EDVOTEK®

The Biotechnology Education Company ®

EDVO-Kit #

371

PCR-based DNA Fingerprinting

Storage: See Page 3 for specific storage instructions

Experiment Objective:

The objective of this experiment is to perform PCR-based DNA fingerprinting on actual DNA samples that are cloned in plasmids and to understand the concept of this technology as applied to forensic science.

Table of Contents

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.

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EVT 002287AM

Experiment Components

Component Quantities:
Experiment # 371 contains reagents to perform five sets of Polymerase Chain Reaction (PCR)* reactions (25 reactions total).

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.

CONTENTS		STORAGE
A	Tubes with PCR reaction pellets™ Each PCR reaction pellet™ contains: • dNTP Mixture • *Taq* DNA Polymerase Buffer • *Taq* DNA Polymerase • MgCl2	Room Temperature
B	Primer Mix	-20°C Freezer
C	200 bp ladder	-20°C Freezer
D	DNA Template #1	-20°C Freezer
E	DNA Template #2	-20°C Freezer
F	DNA Template #3	-20°C Freezer
G	DNA Template #4	-20°C Freezer

REAGENTS & SUPPLIES:

- UltraSpec-Agarose™
- Electrophoresis Buffer (50x)
- 10x Gel Loading Solution
- InstaStain® Ethidium Bromide
- InstaStain® Methylene Blue
- 100 ml graduated cylinder (packaging for samples)
- Microcentrifuge Tubes (0.5 ml)
- PCR tubes (0.2 ml - for thermal cyclers with 0.2 ml template)
- Wax beads (for waterbath option or thermal cyclers without heated lid)

REQUIREMENTS:

- Thermal Cycler (EDVOTEK catalog #541 is recommended)
- Alternative Option: Three waterbaths (94°C, 25°C, and 72°C)
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- UV Transilluminator or UV Photodocumentation system
- UV safety goggles
- Automatic micropipets (5-50 µl) with tips
- Microwave, hot plate or burner
- Pipet pumps or bulbs
- 250 ml flasks or beakers
- Hot gloves
- Disposable vinyl or latex laboratory gloves
- Ice buckets and ice
- Distilled or deionized water

*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.

Technical Service Department

Mon - Fri
9:00 am to 6:00 pm ET

FAX: (301) 340-0582
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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**

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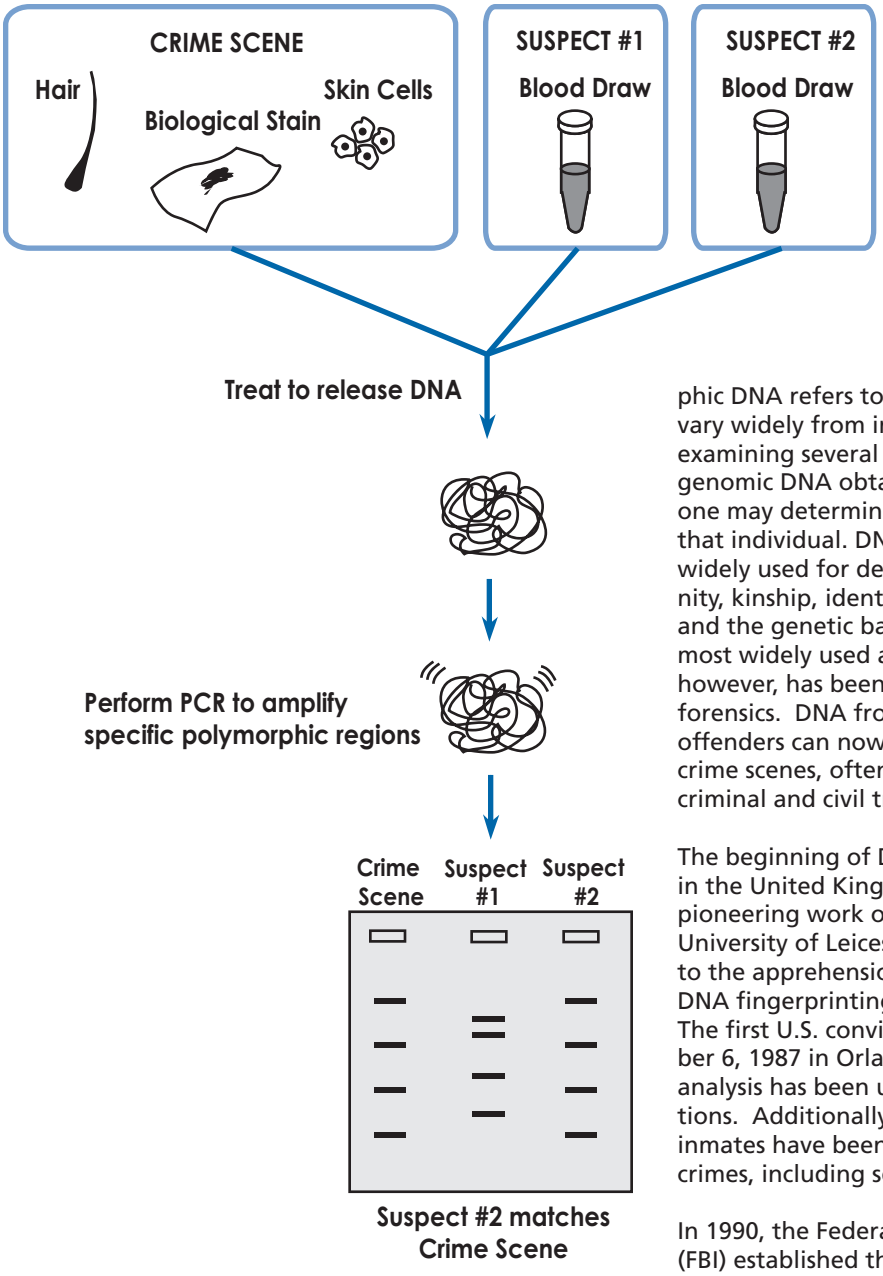
PCR-based DNA Fingerprinting



Background Information

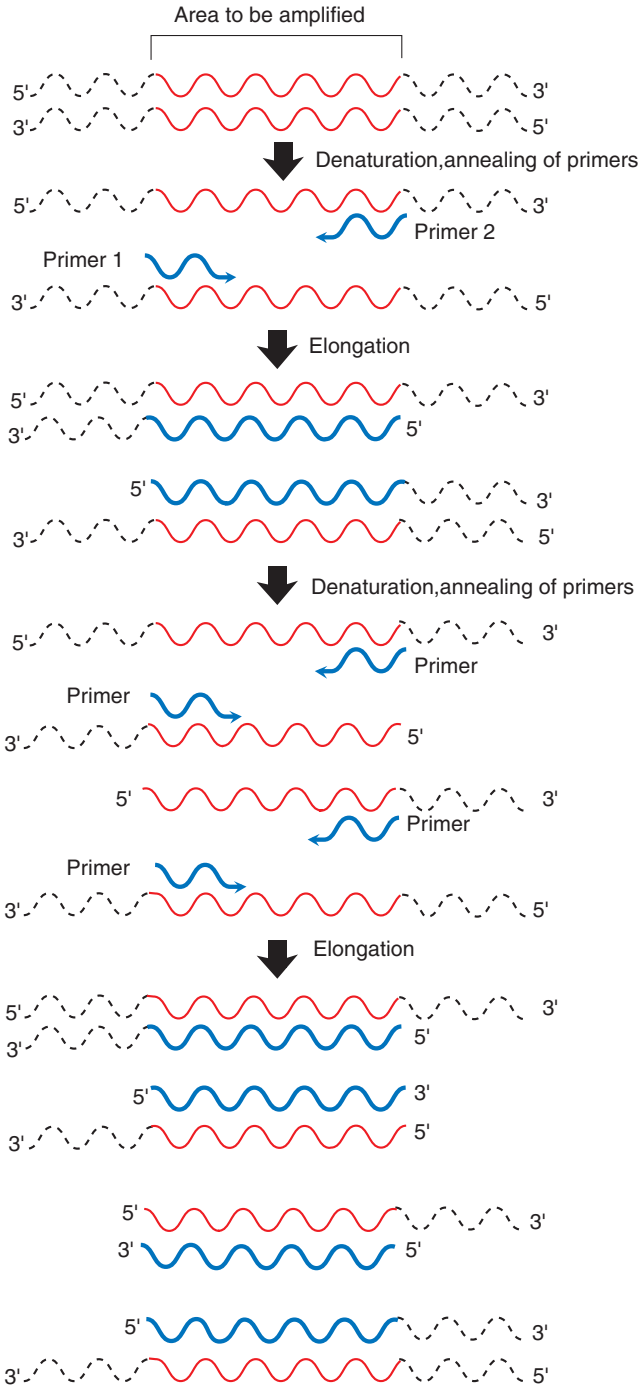
Deoxyribonucleic acid (DNA), present in the nucleus of every living cell, is the genetic material that acts as a blueprint for all of the proteins synthesized by that cell. In mammals, however, a large fraction of the total DNA does not encode protein and serves no obvious function. Polymorphic DNA refers to chromosomal regions that vary widely from individual to individual. By examining several of these regions within the genomic DNA obtained from an individual, one may determine a "DNA Fingerprint" for that individual. DNA polymorphisms are now widely used for determining paternity/maternity, kinship, identification of human remains, and the genetic basis of various diseases. The most widely used and far-reaching application, however, has been to the field of criminal forensics. DNA from both crime victims and offenders can now be definitively matched to crime scenes, often affecting the outcome of criminal and civil trials.

The beginning of DNA fingerprinting occurred in the United Kingdom in 1984, following the pioneering work of Dr. Alex Jeffreys at the University of Leicester. Analysis by Jeffreys led to the apprehension of a murderer in the first DNA fingerprinting case in September 1987. The first U.S. conviction occurred on November 6, 1987 in Orlando, FL. Since then, DNA analysis has been used in thousands of convictions. Additionally, over 100 convicted prison inmates have been exonerated from their crimes, including several death row inmates.

In 1990, the Federal Bureau of Investigation (FBI) established the **Co**mbined **D**NA **I**ndex **S**ystem (CODIS), a system which allows comparison of crime scene DNA to DNA profiles in a convicted offender and a forensic (crime scene) index. A match of crime scene DNA

Background Information

PCR-based DNA Fingerprinting



to a profile in the convicted offender index indicates a suspect for the crime, whereas a match of crime scene DNA to the forensic index (a different crime scene) indicates a serial offender. CODIS has now been used to solve dozens of cases where authorities had not been able to identify a suspect for the crime under investigation.

The first step in forensic DNA fingerprinting is the collection of human tissue from the crime scene or victim. These tissues include blood, hair, skin, and body fluids. The sample, often present as a stain, is treated with a detergent to rupture (lyse) cell membranes and obtain DNA for further analysis (Figure 1). One early method, called Restriction Fragment Length Polymorphism (RFLP) analysis, involves digesting DNA with restriction enzymes, separating the fragments by agarose gel electrophoresis, transferring the DNA to a membrane, and hybridizing the membrane with probes to polymorphic regions. This method is statistically very accurate, but requires relatively large amounts of DNA and takes several days to complete. Because of the time and DNA requirements, the RFLP method is no longer used in forensics, but remains in use in certain medical genetics-based tests.

More recently, the polymerase chain reaction (PCR) has been used in forensics to analyze DNA (Figure 2). This technique requires much less (500-fold) DNA than RFLP analysis and is much less time-consuming. PCR amplification (Figure 2) uses an enzyme known as *Taq* 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 two (15-30 nucleotide) synthetic oligonucleotides, known as "primers" and the extracted DNA, known as the "template". The region of DNA to be amplified is known as the "target". In the first step of the PCR reaction, the template complimentary DNA strands are separated (denatured) from each other at 94˚C, while the *Taq* polymerase remains stable. In the second

PCR-based DNA Fingerprinting

step, known as annealing, the sample is cooled to an intermediate temperature, usually 40˚-65˚C, to allow hybridization of the two primers, one to each of the two strands of the template DNA. In the third step, known as extension, the temperature is raised to 72˚C and the *Taq* polymerase adds nucleotides to the primers to complete the synthesis of the new complementary strands. 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 (Figure 2). PCR is performed in a thermal cycler, an instrument that is programmed to rapidly heat, cool and maintain samples at designated temperatures for varying amounts of time.

In forensics, PCR is used to amplify and examine highly variable (polymorphic) DNA regions. These are regions that vary in length from individual to individual and fall into two categories: 1) variable number of tandem repeats (VNTR) and 2) STR (short tandem repeats). A VNTR is a region that is variably composed of a 15-70 base pair sequence, typically repeated 5-100 times. An STR is similar to a VNTR except that the repeated unit is only 2-4 nucleotides in length. By examining several different VNTRs or STRs from the same individual, investigators obtain a unique DNA profile for that individual which is unlike that of any other person (except for an identical twin).

In this experiment, students and teachers are encouraged to design their own crime scene scenario and come up with a plan to test their crime-solving skills by using tools such as PCR.

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".

   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".
3. Write a hypothesis that reflects the experiment and predict experimental outcomes.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to perform PCR-based DNA fingerprinting on actual DNA samples that are cloned in plasmids and to understand the concept of this technology as applied to forensic science.

BRIEF DESCRIPTION OF THE EXPERIMENT:

In this experiment, students will conduct a PCR-based DNA fingerprinting exercise on DNA from a simulated crime scene and four different suspects. Students will be encouraged to constitute the profiles of the individuals. After PCR, students will analyze the amplified DNA segments on agarose gels.

This experiment has two modules:

I. PCR Amplification of Crime Scene and Suspect DNA
II. Separation of PCR Reactions by Electrophoresis

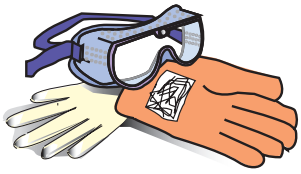
GEL SPECIFICATIONS:

This experiment requires a gel with the following specifications:

Recommended Gel Size:	7 x 14 cm (long tray)
Number of Samples Wells:	6
Placement of the Well-former Template:	First set of notches
Gel Concentration Required:	1.0%

Experiment Overview and General Instructions

LABORATORY SAFETY

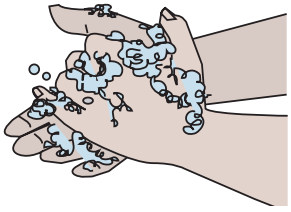


1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical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

    • 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: PCR Amplification of Crime Scene & Suspect DNA

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.

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, add one wax bead to the tube before proceeding to polymerase chain reaction cycling. The wax bead will melt to form a layer of oil that will protect the PCR incubation reaction from evaporation.

1. Each student group should obtain the following items from the instructor:

Crime scene DNA
Suspect #1 DNA
Suspect #2 DNA
Suspect #3 DNA
Suspect #4 DNA
5 PCR beads (in tubes)
Primer Mix

Label tubes containing PCR beads with the different DNAs from the list above. Also include your group designation. Label the tubes accordingly:

Crime scene PCR
Suspect #1 PCR
Suspect #2 PCR
Suspect #3 PCR
Suspect #4 PCR

SETTING UP PCR REACTIONS:

Each individual PCR reaction should be prepared as follows:

Tap the PCR reaction tube to assure that the PCR reaction pellet™ is at the bottom of the tube. Add the following to the PCR tube:

- 20 µl of Primer Mix
- 5 µl of corresponding DNA
- Gently mix the reaction tube.
- Place each sample on ice until the remaining samples are prepared.
- Note – use a clean pipet tip for each individual

Module I: PCR Amplification of Crime Scene & Suspect DNA

POLYMERASE CHAIN REACTION CYCLING

Program the thermal cycler for a total of 35 cycles. Each cycle will be:

Initial Denaturation	35 cycles @	Final Extension
94°C for 3 min.	94°C for 30 sec. 45°C for 30 sec. 72°C for 30 sec.	72°C for 3 min.

After the final extension is complete, add 5 µl of 10x Gel Loading solution to each PCR sample. Store samples on ice until ready for electrophoresis.

NOTE: If your thermal cycler has the capability, you can to link to a program to hold samples at 4°C overnight after completing the final extension.

OPTIONAL STOPPING POINT

The samples can be held in the thermal cycler at 4°C or frozen at -20°C after addition of 5 µl of 10x Gel Loading Solution until ready for electrophoresis.

Module II: Separation of PCR Reactions by Electrophoresis

The Experiment

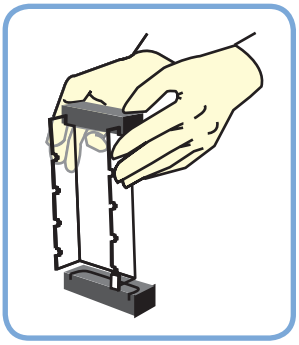
AGAROSE GEL REQUIREMENTS FOR THIS EXPERIMENT

- Recommended gel size: 7 x 14 cm

  To achieve better resolution of the PCR products, 7 x 14 cm gels, which can be shared by several students or groups, are recommended.

- Placement of well-former template: First set of notches
- Agarose gel concentration: 1.0%

Agarose Gel Preparation



PREPARING THE GEL BED

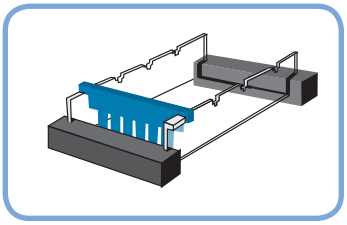
1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.

   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:

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



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.

Module II: Separation of PCR Reactions by Electrophoresis

CASTING THE AGAROSE GEL(S)

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

IMPORTANT

Check with your instructor regarding the concentration of the buffer you are using to prepare your gel. Use the appropriate table (A.1 or A. 2) below.

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

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

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

Diluted buffer is one volume of concentrated buffer to every 49 volumes of distilled or deionized water. See Table B.

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

4. Swirl the mixture to disperse clumps of agarose powder.

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

Module II: Separation of PCR Reactions by Electrophoresis

The Experiment

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

6. Heat the mixture to dissolve the agarose powder. The final solution should appear clear (like water) without any undissolved particles.

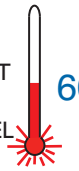
   A. Microwave method:
   - Cover the flask with plastic wrap to minimize evaporation.
   - Heat the mixture on High for 1 minute.
   - Swirl the mixture and heat on High in bursts of 25 seconds until all the agarose is completely dissolved.

   B. Hot plate method:
   - Cover the flask with aluminum foil to prevent excess evaporation.
   - Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.

   Check the solution carefully. If you see "crystal" particles, the agarose is not completely dissolved.

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 on the flask in step 6.

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.

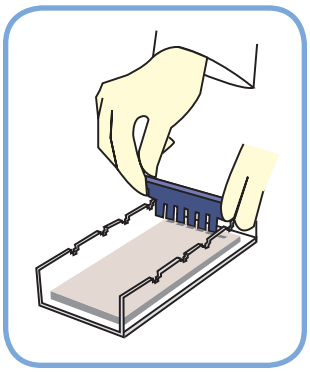
8. Seal the interface of the gel bed and tape to prevent the agarose solution from leaking.
   - Use a transfer pipet to deposit a small amount of cooled agarose to both inside ends of the bed.
   - Wait approximately 1 minute for the agarose to solidify.

9. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.

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

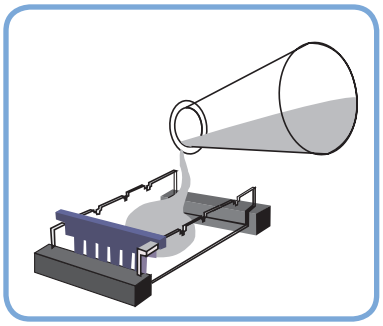
Module II: Separation of PCR Reactions by Electrophoresis

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.



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.

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

IMPORTANT: Check with your instructor to determine if the buffer has previously been diluted. Pour the appropriate amount of 1x buffer into the electrophoresis chamber according to Table B below.

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

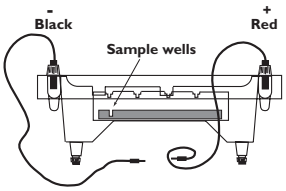
EDVOTEK Model #	Total Volume Required (ml)	Dilution: 50x Conc. Buffer (ml)	+ Distilled Water (ml)
M6+	300	6	294
M12	400	8	392
M36 (blue)	500	10	490
M36 (clear)	1000	20	980

Module II: Separation of PCR Reactions by Electrophoresis

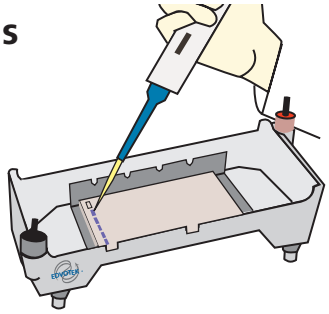
The Experiment

Reminder:

During electrophoresis, the DNA samples migrate through the agarose gel towards the positive electrode. Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



Conducting Electrophoresis



LOAD THE SAMPLES

This experiment requires a 1.0% agarose gel. More than one group can share each gel.

1. 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. Load the DNA ladder in lane 1 of each gel.
3. Load the entire volume (30 µl) of each PCR sample in consecutive wells.

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

RUNNING THE GEL

4. After the DNA samples are loaded, carefully snap the cover down onto the electrode terminals.

   Make sure that the negative and positive color-coded indicators on the cover and apparatus chamber are properly oriented.

5. Insert the plug of the black wire into the black input of the power source (negative input). Insert the plug of the red wire into the red input of the power source (positive input).

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

6. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor. General guidelines are presented in Table C.
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, turn off the power, unplug the power source, disconnect the leads and remove the cover.
9. Remove the gel from the bed for staining.

Staining and Visualization of DNA

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

INSTASTAIN® ETHIDIUM BROMIDE

Optimal visualization of PCR products on gels of 1.0% or higher concentration is obtained by staining with InstaStain® Ethidium Bromide (InstaStain® EtBr) cards.

Caution: Ethidium Bromide 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® METHYLENE BLUE

Alternatively, InstaStain® Methylene Blue (InstaStain® MetBlue) can be used for staining gels in this experiment. However, InstaStain® MetBlue is less sensitive than InstaStain® EtBr and will yield variable results.

Two options are provided for using the InstaStain® Methylene Blue cards.

Method 1: One-step Staining and Destaining with InstaStain® MetBlue

Method 2: Staining with InstaStain® Methylene Blue

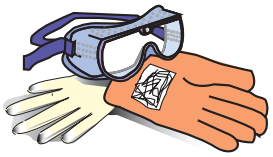
Using Method 1, agarose gels can be stained and destained in one easy step, which can be completed in approximately 3 hours, or can be left in liquid overnight. Method 2, using InstaStain® Methylene Blue cards, requires approximately 5-10 minutes for staining. DNA bands will become visible after destaining for approximately 20 minutes, and will become sharper with additional destaining. For the best photographic results, allow the gel to destain for several hours to 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® Methylene 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® MetBlue cards and destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed down the drain.

InstaStain is a registered trademark of EDVOTEK, Inc. Patents Pending.

The Experiment

Module II: Staining of DNA with InstaStain® Ethidium Bromide



Wear gloves and safety goggles

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

www.edvotek.com

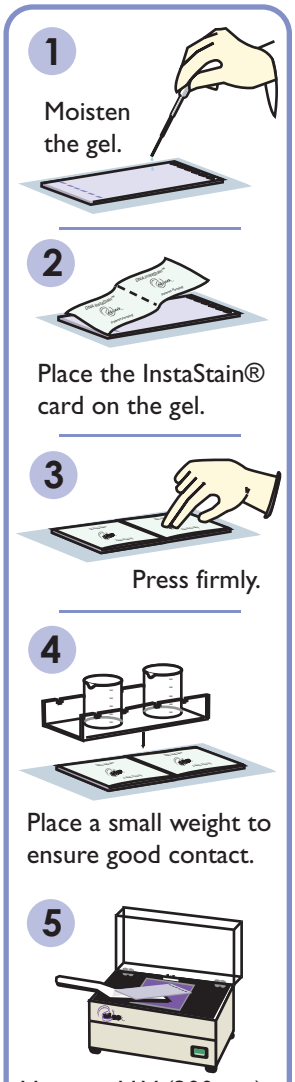
Visit our web site for an animated demonstration of InstaStain® EtBr.

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.

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.
- Gels stained alternatively with InstaStain Methylene Blue or liquid methylene blue may fade with time. Re-stain the gel to visualize the DNA bands.
- 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.

Caution: Ethidium Bromide is a listed mutagen.

Staining and Visualization of DNA - InstaStain® Ethidium Bromide

PHOTODOCUMENTATION OF DNA

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.

The following guidelines are for photographing gels stained with InstaStain® Ethidium Bromide, utilizing the EDVOTEK UV photodocumentation system (Cat. # 555). It is a relatively simple photodocumentation system comprised of a UV transilluminator, a 6 inch safety camera hood, and Polaroid camera fitted with a deep yellow Tiffin 40.5 mm filter. The camera uses Polaroid 667 Black and White film. The recommended settings can be used as a starting point, although optimal conditions for your system may vary.

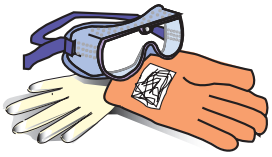
PHOTOGRAPHY GUIDELINES

1. To assemble the camera, screw the handle into the center hole at the base of the camera.
2. Align the hood onto the camera lens.
3. Carefully and firmly push down the buttons on the inside of the hood on both sides of the lens.
4. Load your Polaroid camera with Polaroid 667 Black and White film.
5. Open the safety cover of the transilluminator and place the gel on the surface of the filter.
6. Cover the gel with the camera hood so that the hood is aligned with the camera mounting plate.
7. Turn on the transilluminator and photograph.
   - Recommended camera setting is f 5.6 for 2 seconds.
   - If the photograph is too light, change the aperture to f 8 and expose for 2 seconds.
   - If too dark, reduce the shutter speed to 1 second at f 5.6.

For additional information, refer to the instructions which accompany your photodocumentation system.

Staining and Visualization of DNA - InstaStain® Methylene Blue

The Experiment



Wear gloves and safety goggles

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

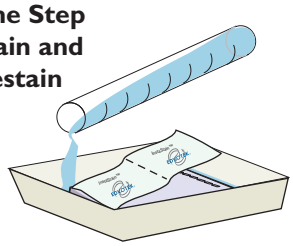
METHOD 1: ONE-STEP STAINING AND DESTAINING WITH INSTASTAIN® METHYLENE BLUE

Agarose gels can be stained and destained in one easy step with InstaStain™ Methylene 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

One Step Stain and Destain



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

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® METHYLENE BLUE CARDS AND GELS

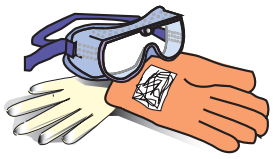
- Stained gels may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid.

  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.

Staining and Visualization of DNA - InstaStain® Methylene Blue



Wear gloves and safety goggles

METHOD 2: STAINING WITH INSTASTAIN® METHYLENE BLUE CARDS

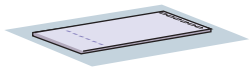
1. After electrophoresis, place the agarose gel on a flat surface covered with plastic wrap.
2. Wearing gloves, place the blue dye side of the InstaStain® Methylene Blue card on the gel.
3. Firmly run your fingers several times over the entire surface of the InstaStain® card to establish good contact between the InstaStain® card and the gel.
4. To ensure continuous contact between the gel and the InstaStain® card, place a gel casting tray and weight, such as a small empty beaker, on top of the InstaStain® card.
5. Allow the InstaStain® Methylene Blue to sit on the gel for 5 to 10 minutes.
6. After staining, remove the InstaStain® card.

   If the color of the gel appears very light, wet the gel surface with buffer or distilled water and place the InstaStain® card back on the gel for an additional 5 minutes.

Destaining and Visualization of DNA

7. Transfer the gel to a large weigh boat or small plastic container.
8. Destain with distilled water.*
   - Add approximately 100 ml of distilled water to cover the gel.

1



Place gel on a flat surface covered with plastic wrap.

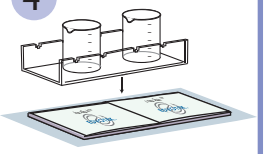
2

Place the InstaStain® card on the gel.

3

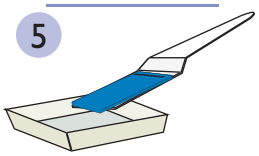
Press firmly.

4



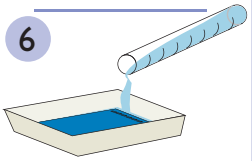
Place a small weight for approx. 5 minutes.

5



Transfer to a small tray for destaining.

6



Destain with 37°C distilled water.

The Experiment

InstaStain is a registered trademark of EDVOTEK, Inc. Patents Pending.

Staining and Visualization of DNA - InstaStain® Methylene Blue

9. Repeat destaining by changing the distilled water as needed.

   The larger DNA bands will initially be visible as dark blue bands against a lighter blue background. When the gel is completely destained, the larger DNA bands will become sharper and the smaller bands will be visible. With additional destaining, the entire background will become uniformly light blue.

10. Carefully remove the gel from the destain solution and examine the gel on a Visible Light Gel Visualization System. To optimize visibility, use the amber filter provided with EDVOTEK equipment.

11. If the gel is too light and bands are difficult to see, repeat the staining and destaining procedures.

* Destaining Notes

- Warmed distilled water at 37°C will accelerate destaining. Destaining will take longer with room temperature water.
- DO NOT EXCEED 37°C ! Warmer temperatures will soften the gel and may cause it to break.
- The volume of distilled water for destaining depends upon the size of the tray. Use the smallest tray available that will accommodate the gel. The gel should be completely submerged during destaining.
- Do not exceed 3 changes of water for destaining. Excessive destaining will cause the bands to be very light.

STORAGE AND DISPOSAL OF INSTASTAIN® METHYLENE 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.

Study Questions

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

1. What is polymorphic DNA? How is it used for identification purposes?
2. What is CODIS? How is it used to solve crimes?
3. What is an STR? A VNTR? Which (STR or VNTR) is predominantly used in law enforcement? Why?

Experiment Notes

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 extract chromosomal DNA, 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 Electrophoresis Experiments are easy to perform and are 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.

Laboratory Extensions and Supplemental Activities

Laboratory extensions are easy to perform using EDVOTEK experiment kits. For laboratory extension suggestions, please check the EDVOTEK website, which is updated on a continuous basis with educational activities and resources.

Online Ordering now available

www.edvotek.com

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

Technical Service Department

EDVO-TECH SERVICE
1-800-EDVOTEK
(1-800-338-6835)
Mon - Fri 9 am - 6 pm ET

Mon - Fri
9:00 am to 6:00 pm ET

FAX: (301) 340-0582
Web: www.edvotek.com
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**

Instructor's Guide

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 two modules:

i. PCR Amplification of Crime Scene & Suspect DNA
II. Separation of PCR Reactions by Electrophoresis

APPROXIMATE TIME REQUIREMENTS

DNA Amplification

DNA Amplification (35 PCR cycles) will take about 70-90 minutes or can be processed overnight and held at 4°C.

Agarose Gel Preparation

There are several options for preparing agarose gels for the electrophoresis experiment. Your schedule will determine when to prepare the gel(s) for the experiment. Whether you choose to prepare the gel(s) or have the students do it, allow approximately 30-40 minutes for this procedure. Generally, 20 minutes of this time is required for gel solidification.

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

Notes to the Instructor:

Agarose Gel Electrophoresis

The approximate time for electrophoresis will vary from 55 minutes to 5 hours, depending on the power supply voltage.

Generally, the higher the voltage applied the faster the samples migrate. However, the maximum amount of voltage significantly depends upon the design of the electrophoresis apparatus and should not exceed manufacturers 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

OPTIONAL STOPPING POINTS

The experiment can be temporarily stopped after the completion of DNA Amplification (Module I) 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 the procedural instructions.

STAINING AND VISUALIZATION OF PCR PRODUCTS AFTER AGAROSE GEL ELECTROPHORESIS

For this experiment, optimal visualization will be obtained by staining gels with InstaStain® Ethidium Bromide cards, which are included in this experiment. Staining of higher concentration agarose gels (1.0% or higher) require more care to obtain visible and clear results.

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 guideline for chemical waste.

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

LABORATORY NOTEBOOKS

It is highly recommended that students maintain a laboratory notebook to formulate hypotheses and to record experimental procedures and results.

Pre-Lab Preparations

NOTE:
There is enough template DNA (Components D-G) for each group to perform a unique forensic crime scene scenario.

There is enough material to perform 25 PCR reactions and 5 gels. Students can be divided into groups of five students per group and samples from each group can be run on a gel.

There are four different DNA samples provided. This can be an open-ended experiment that students can design – you can designate one sample as the crime scene for one group and a different sample as the crime scene for another group. Make sure to repeat the crime scene DNA as one of the suspect DNAs for the respective groups. Alternatively, students can design their own crime scene scenario and designate the DNAs for the crime scene and suspects.

1. For the open-ended experiments, one of the four samples has to be assembled in duplicate - one of the reactions will be designated as the evidence collected at the scene of the crime and the second will be used as a possible suspect.

2. Thaw the frozen materials and immediately place on ice.

3. For the assigned samples for crime scene and suspects, aliquot and gather the materials outlined below (keep DNAs and primers on ice):

   7 µl Crime scene DNA (choose DNA Template #1, #2, #3, or #4)
   7 µl Suspect #1 DNA (DNA Template #1)
   7 µl Suspect #2 DNA (DNA Template #2)
   7 µl Suspect #3 DNA (DNA Template #3)
   7 µl Suspect #4 *DNA (DNA* Template #4)
   120 µl Primer mix
   30 µl 200 base-pair ladder
   5 PCR beads (in tubes)
   50 µl 10X Gel Load Solution

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 ".
- 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".

Quantity Preparations for Agarose Gel Electrophoresis

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

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

Batch Preparation of 1.0% UltraSpec-Agarose™

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

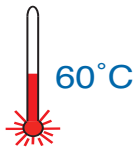
BATCH AGAROSE GELS (1.0%)

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

1. Use a 500 ml (or larger) flask to prepare the diluted gel buffer
2. Pour 4.0 grams of UltraSpec-Agarose™ into 400 ml of prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.

Note: The UltraSpec-Agarose™ kit component is often labeled with the amount it contains. In many cases, the entire contents of the bottle is 3.0 grams. 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.

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.

Experiment Results and Analysis

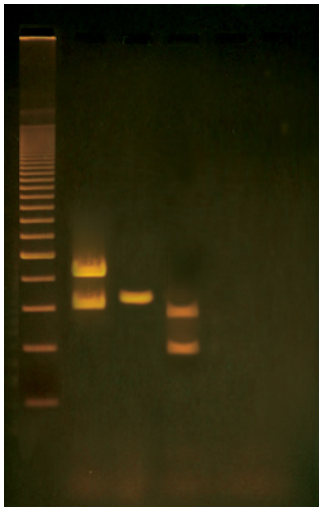


Photo of Gel Results

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.

Study Questions and Answers

1. **What is polymorphic DNA? How is it used for identification purposes?**

   Polymorphic DNA refers to chromosomal regions that vary widely from person to person. This variation is usually in the length of a specific DNA region. By analyzing a number of these regions, one may obtain a "DNA fingerprint" of a person that is extremely unlikely to match the DNA fingerprint of any other individual. DNA fingerprinting is used for the identification of missing persons, human remains, and matching criminal suspects to crime scenes.

2. **What is CODIS? How is it used to solve crimes?**

   CODIS is an acronym for the **Co**mbined **D**NA **I**ndex **S**ystem, a computer-based database containing DNA fingerprints. In the convicted offender database, DNA profiles of convicted felons are maintained. In the forensic database, DNA fingerprints from crime scenes are maintained.

3. **What is an STR? A VNTR? Which (STR or VNTR) is predominantly used in law enforcement? Why?**

   An STR is an acronym for a short tandem repeat, a DNA sequence of 2-4 base pairs that is repeated variably from person to person. VNTRs, or variable number of tandem repeats, have longer repeat units of 15-70 base pairs. STRs are now preferred to VNTRs as the length of their amplified products requires less template DNA, often allowing even degraded samples to be amplified.

Appendix

Appendix: PCR Experimental Success Guidelines

EDVOTEK experiments which involve the extraction and amplification of DNA for fingerprinting 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.

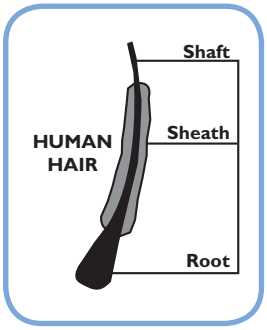
DNA Extraction and Sample Preparation

CELL PREPARATION:

1. **Sufficient Cells:** It is critical that there are sufficient cells to obtain enough DNA that will yield positive DNA fingerprinting 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. **Human (Self) DNA Fingerprinting:** Cells obtained from human sources, such as cheek cells, need to be harvested cautiously. Aerosol can result and cross-contamination among students can be a health hazard. Hair follicles do not pose the same problem and yield sufficient DNA required for the PCR reaction.

3. **Hair Cells:** At least four (4) hair follicles are needed. The **preferred** source is hair from eyebrows. Use only hairs containing a sheath, a barrel-shaped structure (often white in color) encircling the shaft near the base of the hair (see figure at left). Centrifuge the hair follicles to the bottom of the microcentrifuge tube to ensure direct contact with the reagents used in subsequent steps.



4. **Cheek Cells:** A white pellet must be visible after centrifuging the cell suspension obtained from cheek cell swabbing. If necessary, repeat the centrifugation to obtain a visible pellet. After removal of the supernatant, suspend the pellet in the chelating agent by repeated vortexing and pipetting up and down.

5. **Chelating Agent:** Chelating agent removes Mg (required by DNA-degrading nucleases and DNA polymerases). The small beads must be suspended in the buffer prior to delivery to the cells (i.e., mix the chelating agent just before you transfer it to the tube containing the cells.

6. **Boiling:** The boiling step for 10 minutes is required to obtain cell lysis. Boiling will not degrade the DNA and nucleases will NOT degrade DNA in the absence of Mg.

7. **Centrifugation:** Centrifuge the cell suspension carefully after cooling. If the pellet loosens, repeat this 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.

8. **DNA Transfer:** Transfer the DNA to a new microcentrifuge tube very carefully. It is the step prior to the PCR reaction. If any chelating agent beads (as few as one or two) are transferred, they can easily trap the Mg required by the *Taq* DNA polymerase as a cofactor for catalysis. As an additional precaution, centrifuge the supernatant a second time.

   Remember: Any carry-over of chelating agent to the PCR reaction will not yield results.

Appendix: PCR Experimental Success Guidelines

THE PCR REACTION

9. **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.

10. **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.

11. **Oil or Wax:** For certain thermal cyclers which do not have a top heating plate, it is necessary to overlay the reaction in the microcentrifuge tubes with oil or wax to prevent evaporation.

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

GEL PREPARATION AND STAINING

13. **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.

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

15. **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 (300nm) transilluminator. Repeat the staining as required.
    - Gels stained with InstaStain® Methylene Blue or liquid methylene blue stain may fade with time. Re-stain the gel to visualize the DNA bands.

16. **DNA 200 bp markers:** After staining the agarose gel, the DNA 200 bp markers should be visible after staining. If there are visible bands in the markers and control lanes, but bands in the sample lanes are faint or absent, it is possible that the DNA was not successfully extracted from the cells. If markers, control and DNA bands are all faint or absent, problems could potentially be due to improper preparation of the gel, absence of buffer in the gel, improper gel staining or a dysfunctional electrophoresis unit or power source.

Appendix

Appendix:
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.

PREPARATION OF 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™
     (Each pellet contains *Taq* DNA polymerase, four deoxytriphosphates, Mg^{+2} and buffer.)
   - 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. The three-waterbath PCR method requires three separate waterbaths, each set at different temperatures. The PCR reaction sample is sequentially cycled between the three waterbaths 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. A typical PCR cycle might be set up as follows:

   94°C for 1 minute
   45°C for 1 minute
   72°C for 1 minute

   It is imperative that the 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 a tube falls on the lab bench or floor, 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.

   Please refer to the Appendix entitled "PCR Samples with Wax Overlays" for sample handling and preparation tips.

Appendix:
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 or pellet 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™
   (Each pellet contains Taq DNA polymerase, four deoxytriphosphates, Mg^{+2} and buffer.)

   • 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 ELECTROPHORESIS:

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.

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: 07/01/03

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	94 F	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: 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 (General) 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

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 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: 05/01/06

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)
Tris (hydroxymethyl) aminoethane CAS# 77-86-1				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 1)	Not available
Vapor Pressure (mm Hg.)	Negligible	Melting Point	171°C
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	Negligible

Solubility in Water: Soluble

Appearance and Odor: White crystalline solid

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
Not Applicable			

Extinguishing Media: Water spray, dry chemical, carbon dioxide

Special Fire Fighting Procedures: Wear SCBA apparatus and protective clothing

Unusual Fire and Explosion Hazards: Fire or excessive heat may producehazardous decomposition products.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	

Incompatibility: Strong oxidizers

Hazardous Decomposition or Byproducts: Combustion will produce carbon dioxide and probably carbon monoxide.

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Low harard/inhalation, skin, ingestion

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

Signs and Symptoms of Exposure: No data

Medical Conditions Generally Aggravated by Exposure: None

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: Ventilate area and wash spill area

Waste Disposal Method: Dispose by incineration or with licenses chemical waste disposal

Precautions to be Taken in Handling and Storing: None

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Supplimentary ventilation or respiratory protection

Ventilation			
	Local Exhaust	Yes	Special
	Mechanical (General)	Yes	Other

Protective Gloves: Yes Eye Protection: Yes

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): **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: 07/01/03

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: 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): 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: 12-01-05

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: 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.

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® 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: 05/01/06

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)	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			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide, nitrogen oxides, hydrogen bromide 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): 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			Special	
	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): **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: 12-01-05

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_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 - 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			Special	
	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