EDVOTEK®

THE BIOTECHNOLOGY EDUCATION COMPANY®

Edvo-Kit #
112/AP09

AP BIOLOGY

Edvo-Kit #112/AP09

Restriction Enzyme Analysis of DNA

Experiment Objective:

This experiment is designed to develop an understanding of restriction enzyme digestion and separation of DNA fragments using agarose gel electrophoresis.

See page 3 for storage instructions.

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Safety Data Sheets can be found on our website: **www.edvotek.com/safety-data-sheets**

Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Store all components at room temperature.

Components		Check (✓)
A & D	Lambda DNA cut with *Hin*dIII	❑
B & E	Lambda DNA cut with *Eco*RI	❑
C & F	Lambda DNA (uncut)	❑

REAGENTS & SUPPLIES

- UltraSpec-Agarose™ ❑
- 50x Electrophoresis Buffer ❑
- FlashBlue™ DNA Stain ❑
- InstaStain® Blue cards ❑
- 1 ml pipet ❑
- Microtipped Transfer Pipets ❑

Experiment #112/AP09 is designed for 8 gels if stained with FlashBlue™ or InstaStain® Blue (both included) or 16 gels if stained with SYBR® Safe or InstaStain® Ethidium Bromide (not included).

The DNA samples are stable at room temperature for up to one month after receipt. However, if the experiment will not be conducted within this time frame, it is recommended that the samples be stored in the refrigerator.

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

All experiment 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.

Background Information

One of the most significant discoveries of molecular biology is a class of enzymes known as restriction endonucleases. These endonucleases (also known as restriction enzymes) are produced by many species of bacteria to protect themselves from invading viral DNA. Restriction enzymes act like molecular scissors, cutting double-stranded DNA at specific sequences. The utility of restriction enzymes has made molecular cloning, DNA mapping, sequencing and various genome-wide studies possible, launching the era of biotechnology.

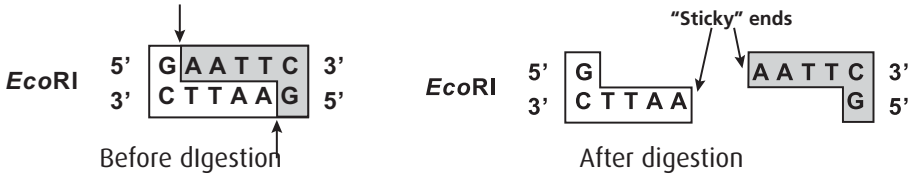
Restriction Enzyme	Genus	Species	Strain	Recognition Site
Ava I	*Anabaena*	*variablis*	n/a	C^YCGUG
Bgl I	*Bacillus*	*globigii*	n/a	GCCNNNN^NGGC
*Eco*RI	*Escherichia*	*coli*	RY 13	G^AATTC
*Hae*III	*Haemophilus*	*aegyptius*	n/a	GG^CC
*Hin*dIII	*Haemophilus*	*influenzae*	R_d	A^AGCTT
Sac I	*Streptomyces*	*achromogenes*	n/a	GAGCT^C

Table 1

Since they were first discovered in the 1970s, over 3,000 restriction enzymes have been identified, each one given a unique acronym describing the organism from which it was first isolated. The first letter of the acronym is the first letter of the genus, the next two letters are the first two letters of the species name of the organism, and additional letters and numerals indicate specific strains and order of discovery. For example, *Eco*RI was the first restriction enzyme isolated from the RY13 strain of the bacterium *Escherchia coli*. (More examples are shown in Table 1.)

Many restriction enzymes require Mg^{2+} for activity and recognize palindromic stretches of DNA, generally 4-8 base pairs in length. The probability that a given enzyme will cut, or "digest", a piece of DNA is directly proportional to the length of its recognition site. Statistically, an enzyme will average one cut for every 4^n base pairs, where n is the length of the recognition site. For instance, an enzyme that recognizes a four base pairs long sequence (e.g., *Hae*III) will cut DNA once every 256 (or 4^4) base pairs, while an enzyme that recognizes a six base pairs long site (e.g., *Eco*RI) will cut once every 4096 (or 4^6) base pairs. Therefore, the longer a DNA molecule is, the greater the probability is that it contains one or more restriction sites. For example, if *Eco*RI is used to digest human chromosomal DNA containing 3 billion base pairs and a plasmid containing 5,000 base pairs, it will cut the chromosomal DNA over 700,000 times (3 billion base pairs, cut every 4096 base pairs), but may only cut the plasmid once (5,000 base pairs, cut every 4096 base pairs).

Digestion by a restriction enzyme generates DNA fragments with one of two types of DNA ends--"sticky" or "blunt". To illustrate this, first consider the recognition site and cleavage pattern of *Eco*RI.



*Eco*RI cleaves between the G and neighboring A, as indicated by the arrows in the left side of the figure. It is important to note that the positions of the cleavage are staggered, so the resulting fragments project short overhangs of single-stranded DNA with complementary sequences. Such overhangs are referred to as "sticky" ends because the single-strands can interact with—or stick to—other overhangs with a complementary sequence. Digestion of the same piece of DNA using different enzymes can produce sticky ends of different lengths and strand orientation (5′ vs. 3′).

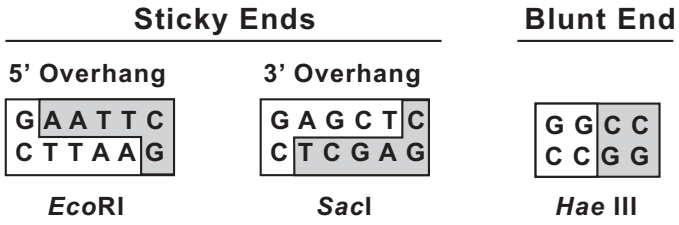
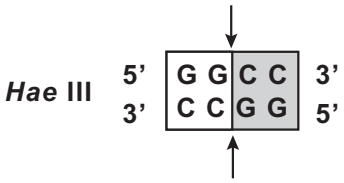
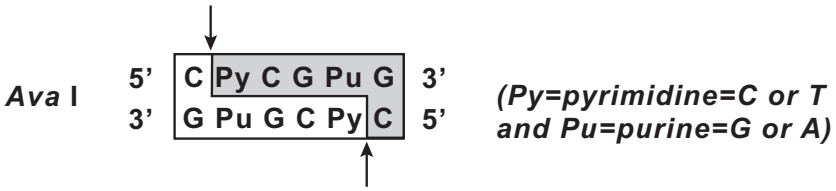


Figure 1: Different types of DNA ends produced by Restriction Enzymes.

In contrast to *Eco*RI, *Hae*III cuts both DNA strands at the same position, which generates fragments without an overhang. These so-called "blunt" ends can be joined with any other blunt end without regard for complementarity.

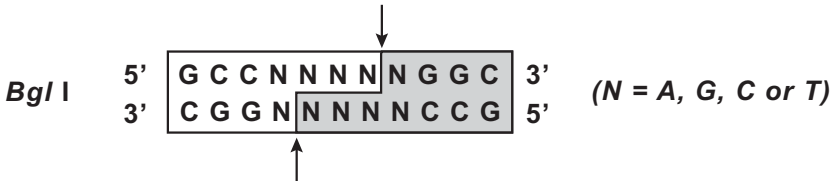


Some restriction enzymes, such as *Ava*I, recognize "degenerate" sites, which contain one or more variable positions.



Consequently, there are four possible sites that *Ava*I will recognize and cut: CCCGGG, CCCGAG, CTCGGG and CTCGAG.

There are even enzymes like *Bgl*I that recognize "hyphenated" sites, which are palindromic sequences separated by a number of completely variable bases.



The six G-C base pairs that *Bgl*I specifically recognizes must be separated by five base pairs of DNA; otherwise the enzyme cannot properly interact with the DNA to cleave its backbone. Because these five base pairs are not required to make up a specific sequence, *Bgl*I can recognize and cleave up to 625 possible sequences!

Depending on the distances between recognition sites, digestion of DNA by a restriction enzyme will produce DNA fragments of varying lengths. In order to analyze such a mixture of DNA fragments, scientists use a technique called agarose gel electrophoresis.

Agarose gel electrophoresis separates DNA fragments according to size (see Figure 2). First, DNA molecules are added into depressions (or "wells") within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the restriction fragments through the gel towards the positive electrode.

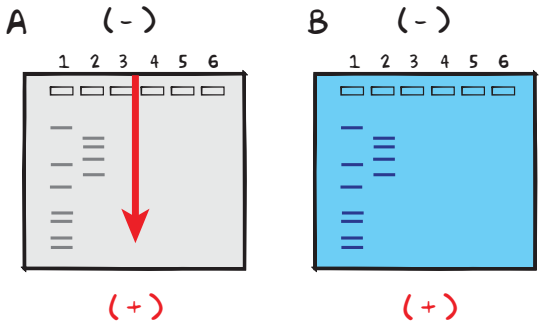


Figure 2:
Overview of Agarose Gel Electrophoresis

At first glance, an agarose gel appears to be a solid at room temperature, but on the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA.

While electrophoresis is a powerful separation technique, it is not without its technical limitations. Most significantly, if two different fragments share a similar size, they will migrate together through the gel and may appear as a single band. In addition, if digestion results in a broad distribution of DNA sizes, the fragments may stain as a smear. Lastly, DNA with a streamlined secondary structure (such as supercoiled DNA) can pass through the gel more quickly than similarly-sized linear DNA, which prevents an accurate comparison of size.

In this experiment, agarose gel electrophoresis is used to analyze Lambda DNA that has been digested with one of two different enzymes (*Eco*RI and *Hin*dIII). Results are analyzed using a semi-logarithmic plot to calculate the length of each DNA fragment.

Experiment Overview

EXPERIMENT OBJECTIVE:

This experiment is designed to develop an understanding of restriction enzyme digestion and separation of DNA fragments using agarose gel electrophoresis. At the end of the activity, students will have experience analyzing observed results and be capable of transforming the abstract concepts of restriction digestion and electrophoresis into an enhanced scientific understanding.

LABORATORY SAFETY

Wear gloves and safety goggles

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

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

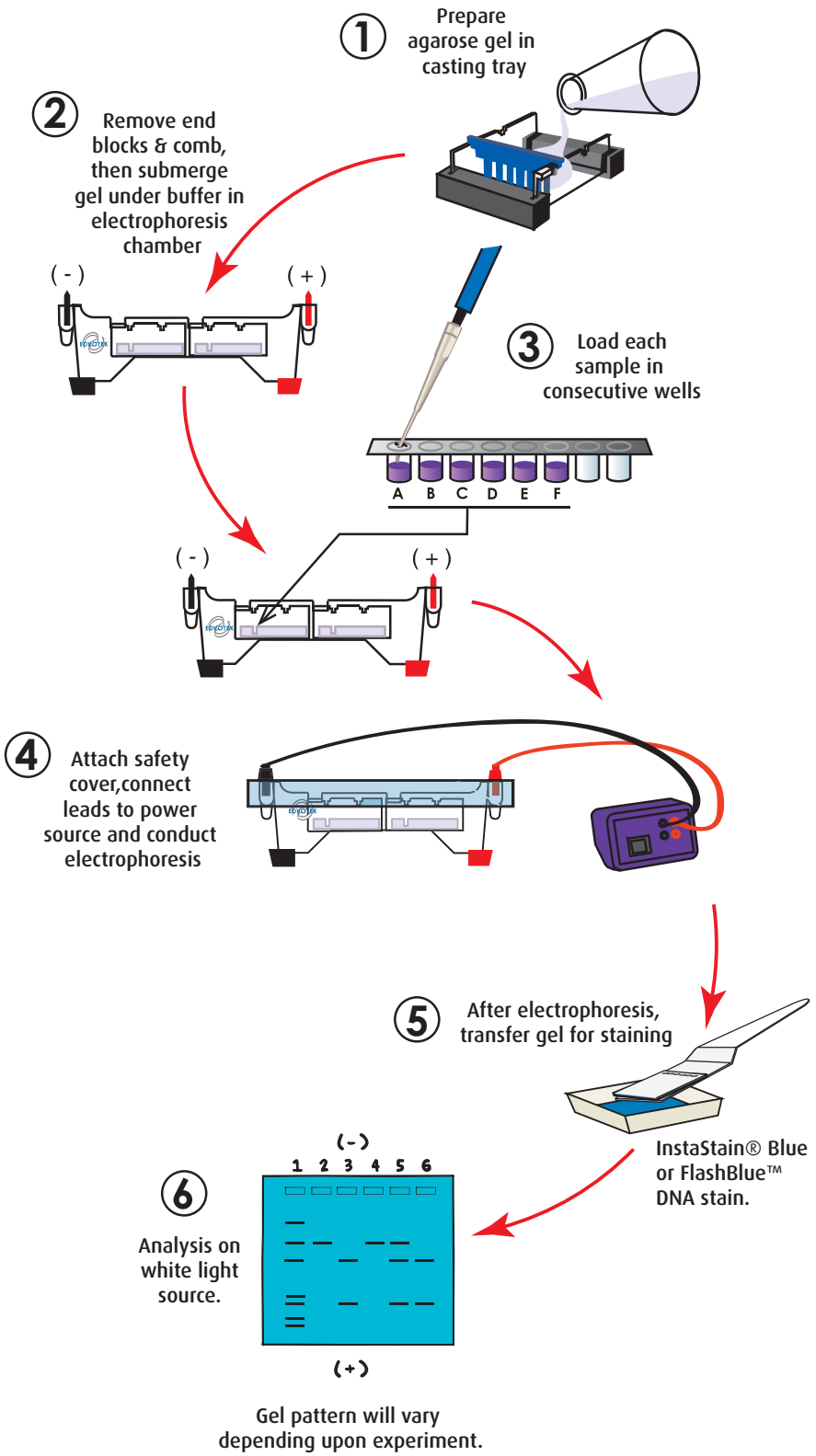
During the Experiment:

- Record your observations.

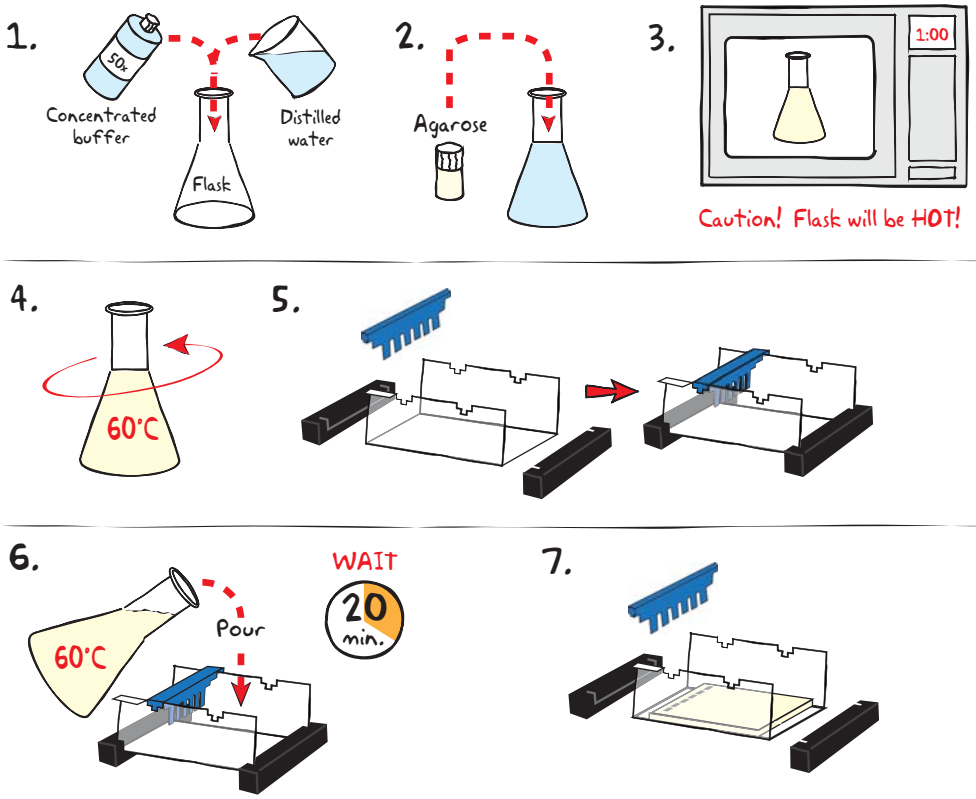
After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Experiment Overview



Module I: Agarose Gel Electrophoresis



IMPORTANT:

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

Wear gloves and safety goggles

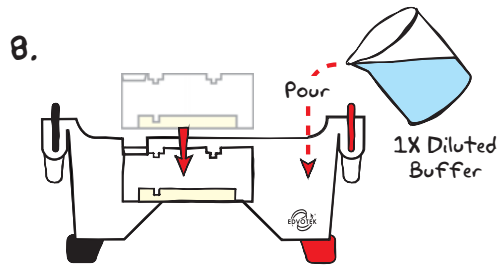
CASTING THE AGAROSE GEL

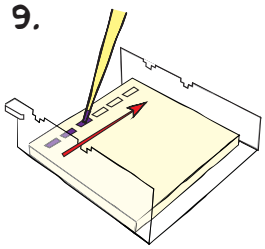
1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 ml flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — Individual 0.8% UltraSpec-Agarose™ Gel

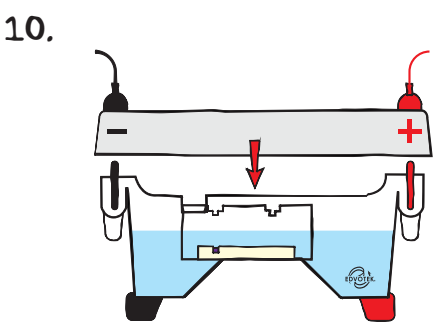
Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 ml	29.4 ml	0.23 g	30 ml
7 x 10 cm	1.0 ml	49.0 ml	0.39 g	50 ml
7 x 14 cm	1.2 ml	58.8 ml	0.46 g	60 ml

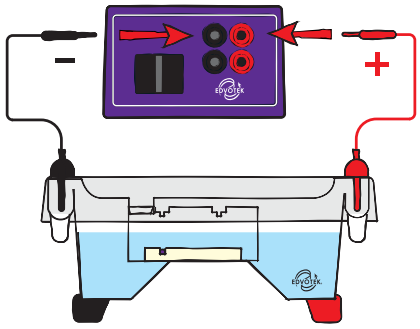
Module I: Agarose Gel Electrophoresis





Wear gloves and safety goggles





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

RUNNING THE GEL

8. **PLACE** the gel (still on the tray) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35 µl) into the well in the order indicated by Table 1, at right.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3.5 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to instructions for **STAINING** the agarose gel.

Table 1: Gel Loading

Lane 1	Tube A or D	Lambda DNA cut with *Hind* III
2	Tube B or E	Lambda DNA cut with *Eco*RI
3	Tube C or F	Lambda DNA (uncut)

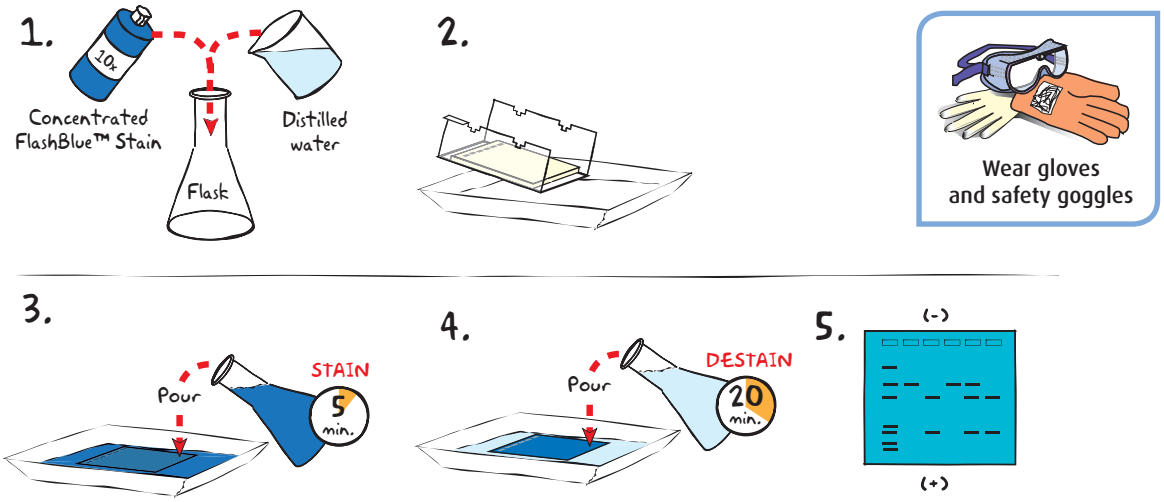
Table B 1x Electrophoresis Buffer (Chamber Buffer)

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

Table C Time & Voltage Guidelines (0.8% Agarose Gel)

	Electrophoresis Model		
	M6+	M12 (new)	M12 (classic) & M36
Volts	Min. / Max.	Min. / Max.	Min. / Max.
150	15/20 min.	20/30 min.	25 / 35 min.
125	20/30 min.	30/35 min.	35 / 45 min.
75	35 / 45 min.	55/70 min.	60 / 90 min.

Module II-A: Staining Agarose Gels Using FlashBlue™

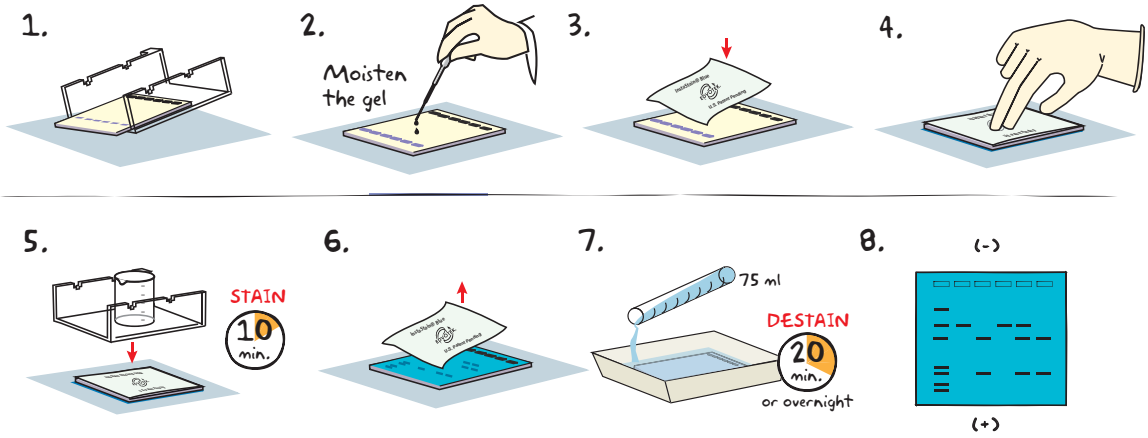


1. **DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 ml of water in a flask and **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.
5. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

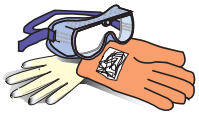
ALTERNATIVE PROTOCOL:

1. **DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH_2O.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.

Module II-B: Staining Agarose Gels Using InstaStain® Blue



1. Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface.
2. **MOISTEN** the gel with a few drops of electrophoresis buffer.
3. Wearing gloves, **PLACE** the blue side of the InstaStain® Blue card on the gel.
4. With a gloved hand, **REMOVE** air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.
5. **PLACE** the casting tray on top of the gel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Blue card is in direct contact with the gel surface. **STAIN** the gel for 10 minutes.
6. **REMOVE** the InstaStain® Blue card. If the color of the gel appears very light, reapply the InstaStain® Blue card to the gel for an additional five minutes.
7. **TRANSFER** the gel to a small, clean gel-staining tray. **COVER** the gel with about 75 mL of distilled water and **DESTAIN** for at least 20 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. To accelerate destaining, warm the distilled water to 37°C and change it frequently.
8. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.



Wear gloves and safety goggles

NOTE:
DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.

ALTERNATIVE PROTOCOL:

1. Carefully **SLIDE** the agarose gel from its casting tray into a small, clean tray containing about 75 ml of distilled/deionized water or used electrophoresis buffer. The gel should be completely submerged.
2. Gently **FLOAT** the InstaStain® Blue card(s) on top of the liquid with the stain (blue side) facing toward the gel. Each InstaStain® Blue card will stain 49 cm^2 of gel (7 x 7 cm).
3. **COVER** the tray with plastic wrap to prevent evaporation. **SOAK** the gel in the staining liquid for at least 3 hours. The gel can remain in the liquid overnight if necessary.
4. Carefully **REMOVE** the gel from the staining tray. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Module III: Size Determination of DNA Restriction Fragments

Agarose gel electrophoresis separates cut DNA into discrete bands, each comprising molecules of the same size. How can these results be used to determine the lengths of fragments in the restriction digests? Remember, as the length of a DNA molecule increases, the distance to which the molecule can migrate decreases because large DNA fragments cannot pass through the channels in the gel with ease. Therefore, the migration rate is inversely proportional to the length of the DNA fragment—more specifically, to the $\log_{10}$ of fragment length. To illustrate this, we ran a sample that contains DNA strands of known lengths called a "standard". We will measure the distance that each of these bands traveled to create a graph, known as a "standard curve", which can then be used to extrapolate the size of unknown DNA fragments.

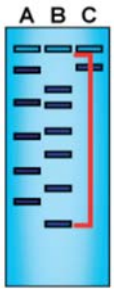


Figure 3: Measure distance migrated from the lower edge of the well to the lower edge of each band.

1. MEASURE AND RECORD DISTANCES
Using Lambda/*Hin*dIII as the Standard

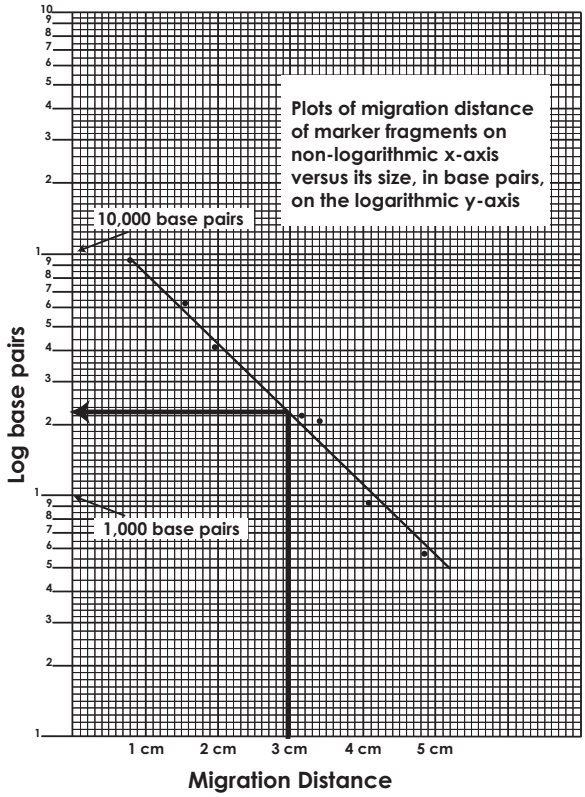
Measure the distance traveled by each Lambda/*Hin*dIII digest from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters (to the nearest millimeter) in your notebook. Repeat this for each DNA fragment in the standard. Do not measure the migration of the largest fragment, as this point will not be used to create the standard curve.

2. GENERATE A STANDARD CURVE

Because migration rate is inversely proportional to the $\log_{10}$ of DNA length, plotting the data as a semi-log plot will produce a straight line and allow us to analyze an exponential range of fragment sizes. You will notice that the vertical axis of the semi-log plot appears atypical at first; the distance between numbers shrinks as the axis progresses from 1 to 9. This is because the axis represents a logarithmic scale. The first cycle on the y-axis corresponds to lengths from 100-1,000 base pairs, the second cycle measures 1,000-10,000 base pairs, and so on. To create a standard curve on the semi-log paper, plot the distance each Standard DNA fragment migrated on the x-axis (in mm) versus its size on the y-axis (in base pairs). Be sure to label the axes!

After all the points have been plotted, use a ruler or a straight edge to draw the best straight line possible through the points. The line should have approximately equal numbers of points scattered on each side of the line. It is okay if the line runs through some points (see Figure 4 for an example).

Figure 4: Semilog graph example



3. DETERMINE THE LENGTH OF EACH UNKNOWN FRAGMENT

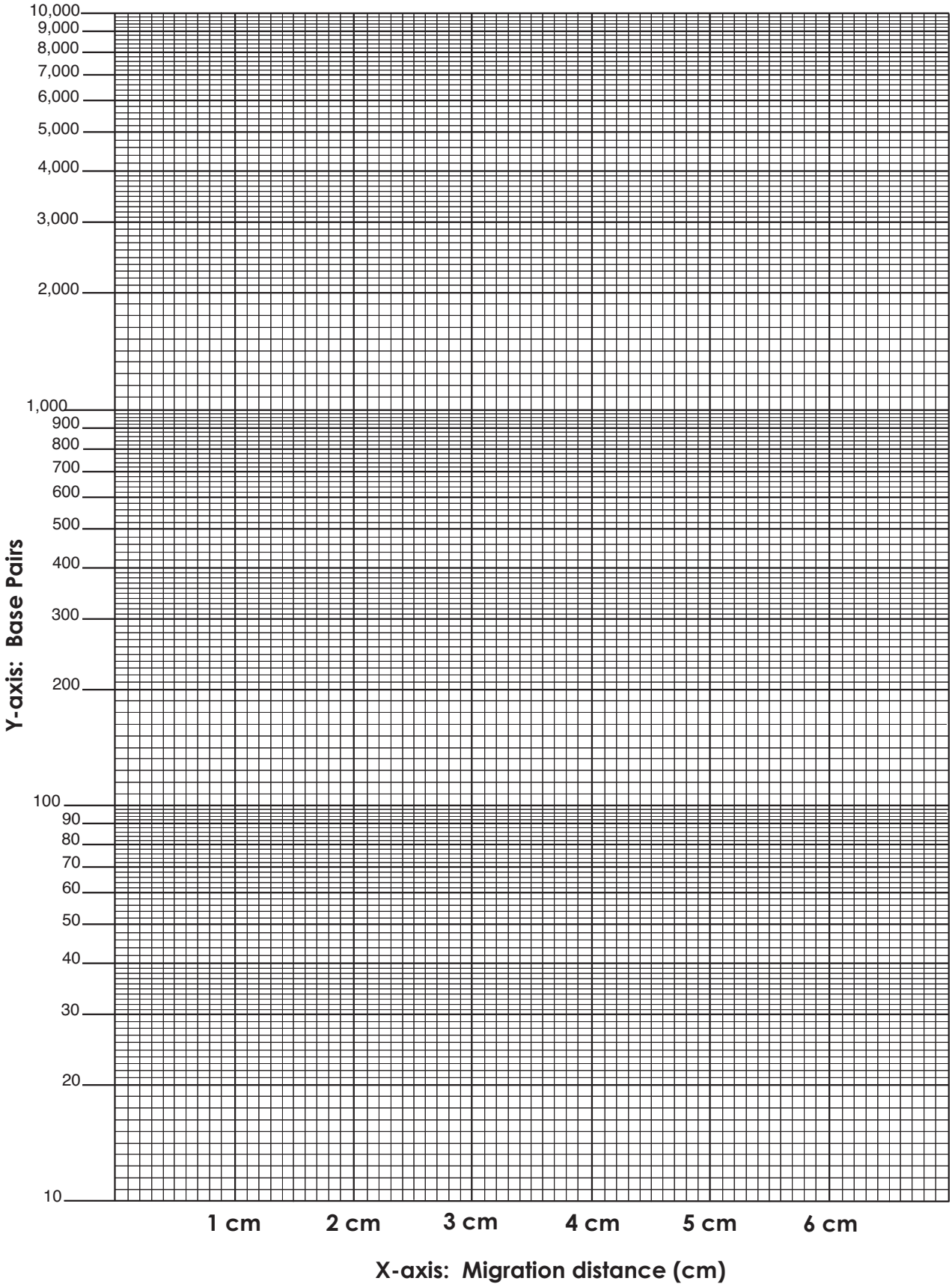
a. Locate the migration distance of the unknown fragment on the x-axis of your semi-log graph. Draw a vertical line extending from that point until it intersects the line of your standard curve.
b. From the point of intersection, draw a second line, this time horizontally, toward the y-axis. The value at which this line intersects the y-axis represents the approximate size of the fragment in base pairs (refer to Figure 4 for an example). Make note of this in your lab notebook.
c. Repeat for each fragment in your unknown sample.

Quick Reference:

Lambda DNA cut with *Hin*dIII using a standard curve will be plotted on semi-log graph paper. The following are the sizes - length is expressed in base pairs.

23130* 9416 6557 4361 2322 2027 564

** NOTE: 23,130 size is not to be used in the creation of the standard curve.*



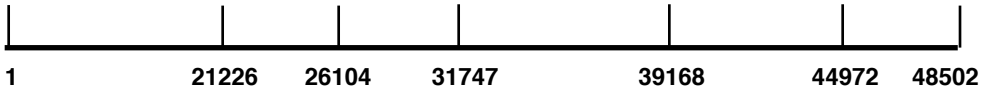
Study Questions

1. The restriction enzyme *Not*I recognizes the following sequence: 5'-GCGGCCGC-3'. On average, how often should this enzyme cleave DNA? The genome of the human malaria parasite *Plasmodium falciparum* is extremely A-T rich – As and Ts comprise about 80% of its genome. Would *Not*I cleave this DNA more or less frequently?

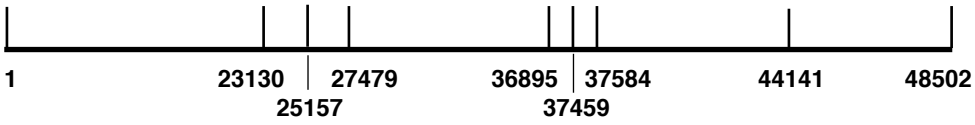
2. Predict the number of DNA fragments and their sizes if Lambda phage DNA were incubated and cleaved simultaneously with both *Hin*dIII and *Eco*RI (refer to the map below).

LAMBDA PHAGE DNA RESTRICTION ENZYME MAP
48,502 Base Pairs

A. *Eco*RI (5 Sites)



B. *Hin*dIII (7 Sites)



Instructor's Guide

ADVANCE PREPARATION:

Preparation for:	What to do:	When?	Time Required:
Module I: Agarose Gel Electrophoresis	Prepare QuickStrips™	Up to one day before performing the experiment	45 min.
	Prepare diluted electrophoresis buffer		
	Prepare molten agarose and pour gels		
Module II: Staining Agarose Gels	Prepare staining components	The class period or overnight after the class period	10 min.

Pre-Lab Preparations: Module I

AGAROSE GEL ELECTROPHORESIS

This experiment requires a 0.8% agarose gel per student group. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

Individual Gel Preparation:

Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module I in the Student's Experimental Procedure. Students will need 50x Electrophoresis Buffer, distilled water and agarose powder.

Batch Gel Preparation:

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. Electrophoresis buffer can also be prepared in bulk. See Appendix B.

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 freeze gels at -20° C as 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 molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

NOTE:
Accurate pipetting is critical for maximizing successful experiment results. EDVOTEK Series 100 experiments are designed for students who have had previous experience with micropipetting techniques and agarose gel electrophoresis.

If students are unfamiliar with using micropipets, we recommended performing Cat. #S-44, Micropipetting Basics or Cat. #S-43, DNA DuraGel™ prior to conducting this advanced level experiment.

FOR MODULE I
Each Student Group should receive:
- 50x Electrophoresis Buffer
- Distilled Water
- UltraSpec-Agarose™
- QuickStrip™ Samples

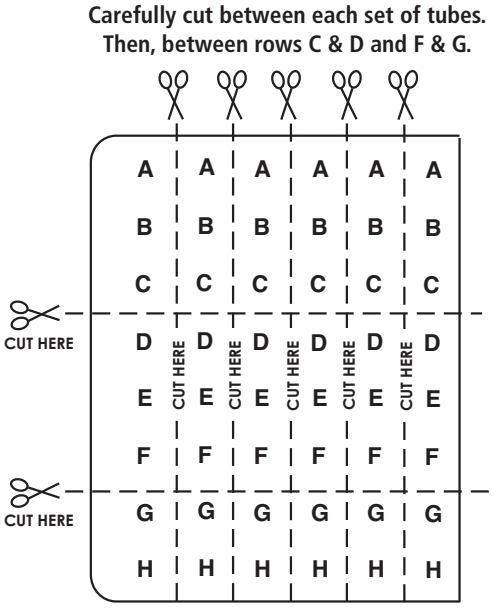
SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

QuickStrip™ tubes consist of a microtiter block covered with a protective overlay. Each well contains pre-aliquoted DNA.

Using sharp scissors, first divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Next, cut each individual strip between wells C and D and wells F and G. Take care not to damage the protective overlay while separating the samples.

Each lab group will receive one set of tubes, either rows A-C or D-F.
- A & D contain Lambda DNA cut with *Hin*dIII
- B & E contain Lambda DNA cut with *Eco*RI
- C & F contain Uncut Lambda DNA
- G & H are intentionally left blank. (Discard these tubes.)

If using SYBR® Safe or InstaStain® Ethidium Bromide for DNA visualization, each QuickStrip™ is shared by two groups. 18 µl of the DNA sample will be loaded into each well. Proceed to visualize the results as specified by the DNA stain literature.



Pre-Lab Preparations: Module II

MODULE II-A: STAINING AGAROSE GELS WITH INSTASTAIN® BLUE

The easiest and most convenient DNA stain available is InstaStain® Blue. InstaStain® Blue does not require the formulation, storage and disposal of large volumes of liquid stain. Each InstaStain® Blue card contains a small amount of blue DNA stain. When the card is placed in water, the DNA stain is released. This solution simultaneously stains and destains the gel, providing uniform gel staining with minimal liquid waste and mess.

You can use a White Light Visualization System (Cat. #552) to visualize gels stained with InstaStain® Blue.

FOR MODULE II-A
Each Student Group should receive:
- 1 InstaStain® card per 7 x 7 cm gel

Wear gloves and safety goggles

MODULE II-B: STAINING AGAROSE GELS WITH FLASHBLUE™

FlashBlue™ stain is optimized to shorten the time required for both staining and de-staining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to "equilibrate" in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (Cat. #552) is recommended for visualizing gels stained with FlashBlue™.

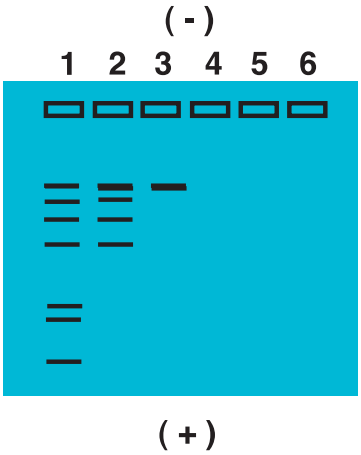
- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

FOR MODULE II-B
Each Student Group should receive:
- 10 ml 10X concentrated FlashBlue OR 100 mL 1x diluted FlashBlue
- Small plastic tray or weight boat
- Distilled or deionized water

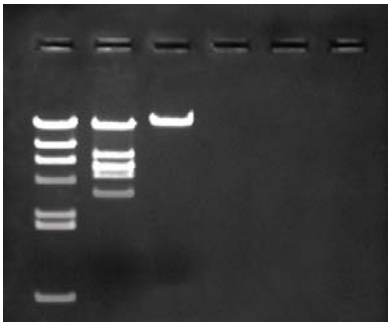
MODULE II: PHOTODOCUMENTATION OF DNA (OPTIONAL)

Once gels are stained, you may wish to photograph your results. 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.

Experiment Results and Analysis



In the idealized schematic, the relative positions of Lambda DNA fragments are shown but are not depicted to scale.



Lane Tube

1 A or D Lambda DNA cut with *Hin*dIII
(expressed in approximate base pairs)

23130 9416 6557 4361 2322 2027 564*

2 B or E Lambda DNA cut with *Eco*RI
Expected *Eco*RI fragment sizes in base pairs:

21226 7421 5804** 5643** 4878 3530

3 C or F Lambda DNA (uncut)

Note: This technique has a ± 10 - 15% margin of error.

** This band may not appear on the gel and likely will not be visualized.*
*** Two bands appear as a single band.*

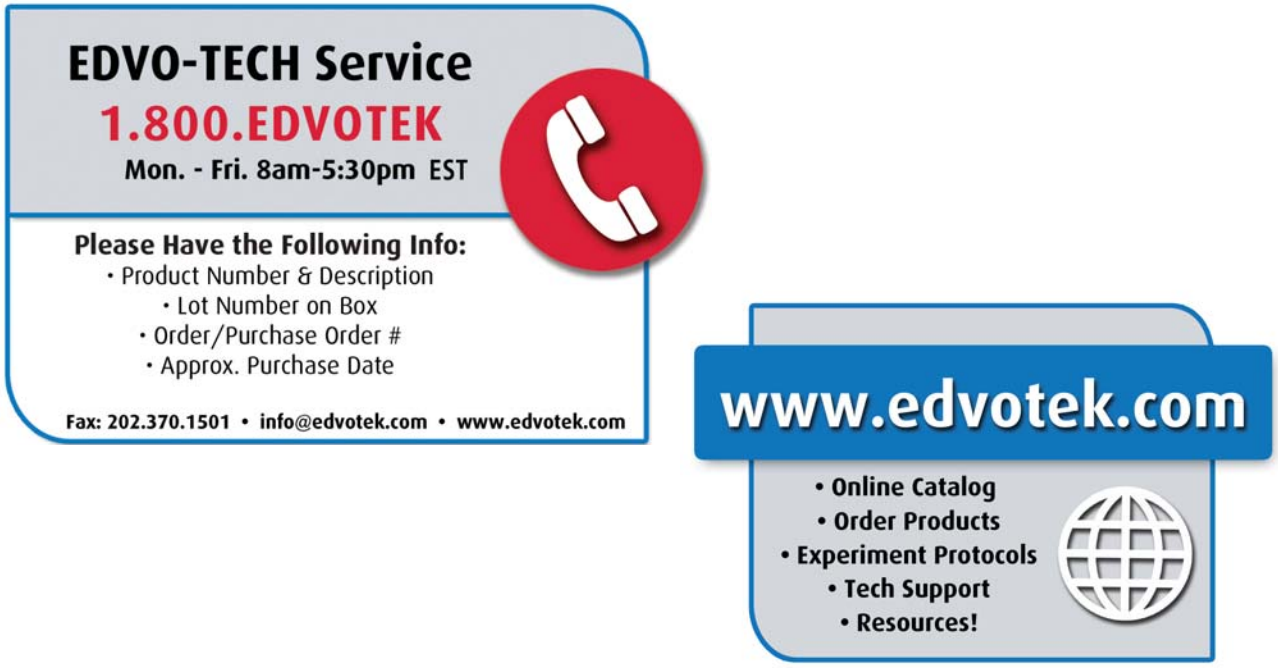
Please refer to the kit insert for the Answers to Study Questions

Appendices

A EDVOTEK® Troubleshooting Guide

B Bulk Preparation of Agarose Gels

Safety Data Sheets can be found on our website:
www.edvotek.com/safety-data-sheets



Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
Bands are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	The gel was not stained properly.	Repeat staining.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
After staining the gel, the DNA bands are faint.	The gel was not stained for a sufficient period of time.	Repeat staining protocol.
	The background of gel is too dark.	Destain the gel for 5-10 minutes in distilled water.
DNA bands were not resolved.	Tracking dye should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation.	Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).
DNA bands fade when gels are kept at 4°C.	DNA stained with FlashBlue™ may fade with time	Re-stain the gel with FlashBlue™
There is no separation between DNA bands, even though the tracking dye ran the appropriate distance.	The wrong percent gel was used for electrophoretic separation.	Be sure to prepare the correct percent agarose gel. For reference, the Ready-to-Load™ DNA samples should be analyzed using a 0.8% agarose gel.
There's not enough sample in my QuickStrip™.	The QuickStrip™ has dried out.	Add 40 µL water, gently pipet up and down to mix before loading.

Appendix B
Bulk Preparation of Agarose Gels

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.

Bulk Electrophoresis Buffer

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

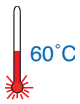
Table D — Bulk Preparation of Electrophoresis Buffer

50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 ml		2,940 ml	3000 ml (3 L)

Batch Agarose Gels (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.

1. Use a 500 ml flask to prepare the diluted gel buffer.
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60° C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. Measure 30 ml for a 7 x 7 cm tray, 50 ml for a 7 x 10 cm tray, and 60 ml for a 7 x 14 cm tray. ***For this experiment, 7 x 7 cm gels are recommended.***
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.

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

Table E — Batch Prep of 0.8% UltraSpec-Agarose™

Amt of Agarose (g)	+	Concentrated Buffer (50X) (ml)	+	Distilled Water (ml)	Total Volume (ml)
3.0		7.5		382.5	390