EDVOTEK®

The Biotechnology Education Company ®

EDVO-Kit

109

DNA Fingerprinting: Identification of DNA Restriction Fragmentation Patterns

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to develop a basic understanding of DNA fingerprinting. Variations in restriction enzyme cleavage patterns obtained from different DNA molecules will be analyzed and the possible perpetrator of a crime will be identified using the logic of DNA fingerprinting.

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EVT 100202AM

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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.

Experiment Components

DNA samples are stable at room temperature. However, if the experiment will not be conducted within one month of receipt, it is recommended that the DNA samples be stored in the refrigerator.

DNA samples do not require heating prior to gel loading.

READY-TO-LOAD™ DNA SAMPLES FOR ELECTROPHORESIS

A DNA from crime scene cut with Enzyme 1
B DNA from crime scene cut with Enzyme 2
C DNA from Suspect 1 cut with Enzyme 1
D DNA from Suspect 1 cut with Enzyme 2
E DNA from Suspect 2 cut with Enzyme 1
F DNA from Suspect 2 cut with Enzyme 2

REAGENTS & SUPPLIES

- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- FlashBlue™ DNA Stain
- InstaStain® Blue cards
- Practice Gel Loading Solution
- 1 ml pipet
- Microtipped Transfer Pipets

Note: If you ordered Experiment #109-Q, the experiment components include InstaStain® Ethidium bromide instead of FlashBlue™ and InstaStain® Blue DNA stains.

Requirements

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

Background Information

DNA typing (also called DNA profile analysis or DNA fingerprinting) is the process whereby the genomic DNA of an organism is analyzed by examining several specific, variable DNA sequences located throughout the genome. In humans, DNA fingerprinting is now used routinely for identification purposes.

Human DNA fingerprinting was pioneered by Dr. Alex Jeffreys at the University of Leicester in 1984 which led to the apprehension of a murderer in the first DNA fingerprinting conviction in September 1987 in the UK. Two months later, the first U.S. conviction based on DNA fingerprinting occurred in Orlando, Florida. Since then, the use of DNA fingerprinting has led to thousands of criminal convictions, as well as dozens of exonerations.

In contrast to earlier methodologies, such as blood typing which can only exclude a suspect, DNA fingerprinting can provide positive identification with great accuracy. In addition to criminal identification cases, DNA fingerprinting is now used routinely in paternity determinations and for the identification of genetic disease "markers". It is also used for the identification of human remains, such as in war casualties, and was used extensively to identify victims of the September 11, 2001 terrorist attacks on the World Trade Center, the Pentagon, and passengers in the plane which crashed in a field near Shanksville, Pennsylvania.

Human cells contain two types of DNA. The first type is cellular chromosomal DNA, which is packaged in 23 sets of chromosomes in the nucleus of the cell. This DNA, obtained from both parents, reflects the combined parental genetic inheritance of an individual. DNA fingerprinting utilizing cellular DNA involves analysis of the sequence of two alleles for a particular gene.

The second type of DNA is different from cellular DNA and is present only in the mitochondria, which are the energy-producing organelles of the cell. Mitochondrial DNA is inherited maternally by both males and females and is extremely useful in the analysis of specific cases where fraternal linkages are important to determine. For example, a brother, sister, half brother or half sister who share the same mother would inherit the same mitochondrial DNA. Identification is determined by sequencing certain regions within mitochondrial DNA, which is a single circular chromosome composed of 16,569 base pairs.

DNA fingerprinting developed by Dr. Jeffreys utilizes cellular chromosomal DNA submitted to restriction enzyme digestion and Southern blot analysis. When human DNA is digested by a restriction enzyme, a very large number of DNA fragments are generated. When separated by agarose gel electrophoresis, the numerous DNA fragments appear as a "smear" on the gel. Labeled probes are used to detect Restriction Fragment Length Polymorphic (RFLP) regions within DNA, which will be described in greater detail. DNA RFLP analysis is statistically very accurate but requires relatively large amounts of DNA and takes several days to perform.

In recent years, the use of the RFLP method has been overtaken by the Polymerase Chain Reaction (PCR) method because of two important advantages. The first is the sensitivity of PCR, which allows for DNA fingerprinting identification using much smaller amounts of DNA. This is because PCR is able to amplify DNA to facilitate analysis. The second advantage is the speed of PCR analysis, which allows critical questions to be answered more quickly compared to Southern Blot analysis. One PCR cycle has three steps, resulting in a doubling of the amount of DNA (see Figure 1).

Background Information

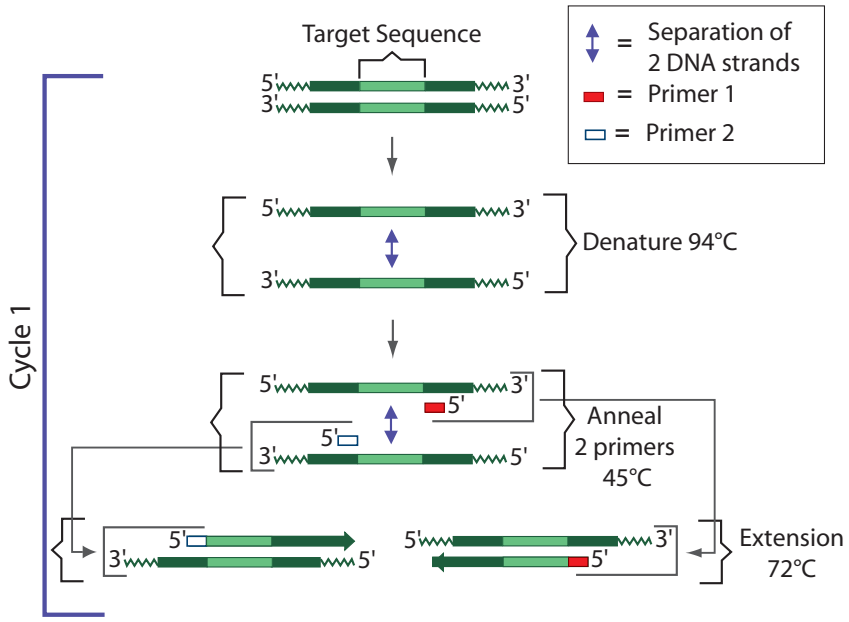


Figure 1: The Polymerase Chain Reaction

The Polymerase Chain Reaction (PCR) method amplifies target sequences of DNA, which are referred to as AMRFLPs. PCR made it possible for very small amounts of DNA found at crime scenes to be amplified for DNA fingerprinting analysis. A specific set of two primers is used to prime DNA polymerase to synthesize many copies of the targeted areas of DNA.

Many important concepts of molecular biology can be conveyed in the context of DNA Fingerprinting methods. In this experiment, emphasis is placed on concepts related to RFLP analysis. The experiment activities will focus on the identification of DNA by analyzing restriction fragmentation patterns separated by agarose gel electrophoresis.

Background Information

USE OF RESTRICTION ENZYMES IN DNA FINGERPRINTING

DNA fingerprinting involves the electrophoretic analysis of DNA fragment sizes generated by restriction enzymes. Restriction enzymes are endonucleases which catalyze the cleavage of phosphodiester bonds within both DNA strands. The sites of cleavage occur in or near very specific palindromic sequences of bases called recognition sites, which are generally 4 to 8 base pairs in length.

The two most commonly used restriction enzymes for DNA profile analysis are *Hae* III and *Hinf* I, which are 4-base and 5-base cutting enzymes. The examples in the figure 2 show recognition sites for various restriction enzymes.

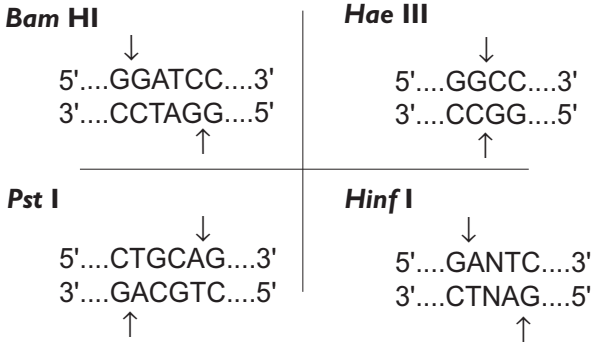


Figure 2: Restriction enzyme recognition sites

The size of the DNA fragments generated depends on the distance between the recognition sites. In general, the longer the DNA molecule, the greater the probability that a given recognition site will occur. Human DNA is very large and contains approximately three billion base pairs. A restriction enzyme having a 6-base pair recognition site, such as *Eco* RI, would be expected to cut human DNA into approximately 750,000 different fragments.

DNA is highly polymorphic - that is, no two individuals have exactly the same pattern of restriction enzyme recognition sites in their DNAs. A large number of alleles exist in the population. Alleles, which are alternate forms of a gene, result in alternative expressions of genetic traits which can be dominant or recessive.

Chromosomes occur in matching pairs, one of maternal and the other of paternal origin. The two copies of a gene (alleles) at a given chromosomal locus represent a composite of the parental genes constituting an individual's unique genotype. It follows that alleles have differences in their base sequences which consequently creates differences in the distribution and frequencies of restriction enzyme recognition sites. Other differences in base sequences between individuals can occur because of mutations and deletions. Such changes can also create or eliminate a recognition site.

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 obtain a "DNA fingerprint" for that individual. The most commonly used polymorphisms are those that vary in length; these are known as Fragment Length Polymorphisms (FLPs). The main reason for the occurrence of RFLPs is because of variations in length of a given segment of genomic DNA between two restriction enzyme recognition sites among individuals of the same species.

Background Information

Likewise, RFLP can occur in "intergenic" or noncoding regions of DNA and is known as Variable Number of Tandem Repeats (VNTRs). In this case, segments of DNA that contain sequences from 2 to 40 bases in length repeat in tandem manner many times. The number of segments or "core unit" repeats varies among individuals of the same species while the restriction enzyme cut sites are not altered. VNTR loci are very polymorphic. There are potentially hundreds of alleles at a single locus and therefore they are very useful in DNA fingerprinting. Ten to fifteen percent of mammalian DNA consists of sets of repeated, short sequences of bases that are tandemly arranged in arrays. The length of these arrays (the amount of repeated sets) varies between individuals at different chromosomal loci.

TGTTTA|TGTTTA|TGTTTA.....variable number

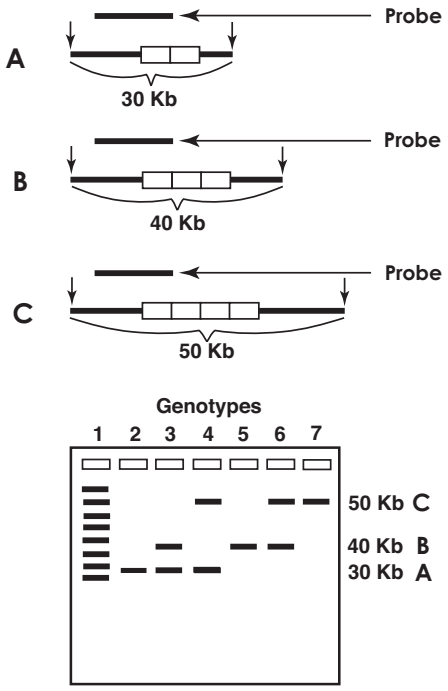
When these sequences in DNA are flanked by recognition sites, the length of the repeat will determine the size of the restriction enzyme fragment generated. There are several types of these short, repetitive sequences and they have been characterized.

DNA FINGERPRINTING USING SOUTHERN BLOTS

Agarose gel electrophoresis is a procedure used to analyze DNA fragments generated by restriction enzymes. The gel consists of microscopic pores that act as a molecular sieve. Samples of DNA are loaded into wells made in the gel during casting. Since DNA has a negative charge at neutral pH, it migrates through the gel towards the positive electrode during electrophoresis. DNA fragments are separated by the gel according to their size. The smaller the fragment the faster it migrates. After electrophoresis, the DNA can be visualized by staining the gel with dyes. Restriction enzyme cleavage of relatively small DNA molecules, such as plasmids and viral DNAs, usually results in discrete banding patterns of the DNA fragments after electrophoresis. However, cleavage of large and complex DNA, such as human chromosomal DNA, generates so many differently sized fragments that the resolving capacity of the gel is exceeded. Consequently, the cleaved DNA is visualized as a smear after staining and has no obvious banding patterns.

RFLP analysis of genomic DNA is facilitated by Southern Blot analysis. After electrophoresis, the DNA fragments in the gel are denatured by soaking in an alkali solution. This causes double-stranded DNA fragments to be converted into single-stranded form (no longer base-paired in a double helix). A replica of the electrophoretic pattern of DNA fragments in the gel is made by transferring (blotting) them to a sheet of nylon membrane. This is done by placing the membrane on the gel after electrophoresis and transferring the fragments to the membrane by capillary action or suction by vacuum. The DNA, which is not visible, becomes permanently adsorbed to the membrane, and can be manipulated easier than gels.

Background Information



Probe overlaps both the variable region, as well as adjacent part of the genome. Arrows show restriction enzyme sites with probe for Southern Blot analysis. PCR can also be used to detect variable nucleotide regions.

Lane 1	DNA Marker
Lane 2	Homozygous Copies
Lane 3	Heterozygous VNTR
Lane 4	Heterozygous VNTR
Lane 5	Homozygous Copies
Lane 6	Heterozygous VNTR
Lane 7	Homozygous Copies

(Lanes 3, 4, and 6 represent different combinations of the three VNTRs.)

Figure 3: RFLP analysis demonstrating Variable Numbers of Nucleotide Tandem Repeats (VNTR).

Analysis of the blotted DNA is done by hybridization with a labeled DNA probe. In forensic RFLP analysis, the probe is a DNA fragment that contains base sequences which are complementary to the variable arrays of tandemly repeated sequences found in the human chromosomes. Probes can be labeled with isotopic or non-isotopic reporter molecules, such as fluorescent dyes used for detection. A solution containing the single-stranded probe is incubated with the membrane containing the blotted, single-stranded (denatured) DNA fragments. Under the proper conditions, the probe will only base pair (hybridize) to those fragments containing the complementary repeated sequences. The membrane is then washed to remove excess probe. If the probe is isotopically labeled to the membrane, it is then placed on an x-ray film for several hours. This process is known as autoradiography. Only DNA fragments that have hybridized to the probe will reveal their positions on the film because the localized areas of radioactivity cause exposure. The hybridized fragments appear as discrete bands (fingerprint) on the film and are in the same relative positions as they were in the agarose gel after electrophoresis. Only specific DNA fragments, of the hundreds of thousands of fragments present, will hybridize with the probe because of the selective nature of the hybridization (base pairing) process.

In forensic cases, DNA samples can be extracted and purified from small specimens of skin, blood, semen, or hair roots collected at the crime scene. DNA that is suitable for analysis can also be obtained from dried stains of semen and blood. The RFLP analyses performed on these samples is then compared to samples obtained from the suspect. If the RFLP patterns match, it is then beyond reasonable doubt that the suspect was at the crime scene. In practice, several different probes containing different types of repetitious sequences are used in the hybridizations in order to satisfy certain statistical criteria for absolute, positive identification. To assure positive identification in criminal cases, 13 different loci are compared between a suspect and evidence DNA obtained from the crime scene.

Background Information

In this experiment, DNAs are pre-digested by restriction enzymes and the fragmentation patterns serve as the individual fingerprint. The DNA fragmentation patterns can be analyzed directly in the stained agarose gel, which eliminates the need for a Southern blot. In this hypothetical case, DNA obtained from two suspects are cleaved with two restriction enzymes in separate reactions. The objective is to analyze and match the DNA fragmentation patterns after agarose gel electrophoresis and determine if Suspect 1 or Suspect 2 was at the crime scene.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA.

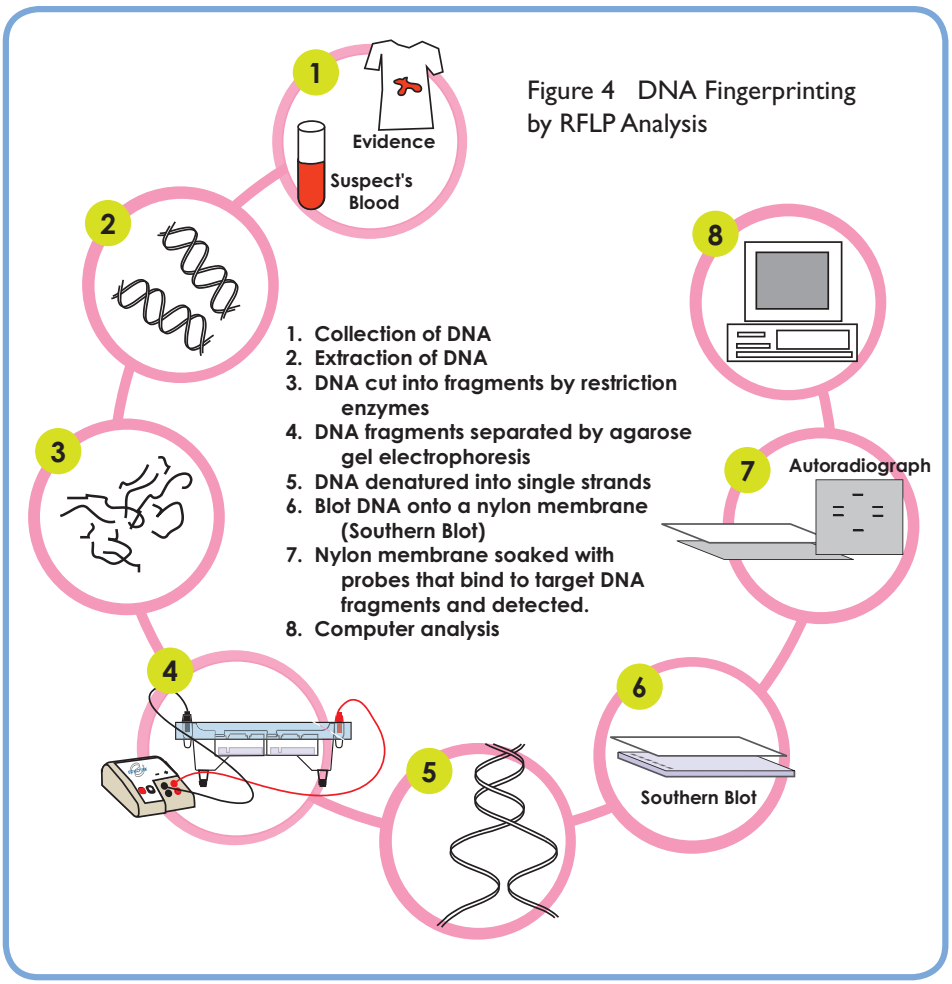


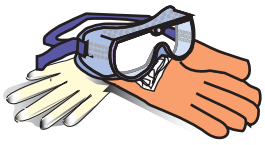
Figure 4 DNA Fingerprinting by RFLP Analysis

Experiment Overview and General Instructions

Experiment Procedure

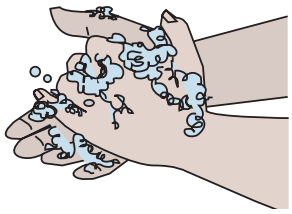
EXPERIMENT OBJECTIVE:

The objective of this experiment is to develop a basic understanding of DNA fingerprinting. Variations in restriction enzyme cleavage patterns obtained from different DNA molecules will be analyzed and the possible perpetrator of a crime will be identified using the logic of DNA fingerprinting.



LABORATORY SAFETY

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



LABORATORY NOTEBOOK RECORDINGS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

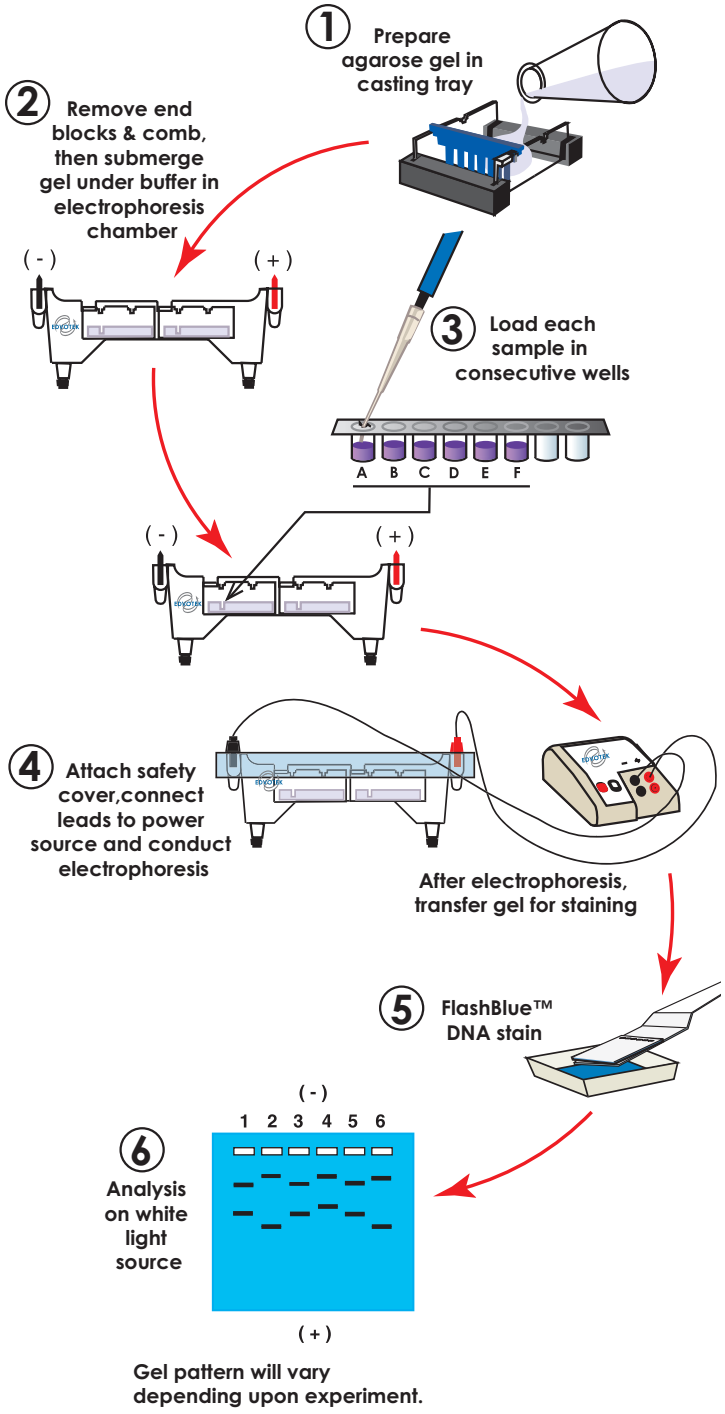
During the Experiment:

- Record (draw) your observations, or photograph the results.

Following the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Experiment Overview: Flow Chart



Experiment Procedure

Agarose Gel Electrophoresis

For gels to be stained with FlashBlue™ or InstaStain® Blue, prepare gels according to Appendix A.

For gels to be stained with InstaStain® Ethidium bromide, prepare gels according to Appendix B.

Step-by-step guidelines for agarose gel preparation are summarized in Appendix D.

Prepare the Gel

1. Prepare an agarose gel with specifications summarized below. Your instructor will specify which DNA stain you will be using.

• Agarose gel concentration required:	0.8%
• Recommended gel size:	7 x 7 cm or 7 x 14 cm (two gels)
• Number of sample wells required:	6
• Placement of well-former template:	first set of notches (7 x 7 cm) first & third set of notches (7 x 14 cm)

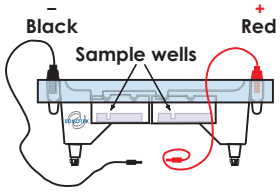
Load the Samples

2. Load the DNA samples in tubes A - F into the wells in consecutive order.
   - For gels to be stained with FlashBlue™ or InstaStain® Blue, fill wells with 35 - 38 µl.
   - For gels to be stained with InstaStain® Ethidium Bromide, fill wells with 18 - 20 µl.

Lane	Tube
A	DNA from crime scene cut with Enzyme 1
B	DNA from crime scene cut with Enzyme 2
C	DNA from Suspect 1 cut with Enzyme 1
D	DNA from Suspect 1 cut with Enzyme 2
E	DNA from Suspect 2 cut with Enzyme 1
F	DNA from Suspect 2 cut with Enzyme 2

Reminders:

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.



Run the Gel

3. After DNA samples are loaded, connect the apparatus to the D.C. power source and set the power source at the required voltage.

4. Check that current is flowing properly - you should see bubbles forming on the two platinum electrodes. Conduct electrophoresis for the length of time specified by your instructor.

5. After electrophoresis is completed, proceed to DNA staining and visualization. Refer to Appendix E, F, G, or H for the appropriate staining instructions.

6. Document the results of the gel by photodocumentation.

   Alternatively, place transparency film on the gel and trace it with a permanent marking pen. Remember to include the outline of the gel and the sample wells in addition to the migration pattern of the DNA bands.

Material Safety Data Sheets

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

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): **Practice Gel Loading Solution**

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

Section I

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

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

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

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

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)

This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_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: 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	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: None

Hazardous Decomposition or Byproducts: Sulfur oxides, and bromides

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

Section VI - Health Hazard Data

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

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

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

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

Medical Conditions Generally Aggravated by Exposure: None reported

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

Section VII - Precautions for Safe Handling and Use

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

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

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

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

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

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Avoid eye and skin contact

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.**

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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	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): None

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

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

Medical Conditions Generally Aggravated by Exposure: None

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

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

Section VII - Precautions for Safe Handling and Use

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

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

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

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

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

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: None

EDVOTEK®

Material Safety Data Sheet

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

IDENTITY (As Used on Label and List): **Agarose**

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

Section I

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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	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: No data available

Hazardous Decomposition or Byproducts:

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Inhalation: No data available Ingestion: Large amounts may cause diarrhea

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

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

Waste Disposal Method: Normal solid waste disposal

Precautions to be Taken in Handling and Storing: None

Other Precautions: None

Section VIII - Control Measures

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

Ventilation	Local Exhaust		Special		
	Mechanical	Gen. dilution ventilation	Other		
Protective Gloves	Yes		Eye Protection	Splash proof goggles	

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

Work/Hygienic Practices: None

EDVOTEK®

Material Safety Data Sheet

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

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

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

Section I

Manufacturer's Name: EDVOTEK, Inc.

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

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 03-26-09

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Methylene Blue			No data available	
3,7 Bis (Dimethylamino) Phenothiazin-5 IUM Chloride CAS # 61-73-4				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity ($H_2$0 = 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): No data available	Flammable Limits: No data	LEL	UEL: 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	
	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	May Occur		Conditions to Avoid	
	Will Not Occur	X		None

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): 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 PER FEAC 47 30420 82

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically

Section VII - Precautions for Safe Handling and Use

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

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

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

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type)	MIOSH/OSHA approved, SCBA				
Ventilation	Local Exhaust		Special		
	Mechanical (General)	Required	Other		
Protective Gloves	Rubber		Eye Protection	Chem. safety goggles	

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices

EDVOTEK®

Material Safety Data Sheet

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

IDENTITY (As Used on Label and List): 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.

Address (Number, Street, City, State, Zip Code): 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_2$0 = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Chemical bound to paper, no odor

Section IV - Physical/Chemical Characteristics N.D. = No data

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

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

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

Unusual Fire and Explosion Hazards: Emits toxic fumes

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid	
	Stable	X		None

Incompatibility: Strong oxidizing agents

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

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Acute: Material irritating to mucous membranes, upper respiratory tract, eyes, skin

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

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

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

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

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

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

Other Precautions: Mutagen

Section VIII - Control Measures

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

Other Protective Clothing or Equipment: Rubber boots

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

EDVOTEK Series 100 Electrophoresis Experiments:

Cat. #	Title
101	Principles and Practice of Agarose Gel Electrophoresis
102	Restriction Enzyme Cleavage Patterns of DNA
103	PCR - Polymerase Chain Reaction
104	Size Determination of DNA Restriction Fragments
105	Mapping of Restriction Sites on Plasmid DNA
109	DNA Fingerprinting - Identification of DNA by Restriction Fragmentation Patterns
112	Analysis of *Eco* RI Cleavage Patterns of Lambda DNA
114	DNA Paternity Testing Simulation
115	Cancer Gene Detection
116	Sickle Cell Gene Detection (DNA-based)
117	Detection of Mad Cow Disease
118	Cholesterol Diagnostiics
124	DNA-based Screening for Smallpox
130	DNA Fingerprinting - Amplification of DNA for Fingerprinting