EDVOTEK®

The Biotechnology Education Company ®

EDVO-Kit

105

Mapping of Restriction Sites on Plasmid DNA

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of the principles of DNA mapping using various restriction enzymes to generate DNA fragments.

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EVT 100202AM

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.

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 Standard DNA Fragments
B Plasmid cut with Enzyme 1
C Plasmid cut with Enzyme 2
D Plasmid cut with Enzyme 1 and 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 #105-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

The Human Genome and other genome projects are extremely significant accomplishments with important applications to biology and medicine. The explosion of this new information is leading to dramatic changes in the way we are able to improve life. Part of the challenge in dealing with the enormous amounts of data is to determine what genes are responsible for different functions. Scientists must determine the location of genes through DNA mapping, and then begin the arduous task of determining what the individual genes do.

Mapping the positions of restriction enzyme cleavage sites on a DNA molecule is an important prerequisite to DNA sequencing, which provides the primary nucleotide sequence information in DNA. Mapping involves the determination of the relative distances between restriction enzyme cleavage sites. An illustrative analogy would be somewhat similar to the following: If DNA mapping were compared to identifying the streets on a city map, then DNA sequencing would be analogous to identifying the specific houses on the streets.

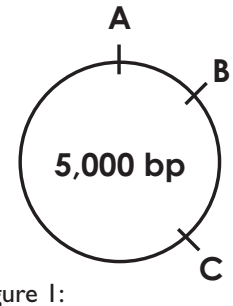


Figure 1:
Plasmid with single restriction enzyme sites for three enzymes - A, B, & C.

DNA mapping is performed by determining the size of the DNA fragments generated by single or combinations of restriction enzyme digestions, and subsequent construction of a DNA map. For example:

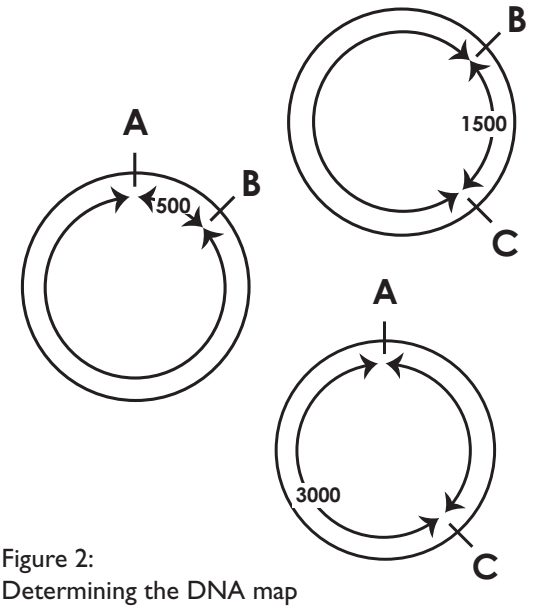


Figure 2:
Determining the DNA map based on experimental DNA fragments obtained.

- Consider a 5000 base pair, circular plasmid DNA containing single recognition sites for enzymes A, B, and C (Figure 1).
- Going in a clockwise direction from A, the distances between

  A and B is 500
  B and C is 1500
  C and A is 3000

These assignments are made based on the size of the entire circular plasmid, which is 5,000 base pairs (Figure 2).

To obtain a reference point, the cleavage site at A will be arbitrarily assigned as position zero. All three enzymes will cleave the plasmid once to produce a linear molecule of 5000 base pairs. Different combinations of these enzymes will produce the following DNA fragments (in base pairs):

A+B	A+C	B+C	A+B+C
4500	3000	3500	3000
500	2000	1500	1500
			500

Background Information

This data shows that the cleavage site at B is closest to A since cleavage A+B generated the smallest fragment (500) out of all the pairs of enzymes. The shortest distance between A and C is 2000 base pairs since the smallest fragment in the A + C pair is 2000. Similarly, the shortest distance between B and C is 1500 base pairs. It remains to be determined if B is in between A and C (Figure 1) or alternatively, B is between C and A (going in a clockwise direction from A around the plasmid, Figure 3).

If C was in between A and B, the 500 base pair fragment would have been cleaved into two smaller fragments. However, when all three enzymes are used, the 500 base pair fragment remains. In addition, only the 2000 base pair fragment found in the A + C pair is cleaved into 1500 and 500 base pair fragments when all three enzymes are used, verifying the location of B. This kind of logic enables the construction of a map, as previously shown, from DNA fragment sizes.

Note that the data from this experiment cannot tell us the absolute orientation of the cleavage sites since it can lead to an alternative map as shown in Figure 2. However, the relative positions are still the same (B is in between A and C). The assignment (Figure 1 or Figure 3) can be made upon further analysis.

Unknown DNA fragment sizes are determined by comparing the relative mobilities of DNA fragments of known size as standards. DNA fragments, from plasmid digests, and standard DNA fragments (also known as markers) are electrophoretically separated in parallel on the same agarose gel. After electrophoretic separation, DNA fragments are stained for visualization, and migration distances of known and unknown fragments are measured.

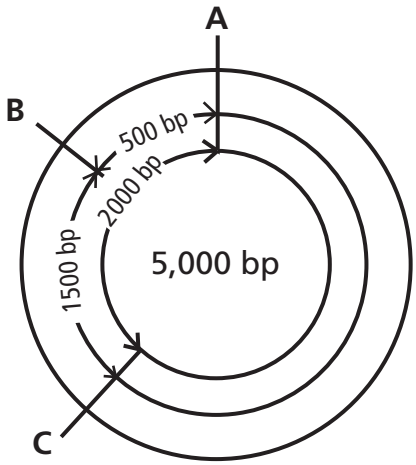


Figure 3: Construction of a plasmid map using three enzymes.

Background Information

Standard fragments are used to make a standard curve by plotting their size on the y-axis versus the migration distance on the x-axis. The size of the fragments on the y-axis are expressed as the log of the number of base pairs they contain or the log of their molecular weight. Most of the plotted data obtained from the markers will yield a straight line. The migration distance of the unknown DNA fragment(s) are located on the X-axis and their size is estimated from the standard curve.

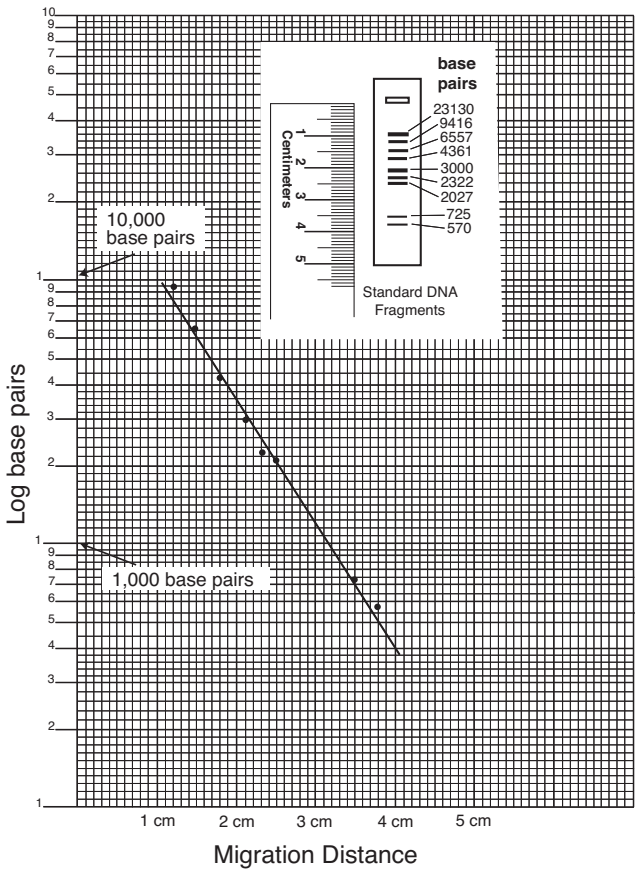
After determining the size of the DNA fragments generated by single and combinations of restriction enzymes, a DNA map is constructed as previously described.

In this experiment, you will determine the relative locations of three restriction enzyme cleavage sites on a circular plasmid DNA. The plasmid has been cleaved with three restriction enzymes. Enzyme 1 cleaves the plasmid once at site A. Assume that the Enzyme 1 site is at position 0. Enzyme 2 and 3 also cut the plasmid once at sites B and C. The objective is to calculate the distances in base pairs between the points of cleavage and to determine whether the Enzyme 1 site is in between the Enzyme 2 sites.

Quick Reference:

A standard curve will be made on semi-log graph paper. The following are the Standard DNA fragment sizes, which are expressed in base pairs.

23130	9416	6557
4361	3000	2322
2027	725	570

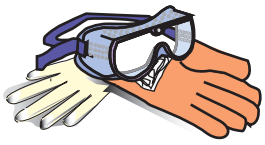


Experiment Overview and General Instructions

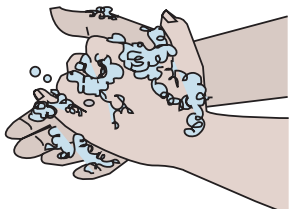
EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of the principles of DNA mapping using various restriction enzymes to generate DNA fragments.

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:

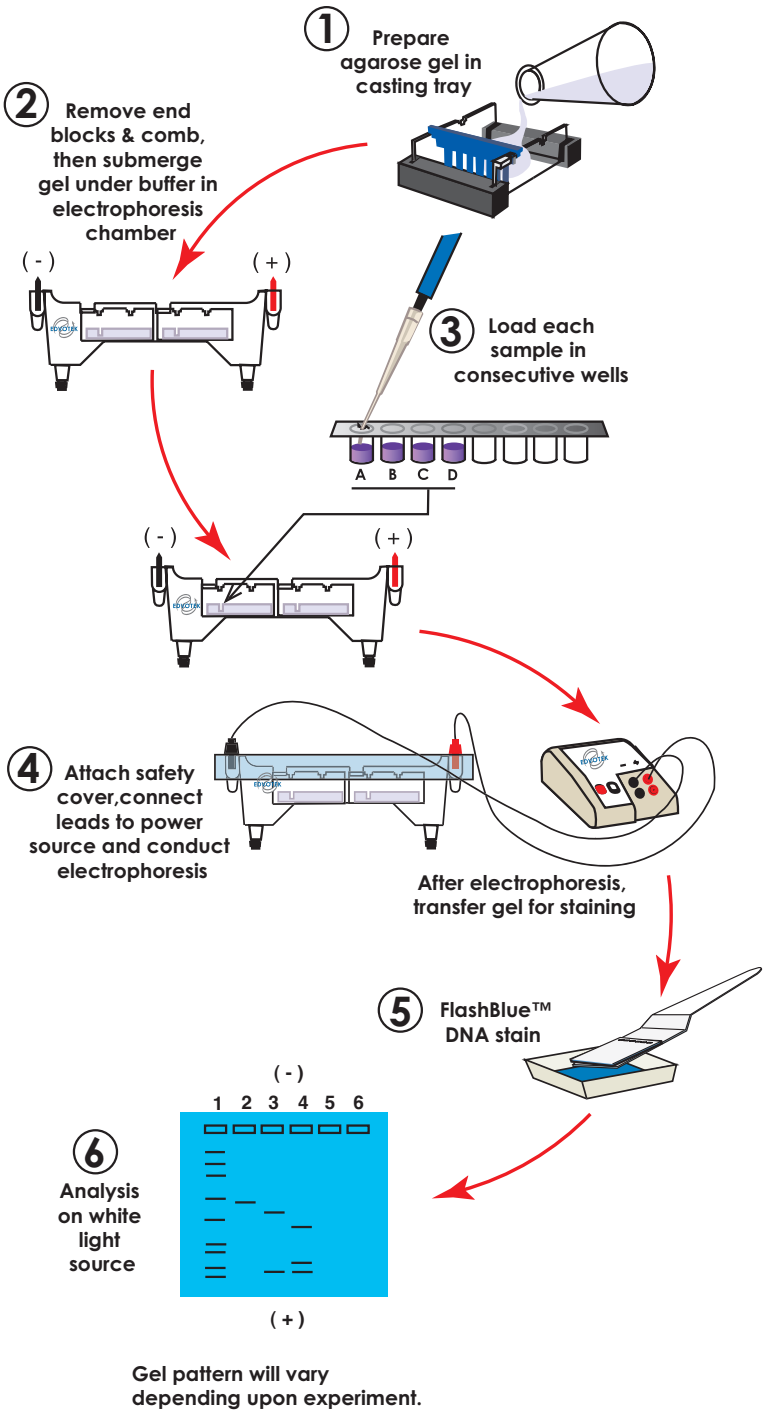
- Record (draw) your observations, or photograph the results.

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

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: 4
   - Placement of well-former template: first set of notches (7 x 7 cm)
     first & third set of notches (7 x 14 cm)

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.

Load the Samples

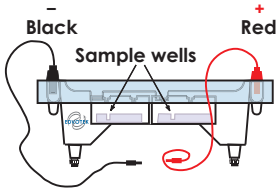
2. Load the DNA samples in tubes A - D into the wells in consecutive order.

   - 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	
1	A	Standard DNA Fragments
2	B	Plasmid cut with Enzyme 1
3	C	Plasmid cut with Enzyme 2
4	D	Plasmid cut with Enzyme 1 and 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.

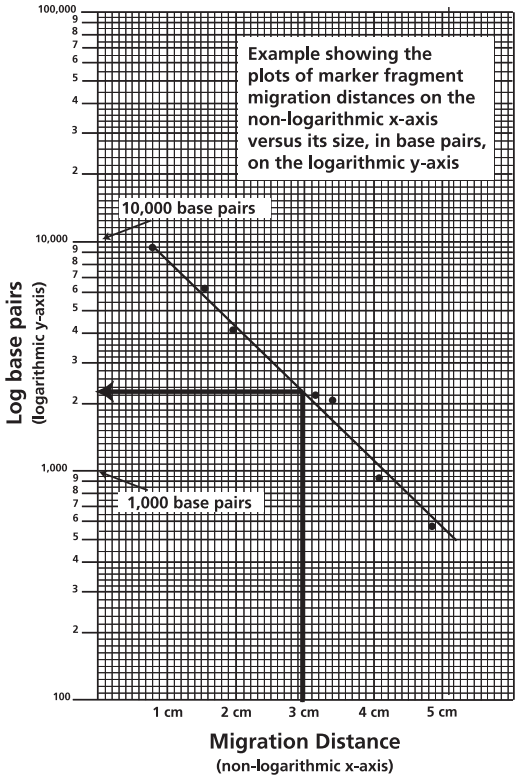
   Alternatively, place transparency film on the gel and trace it with a permanent marking pen. Remember to include the outline of the gel and the sample wells in addition to the migration pattern of the DNA bands.

Experiment Procedure

This exercise focuses on the first step for mapping DNA restriction sites, which is to determine the size of "unknown" DNA fragments generated after electrophoresis. The assignment of sizes for DNA fragments separated by agarose gel electrophoresis can have ± 10% margin of error. The sizes of the "unknowns" will be extrapolated by their migration distances relative to the Standard DNA Fragments (Sample A), for which the fragment sizes are known.

1. Measure and record the distance traveled in the agarose gel by each Standard DNA fragment (except the largest 23,130 bp fragment, which will not fit in a straight line in step 4).

Figure 1



In each case, measure from the lower edge of the sample well to the lower end of each band. Record the distance traveled in centimeters (to the nearest millimeter).

2. Label the semi-log graph paper:

   A. Label the non-logarithmic horizontal x-axis "Migration Distance" in centimeters at equal intervals.
   B. Label the logarithmic vertical y-axis "Log base pairs". Choose your scales so that the data points are well spread out. Assume the first cycle on the y-axis represents 100-1,000 base pairs and the second cycle represents 1,000-10,000 base pairs.

3. For each Standard DNA fragment, plot the measured migration distance on the x-axis versus its size in base pairs, on the y-axis.

4. Draw the best average straight line through all the points. The line should have approximately equal numbers of points scattered on each side of the line. Some points may be right on the line (see Figure 1 for an example).

5. Measure the migration distance of each of the "unknown" fragments from samples B, C, and D.

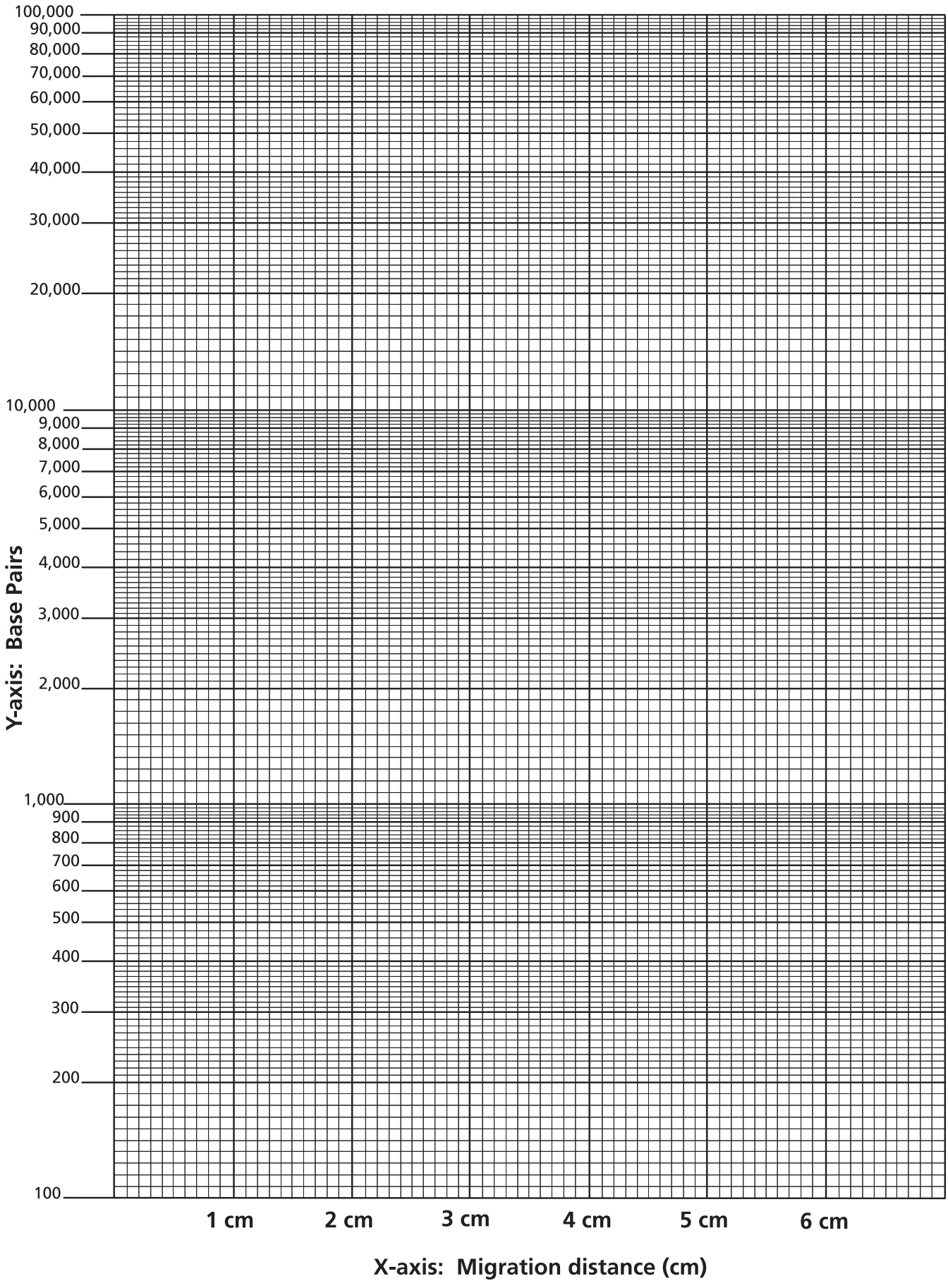
Quick Reference:

Standard DNA fragment sizes - length is expressed in base pairs.

23130	9416	6557
4361	3000	2322
2027	725	570

6. Using the graph of the Standard DNA fragments, determine the sizes in base pairs of each "unknown" fragment.

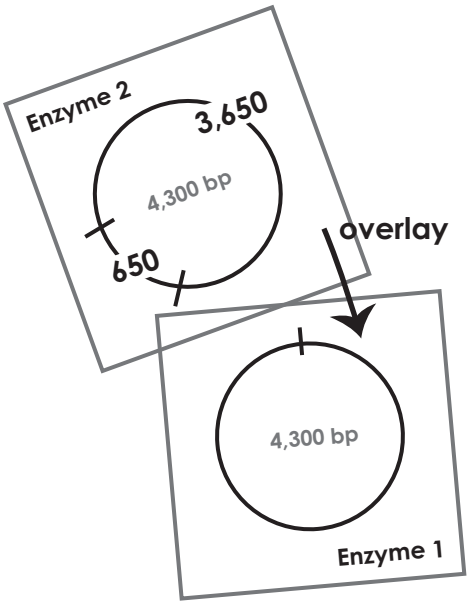
   - Find the migration distance of the unknown fragment on the x-axis. Draw a vertical line from that point until the standard graph line is intersected.
   - From the point of intersection, draw a second line horizontally to the y-axis and determine the approximate size of the fragment in base pairs (refer to Figure 1 for an example).


100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
900
800
700
600
500
400
300
200
100
Y-axis: Base Pairs
1 cm
2 cm
3 cm
4 cm
5 cm
6 cm
X-axis: Migration distance (cm)

Mapping of DNA Restriction Sites

The size of the plasmid used in this experiment is 4300 bp.

1. Draw a circle representing a 4300 bp plasmid on a transparent sheet of acetate.
2. Mark the positions of Enzyme #2 (Lane 3) sites corresponding to the sizes of fragments obtained upon digestion of the plasmid on the gel.
3. Draw a second circle representing a 4300 bp plasmid on a transparent sheet of acetate.
4. Mark the position of the Enzyme #1 (Lane 2) site at the top (12:00 o'clock).



5. To draw a composite map of both enzymes, overlay the Enzyme #2 map on top of the Enzyme #1 map.
6. Keeping the Enzyme #1 site at the 12:00 o'clock position, rotate the Enzyme #2 map until the relative distances between the sites approximate the relative sizes of the fragments of Enzyme #1 and #2 combined.
7. Specify, in base pairs, the distances between all the sites.

Experiment Procedure

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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.**

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

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

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

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

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

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

Section III - Physical/Chemical Characteristics

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

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

Appearance and Odor: Clear, liquid, slight vinegar odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

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

Extinguishing Media: Use extinguishing media appropriate for surrounding fire.

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

Unusual Fire and Explosion Hazards: None identified

Section V - Reactivity Data

Stability	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

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Material Safety Data Sheet

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

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

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

Section I

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

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

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

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

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

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

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

CAS #9012-36-6

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Insoluble - cold

Appearance and Odor: White powder, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

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

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

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

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability	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				
3.7 Bis (Dimethylamino) Phenothiazin 5 IUM Chloride	No data available			
CAS # 61-73-4				

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble - cold

Appearance and Odor: Chemical bound to paper, no odor

Section IV - Physical/Chemical Characteristics

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

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

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

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

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: None
	Stable	X	

Incompatibility: Strong oxidizing agents

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

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

Section VI - Health Hazard Data

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

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

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?
Meets criteria for proposed OSHA medical records rule PEREAC 47.30420.82

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically

Section VII - Precautions for Safe Handling and Use

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

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

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

Other Precautions: None

Section VIII - Control Measures

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

Ventilation	Local Exhaust	Special	
	Mechanical (General) Required	Other	

Protective Gloves	Rubber	Eye Protection	Chem. safety goggles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices:

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: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

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

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble

Appearance and Odor: Chemical bound to paper, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

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

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

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

Unusual Fire and Explosion Hazards: Emits toxic fumes

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: None
	Stable	X	

Incompatibility: Strong oxidizing agents

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

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

Section VI - Health Hazard Data

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

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

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

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

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

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

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

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

Other Precautions: Mutagen

Section VIII - Control Measures

Respiratory Protection (Specify Type): SCBA

Ventilation	Local Exhaust	Yes	Special	Chem. fume hood	
	Mechanical (General)	No	Other	None	

Protective Gloves	Rubber	Eye Protection	Chem. safety goggles

Other Protective Clothing or Equipment: Rubber boots

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

EDVOTEK Series 100 Electrophoresis Experiments:

Cat. #	Title
101	Principles and Practice of Agarose Gel Electrophoresis
102	Restriction Enzyme Cleavage Patterns of DNA
105	Mapping of Restriction Sites on Plasmid DNA
104	Size Determination of DNA Restriction Fragments
105	Mapping of Restriction Sites on Plasmid DNA
105	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