EDVOTEK® • The Biotechnology Education Company®

Edvo-Kit #
AP09/112

BIG IDEA AP BIOLOGY

INVESTIGATION #9:

Restriction Enzyme Analysis of DNA

For 10 groups

Experiment Objective:

The objective of this experiment is to provide students with a hands-on opportunity to understand the process of restriction enzyme digestion and the subsequent separation of DNA fragments through agarose gel electrophoresis.

See page 3 for storage instructions.

Version AP09-112.230619

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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

Components (in QuickStrip™ format)		Check (✓)
Store QuickStrip™ samples in the refrigerator upon receipt.		
A & D	Lambda DNA cut with *Hin*dIII	❑
B & E	Lambda DNA cut with *Eco*RI	❑
C & F	Lambda DNA (uncut)	❑

REAGENTS & SUPPLIES

Store the following at room temperature.

- UltraSpec-Agarose™ ❑
- Electrophoresis Buffer (50x) ❑
- Practice Gel Loading Solution ❑
- FlashBlue™ DNA Stain ❑

Experiment #AP09/112 is designed for 10 groups.

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

ATTENTION:
If you ordered one of our "C" Series kits, bulk samples for 24 gels, simply load 35 µL of sample per well and follow the staining procedures as indicated in this protocol.

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipettes 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

Deoxyribonucleic acid (DNA) is the genetic material present in all living organisms, including bacteria, plants, humans, and certain viruses. It serves as the fundamental blueprint used to construct an organism, where the distinctive sequence of nucleotides, known as genotype, governs the observable characteristics, or phenotype. The proteins synthesized by our genes regulate various processes such as growth, development, cell specification, neuronal function, and metabolism.

DNA is composed of nucleotides, each consisting of three essential components: a phosphate group, a deoxyribose sugar, and a nitrogenous base (adenine, cytosine, guanine, or thymine). The sugar of one nucleotide forms a covalent bond with the phosphate group of its neighboring nucleotide, forming long strands of DNA (Figure 1). Each strand of DNA pairs with a complementary strand in a sequence-specific manner, wherein adenine pairs with thymine and cytosine pairs with guanine through hydrogen bonding between base pairs.

> ***All of today's DNA, strung through all the cells of the earth, is simply an extension and elaboration of [the] first molecule.***
>
> – Lewis Thomas, The Medusa and the Snail: More Notes of a Biology Watcher

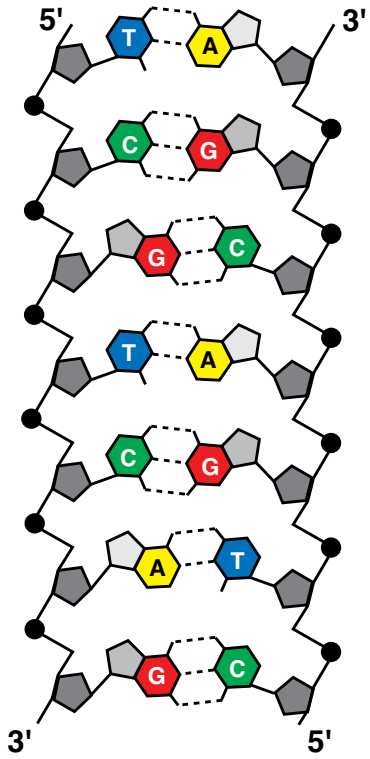


Figure 1 - Structure of DNA

THE DISCOVERY OF RESTRICTION ENZYMES

One of the most significant discoveries in molecular biology belongs to a group of enzymes called restriction endonucleases, also referred to as restriction enzymes. These remarkable enzymes function like molecular scissors by precisely cleaving through the sugar-phosphate DNA backbone based on the specific sequence of nucleotides. The immense utility of restriction enzymes revolutionized numerous scientific endeavors, including molecular cloning, DNA mapping, sequencing, and a wide array of genome-wide studies. This discovery launched the era of modern molecular biotechnology.

TABLE 1

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

Since they were first discovered in the 1970s, over 3,000 restriction enzymes have been identified. Each one is 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.)

Restriction endonucleases can be divided into five distinct classes based on how they cut DNA. Most of the enzymes used to cut DNA in the laboratory are classified as Type II restriction enzymes. Type II enzymes function as homodimers, meaning they form a protein complex consisting of two identical protein subunits that bind together (Figure 2). The complex binds to magnesium ions, an important cofactor for enzyme activity. Each protein subunit contains a DNA binding domain and an endonuclease domain. The DNA binding domain of each subunit recognizes and binds one half of the recognition sequence. Once bound, the nuclease domain cleaves one strand of the DNA backbone.

Restriction enzymes evolved in bacteria as a defense to protect themselves from invading viral DNA. The bacteria protect their DNA from digestion by chemically changing it using specific marks like methylation.

Most type II restriction enzymes 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" (Figure 3).

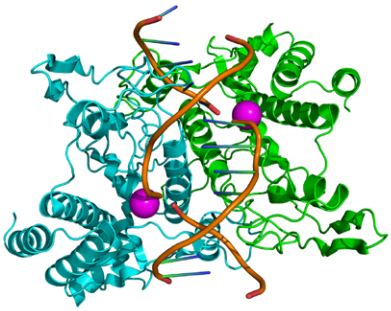


Figure 2 - Structure of the restriction enzyme *Eco*RI. The cyan and green ribbon diagrams represent protein monomers. The brown tubes represent the DNA helix. The magenta circles represent magnesium ion cofactor[1].

In writing, a palindrome is a word that reads the same frontwards and backwards, like racecar or kayak. In DNA, a palindromic sequence is an inverted repeat that reads the same on both strands of DNA, making it complementary to itself.

To illustrate this, first consider the recognition site and cleavage pattern of *Eco*RI. This enzyme 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').

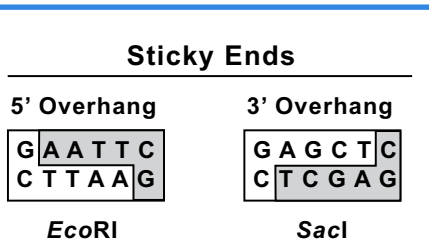
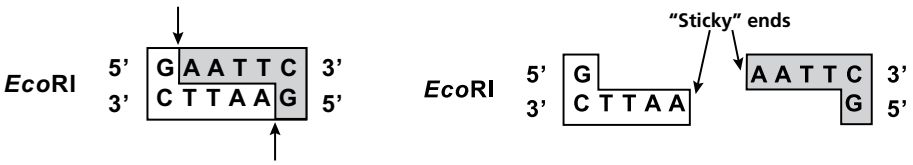
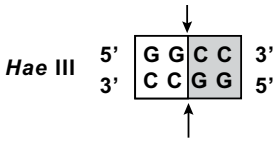


Figure 3: Different types of DNA ends produced by Restriction Enzymes.

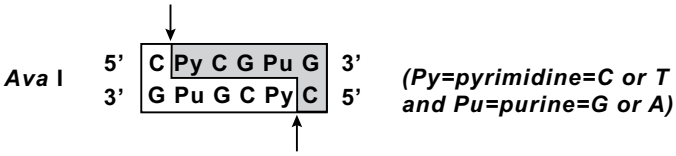


[1]Attribution: Figure 2: Restriction enzyme EcoRI - Boghog2, Public domain, via Wikimedia Commons.

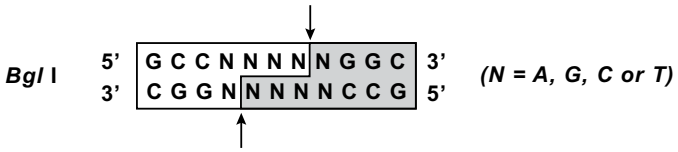
In contrast to *Eco*RI, *Hae*III cuts both DNA strands at the same position, which generates fragments without an overhang. These so-called "blunt" ends can be joined with any other blunt end without regard for complementarity.



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 set 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, BglI can recognize and cleave up to 625 possible sequences!

PERFORMING A RESTRICTION DIGEST

Each restriction enzyme has an ideal set of conditions that allow it to cut DNA efficiently. These conditions can include factors such as incubation temperature, pH, salt concentration, presence of metal ions, and cofactors like ATP. In this experiment, you will be analyzing DNA digested with EcoRI and HindIII, which are commonly used in biotechnology labs. These enzymes have been carefully studied to establish the most efficient and robust DNA digestion reactions possible.

The activity of restriction enzymes is quantified in units (U) based on their effectiveness in cutting DNA. Typically, this activity is defined as the amount of enzyme it takes to digest 1 µg of template DNA in 60 minutes. In most experiments, 10 U of the enzyme is sufficient to accomplish the digestion of the target DNA.

Restriction digestion samples are incubated at a physiologically appropriate temperature for the enzymatic reaction to proceed. The commonly recommended temperature is around 37°C, based on the microbe from which the enzyme was isolated. However, there are exceptions to this rule: some enzymes need temperatures as low as 25°C, and others need temperatures as high as 75°C.

This kit contains predigested DNA. If you are interested in exploring the diversity of enzyme reaction conditions for your future experiments, be sure to refer to the table provided by New England Biolabs! Visit www.neb.com

ANALYSIS OF RESTRICTION DIGESTS

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

This technique uses the combination of a porous gel matrix and an electrical field to separate molecules by size.

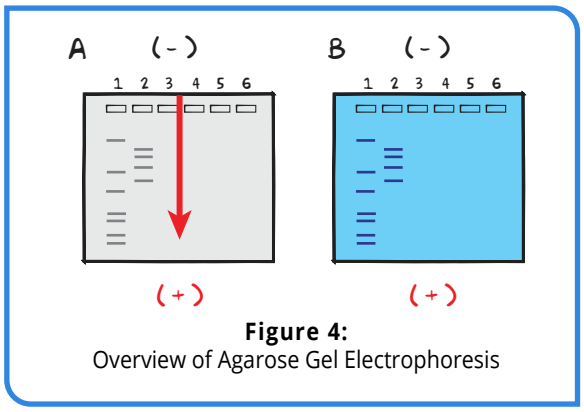


Figure 4:
Overview of Agarose Gel Electrophoresis

First, DNA molecules are added into depressions (or "wells") near the negative electrode of the gel, and then an electrical current is passed through the gel. Due to the chemical structure of the DNA backbone – the sugar phosphate bonds that hold nucleotides together – DNA has a strong negative charge. When we turn on the power supply, the current drives the restriction fragments through the gel towards the positive electrode.

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. As the current is pushing and pulling the DNA fragments through the gel, the molecules must find their way through these pores. Since it is easier for small DNA fragments to move through the pores in the gel than the larger ones, the molecules become separated and form discrete "bands" within the gel (Figure 4A).

After the current is stopped, the results of the experiment can be analyzed. However, since DNA is both clear and colorless, the results remain invisible. To overcome this limitation and visualize the results, we employ a staining technique using dyes such as FlashBlue™ or SYBR® Safe (Figure 4B). These stains label the DNA molecules, rendering them visible and allowing for analysis of the results.

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.

APPLICATIONS OF RESTRICTION ENZYMES IN THE RESEARCH LABORATORY

The utility of restriction enzymes has made molecular cloning, DNA mapping, sequencing, and various genome-wide studies possible, launching the era of biotechnology. For example:

- Molecular Cloning plays a crucial role in enabling researchers to isolate, combine, and reproduce specific DNA sequences with precision.
- DNA Fingerprinting is a technique that differentiates between individuals based on variations in their genome's sequence. This technique was pioneered by Sir Alec Jefferys in the early 1980's, when he noticed that there were similarities and differences in restriction fragment patterns between related people. Although commonly associated with forensic science, DNA fingerprinting serves a broader range of applications. It is utilized for determining parentage, conducting genealogical research, and identifying potential disease-causing alleles in medical investigations.
- Restriction Enzyme Mapping determines the sequence of DNA by identifying the precise locations of restriction enzyme recognition sites. By examining the relationships between these cut sites, including their order and frequency, we can characterize a gene or a specific region of the genome without knowing the complete sequence. Restriction mapping can be use both on small DNA fragments like plasmids and on pieces/regions of genomic DNA.

DIGESTION OF LAMBDA DNA

The bacteriophage lambda is a virus that specifically infects *Escherichia coli* (*E. coli*) bacteria. Early investigations of the viral genome described crucial regions responsible for its cellular entry, production of new viral particles, and subsequent lysis of the host cell (Figure 5). These studies significantly contributed to our understanding of DNA replication, transcription, and translation. As a result of these scholarly efforts, we have the complete 48,500 base pair genome sequence of the lambda phage, allowing us to create a map describing the frequency and locations at which restriction enzymes can cleave the DNA.

In this experiment, lambda genomic DNA has been digested with either the *Eco*RI and *Hin*dIII enzyme, creating a series of DNA fragments. Agarose gel electrophoresis is used to separate the DNA fragments, and length of each DNA fragment is calculated using a semi-logarithmic plot.

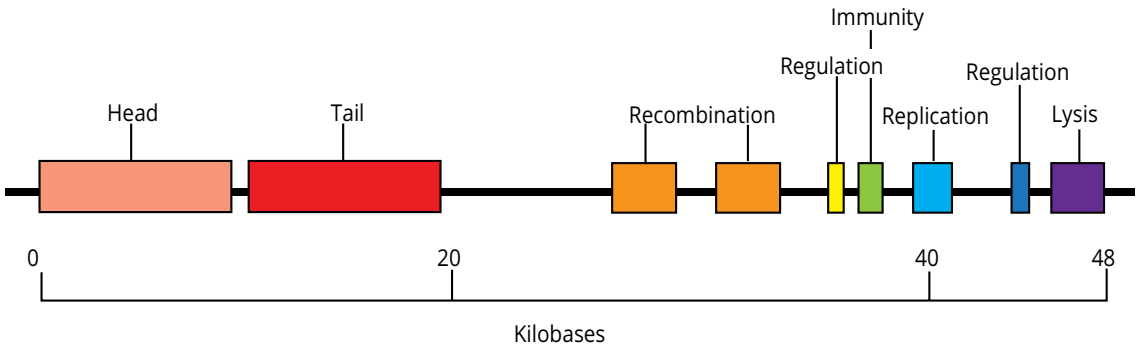


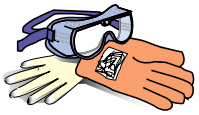
Figure 5: Lambda Phage Map

Experiment Overview

EXPERIMENT OBJECTIVE

The objective of this experiment is to provide students with a hands-on opportunity to understand the process of restriction enzyme digestion and the subsequent separation of DNA fragments through agarose gel electrophoresis. By the end of the activity, students will gain practical experience in analyzing the observed results and develop the ability to translate abstract concepts of restriction digestion and electrophoresis into a more profound 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.
- Record any challenges faced while performing the experiment.

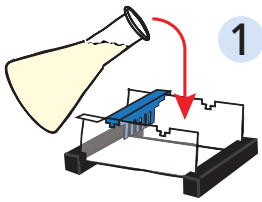
After the Experiment:

- 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.

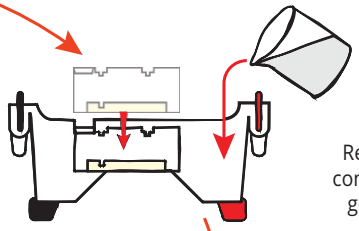
Experiment Overview

MODULE I: Agarose Gel Electrophoresis

Time required: See Table C



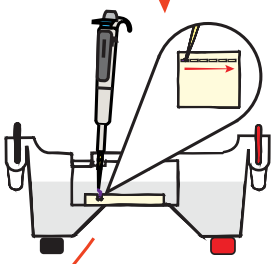
1 Prepare agarose gel in casting tray.



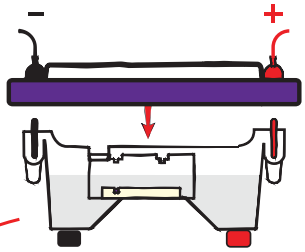
2 Remove end caps & comb, then submerge gel under buffer in electrophoresis chamber.

Quick Reference for EDVO-Kit #112/AP09			
Size of gel casting tray	*Groups per gel*	*Placement of comb*	*Wells required per group*
7 x 7 cm	1 or 2	1st set of notches	3
10 x 7 cm	1 or 2	1st set of notches	3
14 x 7 cm	2 or 4	1st and 3rd sets of notches	3

NOTE: This experiment requires 0.8% agarose gels. This kit provides enough reagents to cast ten 7 x 7 cm gels, eight 10 x 7 cm gels, or five 14 x 7 cm gels. Use the six place well-forming comb.



3 Load each sample in consecutive wells

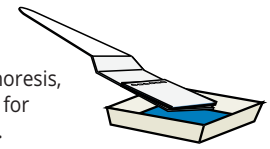


4 Attach safety cover & connect leads to power source to conduct electrophoresis.

MODULE II: Staining Agarose Gels Using FlashBlue™

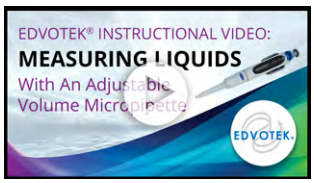
Time required: 30 min.

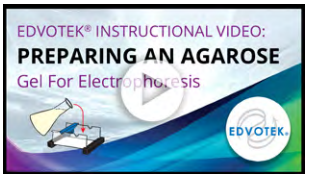
5 After electrophoresis, transfer gel for staining.

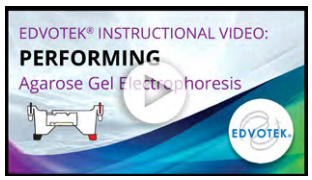


6 Analysis on white light source.

Related EDVOTEK® Instructional Videos

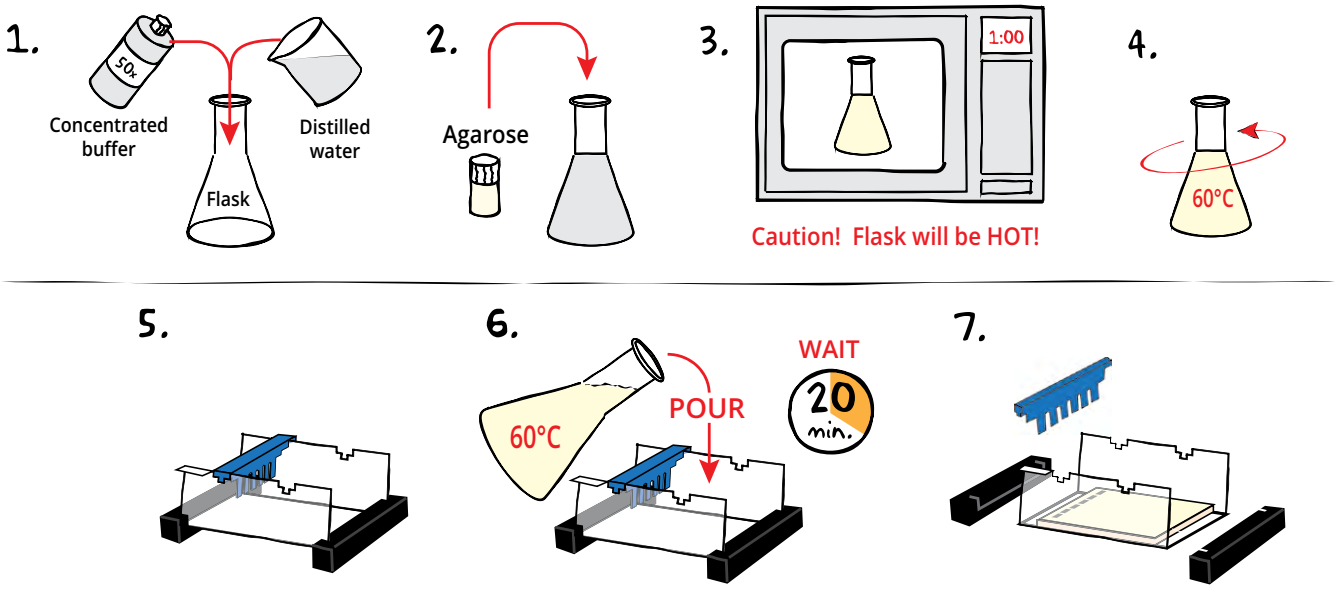






www.youtube.com/edvotekinc

Module I: Agarose Gel Electrophoresis



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.

Wear gloves and safety goggles

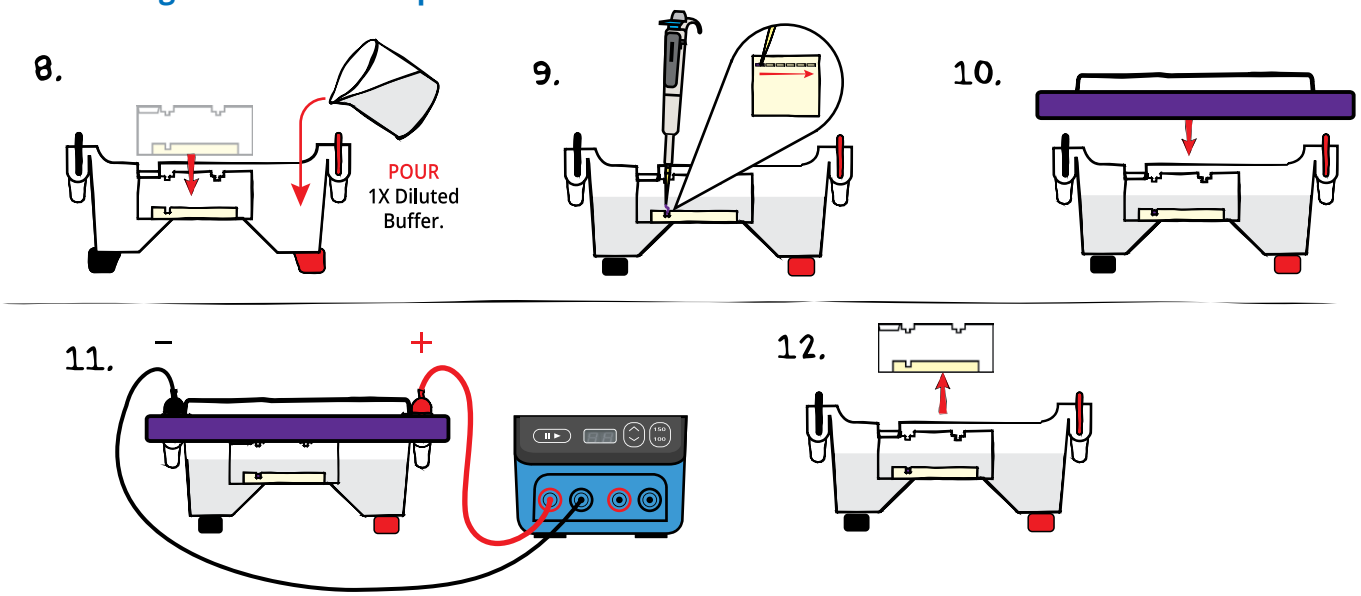
REMINDER:
This experiment requires 0.8% agarose gels cast with 6 wells.

Table A — **Individual 0.8% UltraSpec-Agarose™ Gels**

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	0.24 g	30 mL
10 x 7 cm*	0.9 mL	44.1 mL	0.36 g	45 mL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL

**Recommended gel volume for the EDGE™ Integrated Electrophoresis System. (Cat. #500).*

Module I: Agarose Gel Electrophoresis



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.

PROCEED to Module II: Staining Agarose Gels Using FlashBlue™.

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

TABLE 1: GEL LOADING

Lane 1	Tube A/D	Lambda DNA cut with *Hin*dIII
2	Tube B/E	Lambda DNA cut with *Eco*RI
3	Tube C/F	Lambda DNA (uncut)

Table B — **1x Electrophoresis Buffer (Chamber Buffer)**

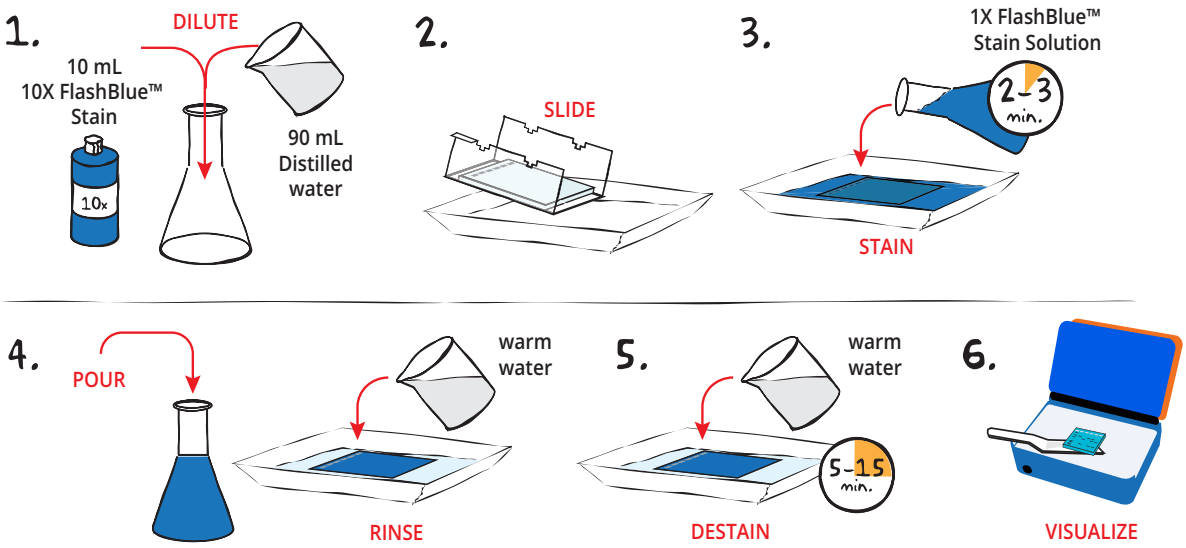
EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

Table C — **Time and Voltage Guidelines (0.8% Agarose Gel)**

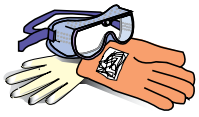
	Electrophoresis Model: EDGE™	M12 & M36
Volts	Min/Max (minutes)	Min/Max (minutes)
150	10/20	20/35
125	N/A	30/45
100	15/25	40/60

*Gels that have previously been removed from their trays should be "anchored" back to the tray with a few drops of molten agarose before placing into the electrophoresis chamber. This will prevent the gels from sliding around in the trays and the chambers.

Module II: Staining Agarose Gels Using FlashBlue™



1. **DILUTE** 10 mL of 10X concentrated FlashBlue™ with 90 mL of distilled water in a flask. **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1X FlashBlue™ stain solution. **STAIN** the gel for 2-3 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 3 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **POUR** the 1X FlashBlue™ back into the flask (the stain can be reused). **COVER** the gel with warm water (40-45 °C). Gently **RINSE** the gel for 20-30 seconds. **POUR** off the water.
5. **COVER** the gel with clean, warm water (40-45 °C). **DESTAIN** for 5-15 minutes with gentle shaking (longer periods will yield better results). DNA bands will start to appear after 5 minutes of destaining. Changing the water frequently will accelerate destaining.
6. 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

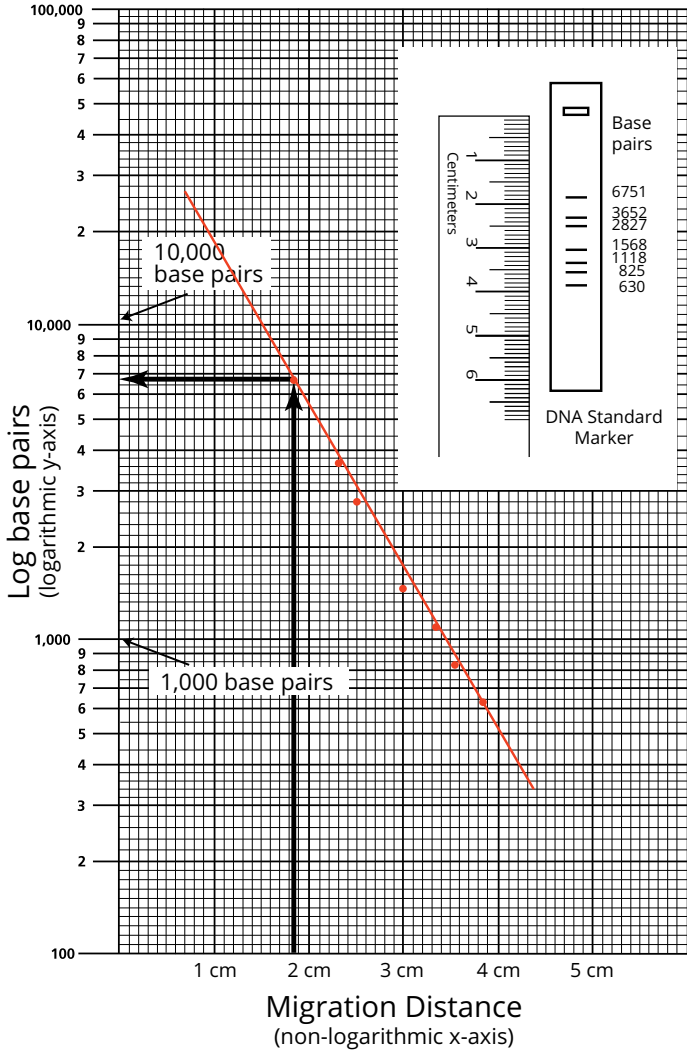
ALTERNATIVE FLASHBLUE™ STAINING PROTOCOL:

1. **DILUTE** 1 mL of 10X FlashBlue™ stain with 149 mL distilled water.
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.
4. Carefully **REMOVE** the gel from the staining liquid. **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 6: Semilog graph example



1. Measure and Record Migration 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 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!

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

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.*

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 6 for an example).

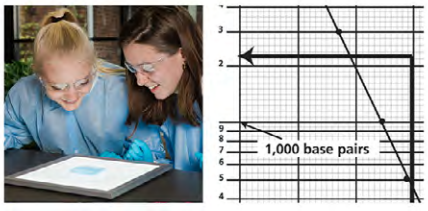
3. Determine the length of each unknown fragment.

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 6 for an example). Make note of this in your lab notebook.

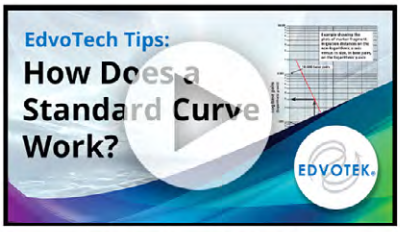
c. Repeat for each fragment in your unknown sample.

Resources:



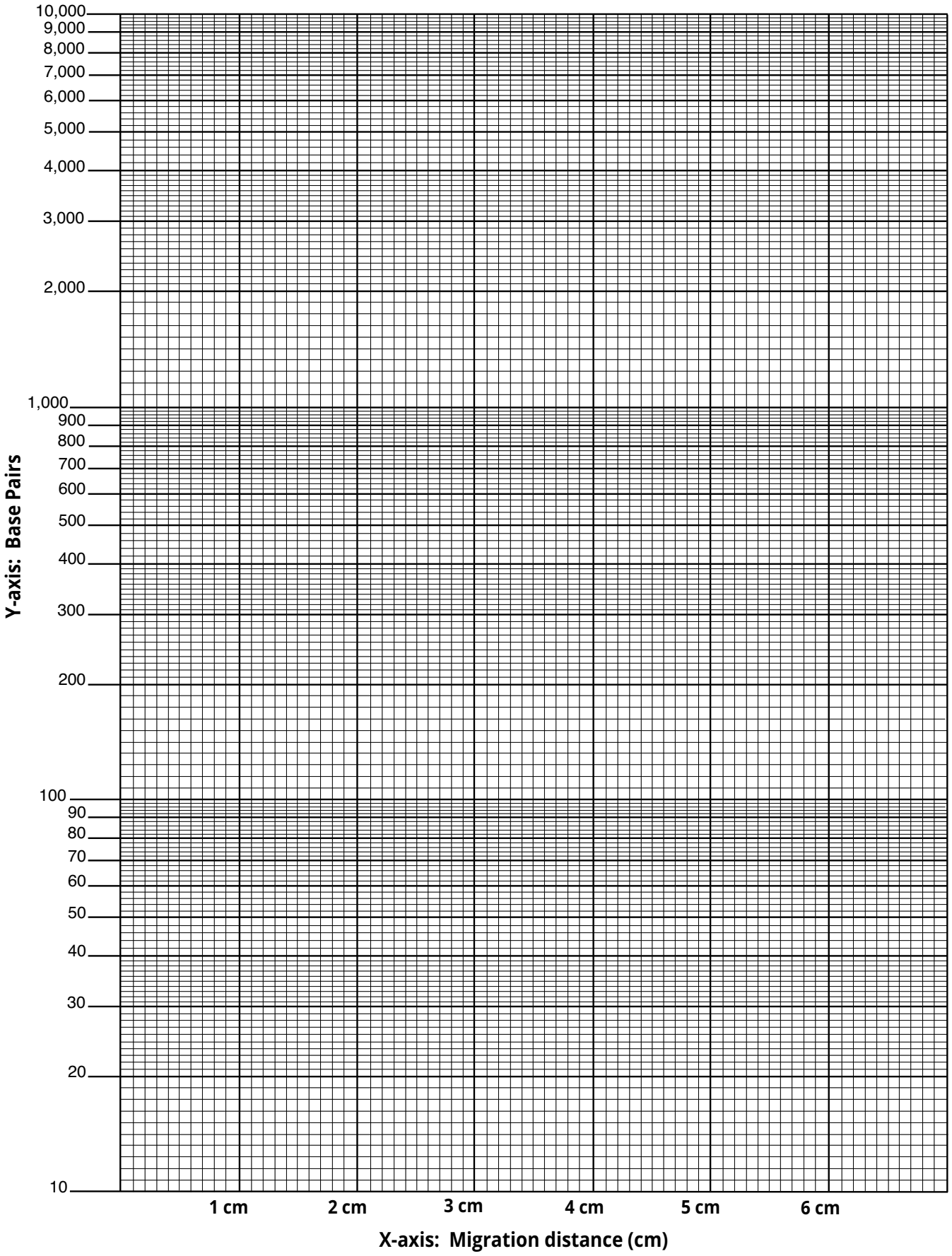
EDVOTEK® QUICK GUIDE:
Using Technology to Create a Standard Curve

READ HERE



EDVOTEK® TIPS VIDEO:
How Does a Standard Curve Work?

WATCH HERE

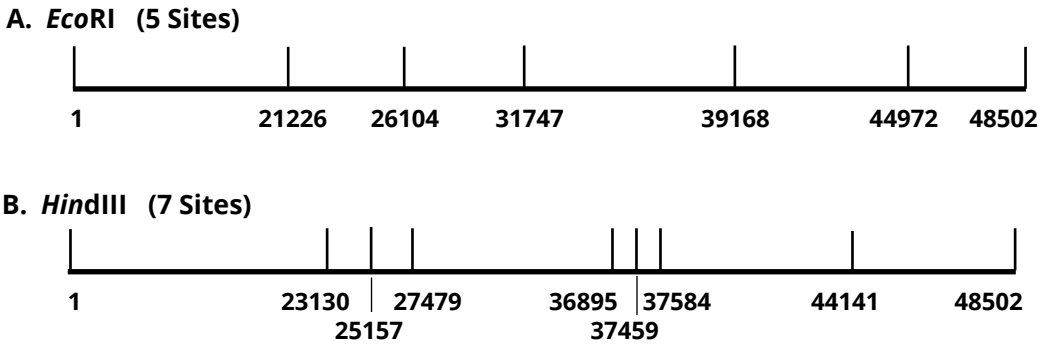


Study Questions

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



3. While working in the lab, you identify a new organism and sequence its genome. A small fragment of the sequence is below.

```
5'-CTCAGGGATCCGACCATGTAGAACATTACTTATAAGTCATCTTGAATTCTTAAACACAATCTTCCTGGGATCCAGCAC-3'
   |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

a) Write in the second strand.
b) Create a restriction map with two enzymes. The *Bam*HI cut sequence is GGATCC. *Eco*RI cuts GAATTC. Label the cut 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 Using FlashBlue™	Prepare staining components.	The class period or overnight after the class period.	10 min.

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 micropipettes, we recommended performing Cat. #S-44, Micropipetting Basics or Cat. #S-43, DNA DuraGel™ prior to conducting this experiment.

Pre-Lab Preparations: Module I

AGAROSE GEL ELECTROPHORESIS

This experiment requires 0.8% agarose gels. This kit provides enough reagents to cast ten 7 x 7 cm gels, eight 10 x 7 cm gels, or five 14 x 7 cm gels. Use the six place well-forming comb. You can choose whether to prepare the gels in advance or have students prepare their own. Allow approximately 30 minutes for this procedure.

FOR MODULE I
Each group will need:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- QuickStrip™ Samples

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.

Quick Reference for EDVO-Kit #112/AP09

Size of gel casting tray	*Groups per gel*	*Placement of comb*	*Wells required per group*
7 x 7 cm	1 or 2	1st set of notches	3
10 x 7 cm	1 or 2	1st set of notches	3
14 x 7 cm	2 or 4	1st and 3rd sets of notches	3

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.

SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

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

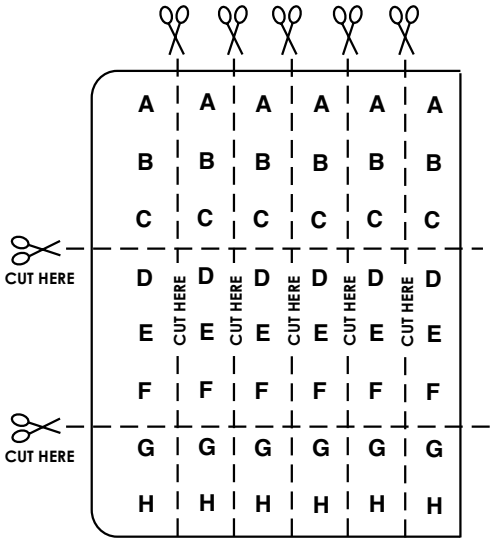
Using sharp scissors, first divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Next, cut each individual strip between wells C and D and wells F and G. Take care not to damage the protective overlay while separating the samples.

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.)

Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube. Puncture the foil overlay of the QuickStrip™ with a pipet tip to aspirate the sample. ***Do not remove the foil as samples can spill.***

Carefully cut between each set of tubes. Then, between rows C & D and F & G.



Pre-Lab Preparations: Module II

STAINING AGAROSE GELS USING 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.

PHOTODOCUMENTATION OF DNA (OPTIONAL)

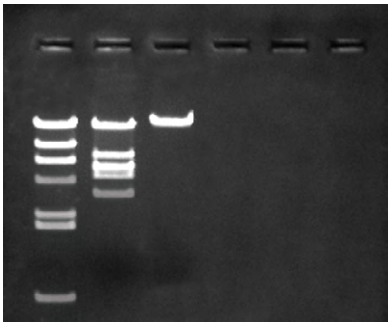
Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

FOR MODULE II
Each group will need:

- 10 mL 10X concentrated FlashBlue™ *OR* 100 mL 1x diluted FlashBlue™
- Small plastic tray or weight boat
- Distilled or deionized water

NOTE:
This kit is compatible with SYBR® Safe Stain (Cat #608, not included). Instructions for preparing gels and visualizing results can be found in Appendix C.

Experiment Results and Analysis



EXPECTED GEL RESULTS			
Lane	**Tube**	**Sample**	**Approx. Base Pairs**
1	A or D	Lambda DNA cut with *Hin*dIII	23130, 9416, 6557, 4361, 2322, 2027, 564*
2	B or E	Lambda DNA cut with *Eco*RI	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 may appear as a single band.*

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

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

Appendices

A EDVOTEK® Troubleshooting Guide

B Bulk Preparation of Electrophoresis Buffer and Agarose Gels

C Using SYBR® Safe Stain (OPTIONAL)

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 3.5 cm before staining and visualizing the DNA (approximately 45 minutes 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.

Visit www.edvotek.com for additional troubleshooting suggestions.

Appendix B

Bulk Preparation of Electrophoresis Buffer and 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)

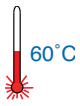
Batch Agarose Gels (0.8%)

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

Table E

Batch Prep of 0.8% UltraSpec-Agarose™			
Amt of Agarose +	Concentrated Buffer (50X) +	Distilled Water	Total Volume
3.0 g	7.5 mL	367.5 mL	375 mL

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, 45 mL for a 10 x 7 cm tray, and 60 mL for a 14 x 7 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. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks. Do not freeze gels.

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.

PROCEED to Loading and Running the Gel (page 12).

Appendix C
Using SYBR® Safe DNA Stain (OPTIONAL)

If desired, the DNA samples in this experiment can be visualized using **SYBR® Safe DNA stain (Cat #608)**.
We recommend adding diluted SYBR® Safe stain to the liquid agarose gels while casting for easy, reproducible results.
A blue light or UV transilluminator is needed for visualizing SYBR® gels. The TruBlu™ 2 (**Cat. #557**) is highly recommended.

PREPARING SYBR® SAFE STAIN

Instructors:

1. Prepare 1x Electrophoresis Buffer by combining 10 µL of 50X Concentrated Buffer with 490 µL of distilled water.
2. Add 20 µL of the SYBR® Safe to the tube of 1X buffer from Step 1 and mix by tapping the tube several times. The diluted SYBR® Safe Stain is now ready to be used during agarose gel preparation.

AGAROSE GEL PREPARATION

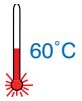
This experiment requires one 0.8% agarose gel for each student group. Instructors can choose whether to prepare the gels in advance (METHOD A) or have the students prepare their own (METHOD B). Allow approximately 30-40 minutes for this procedure.

Table E

Batch Prep of 0.8% UltraSpec-Agarose™			
Amt of Agarose +	Concentrated Buffer (50X) +	Distilled Water	Total Volume
3.0 g	7.5 mL	367.5 mL	375 mL

Instructor Preparation (METHOD A):
For quantity (batch) preparation of 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. Add the entire tube of ***diluted*** **SYBR® Safe** stain to the cooled agarose and mix well.
7. Dispense the required volume of cooled agarose solution for casting each gel. Measure 30 mL for a 7 x 7 cm tray, 45 mL for a 10 x 7 cm tray, and 60 mL for a 14 x 7 cm tray. ***For this experiment, 7 x 7 cm gels are recommended.***
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Solidified gels can be stored in the refrigerator for up to 2 weeks. Place 1-2 mL of electrophoresis buffer in a sealable bag with the gels to prevent them from drying out. Excessive buffer will cause SYBR® Safe to diffuse out of the gels. Do not freeze gels.

PROCEED to Loading and Running the Gel (Steps 8-12 on page 12), followed by the VISUALIZATION procedures on page 29.
NO ADDITIONAL STAINING IS NECESSARY.

Appendix C
Using SYBR® Safe DNA Stain (OPTIONAL)

AGAROSE GEL PREPARATION, CONTINUED

Student Preparation (METHOD B):
For student preparation of agarose gels, see Table A.2.

Table A.2 **Individual 0.8% UltraSpec-Agarose™ with SYBR® Stain**

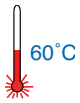
Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume	Diluted SYBR® (Step 6)
7 x 7 cm	0.6 mL	29.4 mL	0.24 g	30 mL	30 µL
10 x 7 cm*	0.9 mL	44.1 mL	0.36 g	45 mL	45 µL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL	60 µL

** Recommended gel volume for the EDGE™ Integrated Electrophoresis System.*

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A.2).

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. Before casting the gel, **ADD *diluted SYBR® Safe*** to the cooled agarose and swirl to mix (see Table A.2).

7. **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.

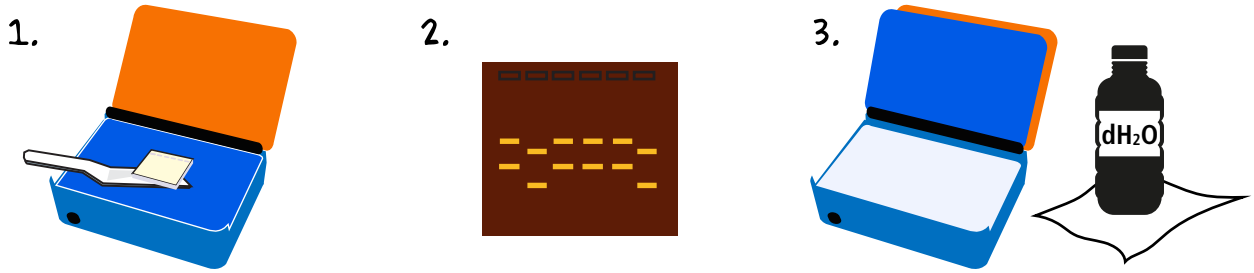
8. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

PROCEED to Loading and Running the Gel (Steps 8-12 on page 12), followed by the VISUALIZATION procedures on page 29.
NO ADDITIONAL STAINING IS NECESSARY.

Appendix C
Using SYBR® Safe DNA Stain (OPTIONAL)

VISUALIZING THE SYBR® GEL

A blue light or UV transilluminator is needed for visualizing SYBR® gels. The TruBlu™ 2 (Cat. #557) is highly recommended.



1. **SLIDE** gel off the casting tray onto the viewing surface of the transilluminator.
2. Turn the unit **ON**. DNA should appear as bright green bands on a dark background. **PHOTOGRAPH** results.
3. Turn the unit **OFF**. **REMOVE** and **DISPOSE** of the gel. **CLEAN** the transilluminator surfaces with distilled water.

Be sure to wear UV goggles if using a UV transilluminator.