EDVOTEK®

The Biotechnology Education Company ®

EDVO-Kit #

962

Identification of Foodstuffs from Genetically Modified Organisms

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

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

This experiment is designed for DNA staining with InstaStain® Ethidium Bromide.

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Experiment Components

This experiment is designed for 10 lab groups.

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

All 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.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

		Storage
A	Tubes with PCR reaction pellets™ Each PCR reaction pellet™ contains • dNTP Mixture • *Taq* DNA Polymerase Buffer • *Taq* DNA Polymerase • $MgCl_2$	Room Temperature
B	GMO Primer mix	-20°C Freezer
C	100 base pair ladder	-20°C Freezer
D	Positive PCR control	-20°C Freezer
E	Tris-EDTA Buffer	-20°C Freezer
F	Proteinase K	Room temperature
G	NaCl	Room temperature
H	DNA extraction buffer	Room temperature

Reagents & Supplies

(Store all components below at room temperature)

- UltraSpec-Agarose™
- Electrophoresis Buffer (50x)
- 10x Gel Loading Solution
- InstaStain® Ethidium Bromide
- InstaStain® Blue
- Microcentrifuge Tubes
- PCR tubes (0.2 ml - for thermal cyclers with 0.2 ml template)
- Calibrated transfer pipets
- Wax beads (for waterbath option or thermal cyclers without heated lid)

Experiment Requirements

*If you do not have a thermal cycler, PCR experiments can be conducted, with proper care, using three waterbaths. However, a thermal cycler assures a significantly higher rate of success.

- Recommended foodstuffs that have worked well in the EDVOTEK testing laboratory include: corn flakes, soybeans, cornmeal, corn chips, soy snacks.
- Thermal cycler (EDVOTEK Cat. # 541 highly recommended) or three waterbaths*
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- Water bath (56°C)
- UV Transilluminator or UV Photodocumentation system
- UV safety goggles
- Automatic micropipets (5-50 µl) with tips
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Disposable vinyl or latex laboratory gloves
- Ice buckets and ice
- Distilled or deionized water
- Isopropanol

Background Information

Tomatoes, soybeans, and corn were among the first genetically modified food products approved by U.S. agencies in the 1990s. Since then food biotechnology continues to grow rapidly. Enormous advances in genetics over the last decade have led to accelerated changes in biotechnology, with ramifications in social and political fields. Genetic research which ushered our understanding about the very basis of life also led the heated debates on what should be done in the area of genetically modified (GM) foods and what may be possible in the future.

Debates on GM foods are world-encompassing, with arguments citing risks and benefits for crops in Brazil, countries in Africa, Europe, Bolivia, India, Japan, Australia and New Zealand, as well as the United States. Articles on the topic have been published in leading science journals, including Scientific American, Nature and Science, and there have been impassioned discussions in government chambers and in environmental, religious and ethical circles. On one side of the controversy are the promises of improved quantity and quality plants, decreasing costs for growers, and benefits to the environment; on the other side are fears of damage to the environment and to other crops, increased allergens, and the creation of other unanticipated dangers to people and the environment.

Adaptation is vital to all living things in nature and is the key to evolution. Nature is dynamic and at all times is changing and as a consequence adaptation to change becomes a prerequisite to survival. In one sense plant genetics is not new to man. Over the centuries selective breeding and conventional hybridization provided mankind a distinct advantage over natural selection. Biotechnology has redirected and accelerated the pace of selective breeding and evolution and introduced possibilities which until recently were not considered to be feasible.

A goal of plant genetics is the development of plants that yield optimum product and have selective advantages. With the advent of biotechnology, cloning and expression of genes in GM plants have increased yields, nutritional value and enhanced quality. Plant biotechnology today offers the possibilities of modification, enhancement or suppression of gene products.

In the last half of the century, the world population more than doubled however agriculture only increased by 10%. In the same time frame world food production per person increased by 25% due to advances in agriculture due to mechanization and biotechnology. For example, in 2002, 74% (80 million acres) of American soybeans were obtained from genetically-modified crops. The benefits of food production have not been equally distributed amongst the world population with the U.S. being both the largest producer and consumer of food.

APPROACHES TO PLANT BIOTECHNOLOGY

Introduction of specific genes through biotechnology can provide advantages. As an example, a genetically modified (GM) plant can protect itself against parasites after the introduction of the endotoxin gene. Golden rice is an example of a GM crop that synthesizes a high value bioproduct. Plants can also be modified to inhibit the expression of specific genes that are involved in the ripening of fruits by maintaining and enhancing fruit flavors and extending their shelf life.

Background Information

There are various biotechnology procedures that can be used in plant genetic engineering. Examples include the use of gene guns, Ti plasmid based gene introduction and antisense technology. The gene gun approach uses tiny metal beads coated with the specific DNA that is targeted to be introduced in plant cells. The pellets are shot into embryonic plant cells, and treated cells are screened for the gene of interest through the use of specific markers. An example of the use of the gene gun for the production of a recombinant plant is the Bt corn. Genes isolated from *Bacillus thuringiensis* together with the CaMV/35S promoter are fired and enter the cells resulting in the expression of an endotoxin. The bacterial endotoxin serves as a pesticide for the corn plant. Parasitic insects that feed on the corn plant ingest the bacterial endotoxin which causes a break down of their gut wall and results in eventual death.

The Ti plasmid from the soil bacterium *Agrobacterium tumefaciens* is used as a vector for transferring DNA into dicotyledonous plants. These plants have two seed halves such as tomatoes, apple and soybean. Monocotyledonous plants, grow from a single seed embryo such as corn and wheat and are genetically manipulated by different technologies.

In some cases, antisense technology is employed for improving plant products. The Flavr Savr tomatoes contain an anti-complementary copy of the gene that codes for the enzyme polygalacturonase (PG). The antisense mRNA binds and inactivates the normal mRNA, thus inhibiting translation and shutting down the production of PG. As a result pectin is not digested and ripening is slowed.

Golden rice, named for its appearance, is genetically modified to produce precursor metabolites of vitamin A. This vitamin is essential for nutrition and is chronically deficient in diets in developing countries. Rice normally lacks beta carotene but contains geranyl diphosphate a (precursor of β-carotene and of vitamin A). Rice is bombarded with cDNA from bacteria that contain the phyteone synthase gene. This enzyme condenses two C20 to C40, which after several enzymatic steps, is converted to β-carotene (C40). Upon ingestion in the presence of fat, the pro-vitamin β-carotene is converted into Vitamin A. Delivery of a vitamin in rice (which is the staple food for a large segment of the world population) is a major step forward in supplementing food with key high value targeted nutritional components.

A future approach to food Agro-biotechnology will include the introduction of genes in chloroplasts which can accept several different genes. Chloroplast genetic engineering is rapidly becoming a viable focus for researchers. Like mitochondria, chloroplast genes typically have high expression, and are not passed through the pollen. With high protein expression levels and little chance of non-target exposure to the product, chloroplast engineering has made plant-based expression of pharmaceuticals a real possibility. Biopharming to produce Farmaceuticals has the potential to use any number of crops such as tobacco, carrots, tomatoes, soybeans and rice for their plant-made pharmaceuticals.

The responsibility of public health and policy concerning agro-biotechnology rests on the shoulders of both the public and the biotechnology industry. It remains to be seen what long-term effects altered plants will have on the ecosystem and overall biodiversity. To gain acceptance, the plant biotechnology

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industry needs to better communicate its research and development of new GM foods products. In the future one can foresee allergy free peanuts, low protein rice that would help kidney disease compromised individuals, low fat and high protein GM foods. Genetically modified foods may also be foreseen to serve as vehicles for the delivery of various pharmaceuticals.

There are several Federal agencies in the United States that oversee various aspects of food safety. The Federal Drug Administration (FDA) is responsible for the safety of food and animal feed products. The U.S. Department of Agriculture (USDA) oversees new plant varieties that are safe for the environment. Lastly, the Environmental Protection Agency (EPA) monitors and sets standards for pesticide levels in plants and determines what is acceptable for human consumption.

ABOUT THE POLYMERASE CHAIN REACTION

The polymerase chain reaction (PCR) is a relatively new tool for DNA amplification that has revolutionized almost all aspects of biological research. PCR was invented in 1984 by Dr. Kary Mullis while at Cetus Corporation. Mullis was awarded a Nobel Prize for his work in 1994. PCR allows for the amplification of a small quantity of DNA over one million-fold in a few hours. The enormous utility of PCR is based on its procedural simplicity and specificity. Since the first application of PCR to diagnose sickle cell anemia, a large number of diagnostic tests have been developed. Many such diagnostic tests have now become routine. PCR has also made amplification of DNA an alternate approach to cloning experiments. It is used extensively in plant agrobiotechnology as well as various other fields of biotechnology. For example biomedical applications include diagnostic test for infectious agents and inherited or acquired genetic conditions including various forms of cancer.

PCR amplification requires the use of a thermostable DNA polymerase. *Taq* polymerase is the most commonly used polymerase which is purified from a bacterium known as *Thermus Aquaticus* that inhabits hot springs. This enzyme remains stable at near-boiling (95°C) temperatures. Also required in the PCR reaction are the four deoxynucleotides (dATP, dCTP, dGTP, and dTTP) and two synthetic oligonucleotides, typically 15-30 base pairs in length, known as "primers". These components, together with the DNA to be amplified, are incubated in an appropriate buffer that contains Mg2+. The primers are designed to correspond to the start and end of the amplified sequence, known as the "template" or "target". If the template DNA is prepared from biological tissue, freshly isolated DNA will give the best amplification results. DNA extracted from older specimens may be degraded and be less suitable for amplification.

The PCR reaction mixture that contains *Taq* polymerase, buffer, the four deoxytriphosphate nucleotides, primers, and template is sequentially subjected to three defined temperatures. The reaction mixture is held at each temperature step for defined amounts of time that usually range from 30 to 60 seconds. In the first step, the reaction mixture is heated to near boiling (94 - 96°C) to denature or "melt" the DNA. This step, known as "denaturation" disrupts the

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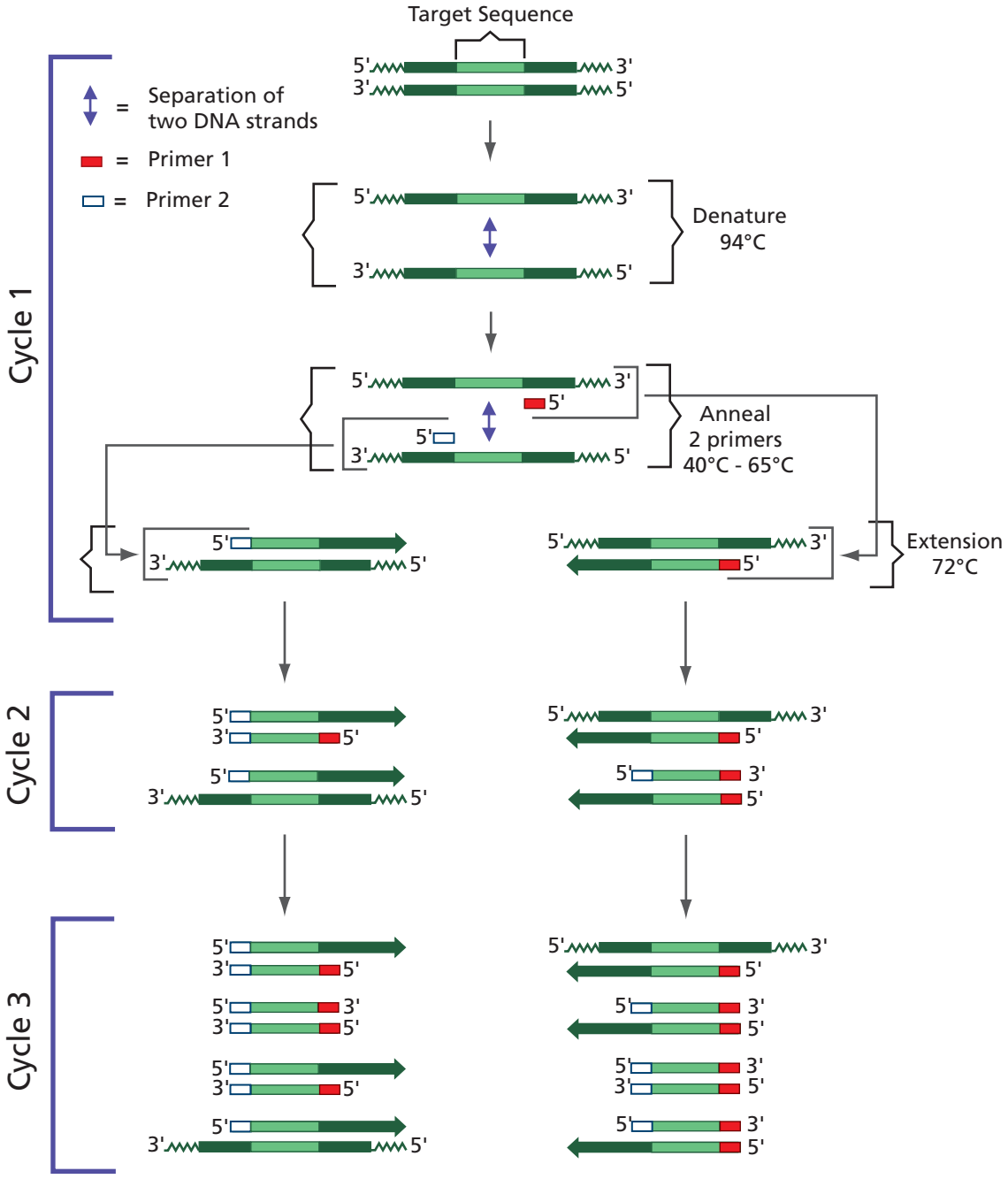
hydrogen bonds between the two complementary DNA strands and causes their separation. In the second PCR step, the mixture is cooled to a temperature that is typically in the range of 45°– 65°. In this step, known as "annealing", the primers, present in great excess of the template, bind to the separated DNA strands. In the third PCR step, known as "extension", the temperature is raised to an intermediate value, usually 72°C. At this temperature the *Taq* polymerase is maximally active and adds nucleotides to the primers to complete the synthesis of the new complementary strands.

The exact temperature and incubation time required at each step depends on several factors, including the length and sequence of the DNA to be amplified the length and GC content of the primers, and the primer/template ratio.

The three PCR steps of denaturation, annealing, and extension constitute one "cycle" and result in a doubling of the number of copies of the template to be amplified. The process is typically repeated for 20-40 cycles. Theoretically, if the reaction is subjected to 30 PCR cycles, the anticipated number of DNA templates copies will be over a million copies. The amplified DNA is then subjected to agarose gel electrophoresis for analysis.

Background Information



Experiment Overview and General Instructions

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. If you will be conducting PCR using a thermal cycler without a heated lid, also read the Appendix entitled "Preparation and Handling PCR Samples with Wax ".
3. If you will be using three waterbaths to conduct PCR, read the two appendices entitled "Polymerase Chain Reaction Using Three Waterbaths" and "Handling samples with wax overlays".
4. Write a hypothesis that reflects the experiment and predict experimental outcomes.

EXPERIMENT OBJECTIVE:

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

BRIEF DESCRIPTION OF EXPERIMENT:

In this experiment, students will identify food that may contain the CaMV 35S promoter region, the NOS terminator, and/or the plant chloroplast gene. Amplification of the PCR products will be performed and analyzed by agarose gel electrophoresis.

Note:
Not all foods will consist of GMO material and may result in negative PCR products. Also, the quality of DNA obtained from processed food will vary widely. Consequently, positive PCR results (bands) are not guaranteed. Foods that have worked well in the EDVOTEK testing laboratory include corn flakes, soy crisps and corn chips.

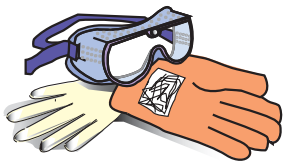
GEL SPECIFICATIONS

This experiment requires a gel with the following specifications:

• Recommended gel size	7 x 14 cm (long tray)
• Number of sample wells required	6
• Placement of well-former template	first set of notches
• Gel concentration required	2.0%

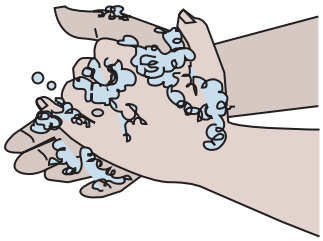
Experiment Overview and General Instructions

LABORATORY SAFETY



Wear gloves
and safety goggles

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.
   - Although electrical current from the power source is automatically disrupted when the cover is removed from the apparatus, first turn off the power, then unplug the power source before disconnecting the leads and removing the cover.
   - Turn off power and unplug the equipment when not in use.
5. EDVOTEK injection-molded electrophoresis units do not have glued junctions that can develop potential leaks. However, in the unlikely event that a leak develops in any electrophoresis apparatus you are using, IMMEDIATELY SHUT OFF POWER. Do not use the apparatus.
6. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.



Module I: Isolation of DNA from Food

1. Weigh 50-100 mg of food and transfer it into a test tube.
2. Add 400 µl of extraction buffer to the tube.
3. Mash the food material well with a micropestle.
4. Incubate the tube at 56°C for at least one hour, or overnight.

OPTIONAL STOPPING POINT - Incubate at 56°C overnight.

5. Add 300 µl of NaCl solution to the tube.
6. Mix well for 30 seconds by vortexing or vigorous tapping with your finger.
7. Centrifuge for 15 - 30 minutes at full speed.
8. Remove the supernatant and transfer it to a fresh tube. Discard the tube with the pellet.
9. Add an equal volume of 100% isopropanol or 91% isopropyl (rubbing) alcohol to the tube containing the supernatant.
10. Incubate the tube in the freezer for at least one hour, or overnight.

OPTIONAL STOPPING POINT - Incubate in the freezer overnight.

11. Spin the tube at full speed for 20 minutes.
12. Using care to avoid disturbing the pellet, completely remove and discard the supernatant from the pellet.
13. Wash the pellet with 1.5 ml of 70% ethanol or isopropanol by slowly adding the alcohol and then removing it.
14. If the pellet becomes dislodged, spin at full speed for two minutes.
15. Remove and discard the alcohol and allow the pellet to dry completely.
16. Dissolve the DNA pellet in 300 µl of 1x TE buffer. Place the DNA sample on ice or in the freezer until sample preparation for amplification.

OPTIONAL STOPPING POINT - Store in the freezer until ready for PCR.

Module II: Amplification of DNA

PREPARE SAMPLES FOR POLYMERASE CHAIN REACTION

The PCR reaction pellet™ contains *Taq* DNA polymerase, the four deoxytriphosphates, Mg^{+2} and buffer.

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

1. Transfer the PCR Reaction pellet™ to the appropriate sized tube (e.g. 0.5 ml or 0.2 ml) for your thermal cycler.
2. Tap the PCR tube to assure the PCR reaction pellet™ is at the bottom of the tube.
3. Label the tube with the sample and with your initials.
4. To prepare the PCR reaction mix, add the following to the pellet:

Food DNA Template for Amplification	5 µl
Primer Mix (two primers)	20 µl

5. Gently mix the reaction tube. Make sure the PCR reaction pellet™ is completely dissolved.
6. If your thermal cycler is equipped with a heated lid, proceed directly to polymerase chain reaction cycling.

   If your thermal cycler does not have a heated lid, or if you are cycling manually with three water baths, add one wax bead to the tube before proceeding to polymerase chain reaction cycling.

Control Reaction:
This kit provides reagents for 21 reactions which includes the control reaction. A control tube "C" (Control) will be assembled to be used for the entire class or per gel. Your instructor will provide that information to the class. To assemble the control reaction, add 5 µl of control DNA Template for Amplification and 20 µl of Primer Mix to the tube containing the pellet. Place this tube in the thermal cycler with the samples from the class.

Module II: Amplification of DNA

POLYMERASE CHAIN REACTION CYCLING

7. Each group should place their tube(s) in the thermal cycler (and the optional control reactions) for automatic cycling as programmed.

Initial Denaturation	50 cycles @	Final Extension
94°C for 10 min.	94°C for 1 min. 63°C for 1 min. 72°C for 1 min.	72°C for 10 min.

8. Once the PCR process is complete, add 5 µl of 10x gel loading solution to each sample. Store on ice until ready for electrophoresis.

9. Proceed to instructions for preparing a 2.0% agarose gel (7 x 14 cm) and separating the PCR products by electrophoresis.

OPTIONAL STOPPING POINT

Samples may be placed in the freezer until ready for agarose gel electrophoresis.

Module III: Separation of PCR Reactions by Agarose Gel Electrophoresis

If you are unfamiliar with agarose gel preparation step by step guidelines are outlined in Appendix F.

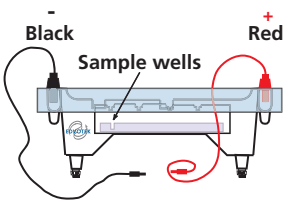
PREPARE THE GEL

1. Prepare a 2.0% agarose gel and is designed for staining with InstaStain® Ethidium Bromide. Refer to Appendix D.
   - Recommended gel size: 7 x 14 cm

     7 x 14 cm gels are recommended to achieve better resolution of the PCR products. Each gel can be shared by several students or groups.
   - Placement of well-former template: first set of notches
   - Agarose gel concentration: 2.0%

Reminder:

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



LOAD DNA SAMPLES

2. (Optional step) Heat the 100 bp DNA ladder and PCR samples for two minutes at 50°C. Allow the samples to cool for a few minutes.
3. Make sure the gel is completely submerged under buffer before loading the samples. Load the DNA ladder in lane 1 of each gel.
4. Load the entire volume (approx. 30 µl) of each PCR sample in consecutive wells.

   Remember to note the wells in which your group's samples are loaded.

RUN THE GEL

5. After the DNA samples are loaded, set the power source at the required voltage and conduct electrophoresis for the length of time specified by your instructor.

STAIN AND VISUALIZE DNA

6. After the electrophoresis is completed, proceed to DNA staining and visualization (see Appendices G, H, or I for staining instructions).

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. How are gene guns used in plant genetics? Discuss an example of the process.
2. What are common potential health concerns about foodstuffs obtained from GM plants?
3. Which Federal agencies are responsible for oversight on GM plants and foodstuffs?
4. How does antisense technology assist in delaying the ripening of fruit and other plant products?
5. How are primers designed to assure that the entire target is amplified by PCR?

Instructor's Guide

Notes to the Instructor

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

NATIONAL CONTENT AND SKILL STANDARDS

By performing this experiment, students will learn to load samples and run agarose gel electrophoresis. Analysis of the experiments will provide students the means to transform an abstract concept into a concrete explanation. Please visit our website for specific content and skill standards for various experiments.

EDUCATIONAL RESOURCES

Electrophoresis Hints, Help and Frequently Asked Questions

EDVOTEK experiments are easy to perform and designed for maximum success in the classroom setting. However, even the most experienced students and teachers occasionally encounter experimental problems or difficulties. The EDVOTEK web site provides several suggestions and reminders for conducting electrophoresis, as well as answers to frequently asked electrophoresis questions.

Visit the EDVOTEK web site often for continuously updated information.

Notes to the Instructor

PCR EXPERIMENTAL SUCCESS GUIDELINES

Please refer to the Appendices section for a summary of important hints and reminders which will help maximize successful implementation of this experiment. This experiment has three modules:

I. Isolation of DNA
II. PCR Amplification of DNA
III. Separation of PCR Reactions by Electrophoresis

MICROPIPETTING BASICS AND PRACTICE GEL LOADING

Accurate pipeting is critical for maximizing successful experiment results. EDVOTEK Series 300 experiments are designed for students who have had previous experience with agarose gel electrophoresis and micropipeting techniques. If your students are unfamiliar with using micropipets, EDVOTEK highly recommends that students perform Experiment # S-44, Micropipetting Basics, or other Series 100 or 200 electrophoresis experiment prior to conducting this advanced level experiment.

APPROXIMATE TIME REQUIREMENTS

1. The PCR step (35 cycles) will take about 150-180 minutes or can be processed overnight and held at 4°C.

2. The experiment can be temporarily stopped after the completion of Module 1 or Module II and later resumed. Experimental results will not be compromised if instructions are followed as noted under the heading "Optional Stopping Point".

Table C
Time and Voltage Guidelines
2% UltraSpec-Agarose Gel

Gel Size (cm) Volume	Volts	Time	Approx. migration distance of tracking dye
7 x 7 25 ml	125 70 50	40 min 2.0 hrs 3.0 hrs	4.5 cm
7 x 14 50 ml	125 70 50	60 min 2.5 hrs 3.5 hrs	6.0 cm

3. Whether you choose to prepare the gel(s) in advance or have the students prepare their own, allow approximately 30-40 minutes for this procedure. Generally, 20 minutes of this time is required for gel solidification. See section "Options for Preparing Agarose Gels" below.

4. The approximate time for electrophoresis will vary from 40 minutes to 3.5 hours. Generally, the higher the voltage applied, the faster the samples migrate. However, depending upon the apparatus configuration and the distance between the two electrodes, individual electrophoresis units will separate DNA at different rates. Follow manufacturer's recommendations. Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C.

Notes to the Instructor

OPTIONS FOR PREPARING AGAROSE GELS

This experiment is designed for DNA staining after electrophoresis with InstaStain® Ethidium Bromide. There are several options for preparing agarose gels for the experiment.

1. Individual Gel Casting:
   Each student lab group can be responsible for casting their own individual gel prior to conducting the experiment.

2. 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 store gels at -20°C. 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 hot, molten agarose before placing the gels into the apparatus for electrophoresis. This will prevent the gels from sliding around in the trays and the chambers.

3. Batch Gel Preparation:
   A batch of agarose gel can be prepared for sharing by the class. To save time, a larger quantity of UltraSpec-Agarose can be prepared for sharing by the class. See instructions for "Batch Gel Preparation".

GEL CONCENTRATION AND VOLUME

The gel concentration required for this experiment is 2.0%. Prepare gels according to Table A.1 or A.2 in Appendix D.

Notes to the Instructor

GEL STAINING AND DESTAINING AFTER ELECTROPHORESIS

After electrophoresis, the agarose gels require staining in order to visualize the separated DNA samples. This experiment features a proprietary stain called InstaStain®.

InstaStain® EtBr (Appendix F)

Optimal visualization of PCR products on gels of 1.0% or higher concentration is obtained by staining with InstaStain® Ethidium Bromide (InstaStain® EtBr) cards. Exercise caution when using Ethidium Bromide, which is a listed mutagen. Disposal of the InstaStain® EtBr cards, which contain only a few micrograms of ethidium bromide, is minimal compared to the large volume of liquid waste generated by traditional ethidium bromide staining procedures. Disposal of InstaStain® cards and gels should follow institutional guidelines for chemical waste.

InstaStain® Blue: One-step Staining and Destaining (Appendix G)

InstaStain® Blue can be used as an alternative for staining gels in this experiment. However, InstaStain® Blue is less sensitive than InstaStain® EtBr and will yield variable results.

Agarose gels can be stained and destained in one easy step, which can be completed in approximately 3 hours, or can be left in liquid overnight. For the best photographic 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.

Gels stained with InstaStain® Blue may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid. DO NOT FREEZE AGAROSE GELS! Used InstaStain® Blue cards and destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed down the drain.

PHOTODOCUMENTATION OF DNA (OPTIONAL)

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.

Pre-Lab Preparations

There is enough material for ten student groups sharing five gels (two groups per gel). There is material to perform one positive control DNA reaction for PCR.

Materials for DNA Isolation
Each student group should receive:

1 ml	DNA extraction buffer
1 ml	NaCl solution
1 ml	TE buffer
2	Microcentrifuge tubes with pestles
4	1.5 ml microcentrifuge tubes with caps

ISOLATION OF DNA
(Components E-H)

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

1. Add 200 µl of DNA Extraction Buffer (H) to each tube of Proteinase K (F) and allow the pellets to hydrate for a couple of minutes. Add the dissolved Proteinase K back to the 10 ml of DNA Extraction Buffer and mix. Aliquot 1 ml for each group and keep tubes on ice.
2. Aliquot 1 ml of NaCl solution (G) for each group.
3. Aliquot 1 ml of TE buffer (E) for each group
4. Place bottles of 95% and 70% Isopropyl alcohol on ice or in the freezer. Chill thoroughly.

Materials for PCR
Each student group should receive:

50 µl	GMO Primer Mix
20 µl	Gel load solution
2	PCR beads
35 µl	100 bp ladder

POLYMERASE CHAIN REACTION
(Components A-D)

Aliquot reagents for PCR.

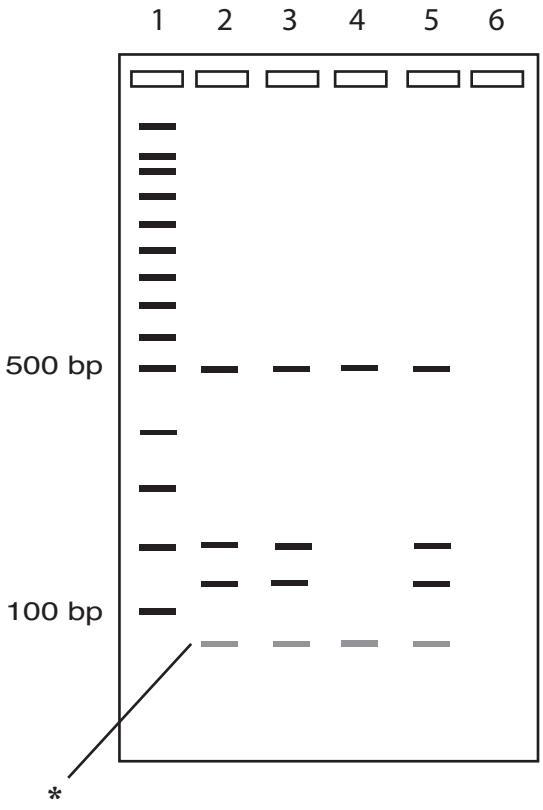
1. GMO Primer Mix (B)
   - Aliquot 50 µl for each group
   - For the group performing the control reaction, aliquot an additional 20 µl.
2. Aliquot 20 µl gel load solution for each group
3. Aliquot 35 µl of 100 bp ladder (C) for each student group.
4. Thaw the positive control sample (D) and dispense 7 µl for the one group performing the control. Place on ice.

Notes and Reminders:

- Accurate temperatures and cycle times are critical. A pre-run for one cycle (approx. 3 to 5 min) is recommended to check that the thermal cycler is properly programmed.
- For thermal cyclers which do not have a top heating plate, it is necessary to place a layer of wax above the PCR reactions in the microcentrifuge tubes to prevent evaporation. See Appendix entitled "Preparation and Handling PCR Samples with Wax ".

Experiment Results and Analysis

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



Lane 1 100 bp ladder

Lane 2 GMO marker

(500 bp plant chloroplast, 200 bp CMV 35S and 127 bp NOS terminator)

Lane 3 GMO food (corn flakes)

(Positive for plant chloroplast, CMV 35S and NOS terminator)

Lane 4 non-GMO food

(Positive for plant chloroplast only)

Lane 5 GMO food (soy chips)

(positive for plant chloroplast, CMV 35S and NOS Terminator)

*** Note:**
Depending on the PCR conditions used, a diffuse, small-molecular weight band, known as a "primer dimer", may be present below the 100 bp marker (shown in the idealized schematic at the bottom of the gel). This is a PCR artifact and can be ignored. Other minor bands may also appear due to nonspecific primer binding and the subsequent amplification of these sequences.

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

Notes

Appendices

A PCR Success Guidelines

B Polymerase Chain Reaction Using Three Waterbaths

C Preparation and Handling of PCR Samples With Wax

D 2.0% Agarose Gel Electrophoresis Reference Tables

E Buffer and Agarose Quantity Preparations

F Agarose Gel Preparation

G Staining and Visualization of DNA with InstaStain® Ethidium Bromide Cards

H Staining and Visualization of DNA - FlashBlue™ Liquid Stain

I InstaStain® Blue: One Step Staining and Destaining

J Electrophoresis Hints and Help

Material Safety Data Sheets

Appendix
A

PCR Experimental Success Guidelines

EDVOTEK experiments which involve the amplification of DNA are extremely relevant, exciting and stimulating classroom laboratory activities. These experiments have been performed successfully in many classrooms across the country, but do require careful execution because of the small volumes used. The following guidelines offer some important suggestions, reminders and hints for maximizing success.

THE PCR REACTION

1. **Add Primers and DNA to the PCR Reaction Bead:** Add the primer mixture (forward and reverse primers) and the cell DNA (supernatant) as specified in the experimental procedures to the microcentrifuge tube containing the PCR reaction bead. Make sure that the bead (which contains the *Taq* DNA polymerase, the 4XdTPs, Mg and the PCR reaction buffer) is completely dissolved. Do a quick spin in a microcentrifuge to bring the entire sample to the bottom of the tube. Prepare the control reaction similarly.

2. **The Thermal cycler:** It is critical that the thermal cycler be accurately programmed for the correct cycle sequence, temperatures and the time for each of the cycles.

3. **Oil or Wax:** For thermal cyclers that do not have a top heating plate, the reaction in the tubes must be overlaid with oil or wax to prevent evaporation.

4. **Manual Water Bath PCR:** Three water baths can be used as an alternative to a thermal cycler for PCR, but results are more variable. Samples require oil or wax layers. This method requires extra care and patience.

GEL PREPARATION AND STAINING

5. **Concentrated agarose:** Gels of higher concentration (> 0.8%) require special attention when dissolving or re-melting. Make sure that the solution is completely clear of "clumps" or glassy granules. Distorted electrophoresis DNA band patterns will result if the gel is not properly prepared.

6. **Electrophoretic separation:** The tracking dye should travel at least 4 cm from the wells for adequate separation before staining.

7. **Staining:** Staining of higher concentration gels (> 0.8%) require special care to obtain clear, visible results. After staining (15 to 30 min.) with InstaStain® Ethidium Bromide, examine the results using a UV (300 nm) transilluminator. Repeat the staining as required.

8. **DNA ladders or markers:** After staining the agarose gel, the DNA 100 bp ladder, 200 bp ladder, or Standard DNA Markers should be visible. If bands are visible in the ladder or marker and control lanes, but bands in the sample lanes are faint or absent, it is possible that DNA was not successfully extracted from the cells. If the ladder or marker, control and DNA bands are all faint or absent, potential problems could include improper gel preparation, absence of buffer in the gel, improper gel staining or a dysfunctional electrophoresis unit or power source.

Polymerase Chain Reaction Using Three Waterbaths

Appendix
B

Superior PCR results are obtained using an automated thermal cycler. However, if you do not have a thermal cycler, this experiment can be adapted to use three waterbaths (Cat. # 544). Much more care needs to be taken when using the three-waterbath PCR method. The PCR incubation sample is small and can easily be evaporated. Results using three waterbaths are often variable. **Please refer to the Appendix entitled "PCR Samples with Wax Overlays" for sample handling and preparation tips.**

PREPARATION OF THE PCR REACTION:

Each PCR Reaction pellet contains *Taq* DNA polymerase, four deoxytriphosphates, Mg^{+2} and buffer.

1. The PCR reaction sample should be prepared as specified in the experiment instructions. Each PCR reaction sample contains three critical components:
   - PCR Reaction pellet™
   - Primer mix
   - DNA for amplification

2. After adding the components of the PCR reaction sample, use clean forceps to transfer one wax bead to the PCR tube. At the start of the PCR reaction, the wax will melt and overlay the samples to prevent evaporation during heating.

POLYMERASE CHAIN REACTION CYCLING

3. In the three-waterbath PCR method, the PCR reaction sample is sequentially cycled between three separate waterbaths, each set at different temperatures, for a specified period of time. The sequential placement of the reaction sample in the waterbaths maintained at three different temperatures constitutes one PCR cycle. One example of a PCR cycle might be as follows:

   94°C for 1 minute
   50°C for 1 minute
   72°C for 1 minute

   See experiment instructions for specific program requirements.

Important Note

It is imperative that temperatures are accurately maintained throughout the experiment.

The PCR tube must be handled carefully when sequentially cycled between the three waterbaths. For each cycle:

- Carefully place the PCR tube in a waterbath float. Make sure that the sample volume is at the bottom of the tube and remains undisturbed. If necessary, pulse spin the tube in a balanced microcentrifuge, or shake the tube to get all of the sample to the bottom of the tube.
- Use forceps to carefully lower the waterbath float (with tubes) sequentially into the waterbaths.

5. Process the PCR reaction sample for the total number of cycles specified in the experiment instructions. On the final cycle the 72°C incubation can be extended to 5 minutes.

6. After all the cycles are completed, the PCR sample is prepared for electrophoresis.

Appendix
C

Preparation and Handling of PCR Samples With Wax

For Thermal Cyclers without Heated Lids, or PCR Using Three Waterbaths

Automated thermal cyclers with heated lids are designed to surround the entire sample tube at the appropriate temperature during PCR cycles. Heating the top of the tubes during these cycles prevents the very small sample volumes from evaporating. For thermal cyclers without heated lids, or when conducting PCR by the three-waterbath method, it is necessary to add a wax bead to the reaction sample. During the PCR process, the wax will melt and overlay the samples to prevent evaporation during heating.

Each PCR Reaction pellet contains *Taq* DNA polymerase, four deoxytriphosphates, Mg^{+2} and buffer.

PREPARING THE PCR REACTION:

1. The PCR reaction sample should be prepared as specified in the experiment instructions. Each PCR reaction sample contains the following three critical components:
   - PCR Reaction pellet™
   - Primer mix
   - DNA for amplification
2. After adding the components of the PCR reaction sample, use clean forceps to transfer one wax bead to the PCR tube.
3. Process the PCR reaction sample for the total number of cycles specified in the experiment instructions.

PREPARING THE PCR REACTION FOR ELECTROPHPORESIS:

4. After the cycles are completed, transfer the PCR tube to a rack and prepare the PCR sample for electrophoresis.
   - Place the PCR tube in a 94°C waterbath long enough to melt the wax overlay. Use a clean pipet to remove most of the melted wax overlay.
   - Allow a thin layer of the wax to solidify.
   - Use a clean pipet tip to gently poke a hole through the solidified wax. Remove the tip.
   - Use another clean pipet tip to enter the hole to remove the volume of mixture specified in the experiment instructions. Transfer this volume to a clean tube.
   - Add other reagents according to experiment instructions, if applicable,.
   - Add 5 µl of 10x Gel Loading solution to the sample and store on ice.
5. Proceed to delivery of the sample onto an agarose gel for electrophoresis as specified in the experiment instructions.

2.0% Agarose Gel Electrophoresis Reference Tables

Appendix
D

If preparing a 2.0% gel with concentrated (50x) buffer, use Table A.7.

If preparing a 2.0% gel with diluted (1x) buffer, use Table A.8.

Table A.7
Individual 2.0% UltraSpec-Agarose™ Gel

Size of Gel (cm)	Amt of Agarose (g)	+	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
7 x 7	0.5		0.5		24.5		25
7 x 14	1.0		1.0		49.0		50

Table A.8
Individual 2.0% UltraSpec-Agarose™ Gel

Size of Gel (cm)	Amt of Agarose (g)	+	Diluted Buffer (1x) (ml)
7 x 7	0.5		25
7 x 14	1.0		50

For DNA analysis, the recommended electrophoresis buffer is Tris-acetate-EDTA, pH 7.8. The formula for diluting EDVOTEK (50x) concentrated buffer is one volume of buffer concentrate to every 49 volumes of distilled or deionized water. Prepare buffer as required for your electrophoresis unit.

Table B
Electrophoresis (Chamber) Buffer

EDVOTEK Model #	Total Volume Required (ml)	Dilution: 50x Conc. Buffer (ml)	+	Distilled Water (ml)
M6+	300	6		294
M12	400	8		392
M36	1000	20		980

Table C.3
Time and Voltage Guidelines (2.0% Gel)

Gel Size (cm) Volume	Volts	Time	Approx. migration distance of tracking dye
7 x 7 25 ml	125 70 50	40 min 2.0 hrs 3.0 hrs	4.5 cm
7 x 14 50 ml	125 70 50	60 min 2.5 hrs 3.5 hrs	6.0 cm

Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C.3 for 2.0% agarose gels. The time for electrophoresis will vary from approximately 15 minutes to 2 hours depending upon various factors. Conduct the electrophoresis for the length of time determined by your instructor.

Appendix
E

Buffer and Agarose Quantity Preparations

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

Table D
Bulk Preparation of Electrophoresis Buffer

Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
60		2,940		3000 (3 L)

BULK ELECTROPHORESIS BUFFER

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

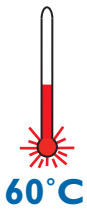
Table E.3
Batch Preparation of 2.0% UltraSpec-Agarose™

Amt of Agarose (g)	+	Concentrated Buffer (50X) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
8.0		8.0		392		400

PREPARING AGAROSE GELS BY BATCH

For quantity (batch) preparation of agarose gel solution, refer to Table E.3.

1. Use a 500 ml Pyrex flask or beaker to prepare the diluted gel buffer
2. Pour the appropriate amount 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 in the same manner as described for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.
6. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.



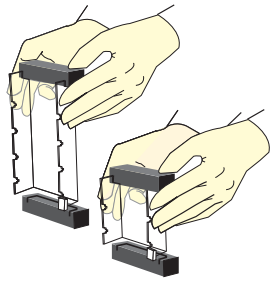
Agarose Gel Preparation
Step by Step Guidelines

Appendix
F

Preparing the Gel bed

EDVOTEK electrophoresis units include 7 x 7 cm or 7 x 14 cm gel casting trays.

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.



A. Using Rubber dams:

- Place a rubber dam on each end of the bed. Make sure the rubber dam fits firmly in contact with the sides and bottom of the bed.

B. Taping with labeling or masking tape:

- Extend 3/4 inch wide tape over the sides and bottom edge of the bed.
- Fold the extended tape edges back onto the sides and bottom. Press contact points firmly to form a good seal.

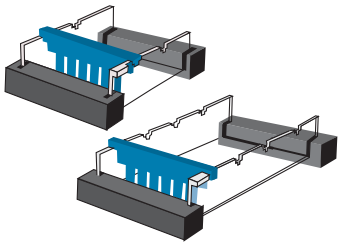
If gel trays and rubber end caps are new, they may be somewhat difficult to assemble. Here is a helpful hint:

Place one of the black end caps with the wide "u" shaped slot facing up on the lab bench.

Push one of the corners of the gel tray into one of the ends of the black cap. Press down on the tray at an angle, working from one end to the other until the end of the tray completely fits into the black cap. Repeat the process with the other end of the gel tray and the other black end cap.

At high altitudes, it is recommended to use a microwave oven to reach boiling temperatures.

2. Place a well-former template (comb) in the first set of notches at the end of the bed. Make sure the comb sits firmly and evenly across the bed.



Casting Agarose Gels

3. Use a 250 ml flask or beaker to prepare the gel solution.

4. Refer to the appropriate Reference Table (i.e. 0.8%, 1.0% or 2.0%) for agarose gel preparation. Add the specified amount of agarose powder and buffer. Swirl the mixture to disperse clumps of agarose powder.

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

6. Heat the mixture to dissolve the agarose powder.

   A. Microwave method:

   - Cover the flask with plastic wrap to minimize evaporation.
   - Heat the mixture on High for 1 minute.
   - Swirl the mixture and heat on High in bursts of 25 seconds until all the agarose is completely dissolved.

   B. Hot plate method:

   - Cover the flask with aluminum foil to minimize evaporation.
   - Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.

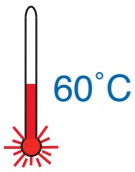
Continue heating until the final solution appears clear (like water) without any undissolved particles. Check the solution carefully. If you see "crystal" particles, the agarose is not completely dissolved.

Appendix
F

Agarose Gel Preparation
Step by Step Guidelines, continued

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

DO NOT POUR BOILING HOT AGAROSE INTO THE GEL BED.

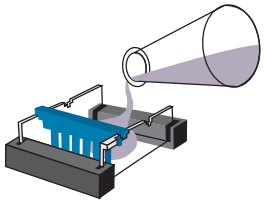


Hot agarose solution may irreversibly warp the bed.

After the gel is cooled to 60°C:

- **If you are using rubber dams, go to step 9.**
- **If you are using tape, continue with step 8.**

8. Seal the interface of the gel bed and tape to prevent agarose solution from leaking.
   - Use a transfer pipet to deposit a small amount of the cooled agarose to both inside ends of the bed.
   - Wait approximately 1 minute for the agarose to solidify.

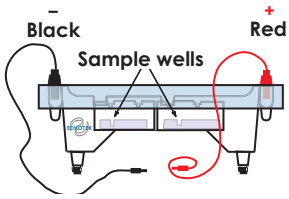


9. Place the bed on a level surface and pour the cooled agarose solution into the bed.

10. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

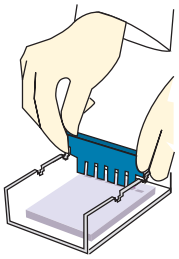
Preparing the gel for electrophoresis

11. After the gel is completely solidified, carefully and slowly remove the rubber dams or tape from the gel bed. Be especially careful not to damage or tear the gel wells when removing the rubber dams. A thin plastic knife, spatula or pipet tip can be inserted between the gel and the dams to break possible surface tension.



During electrophoresis, the DNA samples migrate through the agarose gel towards the positive electrode.

12. Remove the comb by slowly pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.



13. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.

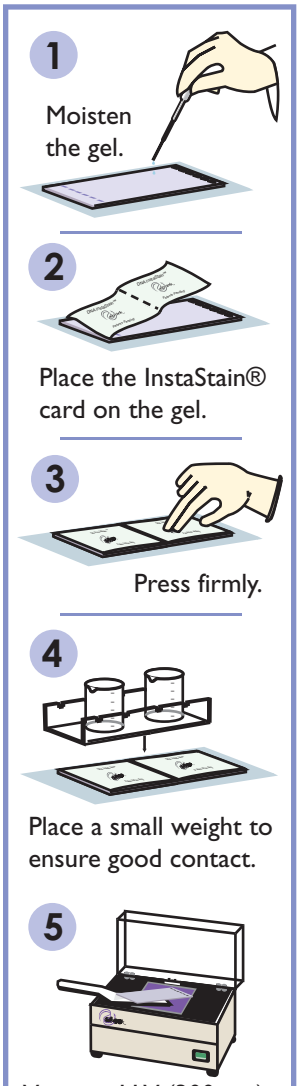
14. Fill the electrophoresis apparatus chamber with the appropriate amount of diluted (1x) electrophoresis buffer (refer to Table B on the Appendix page provided by your instructor).

15. Make sure that the gel is completely submerged under buffer before proceeding to loading the samples and conducting electrophoresis.

Do not stain gel(s) in the electrophoresis apparatus.

Staining and Visualization of DNA InstaStain® Ethidium Bromide Cards

Appendix
G



1. After electrophoresis, place the gel on a piece of plastic wrap on a flat surface. Moisten the gel with a few drops of electrophoresis buffer.

2. If staining a 7 x 14 cm gel, use two 7 x 7 cm InstaStain® EtBr cards. If staining a 7 x 7 cm gel, use one card.

   Wearing gloves, remove the clear plastic protective sheet from the InstaStain® EtBr card(s).

   Place the unprinted side of the InstaStain® EtBr card(s) on the gel.

Wear Gloves and UV Safety Goggles

3. Firmly run your fingers over the entire surface of the InstaStain® EtBr. Do this several times.

4. Place the gel casting tray and a small empty beaker on top to ensure that the InstaStain® card maintains direct contact with the gel surface.

   Allow the InstaStain® EtBr card to stain the gel for 10-15 minutes.

5. After 10-15 minutes, remove the InstaStain® EtBr card. Transfer the gel to a ultraviolet (300 nm) transilluminator for viewing. Be sure to wear UV protective goggles.

Visit our web site for an animated demonstration of InstaStain® EtBr.

www.edvotek.com

Caution: Ethidium Bromide is a listed mutagen.

Disposal of InstaStain

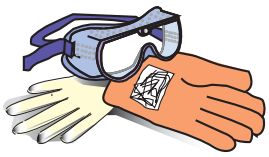
Disposal of InstaStain® cards and gels should follow institutional guidelines for chemical waste.

Additional Notes About Staining

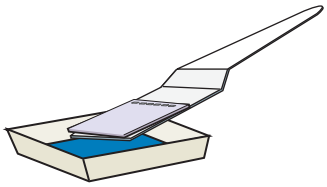
- If bands appear faint, or if you are not using EDVOTEK UltraSpec-Agarose™, gels may take longer to stain with InstaStain® EtBr. Repeat staining and increase the staining time an additional 10-15 minutes.
- Markers (Standard DNA Fragments, DNA 100 bp ladder or DNA 200 bp ladder) should be visible after staining even if the DNA samples are faint or absent. If markers are not visible, troubleshoot for problems with the electrophoretic separation.

Appendix
H

Staining and Visualization of DNA
FlashBlue™ Liquid Stain



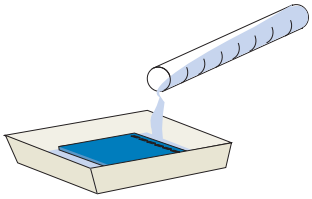
Do not stain gel(s) in the electrophoresis apparatus.



Staining and Destaining

1. Remove the agarose gel from its bed and completely submerse the gel in a small, clean tray containing 75 ml of 1x FlashBlue™ stain. Add additional stain if needed to completely submerge the gel.
2. Stain the gel for no more than 5 minutes.
3. Transfer the gel to another small tray and fill it with 250 - 300 ml of distilled water.
4. Gently agitate the tray every few minutes. Alternatively, place it on a shaking platform.
5. Destain the gel for 20 minutes.

   Dark blue bands will become visible against a light blue background. Additional destaining may yield optimal results.



6. Carefully remove the gel from the destaining liquid and examine the gel on a Visible Light Gel Visualization System. To optimize visibility, use the amber filter provided with EDVOTEK equipment.
7. If the gel is too light and bands are difficult to see, repeat the staining and destaining procedures.

Storage and Disposal of stain and gel

- Gels stained with FlashBlue™ may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid.

  DO NOT FREEZE AGAROSE GELS.
- Stained gels which are not kept can be discarded in solid waste disposal. FlashBlue™ stain and destaining solutions can be disposed down the drain.

Appendix
I

Staining and Visualization of DNA

InstaStain® Blue
One-step Staining and destaining

Do not stain gel(s) in the electrophoresis apparatus.

Agarose gels can be stained and destained in one easy step with InstaStain® Blue cards. This one-step method can be completed in approximately 3 hours, or can be left overnight.

1. Remove the 7 x 7 cm agarose gel from its bed and completely submerse the gel in a small, clean tray containing 75 ml of distilled or deionized water, or used electrophoresis buffer. The agarose gel should be completely covered with liquid.

   Examples of small trays include large weigh boats, or small plastic food containers

2. Gently float a 7 x 7 cm card of InstaStain® Blue with the stain side (blue) facing the liquid.

   Note: If staining a 7 x 14 cm gel, use two InstaStain® Blue cards.

3. Let the gel soak undisturbed in the liquid for approximately 3 hours. The gel can be left in the liquid overnight (cover with plastic wrap to prevent evaporation).

4. After staining and destaining, the gel is ready for visualization and photography.

Storage and Disposal of InstaStain® Blue Cards and Gels

- Stained gels may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid.

  DO NOT FREEZE AGAROSE GELS!

- Used InstaStain® cards and destained gels can be discarded in solid waste disposal.

- Destaining solutions can be disposed down the drain.

Appendix
J

Agarose Gel Electrophoresis Hints and Help

TO MAXIMIZE SUCCESS:

1. The approximate time for electrophoresis will vary from experiment to experiment. A variety of factors, including gel concentration, will influence electrophoresis time. Generally, the higher the voltage applied, the faster the samples migrate. However, depending upon the apparatus configuration and the distance between the two electrodes, individual electrophoresis units will separate DNA at different rates.

2. Do not move the apparatus after the samples have been loaded.

   - Moving the apparatus will dislodge the samples from the wells into the buffer and will compromise results.

   - If it is absolutely necessary to move the apparatus during electrophoresis, you may safely do so after the tracking dye has migrated at least 1 cm from the wells into the gel.

3. For optimal DNA fragment separation, do not use voltages higher than 125 volts for agarose gel electrophoresis. Higher voltages can overheat and melt the gel.

4. The DNA samples contain tracking dye, which moves through the gel ahead of most DNA (except extremely small fragments). Migration of the tracking dye will become clearly visible in the gel after approximately 10-15 minutes.

5. If DNA fragments are similar in size, fragments will migrate close to one another.

   - In general, longer electrophoretic runs will increase the separation between fragments of similar size.

   - Experiments which involve measurement of fragment molecular size or weight should be run at the recommended optimal time to ensure adequate separation.

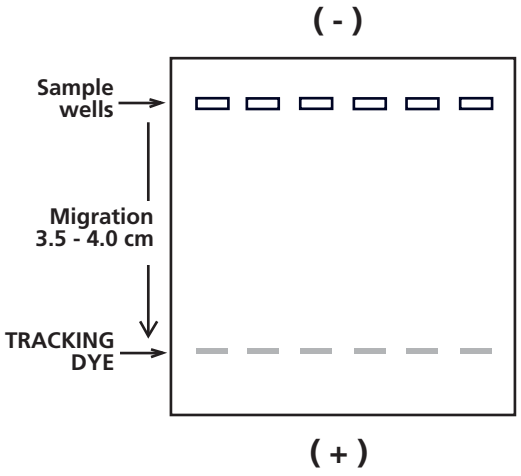


Figure is not drawn to scale.

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9:00 am to 6:00 pm ET

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Web: www.edvotek.com
email: info@edvotek.com

Please have the following information ready:

- **Experiment number and title**
- **Kit lot number on box or tube**
- **Literature version number (in lower right corner)**
- **Approximate purchase date**

Agarose Gel Electrophoresis Hints and Help continued

Appendix
J

6. Electrophoresis should be terminated when the tracking dye has moved a minimum of 3.5 to 4 centimeters from the wells for 7 x 7 cm gels, or 5-8 centimeters for 7 x 14 cm gels. Terminate the electrophoresis before the tracking dye moves off the end of the gel.
    - For optimal results, stain the gel immediately after electrophoresis.
    - For convenience, the power source can be connected to a household automatic light timer to terminate the electrophoretic separation and avoid running samples off the end of the gel.

AVOIDING COMMON PROBLEMS

To avoid potential problems, some suggestions and reminders are listed below.

7. Use only distilled or deionized water to prepare buffers and gels. Do not use tap water.

8. To ensure that DNA bands are well resolved, make sure the gel formulation is correct and that electrophoresis is conducted for the recommended optimal amount of time.

9. Correctly dilute the concentrated buffer for preparation of both the gel and electrophoresis (chamber) buffer. Remember that without buffer in the gel, there will be no DNA mobility. Check that the gel is completely submerged under buffer during electrophoresis.

10. For optimal results, use fresh electrophoresis buffer prepared according to instructions.

11. Before performing the actual experiment, practice sample delivery techniques to avoid diluting the sample with buffer during gel loading.

12. To avoid loss of DNA fragments into the buffer, make sure the gel is properly oriented so the samples are electrophoresed from the negative electrode (cathode) towards the positive electrode (anode).

13. To avoid obtaining gel results that are missing small DNA fragments (small fragments move faster), remember that the tracking dye in the sample moves through the gel ahead of the smallest DNA fragments. Terminate the electrophoresis before the tracking dye moves off the end of the gel.

14. If DNA bands appear faint after staining and destaining, repeat the procedure. Staining for a longer period of time will not harm the gel. Re-stained gels may require longer destaining.

CARE AND MAINTENANCE OF THE ELECTROPHORESIS APPARATUS

15. The temperature of the melted agarose which is poured into the bed during gel casting should not exceed 60°C. Hot agarose solution may irreversibly warp the casting tray.

16. Avoid touching the fragile platinum electrodes.

17. Power should always be turned off and leads disconnected from the power source when the cover is removed from the apparatus.

18. To clean the apparatus chamber, gel casting tray and combs, rinse thoroughly with tap water. Give the items a final rinse with distilled water. Let them air dry. Do not use detergents of any kind, or expose the apparatus to alcohols.

19. EDVOTEK injection-molded electrophoresis units do not have glued junctions that can develop potential leaks. In the unlikely event that a leak develops in any electrophoresis apparatus you are using, IMMEDIATELY SHUT OFF POWER. Do not use the apparatus.

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): InstaStain® Blue, FlashBlue™

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 03-26-09

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Methylene Blue				
3.7 Bis (Dimethylamino) Phenothiazin 5 IUM Chloride	No data available			
CAS # 61-73-4				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble - cold

Appearance and Odor: Chemical bound to paper, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data available	No data		No data

Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam

Special Fire Fighting Procedures: Self contained breathing apparatus and protective clothing to prevent contact with skin and eyes

Unusual Fire and Explosion Hazards: Emits toxid fumes under fire conditions

Section V - Reactivity Data

Stability		Conditions to Avoid
Unstable		
Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Toxic fumes of Carbon monoxide, Carbon dioxide, nitrogen oxides, sulfur oxides, hydrogen, chloride gas

Hazardous Polymerization		Conditions to Avoid
May Occur		
Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Skin: May cause skin irritation Eyes: May cause eye irritation Inhalation: Cyanosis

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Meets criteria for proposed OSHA medical records rule PEREAC 47.30420.82

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Ventilate area and wash spill site

Waste Disposal Method: Mix material with a combustible solvent and burn in chemical incinerator equipped with afterburner and scrubber. Check local and state regulations.

Precautions to be Taken in Handling and Storing: Keep tightly closed. Store in cool, dry place

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): MIOSH/OSHA approved, SCBA

Ventilation			
Local Exhaust		Special	
Mechanical (General)	Required	Other	

Protective Gloves: Rubber Eye Protection: Chem. safety goggles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices:

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): **50x Electrophoresis Buffer**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Appreciable, (greater than 10%)

Appearance and Odor: Clear, liquid, slight vinegar odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		N.D.	N.D.

Extinguishing Media: Use extinguishing media appropriate for surrounding fire.

Special Fire Fighting Procedures: Wear protective equipment and SCBA with full facepiece operated in positive pressure mode.

Unusual Fire and Explosion Hazards: None identified

Section V - Reactivity Data

Stability		Conditions to Avoid
Unstable		
Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide

Hazardous Polymerization		Conditions to Avoid
May Occur		
Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): None

Carcinogenicity: None identified NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Irritation to upper respiratory tract, skin, eyes

Medical Conditions Generally Aggravated by Exposure: None

Emergency First Aid Procedures: Ingestion: If conscious, give large amounts of water

Eyes: Flush with water Inhalation: Move to fresh air Skin: Wash with soap and water

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear suitable protective clothing. Mop up spill and rinse with water, or collect in absorptive material and dispose of the absorptive material.

Waste Disposal Method: Dispose in accordance with all applicable federal, state, and local enviromental regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation			
Local Exhaust	Yes	Special	None
Mechanical (General)	Yes	Other	None

Protective Gloves: Yes Eye Protection: Safety goggles

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: None

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Agarose

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				
CAS #9012-36-6				

Section III - Physical/Chemical Characteristics

Boiling Point For 1% solution 194 F		Specific Gravity (H 0 = 1) 2	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Insoluble - cold

Appearance and Odor: White powder, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		N.D	N.D.

Extinguishing Media: Water spray, dry chemical, carbon dioxide, halon or standard foam

Special Fire Fighting Procedures: Possible fire hazard when exposed to heat or flame

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability		Conditions to Avoid
Unstable		
Stable	X	None

Incompatibility: No data available

Hazardous Decomposition or Byproducts:

Hazardous Polymerization		Conditions to Avoid
May Occur		
Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic)

Inhalation: No data available Ingestion: Large amounts may cause diarrhea

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Sweep up and place in suitable container for disposal

Waste Disposal Method: Normal solid waste disposal

Precautions to be Taken in Handling and Storing: None

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full facepiece.

Ventilation			
Local Exhaust		Special	
Mechanical	Gen. dilution ventilation	Other	

Protective Gloves: Yes Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Impervious clothing to prevent skin contact

Work/Hygienic Practices: None

Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): **Extraction Buffer**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 10/10/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
5144-89-8				
60-00-4				
57-09-0				

Section III - Physical/Chemical Characteristics

Boiling Point	100°C	Specific Gravity (H_20 = 1)	1.020
Vapor Pressure (mm Hg.)	No data	Melting Point	100°C
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Yes

Appearance and Odor: Clear liquid

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)		Flammable Limits	LEL / UEL

Extinguishing Media: Water spray

Special Fire Fighting Procedures: Wear SCBA and protective clothing to prevent contact w/ skin and eyes.

Unusual Fire and Explosion Hazards: Emits toxic fumes under fire conditions

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	

Incompatibility: Strong oxidizing agents, protect from moisture

Hazardous Decomposition or Byproducts: Carbon monoxide, carbon dioxide, nitrogen oxides, hydrogen bromide gas

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Harmful if swallowed, inhaled, or absorbed through skin. Material is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes and skin.

Carcinogenicity: NTP? IARC Monographs? N/A OSHA Regulation?

Signs and Symptoms of Exposure

Medical Conditions Generally Aggravated by Exposure

Emergency First Aid Procedures: Immediately flush eyes or skin w/ copious amounts of water for at least 15 minutes while removing

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Evacuate area. Cover with dry lime or soda ash-pick up. keep in a closed container and hold for waste disposal.

Waste Disposal Method: Dissolve or mix the material w/ combustible solvent and burn in a chemical incinerator equipped with an afterburner. Ventilate area and wash spill site after material pick up is complete

Precautions to be Taken in Handling and Storing: Observe all federal, state, and local laws.

Other Precautions: Use only in a chemical fume hood.

Section VIII - Control Measures

Respiratory Protection (Specify Type): Wear appropriate NIOSH/MSHA aprroved respirator.

Ventilation	Local Exhaust	Special	
	Mechanical (General)	Other	
Protective Gloves	safety gloves	Eye Protection	safety goggles

Other Protective Clothing or Equipment: Safety shower and eye bath, face shield

Work/Hygienic Practices: Wash thoroughly after handling, keep tightly closed.

Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): **Practice Gel Loading Solution**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	No data	Flammable Limits	LEL: No data / UEL: No data

Extinguishing Media: Dry chemical, carbon dioxide, water spray or foam

Special Fire Fighting Procedures: Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.

Unusual Fire and Explosion Hazards: Unknown

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: None

Hazardous Decomposition or Byproducts: Sulfur oxides, and bromides

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Acute eye contact: May cause irritation. No data available for other routes.

Carcinogenicity: No data available NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Treat symptomatically and supportively. Rinse contacted area with copious amounts of water.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear eye and skin protection and mop spill area. Rinse with water.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation	Local Exhaust	Yes	Special	None	
	Mechanical (General)	Yes	Other	None	
Protective Gloves	Yes	Eye Protection	Splash proof goggles		

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Avoid eye and skin contact

Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): **InstaStain® Ethidium Bromide**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **InstaStain, Inc. P.O. Box 1232 West Bethesda, MD 20827**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Ethidium Bromide		Data not available		
(2,7-Diamino-10-Ethyl-9-Phenylphenanthridinium Bromide)				
CAS# 139-33-3				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Chemical bound to paper, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	No data	Flammable Limits	LEL: N.D. / UEL: N.D.

Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam

Special Fire Fighting Procedures: Wear protective clothing and SCBA to prevent contact with skin & eyes

Unusual Fire and Explosion Hazards: Emits toxic fumes

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide, nitrogen oxides, hydrogen bromide gas

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Chronic: May alter genetic material. Acute: Material irritating to mucous membranes, upper respiratory tract, eyes, skin

Carcinogenicity: No data available NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Irritation to mucous membranes and upper respiratory tract

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear SCBA, rubber boots, rubber gloves

Waste Disposal Method: Mix material with combustible solvent and burn in a chemical incinerator equipped afterburner and scrubber

Precautions to be Taken in Handling and Storing: Use in chemical fume hood with proper protective lab gear.

Other Precautions: Mutagen

Section VIII - Control Measures

Respiratory Protection (Specify Type): SCBA

Ventilation	Local Exhaust	Yes	Special	Chem. fume hood	
	Mechanical (General)	No	Other	None	
Protective Gloves	Rubber	Eye Protection	Chem. safety goggles		

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices: Use in chemical fume hood with proper protective lab gear.

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Tris-EDTA Buffer (TE)

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 10/10/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
CAS # 139-33-3	---------- No data ----------			

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Clear, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data			

Extinguishing Media: Dry chemical, carbon dioxide, halon, water spray or standard foam

Special Fire Fighting Procedures: Move container from fire area if possible

Unusual Fire and Explosion Hazards: Thermal decomposition products may include toxic and hazardous oxides of carbon, nitrogen, and sodium.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	Excessive heat, sparks or open flame

Incompatibility: Acids, aluminum, metals, oxidizers (strong)

Hazardous Decomposition or Byproducts: Thermal decomposition products of toxic and hazardous oxides of C, N, & Na

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes

Health Hazards (Acute and Chronic): Moderately toxic by ingestion. Systemic toxicity may result. May chelate lead magnesium, zinc, trace metals if present in intestine poss. causing incr.absorption.

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No data	No data	No data

Signs and Symptoms of Exposure: Mucous membrane irritation, eye/skin irritation, irritating to gastrointestinal system.

Medical Conditions Generally Aggravated by Exposure: Renal or heart disease, potassium deficiency, insulin dependent, diabetes, seizures or intracranial lesions.

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Mop up with absorptive material. Containerize to dispose or properly

Waste Disposal Method: Observe federal, state, and local laws.

Precautions to be Taken in Handling and Storing: Stores away from strong oxidizers or heat. Avoid skin/eye contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full facepiece and organic vapor cartridge

Ventilation			Special	
	Local Exhaust	Yes	Special	None
	Mechanical (General)	Vent. Sys.	Other	None

Protective Gloves		Eye Protection	
	Yes		Splash proof goggles

Other Protective Clothing or Equipment: Impervious clothing to prevent skin contact

Work/Hygienic Practices: Emergency eye wash should be available

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Sodium Chloride

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 10/10/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
CAS # 7647-14-5	No Data			

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	2.165
Vapor Pressure (mm Hg.)	No data	Melting Point	801°C
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Clear liquid

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
	-------Non-combustible--------		

Extinguishing Media: Use extinguishing media appropriate to surrounding fire conditions.

Special Fire Fighting Procedures: None

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	

Incompatibility: Strong oxidizing agents, strong acids

Hazardous Decomposition or Byproducts:

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes

Health Hazards (Acute and Chronic):

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
	---------- No data ----------		

Signs and Symptoms of Exposure: Irritation

Medical Conditions Generally Aggravated by Exposure: Unknown

Emergency First Aid Procedures: Inhaled: Move to fresh air Eyes/Skin: Flush with water for 15 minutes
Ingestion: Wash mouth with water and call physician

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Place in bag. Ventilate area and wash spill site

Waste Disposal Method: Observe federal, state, and local regulations

Precautions to be Taken in Handling and Storing: Avoid skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation			Special	
	Local Exhaust		Special	
	Mechanical (General)	Yes	Other	

Protective Gloves	Rubber	Eye Protection	Chemical safety

Other Protective Clothing or Equipment: Wear NIOSH/MSHA respirator

Work/Hygienic Practices: Do not ingest. Avoid contact with skin, eyes and clothing.

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Gel loading solution concentrate, 10x

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 10/10/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	N/A
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: soluble

Appearance and Odor: Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data	No data	No data	

Extinguishing Media: Dry chemical, carbon dioxide, water spray or foam

Special Fire Fighting Procedures: Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.

Unusual Fire and Explosion Hazards: Unknown

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: None known

Hazardous Decomposition or Byproducts: Sulfur oxides and bromides

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes

Health Hazards (Acute and Chronic)
Acute eye contact: May cause irritation No data available for other routes

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No data	No data	No data

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Treat symptomatically and supportively
Rinse contacted area with copious amounts of water.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Rinse contacted area with copious amounts of water.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with organic vapor cartridge.

Ventilation			Special	
	Local Exhaust	Yes	Special	Yes
	Mechanical (General)	Yes	Other	None

Protective Gloves	yes	Eye Protection	Splash proof goggles

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Do not ingest. Avoid contact with skin, eyes and clothing. Wash thoroughly after handling.