



THE BIOTECHNOLOGY
EDUCATION COMPANY®

Edvo-Kit #

102

Edvo-Kit #102

Restriction Enzyme Cleavage of Plasmid and Lambda DNA

Experiment Objective:

The objective of this experiment is to develop an understanding of the use of restriction endonucleases as tools to cut DNA at specific sequences.

See page 3 for storage instructions.

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Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Store all components at room temperature.

Components (in QuickStrip™ format)

- A Plasmid DNA (uncut)
- B Plasmid cut with *Bgl* I
- C Plasmid cut with *Eco* RI
- D Lambda DNA (uncut)
- E Lambda DNA cut with *Eco* RI
- F Lambda DNA cut with *Bgl* I

Check (✓)

- ☐
- ☐
- ☐
- ☐
- ☐
- ☐

REAGENTS & SUPPLIES

- UltraSpec-Agarose™
- Electrophoresis Buffer (50x)
- 10x Gel Loading Solution
- FlashBlue™ DNA Stain
- InstaStain® Blue cards
- 1 ml pipet
- Microtipped Transfer Pipets

- ☐
- ☐
- ☐
- ☐
- ☐
- ☐
- ☐

Experiment #102 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).

Store QuickStrip™ samples in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

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.

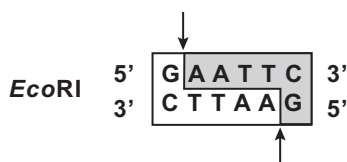
Background Information

The discovery of restriction enzymes ushered in a new era of molecular genetics. These enzymes cut the DNA molecule in a highly specific and reproducible way. This, in turn, has led to the development of molecular cloning and the mapping of genes.

Restriction enzymes are endonucleases which catalyze the cleavage of the phosphodiester bonds within both strands of DNA. They require Mg^{+2} for activity and generate a 5 prime (5') phosphate and a 3 prime (3') hydroxyl group at the point of cleavage. The distinguishing feature of restriction enzymes is that they only cut at very specific sequences of bases. Restriction enzymes are obtained from many different species of bacteria (including blue-green algae). To date, over 3,000 restriction enzymes have been discovered and catalogued.

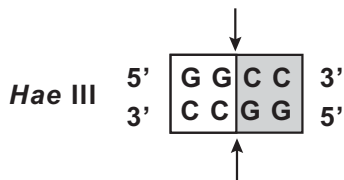
Restriction enzymes are named according to the organism from which they are isolated. This is done by using the first letter of the genus followed by the first two letters of the species. Only certain strains or sub-strains of a particular species may produce restriction enzymes. The type of strain or substrain sometimes follows the species designation in the name. Finally, a Roman numeral is always used to designate one out of possibly several different restriction enzymes produced by the **same** organism or by different substrains of the **same** strain.

A restriction enzyme requires a specific double stranded recognition sequence of nucleotides to cut DNA. Recognition sites are usually 4 to 8 base pairs in length. Cleavage occurs within or near the site. The cleavage positions are indicated by arrows. Recognition sites are frequently symmetrical, i.e., both DNA strands in the site have the same base sequence when read 5' to 3'. Such sequences are called palindromes. Consider the recognition site and cleavage pattern of *Eco* RI as an example.



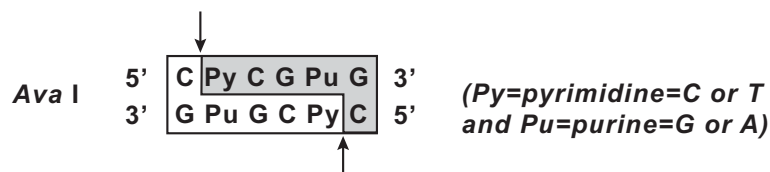
As shown above, *Eco* RI causes staggered cleavage of its site. The ends of the DNA fragments are called “sticky” or “cohesive” ends because the single-stranded regions of the ends are complementary.

Some restriction enzymes, such as *Hae* III, introduce cuts that are opposite each other. This type of cleavage generates “blunt” ends.



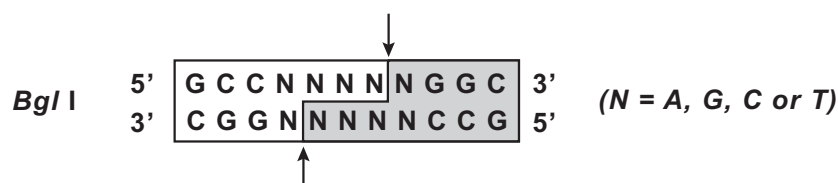
Restriction Enzyme	Organism
<i>Bgl</i> I	<i>Bacillus globigii</i>
<i>Bam</i> HI	<i>Bacillus amyloliquefaciens</i> H
<i>Eco</i> RI	<i>Escherichia coli</i> , strain RY 13
<i>Eco</i> RII	<i>Escherichia coli</i> , strain R 245
<i>Hae</i> III	<i>Haemophilus aegyptius</i>
<i>Hind</i> III	<i>Haemophilus influenzae</i> R _d

The recognition sites of some restriction enzymes contain variable base positions. For example, *Ava* I recognizes:



Keep in mind that A pairs with T and G pairs with C. Consequently, there are four possible sequences *Ava* I recognizes. Recognition sites of this type are called degenerate.

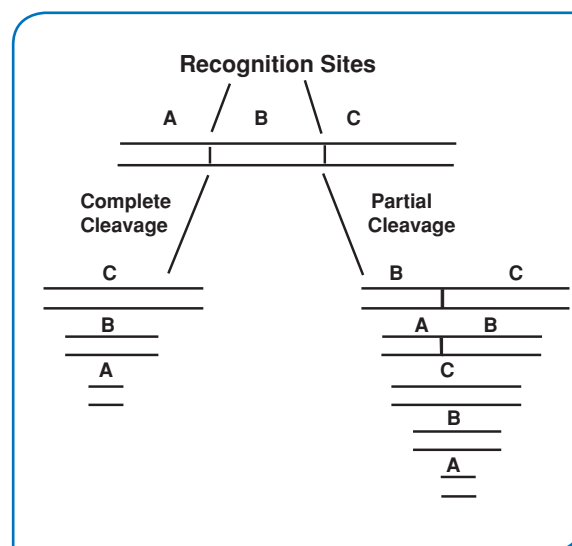
There are some recognition sites that are divided by a certain number of totally variable bases. For example, *Bgl* I recognizes:



There are 625 possible sequences *Bgl* I can cleave. The only bases the enzyme truly "recognizes" are the six G-C base pairs at the ends, which forms a palindrome. In the case of *Bgl* I, these true recognition bases must **always** be separated by 5 base pairs of DNA, otherwise the enzyme cannot properly interact with the DNA and cleave it. Recognition sites like that of *Bgl* I are called hyphenated sites.

In general, the longer the DNA molecule, the greater the probability that a given recognition site will occur. Therefore, human chromosomal DNA, which contains three billion base pairs, has many more recognition sites than a plasmid DNA containing only several thousand base pairs. However, very large DNA is difficult to isolate intact. During handling, it is randomly sheared to fragments in the range of 50,000 to 100,000 base pairs.

Plasmids and many viral DNAs are circular molecules. If circular DNA contains one recognition site for a restriction enzyme, then it will open up to form a linear molecule when cleaved. By contrast, if a linear DNA molecule contains a single recognition site, when cleaved once it will generate two fragments. The size of the fragments produced depends on how far the sites are from each other. If a DNA molecule contains several recognition sites for a restriction enzyme, then under certain experimental conditions, it is possible that certain sites are cleaved and not others. These incompletely cleaved fragments of DNA are called partials. Partials can arise if low amounts of enzyme are used or the reaction is stopped after a short time. In reality, reactions containing partials also contain some molecules that have been completely cleaved.



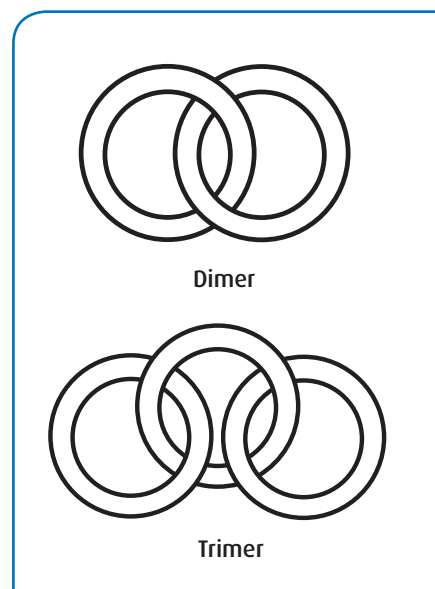
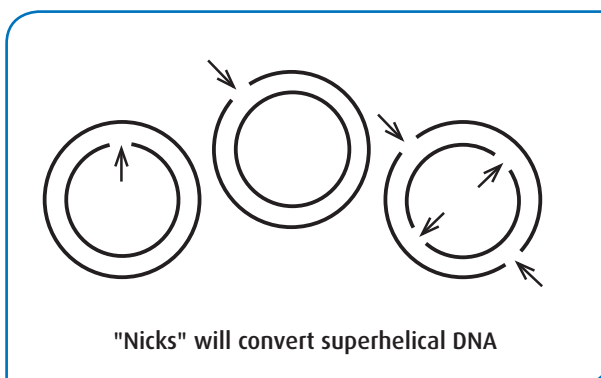
Agarose gel electrophoresis is a powerful separation method used to analyze DNA fragments generated by restriction enzymes. The agarose gel consists of microscopic pores that act as a molecular sieve. Samples of DNA are loaded into wells made in the gel during casting. Since DNA has a strong negative charge at neutral pH, it migrates through the gel towards the positive electrode during electrophoresis. DNA molecules are separated in the gel according to their size and shape. The smaller linear fragments migrate the fastest. If the size of two fragments are similar or identical, they will migrate together in the gel. If DNA is cleaved many times, the wide range of fragments produced will appear as a smear after electrophoresis.

Circular DNAs such as plasmids are supercoiled. Supercoiled DNA has a more compact and entangled shape (like a twisted rubber band) than its corresponding non-supercoiled forms (linear, nicked and relaxed circles).

When supercoiled DNA is cleaved once by a restriction enzyme, it unravels to its linear form. If supercoiled DNA is nicked (a phosphate bond is cleaved anywhere in the molecule, in either strand) it completely unravels to a circular form. Under the electrophoresis conditions being used in this experiment, supercoiled DNA migrates faster than its linear form and linear DNA migrates faster than its nicked circular form.

During replication, several plasmid molecules can form interlocking structures. These forms are called catenanes. Catenanes can contain two plasmid molecules (dimer), three molecules (trimer), etc. Catenanes migrate more slowly than single circles that are nicked during electrophoresis. Dimers migrate faster than trimers, which migrate faster than tetramers, etc. Catenanes give rise to the same final restriction enzyme cleavage patterns as their uncatenated single forms.

In this experiment, restriction enzyme cleavage products will be analyzed by agarose gel electrophoresis. The supercoiled plasmid DNA contains approximately 4,500 base pairs and has one recognition site for *Bgl* I and two for *Eco* RI. The second DNA is isolated from the *E. coli* bacteriophage lambda, which is a linear molecule containing 49,000 base pairs. Lambda DNA contains 5 recognition sites for *Eco* RI and 29 for *Bgl* I. The restriction enzyme digestions demonstrate that a specific restriction enzyme will yield distinctly different patterns when digesting different DNAs. For example, *Bgl* I will produce one fragment when digesting the plasmid DNA, compared to a significantly different pattern when digesting lambda DNA.



Experiment Overview

EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of the use of restriction endonucleases as tools to cut DNA at specific sequences.

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 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.

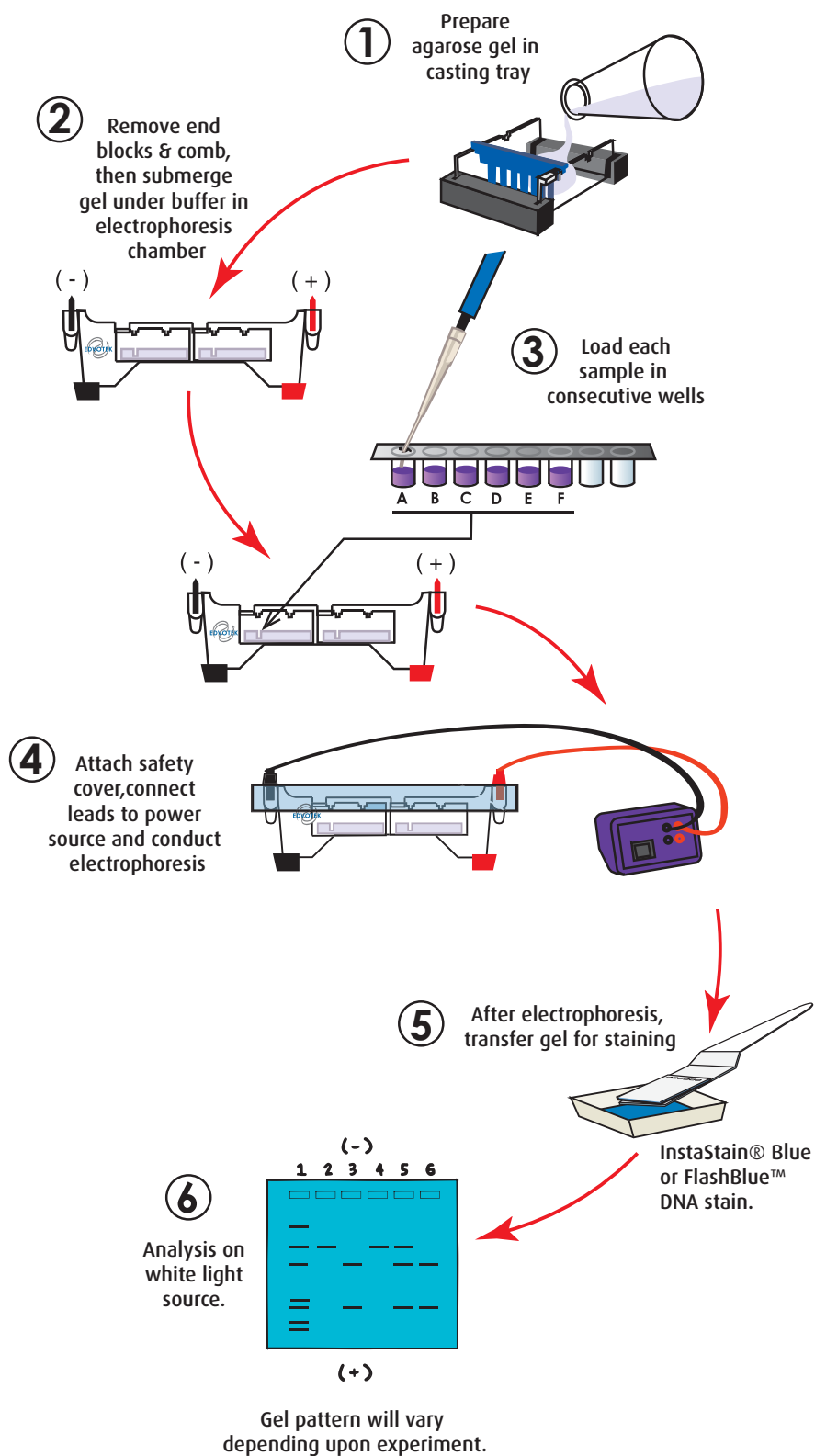
During the Experiment:

- Record your observations.

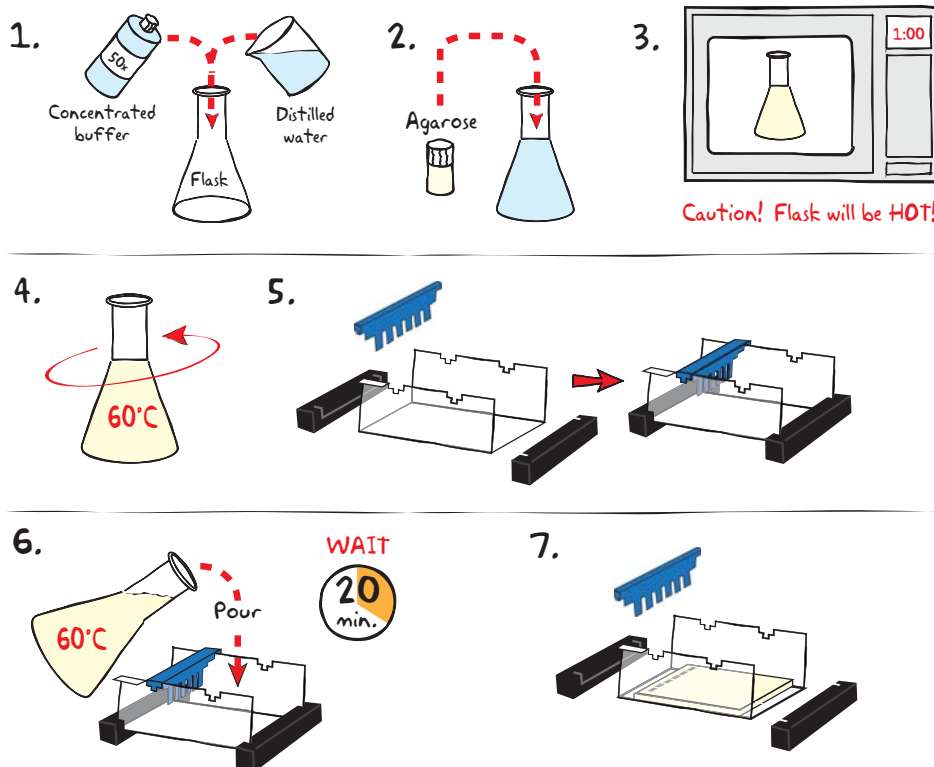
After the Experiment:

- 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

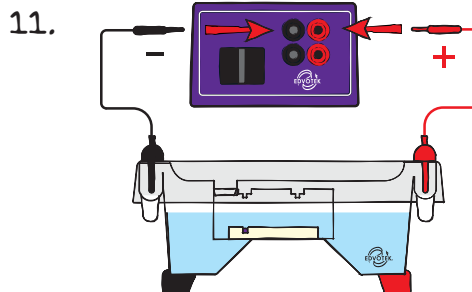
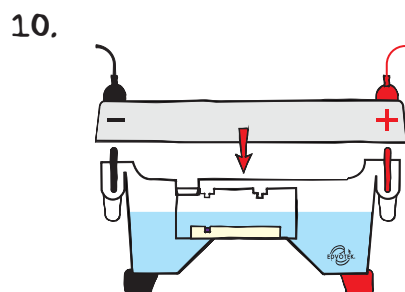
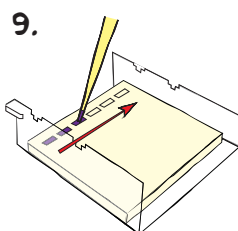
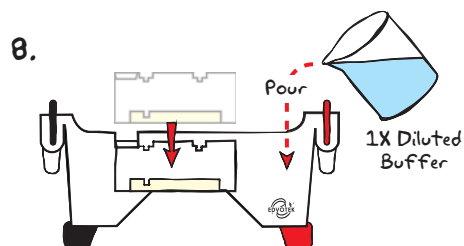
1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask 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

**Reminder:**

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

8. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **LOAD** the entire sample (35-38 μ L) into the well in the order indicated by Table 1, at right.
10. **PLACE** safety cover. **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).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to **STAINING** the agarose gel.

Table 1: Gel Loading

Lane	Tube	Sample
1	Tube A	Plasmid DNA (uncut)
2	Tube B	Plasmid cut with <i>Bgl</i> I
3	Tube C	Plasmid cut with <i>Eco</i> RI
4	Tube D	Lambda DNA (uncut)
5	Tube E	Lambda DNA cut with <i>Eco</i> RI
6	Tube F	Lambda DNA cut with <i>Bgl</i> I

Table
B

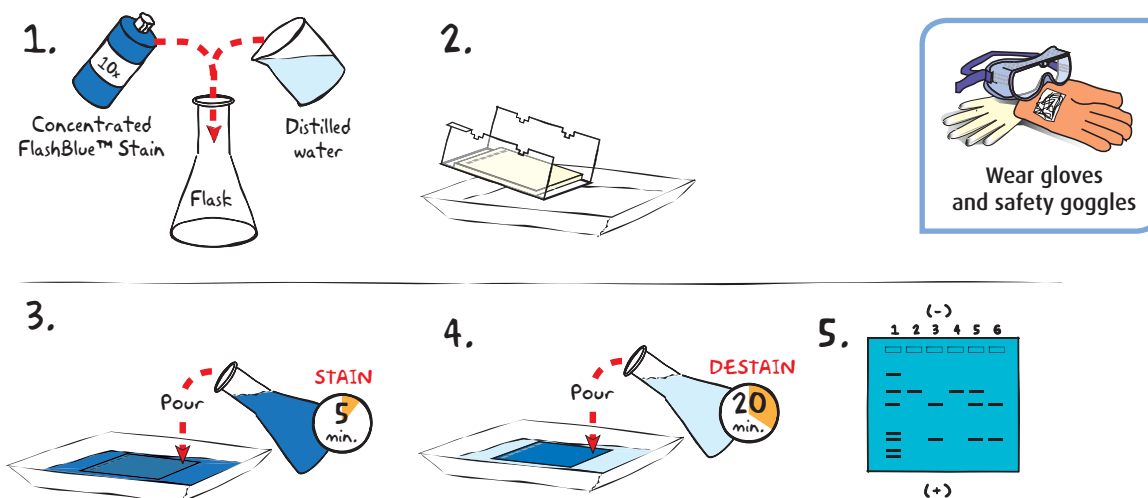
1x Electrophoresis Buffer (Chamber Buffer)

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

Table
CTime and Voltage Guidelines
(0.8% Agarose Gel)

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

Module II-A: Staining Agarose Gels Using FlashBlue™

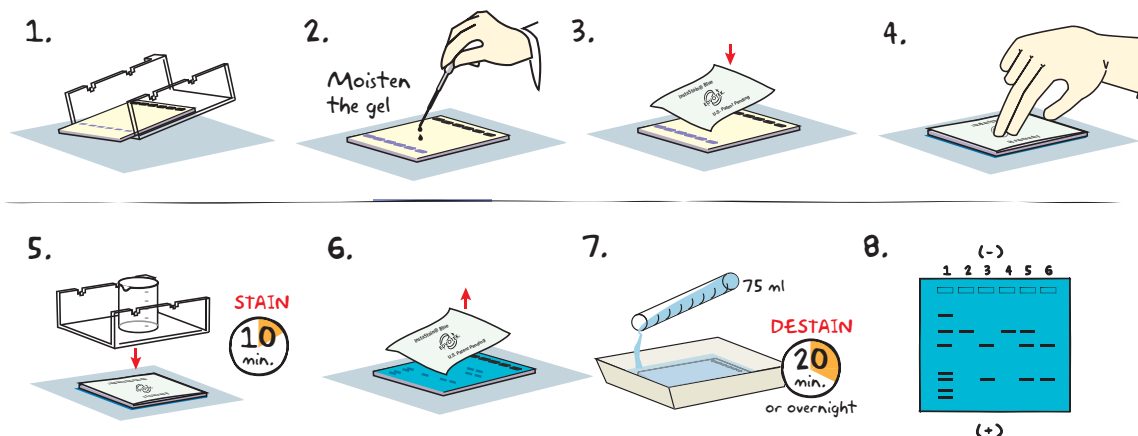


- DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 ml of water in a flask and **MIX** well.
- REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
- COVER** the gel with the 1x FlashBlue™ stain 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.**
- 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.
- 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.

Alternate Protocol:

- DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH₂O.
- COVER** the gel with diluted FlashBlue™ stain.
- 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 cm² 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.

Study Questions

1. To which electrode does DNA migrate and why?
2. Why has the discovery of restriction enzymes been so important?
3. How are restriction enzymes named?
4. Briefly explain the function of restriction enzymes.

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 TAE 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.

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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 concentrated 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. 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 recommend 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 concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- QuickStrip™ Samples

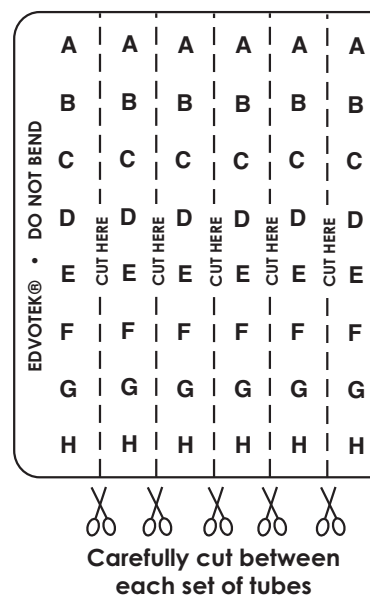
SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

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

Using sharp scissors, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the protective overlay while separating the samples.

Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube.

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 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.

MODULE II-B: STAINING 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.

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.

FOR MODULE II-A Each Student Group should receive:

- 1 InstaStain® card per 7 x 7 cm gel

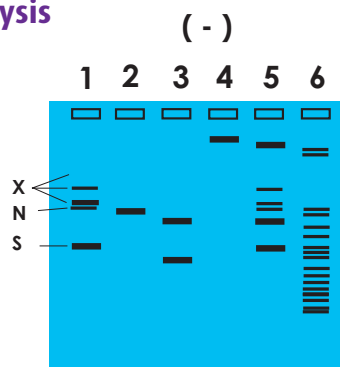
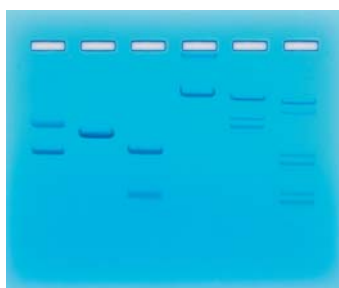


Wear gloves
and safety goggles

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

Experiment Results and Analysis



(+)

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

Lane	Tube	Sample	Molecular Weights (in bp)
1	A	Plasmid DNA (uncut)	S - Supercoiled N - Nicked X - Dimers or Higher catenanes
2	B	Plasmid cut with <i>Bgl</i> I	4282
3	C	Plasmid cut with <i>Eco</i> RI	3000 1282
4	D	Lambda DNA (uncut)	48502
5	E	Lambda DNA cut with <i>Eco</i> RI	21226 7421 5804 5643 4878 3530
6	F	Lambda DNA with <i>Bgl</i> I	*

* Smaller DNA fragments may not be visible after staining since they will bind only a small amount of the stain. Fragments that are close in size may not be resolved as individual bands. Molecular weights for Lambda DNA cut with *Bgl* I are: 16179, 9649, 3009, 2481, 2256, 1650, 1446, 1441, 1249, 1203, 1138, 790, 773, 669, 621, 562, 499, 489, 447, 404, and 366.

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

Appendices

- A EDVOTEK® Troubleshooting Guide
- B Bulk Preparation of Agarose Gels
- C Data Analysis Using a Standard Curve

Material Safety Data Sheets:

Now available for your convenient download on www.edvotek.com.

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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 uL 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.

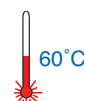


Table E Batch Prep of 0.8% UltraSpec-Agarose™					
Amt of Agarose (g)	+	Concentrated Buffer (50X) (ml)	+	Distilled Water (ml)	Total Volume (ml)
3.0		7.5		382.5	390

Appendix C

Data Analysis Using a Standard Curve

Agarose gel electrophoresis separates biomolecules into discrete bands, each comprising molecules of the same size. How can these results be used to determine the lengths of different fragments? Remember, as the length of a biomolecule increases, the distance to which the molecule can migrate decreases because large molecules cannot pass through the channels in the gel with ease. Therefore, the migration rate is inversely proportional to the length of the molecules—more specifically, to the \log_{10} of molecule's length. To illustrate this, we ran a sample that contains bands 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 molecule(s).



Figure 1:
Measure distance migrated from the lower edge of the well to the lower edge of each band.

1. Measure and Record Migration Distances

Measure the distance traveled by each Standard DNA Fragment 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.

Measure and record the migration distances of each of the fragments in the unknown samples in the same way you measured the standard bands.

2. Generate a Standard Curve.

Because migration rate is inversely proportional to the \log_{10} of band 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!

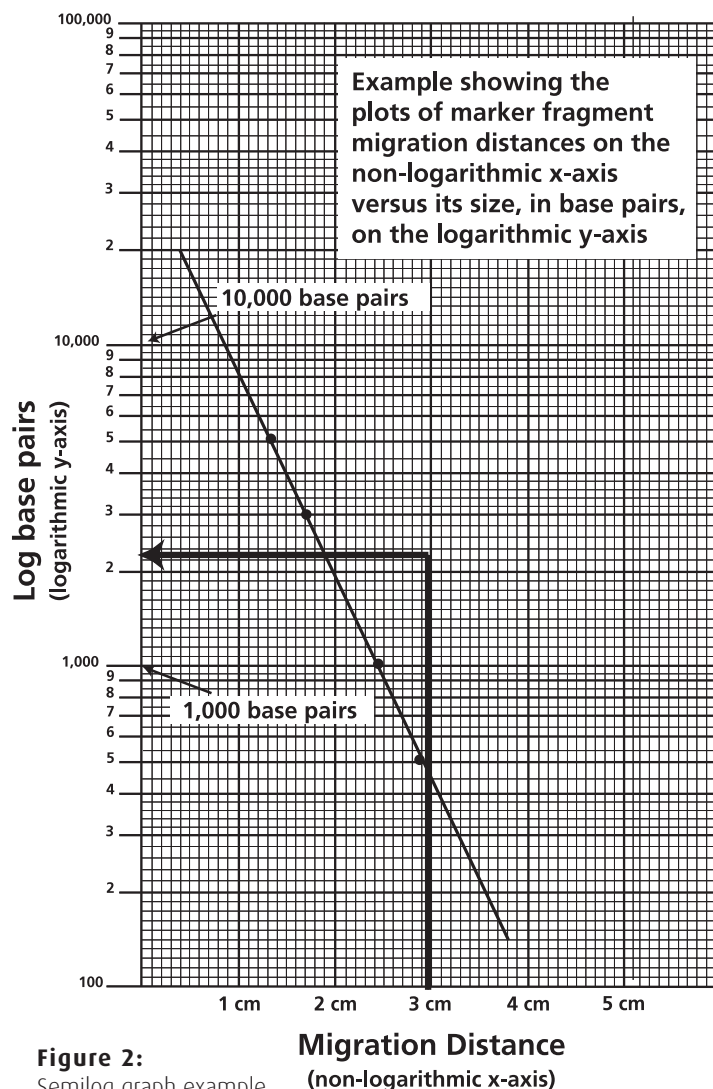


Figure 2:
Semilog graph example

Appendix C

Data Analysis Using a Standard Curve

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 2 for an 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 2 for an example). Make note of this in your lab notebook.
- c. Repeat for each fragment in your unknown sample.

Appendix C

