel, the DNA bands are faint.</td>
<td>The gel was not stained for a sufficient period of time.</td>
<td>Repeat staining protocol.</td>
</tr>
<tr>
<td></td>
<td>The background of gel is too dark.</td>
<td>Destain the gel for 5-10 minutes in distilled water.</td>
</tr>
<tr>
<td>DNA bands were not resolved.</td>
<td>Tracking dye should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation.</td>
<td>Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).</td>
</tr>
<tr>
<td>DNA bands fade when gels are kept at 4°C.</td>
<td>DNA stained with FlashBlue™ may fade with time</td>
<td>Re-stain the gel with FlashBlue™</td>
</tr>
<tr>
<td>There is no separation between DNA bands, even though the tracking dye ran the appropriate distance.</td>
<td>The wrong percent gel was used for electrophoretic separation.</td>
<td>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.</td>
</tr>
<tr>
<td>There's not enough sample in my QuickStrip™.</td>
<td>The QuickStrip™ has dried out.</td>
<td>Add 40 µL water, gently pipet up and down to mix before loading.</td>
</tr>
</tbody>
</table>
Appendix B

Bulk Preparation of Agarose Gels

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

**Bulk Electrophoresis Buffer**

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

<table>
<thead>
<tr>
<th>Table Bulk Preparation of Electrophoresis Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>50X Conc. Buffer</td>
</tr>
<tr>
<td>60 ml</td>
</tr>
</tbody>
</table>

**Batch Agarose Gels (0.8%)**

For quantity (batch) preparation of 0.8% 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. Dispense the required volume of cooled agarose solution for casting each gel. Measure 30 ml for a 7 x 7 cm tray, 50 ml for a 7 x 10 cm tray, and 60 ml for a 7 x 14 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. Then proceed with preparing the gel for electrophoresis.

**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.

<table>
<thead>
<tr>
<th>Table Batch Prep of 0.8% UltraSpec-Agarose™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amt of Agarose (g)</td>
</tr>
<tr>
<td>3.0</td>
</tr>
</tbody>
</table>
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 \( \log_{10} \) 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).

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 \( \log_{10} \) 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 7:** Measure distance migrated from the lower edge of the well to the lower edge of each band.

**Figure 8:** Semilog graph example
Appendix C: Data Analysis Using a Standard Curve, continued

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).

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.