EDVOTEK®

The Biotechnology Education Company ®

EDVO-Kit #
336

Determining QuickPlant™ Genetics Using PCR

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

The object of this experiment is to introduce students to the concept of genetic linkage by using the polymerase chain reaction to amplify DNA from wild type and mutant *Arabidopsis* plants.

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

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EVT 2010-04-19

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Components & Requirements

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.	Primer mix	-20°C Freezer
C.	200 base pair ladder	-20°C Freezer
D.	UltraPure H_2O	-20°C Freezer
E.	Tris 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)

- Wild type and glabra *Arabidopsis* seeds
- Potting soil pellets
- Plant homogenization pestles with tubes
- UltraSpec-Agarose™
- Electrophoresis Buffer (50x)
- 10x Gel Loading Solution
- InstaStain® Ethidium Bromide
- 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)

Requirements

- Thermal cycler (EDVOTEK Cat. # 541 highly recommended) or three waterbaths*
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- Water bath (56°C)
- Plant lights (optional)
- 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
- Ethanol

*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.

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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

QuickPlants™ - *Arabidopsis Thaliana*

Arabidopsis thaliana is a small, weed-like plant from the mustard family, *Brassicaceae* (*Cruciferae*). In spite of its humble appearance, *Arabidopsis* has become a superstar for plant geneticists and molecular biologists. There are several reasons for its success. First, the small size of the plants allows for large numbers to be grown in a small space in the laboratory, growth chamber or greenhouse. Second, *Arabidopsis* has a very short life cycle. Plants from seeds planted today will begin flowering in only three to four weeks. This is an advantage for geneticists because they can make experimental crosses and raise many generations in a very short period of time. Third, *Arabidopsis* has a very small genome consisting of 5 chromosomes. The amount of DNA normally found in *Arabidopsis* cells is small compared to that of other plants. Some plant species are known to contain 10,000 times as much DNA per cell as *Arabidopsis*. The small size of the *Arabidopsis* genome has made it possible to determine its entire nucleotide sequence. This task was completed in 2000. Annotating and identifying the genes in the sequence and assigning functions to them, will probably take many more years.

The same features of *Arabidopsis* that make it an attractive organism for research also make it useful in the classroom. The plant can be grown in large numbers and in a small space under classroom conditions. Genetics experiments can be completed in a single semester. Large numbers of interesting mutants have been identified and characterized, and several have been selected as especially useful for education.

Examples of mutant characteristics are described below:

- gai1 is a gibberellic acid insensitive dwarf. This *Arabidopsis* plant is much smaller than the wild type.
- ap1-1 and ap3-3, are homeotic mutants. Homeotic mutations have the effect of converting one organ or body part into another; ap stands for apetala. The name refers to the phenotype of the mutants, lacking petals because they have been converted into other flower parts. Note that although both of these mutations produce similar phenotypes, they are defects in different genetic loci as indicated by their numbering.
- fus3-3 , fusca, is a mutant in which the germinating seeds are splotched with reddish brown color. Normally, seedlings are expected to be a uniform light green color.
- var mutants are variegated. Leaves are splashed with patches of white.
- yi1 mutants have a yellow inflorescence. The flower buds of this mutant are a very pale green – yellowish color and the flower petals are off-white.

Background Information

QuickPlants™ - *Arabidopsis Thaliana*

Figure 1:
Wild and mutant Glabra strains. The gl1-1 glabra are hairless mutants that lack fine glandular hairs (trichomes).

- tt2-1 mutants have a transparent testa (or uncolored seed coat). Normally, seed coats are brown and this makes the seeds brown. Transparent testa mutants, therefore, produce yellow, rather than brown seeds. Since the seed coat has no color, the seeds show the color of the embryo inside.

The gl1-1 glabra are hairless mutants that are selected for inclusion in this mapping experiment. This mutant lacks the fine glandular hairs (trichomes) normally found covering the surface of an *Arabidopsis* leaf.

MAPPING STRATEGY

There are many advantages of genetic mapping vs. classical plant breeding. With classical plant breeding/genetics, many crosses are required and many f1 lines must be maintained to reach a final result. With genetic mapping, an assay from the DNA of a single cross will yield many DNA polymorphic markers.

Traditionally, genes have been located, or mapped to specific loci on chromosomes by the technique of recombination mapping. This technique takes advantage of the fact that genes located very close together on a chromosome are often inherited together as a package. The closer two genes are to one another, the less likely they are to be separated by recombination. So, a gene is mapped by measuring the frequency of recombination between the gene of interest and other genes that have already been placed on the chromosome.

This strategy for mapping genes is limited however, by the number of genes that have already been mapped. Producing a very detailed map by recombination analysis requires many genes. Molecular biology has extended our ability to map genes by providing convenient genetic markers in numbers that literally saturate the chromosomes. Using molecular markers rather than Mendelian traits as chromosomal landmarks for mapping means that genes can be placed very precisely on the genetic map.

DNA EXTRACTION

Every method for extraction of DNA includes some common features: tissues are disrupted to release DNA, cellular debris is removed, and DNA is precipitated to separate it from other cellular components. The method outlined in this experiment includes each of these steps. First, small amounts of plant leaf tissue from *Arabidopsis* plants carrying the gene to be mapped and from mutant *Arabidopsis* plants are ground into a fine suspension in extraction

buffer. This buffer contains a chelating agent (EDTA) to protect DNA from the activity of nucleases released from the tissue as the cells are disrupted. It also includes salt and a detergent (SDS) which will disrupt cellular membranes. Second, the plant tissue is incubated in a hot water bath to facilitate cell lysis. Cell debris is removed from the preparation by centrifugation and the pelleted material is ground a second time to maximize DNA yield. After centrifuging a second time, DNA is precipitated from the clarified supernatant with isopropanol. The DNA prepared by this method is sufficiently purified to work as a template in the polymerase chain reaction step that follows.

POLYMERASE CHAIN REACTION

Since its discovery in the mid 1980s, the polymerase chain reaction (PCR) has revolutionized biological science. The enormous utility of PCR is based on its ease of use and its ability to amplify DNA. PCR amplification uses an enzyme known as *Taq* DNA polymerase. This enzyme, originally purified from a bacterium that inhabits hot springs, is stable at very high (near boiling) temperatures. Also included in the PCR reaction mixture are short (15-30 nucleotide) synthetic oligonucleotides, known as primers and the extracted DNA that contains the region to be amplified, known as the "target".

In the first step of the PCR reaction (Figure 2), known as denaturation, the target complementary DNA strands are melted (separated) from each other at 94°C, while the *Taq* DNA polymerase remains stable. In the second step, known as annealing, the sample is cooled to an intermediate temperature (usually between 37°C and 65°C) to allow hybridization of the two primers to the two strands of the target DNA. In the third PCR step (Figure 2), known as extension, the temperature is raised to 72°C. At this temperature, the *Taq* DNA polymerase is maximally active and adds nucleotides to the primers to complete the synthesis of the new complementary strands to the target region. These three steps - denaturation, annealing, and extension– constitute one PCR "cycle". This process is typically repeated for 20-40 cycles, amplifying the target sequence exponentially. PCR is performed in a thermal cycler that is programmed to heat, cool and maintain samples at precise temperatures for varying time intervals.

The Experiment

QuickPlants™ - *Arabidopsis Thaliana*

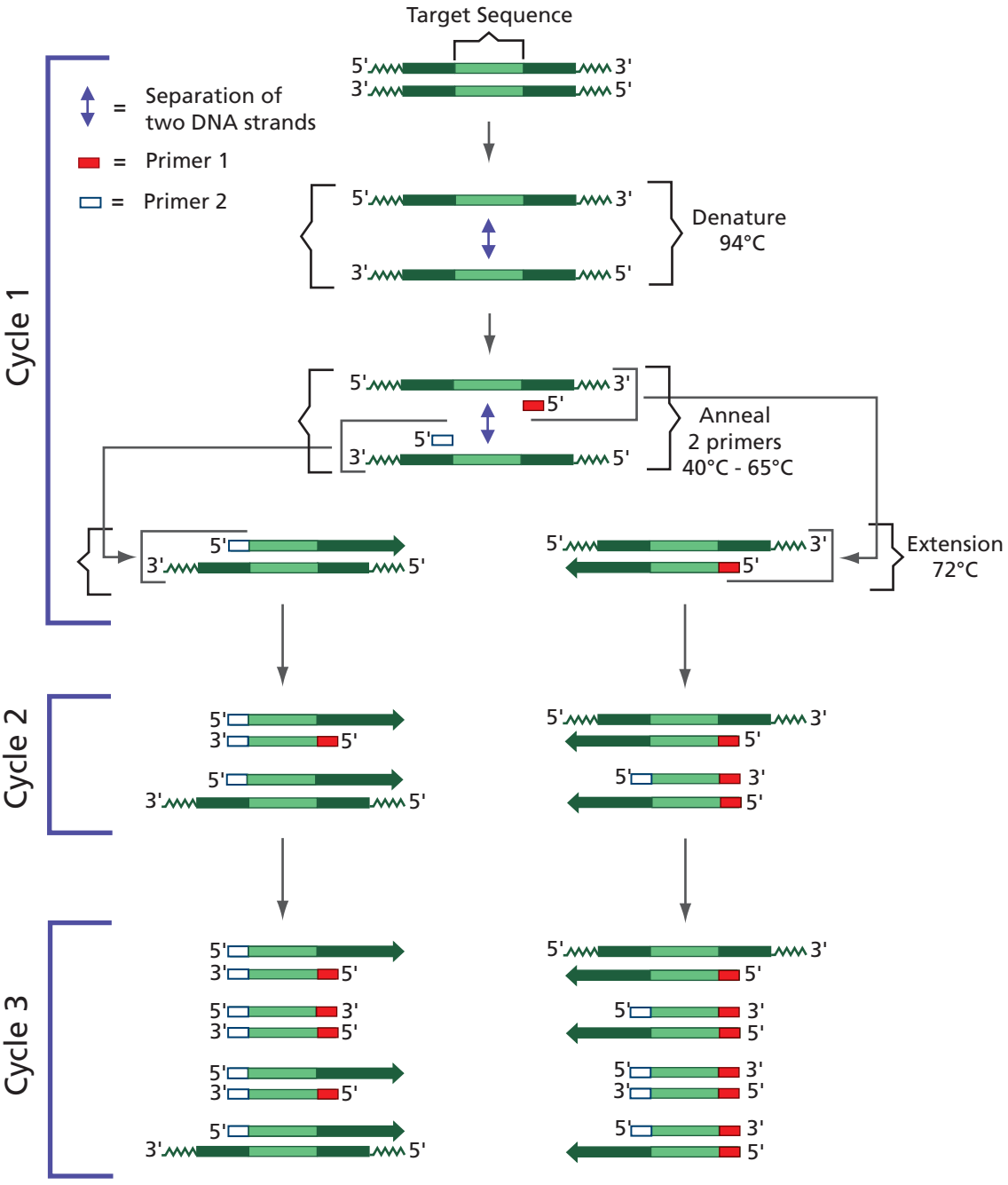


Figure 2: Polymerase Chain Reaction

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 object of this experiment is to introduce students to the concept of genetic linkage by using the polymerase chain reaction to amplify DNA from wild type and mutant *Arabidopsis* plants.

BRIEF DESCRIPTION OF EXPERIMENT:

In this experiment, the extracted *Arabidopsis* (glabra and wild type) DNA will be amplified at two separate target sequences on chromosomes 1 and 3. The amplified region (519 base pairs) on chromosome 3 is unlinked to the glabra gene, while the target on chromosome 1 (1481 base pairs) is linked. Comparison of the wild type and glabra PCR products experimentally demonstrate the concept of genetic linkage. This experiment has three modules:

Module I: Module I: Growing QuickPlants™ - *Arabidopsis Thaliana*
Module II: Isolation of Genomic DNA from *Arabidopsis*
Module III: PCR of Genomic DNA from *Arabidopsis*
Module IV: Agarose Gel Electrophoresis

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	1.0%

Laboratory Safety

1. Gloves and goggles should be worn routinely as good laboratory practice.

2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.

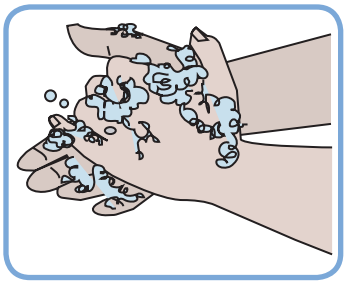
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.

Wear gloves and safety goggles

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: Growing QuickPlants™ - *Arabidopsis Thaliana*

1. Sow the seeds thinly on the surface of a moist peat-based potting mix or on moistened peat pods.

   Alternatively, sprinkle several seeds into a tube and add 0.5 ml tap water to the tube. Use a small transfer pipet to disperse the seeds evenly on the soil surface.

2. Do not cover the seeds; the seeds need light for germination.

3. Place the seeds directly under fluorescent lights or on the sill of a bright window.

4. Keep the potting medium moist to wet while the seeds germinate. This will take approximately 3-4 days.

5. After the seeds germinate, they can tolerate some drying, but don't let them dry completely. Mist the plants daily with a dilute (1/4 strength) solution of balanced commercial fertilizer.

Helpful Hints and Notes:

Quick Plants™ are amazingly hardy and tolerant of abuse once they become established.

Soil and planting: Soil can be mixed from standard greenhouse components. Use light soil mixtures with ample peat moss, and sterilize before planting in order to avoid any pest contamination. Alternatively, use commercially prepared mixes, such as Metromix 350 or ProMix BX. The surface of the soil should be approximately 1 cm from the top of the pot. Several pots can be put together in a tub or similar container. Cover with clear plastic wrap. Perforate the wrap to maintain enough humidity for germination.

Temperature: Quick Plants™ thrive under cool conditions. Optimum temperature is 25°C. Room temperature works great.

Lighting: More than any other factor, light determines how quickly the plants will grow and develop. Fastest growth is under continuous fluorescent light (shop lights). These can be easily and inexpensively configured in a classroom or lab. These conditions also produce compact sized plants. On a bright windowsill, or in a cool greenhouse, the plants take one to several weeks longer to develop, but are larger in size. Slowest growth occurs under low light conditions, such as a poorly lit windowsill.

Watering: After germination, water plants as needed to avoid water stress. Avoid over-watering to prevent the potential for algal or fungal growth on the soil surface. If algae does appear, allow the pots to dry and scrape the algae from the soil surface with care.

Module II: Isolation of Genomic DNA from *Arabidopsis*

The Experiment

1. Harvest 4-6 seedlings (~ 1 cm tall, 1-3 weeks old), or a leaf from a mature plant (~1 x 1 cm) into a microfuge tube with pestle.
   - Place Glabra mutant seedlings in one tube and wild type seedlings in another.
   - Keep each tube separate throughout the entire experiment.
2. Use the pestle to partially mash the tissue.
3. Add 100 µl of DNA Extraction Buffer to each tube and continue grinding the tissue.

WARNING!
Use only screw-cap tubes when incubating in the waterbath for DNA isolation. Do not use snap-top tubes.

4. Add an additional 200 µl of DNA Extraction Buffer to each tube and grind the tissue again.
5. Incubate the tubes in a waterbath at 56°C for one hour.

Use caution not to cross-contaminate plant tissue and DNA - this will yield false positive results.

After the 56°C Incubation:

6. Add 250 µl NaCl solution to each tube and mix well for 30 seconds.
7. Centrifuge the tubes at 13,000 rpm (microcentrifuge maximum speed) for 15-30 minutes.
8. Re-grind the pelleted material in each tube and centrifuge the tube at 13,000 rpm for 5 minutes.
9. Carefully transfer the supernatant from each tube into a fresh labeled microcentrifuge tube being careful not to disturb the pellet. Discard the tubes with pellets.
10. Precipitate the DNA in the supernatant by adding an equal amount of ice-cold isopropanol.
11. Incubate the tubes in the freezer for at least one hour to overnight.

After Incubation in the freezer:

12. Collect the precipitated DNA by centrifugation at 13,000 rpm for 10 minutes.
13. Carefully remove and discard all the supernatant and leave the pelleted DNA at the bottom of the tube.

Module II: Isolation of Genomic DNA from *Arabidopsis*

14. Wash the pellet with 1.5 ml of 70% ethanol or isopropanol.
15. If the pellet becomes dislodged, spin at full speed for 2 minutes.
16. Discard the supernatant and allow the DNA pellet to dry for 5 minutes.
17. Completely resuspend the pellet in 100 µl of TE (10 mM Tris, pH 8.0, 0.1 mM EDTA) by pipetting up and down several times and by vortexing or tapping vigorously.
18. Proceed with the PCR preparations or store the DNA at -20°C if it will not be used immediately.

OPTIONAL STOPPING POINT

The supernatant may be stored at -20°C until the experiment is continued.

Module III: PCR of Genomic DNA from *Arabidopsis*

The Experiment

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.

Perform one PCR reaction for <u>each</u> plant type.

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. Label each PCR tube with the appropriate name ("glabra" or "wild").
3. To each tube, add and mix the following:

   PCR Reaction pellet™,
   10 µl UltraPure water
   10 µl the appropriate *Arabidopsis* DNA
   5 µl primer mixture.

4. 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.

5. Process the assembled reactions for polymerase chain reaction cycling in a thermal cycler as follows:

<u>1 cycle</u>	<u>35 cycles</u>	<u>1 cycle</u>
94°C for 5 minutes	94°C for 1 minute 54°C for 30 seconds 72°C for 90 seconds	72°C for 4 minutes

6. After the completion of the cycling, add 5 microliters of 10x gel load solution to each tube.
7. Proceed to instructions for preparing a 1.0% agarose gel (7 x 14 cm) and separating the PCR products by electrophoresis.

OPTIONAL STOPPING POINT

The samples can be held in the thermal cycler at 4°C or frozen after addition of 5 µl of 10x Gel Loading Solution until ready for electrophoresis.

Agarose Gel Electrophoresis

If you are unfamiliar with agarose gel preparation and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

AGAROSE GEL REQUIREMENTS

- 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: 1.0%

PREPARING THE AGAROSE GEL

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
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.
3. To a 250 ml flask or beaker, add agarose powder and buffer as indicated in the Reference Tables (Appendix A) provided by your instructor. Swirl the mixture to disperse clumps of agarose powder.
4. With a marking pen, indicate the level of the solution volume on the outside of the flask.

Important Note

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.

5. Heat the mixture using a microwave oven or burner to dissolve the agarose powder.
6. 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 4.

After the gel is cooled to 60°C:

7. Place the bed on a level surface and pour the cooled agarose solution into the bed.
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.
9. After the gel is solidified, be careful not to damage or tear the wells while removing the rubber dams or tape and comb(s) from the gel bed.
10. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.
11. Fill the electrophoresis apparatus chamber with the appropriate amount of diluted (1x) electrophoresis buffer (refer to Table B on the instruction Appendix provided by your instructor).

Agarose Gel Electrophoresis

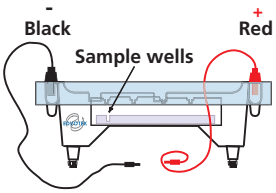
The Experiment

BEFORE LOADING THE SAMPLES

This experiment requires a 1.0% agarose gel and is designed for staining with InstaStain® Ethidium Bromide.

Reminder:

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



LOADING DNA SAMPLES

1. (Optional Step) Heat the 200 bp DNA ladder and PCR samples for two minutes at 50°C. Allow the samples to cool for a few minutes.

2. Make sure the gel is completely submerged under buffer before loading the samples. Load the entire volume (30 µl) of the samples in the following sequence.

Lane	
1	200 bp ladder
2	Wild type PCR DNA
3	Glabra PCR DNA

3. Record the position of your sample in the gel for easy identification after staining.

RUNNING THE GEL

4. After the DNA samples are loaded, properly orient the cover and carefully snap it onto the electrode terminals.

5. Insert the plugs of the black and red leads into the corresponding inputs of the power source.

6. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor.

7. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.

8. After the electrophoresis is completed, disconnect the power and remove the gel from the bed for staining.

STAINING AND VISUALIZATION OF DNA

After electrophoresis, agarose gels require staining to visualize the separated DNA samples. Your instructor will provide instructions for DNA staining with InstaStain® Ethidium Bromide.

Study Questions

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

1. Describe the methods involved in amplification of plant DNA from start to finish.
2. What can interfere with obtaining successful PCR results.
3. What are the advantages of using a genetic mapping strategy vs. traditional plant breeding/crossing?
4. How can mapping a plant such as *Arabidopsis* help with other plant species or in other areas of plant breeding and genetics?


EDVOTEK®

Instructor's Guide

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.

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email: edvotek@aol.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

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.

Online Ordering now available

Visit our web site for information about EDVOTEK's complete line of "hands-on" experiments for biotechnology and biology education.

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:

Module I: Growing QuickPlants™ - *Arabidopsis Thaliana*
Module II: Isolation of Genomic DNA from *Arabidopsis*
Module III: PCR of Genomic DNA from *Arabidopsis*
Module IV: Agarose Gel 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 100-120 minutes or can be processed overnight and held at 4°C.

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

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 1 - 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.

Table C
Time and Voltage
(1.0% - 7 x 14 cm gel)

Volts	Recommended Time	
	Minimum	Maximum
125	55 min	1 hr 15 min
70	2 hrs 15 min	3 hrs
50	3 hrs 25 min	5 hrs

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 1.0%. 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

MODULE I: GROWING QUICKPLANTS™

Plan to have plants ready for harvest on the day of the lab. Allow 2- 3 weeks for adequate growth. See Growing EDVOTEK QuickPlants™ in the Experiment Procedures section.

MODULE II: ISOLATION OF GENOMIC DNA FROM *ARABIDOPSIS*

1. If a precipitate has formed in the DNA extraction buffer, warm at 37°C to redissolve.
2. Prepare Proteinase K solution:
   - Add 200 µl of DNA Extraction Buffer (H) to each tube of Proteinase K 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 on ice.
3. Aliquot 1 ml of NaCl solution (G) for each group.
4. Aliquot 1 ml of Tris Buffer (E) for each group.
5. Place bottles of 95% and 70% Isopropyl alcohol on ice or in the freezer. Chill thoroughly.

Each student group will require:

- *Arabidopsis* glabra plants
- *Arabidopsis* wild type plants
- 2 microcentrifuge tubes with pestle
- 1 ml DNA Extraction Buffer (H)
- Ice-cold isopropanol and ethanol
- 1 ml Tris buffer
- 1 ml NaCl solution
- Additional microcentrifuge tubes

Instructor's Guide

Pre-Lab Preparations

MODULE III: PCR OF GENOMIC DNA FROM *ARABIDOPSIS*

- Thaw the primer mix (B) and place on ice. Aliquot 15 µl for each student group.

Each student group will require:

- 2 PCR beads (in tubes)
- 50 µl UltraPure water
- 15 µl primer mixture
- 20 µl 10x Gel Load solution

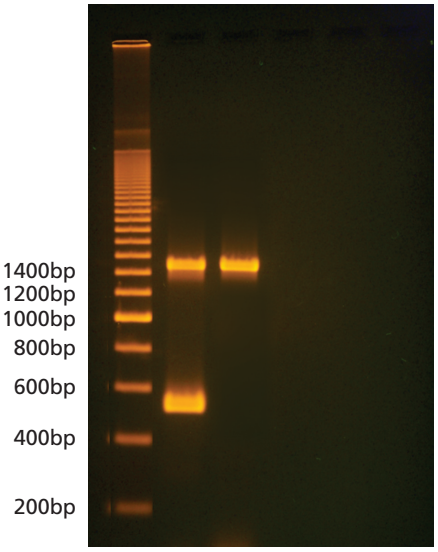
Notes and Reminders:

- Accurate temperatures and cycle times are critical for PCR. A pre-run for one cycle (approx. 3 to 5 minutes) is recommended to check that the thermal cycler is properly programmed.
- For thermal cyclers that 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 ".
- Three water baths can be used for PCR if a thermal cycler is unavailable. The experiment will require great care and patience. Samples will require wax layers. See appendices entitled "Polymerase Chain Reaction Using Three Waterbaths" and "Handling samples with wax overlays".

MODULE IV: AGAROSE GEL ELECTROPHORESIS

When students are ready to perform the electrophoresis, thaw the 200 bp DNA ladder (C). Aliquot 30 µl of the 200 bp DNA ladder for each gel to be run. Place on ice until students are ready to load the gels.

Experiment Results and Analysis



The amplified region (519 base pairs) on chromosome 3 is unlinked to the glabra gene, while the target on chromosome 1 (1481 base pairs) is linked. Comparison of the wild type and glabra PCR products experimentally demonstrate the concept of genetic linkage.

Lane 1 - 200 bp ladder
Lane 2 - Wild Type PCR DNA
Lane 3 - Glabra PCR DNA

Note:
Depending on the PCR conditions used, a diffuse, small-molecular weight band, known as a "primer dimer", may be present below the 200 bp marker. This is a PCR artifact and can be ignored. Other minor bands may also appear due to nonspecific primer binding and the subsequent amplification of these sequences.

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

Appendices

A PCR Experimental Success Guidelines
B Polymerase Chain Reaction Using Three Waterbaths
C Preparation and Handling of PCR Samples With Wax
D 1.0% Agarose Gel Preparation
E 1.0% Agarose Gels - Quantity Preparations
F Staining and Visualization of DNA with InstaStain® Ethidium Bromide Cards
G InstaStain® Blue: One Step Staining and Destaining

Material Safety Data Sheets

EDVO-Kit # 336

Appendix A

PCR Experimental Success Guidelines

EDVOTEK experiments which involve the extraction and 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.

DNA EXTRACTION AND SAMPLE PREPARATION:

1. **Sufficient Cells:** It is critical that there are sufficient cells to obtain enough DNA that will yield positive results. Cell sources include human, plant, drosophila and bacterial cells. Without enough cells, there will not be enough DNA template for the PCR reaction.

2. **Centrifugation:** Centrifuge the cell suspension carefully. If the pellet loosens, repeat the step. The supernatant should be clear, not cloudy, and the pellet should be solid at the bottom of the tube. Repeat centrifugation for a longer period of time, if necessary.

THE PCR REACTION

3. **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.

4. **The Thermal cycler:** The thermal cycler must be programmed for the correct cycle sequence. It is critical that the temperatures and the time for each of the cycles are accurate.

5. **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.

6. **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.

Appendix A

EDVO-Kit # 336

PCR Experimental Success Guidelines
(continued)

GEL PREPARATION AND STAINING

7. **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.

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

9. **Staining:** Staining of higher concentration gels (> 0.8%) require additional care to obtain clear, visible results.
   - After staining (15 to 30 min.) with InstaStain® Ethidium Bromide or liquid ethidium bromide, examine the results using a UV (300 nm) transilluminator. Repeat the staining as required.
   - Gels stained with InstaStain® Blue or other liquid blue stain may fade with time. Re-stain the gel to visualize the DNA bands.

10. **DNA 200 bp ladder:** After staining the agarose gel, the DNA 200 bp ladder (markers) should be visible. If bands are visible in the markers 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, 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.

EDVO-Kit # 336

Appendix B

Polymerase Chain Reaction Using Three Waterbaths

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:

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

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

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.

4. 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

EDVO-Kit # 336

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.

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

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

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.

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Appendix D

1.0% Agarose Gel Preparation

If preparing the gel with concentrated (50x) buffer, use Table A.1.

If preparing the gel with diluted (1x) buffer, use Table A.2.

Table A.1 Individual 1.0% UltraSpec-Agarose™ Gel

Size of Gel (cm)	Amt of Agarose (g)	+ Concentrated Buffer (50X) (ml)	+ Distilled Water (ml)	= **Total Volume (ml)**
7 x 7	0.25	0.5	24.5	**25**
7 x 14	0.5	1.0	49.0	**50**

Table A.2 Individual 1.0% UltraSpec-Agarose™ Gel

Size of Gel (cm)	Amt of Agarose (g)	+ Diluted Buffer (1x) (ml)
7 x 7	0.25	25
7 x 14	0.5	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 (blue)	500	10	490
M36 (clear)	1000	20	980

Table C Time and Voltage (1.0% - 7 x 14 cm gel)

Volts	Recommended Time: Minimum	Maximum
125	55 min	1 hr 15 min
70	2 hrs 15 min	3 hrs
50	3 hrs 25 min	5 hrs

Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C. The approximate time for electrophoresis will vary from approximately 1 - 5 hours depending upon various factors. Conduct electrophoresis for the length of time determined by your instructor.

Appendix E

EDVO-Kit # 336

1.0% Agarose Gels - 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 used at a later time and solidified agarose gel 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)

Table E
Batch Preparation of 1.0% UltraSpec-Agarose™

Amt of Agarose (g)	+	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
3.0		6.0		294		300
4.0		8.0		392		400

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

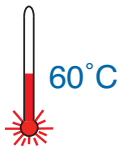
BULK ELECTROPHORESIS BUFFER

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

BATCH AGAROSE GELS (1.0%)

For quantity (batch) preparation of 1.0% agarose gels, see Table E.

1. Use a 500 ml flask to prepare the diluted gel buffer
2. Pour 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 as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.

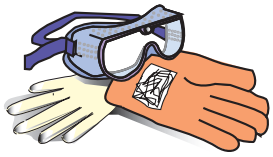
EDVO-Kit # 336

Appendix F

Staining and Visualization of DNA

INSTASTAIN® ETHIDIUM BROMIDE CARDS

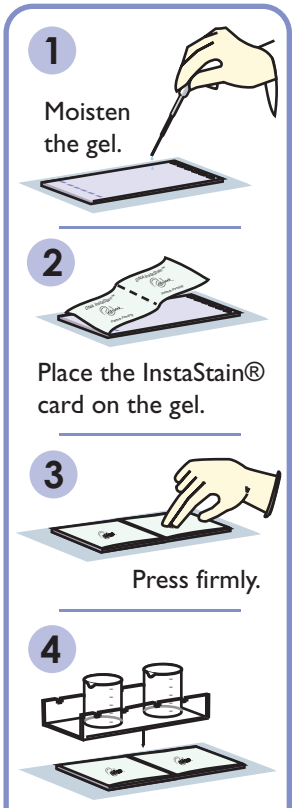
Do not stain gel(s) in the electrophoresis apparatus.



Wear gloves and safety goggles

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. Wearing gloves, remove the clear plastic protective sheet, and place the unprinted side of the InstaStain® EtBr card on the gel.
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.
- DNA 200 bp markers should be visible after staining even if the amplified DNA samples are faint or absent. If markers are not visible, troubleshoot for problems with the electrophoretic separation.

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		None
Stable	X	

Incompatibility: No data available

Hazardous Decomposition or Byproducts:

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

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		Special
Local Exhaust		
Mechanical	Gen. dilution ventilation	Other

Protective Gloves		Eye Protection	
Yes		Splash proof goggles	

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

Work/Hygienic Practices: None

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		None
Stable	X	

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide

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

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

Protective Gloves		Eye Protection	
Yes		Safety goggles	

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: None

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		None
Stable	X	

Incompatibility: None known

Hazardous Decomposition or Byproducts: Sulfur oxides and bromides

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

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: None NTP? No data IARC Monographs? No data OSHA Regulation? 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		Eye Protection	
yes		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.

Material Safety Data Sheets

Full size (8.5 x 11") pdf copy of MSDS available at www.edvotek.com or by request.

EDVO-Kit # 336

EDVO-Kit # 336

Material Safety Data Sheets

Full size (8.5 x 11") pdf copy of MSDS available at www.edvotek.com or by request.

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_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: Chemical bound to paper, 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, 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: None
	Stable	X	
Incompatibility	Strong oxidizing agents		
Hazardous Decomposition or Byproducts	Carbon monoxide, Carbon dioxide, nitrogen oxides, hydrogen bromide gas		
Hazardous Polymerization	May Occur		Conditions to Avoid: None
	Will Not Occur	X	

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.

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): 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	Unstable		Conditions to Avoid: Excessive heat, sparks or open flame
	Stable	X	
Incompatibility	Acids, aluminum, metals, oxidizers (strong)		
Hazardous Decomposition or Byproducts	Thermal decomposition products of toxic and hazardous oxides of C, N, & Na		
Hazardous Polymerization	May Occur		Conditions to Avoid: None
	Will Not Occur	X	

Section VI - Health Hazard Data

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

Carcinogenicity: None NTP? IARC Monographs? No data OSHA Regulation? 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	Local Exhaust	Yes	Special	None	
	Mechanical (General)	Vent. Sys.	Other	None	
Protective Gloves	Yes		Eye Protection	Splash proof goggles	

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

Work/Hygienic Practices: Emergency eye wash should be available

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® Methylene Blue, Methylene Blue Plus

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)
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	Unstable		Conditions to Avoid: None
	Stable	X	
Incompatibility	Strong oxidizing agents		
Hazardous Decomposition or Byproducts	Toxic fumes of Carbon monoxide, Carbon dioxide, nitrogen oxides, sulfur oxides, hydrogen, chloride gas		
Hazardous Polymerization	May Occur		Conditions to Avoid: None
	Will Not Occur	X	

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:

Material Safety Data Sheets

Full size (8.5 x 11") pdf copy of MSDS available at www.edvotek.com or by request.

EDVO-Kit #
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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): **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				
5743-0				

Section III - Physical/Chemical Characteristics

Boiling Point	100°C	Specific Gravity (H_2O = 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? N/A 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 approved 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.

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: Non-combustible	UEL: ----

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	Unstable		Conditions to Avoid
	Stable	X	

Incompatibility: Strong oxidizing agents, strong acids

Hazardous Decomposition or Byproducts

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)

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

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	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.