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.

Version 102.210628
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Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Components (in QuickStrip™ format)  Check (√)

Store QuickStrip™ samples in the refrigerator upon receipt.

A  Plasmid DNA (uncut)  
B  Plasmid cut with *Bgl* II  
C  Plasmid cut with *EcoR* I  
D  Lambda DNA (uncut)  
E  Lambda DNA cut with *EcoR* I  
F  Lambda DNA cut with *Bgl* II

REAGENTS & SUPPLIES

Store the following at room temperature.

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

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.

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The discovery of restriction enzymes ushered in a new era of molecular ge-netics. These enzymes cut the DNA molecule in a highly specific and reproducible way. This, in turn, has lead 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.
The recognition sites of some restriction enzymes contain variable base positions. For example, **Ava I** recognizes:

\[
\text{Ava I} \\
5' \quad \text{C Py C G Pu G} \quad 3' \\
3' \quad \text{G Pu G C Py C} \\
\]

(Py=pyrimidine=C or T and Pu=purine=G or A)

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:

\[
\text{Bgl I} \\
5' \quad \text{G C C N N N N N G G C} \quad 3' \\
3' \quad \text{C G G N N N N N C C G} \\
\]

(N = A, G, C or T)

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. Partial cleavage 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**

*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.

3. Load each sample in consecutive wells.

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

5. After electrophoresis, transfer gel for staining.

6. Analysis on white light source.

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**Quick Reference for EDVO-Kit #102**

<table>
<thead>
<tr>
<th>Size of gel casting tray</th>
<th>Groups per gel</th>
<th>Placement of comb</th>
<th>Wells required per group</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7 cm</td>
<td>1 group</td>
<td>1st set of notches</td>
<td>6</td>
</tr>
<tr>
<td>10 x 7 cm</td>
<td>1 group</td>
<td>1st set of notches</td>
<td>6</td>
</tr>
<tr>
<td>14 x 7 cm</td>
<td>2 groups</td>
<td>1st and 3rd sets of notches</td>
<td>6</td>
</tr>
</tbody>
</table>

**MODULE II: Staining Agarose Gels Using FlashBlue™**

*Time required: 30 min.*

5. After electrophoresis, transfer gel for staining.

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**Related EDVOTEK® Instructional Videos**

[www.youtube.com/edvotekinc](http://www.youtube.com/edvotekinc)
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.

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

<table>
<thead>
<tr>
<th>Size of Gel Casting Tray</th>
<th>Concentrated Buffer (50X)</th>
<th>Distilled Water</th>
<th>Amt of Agarose</th>
<th>TOTAL Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7 cm</td>
<td>0.6 mL</td>
<td>29.4 mL</td>
<td>0.24 g</td>
<td>30 mL</td>
</tr>
<tr>
<td>10 x 7 cm*</td>
<td>0.9 mL</td>
<td>44.1 mL</td>
<td>0.36 g</td>
<td>45 mL</td>
</tr>
<tr>
<td>14 x 7 cm</td>
<td>1.2 mL</td>
<td>58.8 mL</td>
<td>0.48 g</td>
<td>60 mL</td>
</tr>
</tbody>
</table>

*Recommended gel volume for the EDGE™ Integrated Electrophoresis System. (Cat. #500).
Module I: Agarose Gel Electrophoresis

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 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™.

**Running the Gel**

*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.

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.
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.
ADVANCE PREPARATION:

<table>
<thead>
<tr>
<th>PREPARATION FOR:</th>
<th>WHAT TO DO:</th>
<th>WHEN?</th>
<th>TIME REQUIRED:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Module I: Agarose Gel Electrophoresis</td>
<td>Prepare QuickStrips™.</td>
<td>Up to one day before performing the experiment.</td>
<td>45 min.</td>
</tr>
<tr>
<td></td>
<td>Prepare diluted electrophoresis buffer.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prepare molten agarose and pour gels.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Module II: Staining Agarose Gels</td>
<td>Prepare staining components.</td>
<td>The class period or overnight after the class period.</td>
<td>10 min.</td>
</tr>
<tr>
<td>Using FlashBlue™</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Pre-Lab Preparations: Module I

AGAROSE GEL ELECTROPHORESIS

This experiment requires 0.8% agarose gels. Enough reagents are provided to cast either eight 7 x 7 cm gels, eight 10 x 7 cm gels, or four 14 x 7 cm gels. You can choose whether to prepare the gels in advance or have students prepare their own. Allow approximately 30 minutes for this procedure.

<table>
<thead>
<tr>
<th>Size of gel casting tray</th>
<th>Groups per gel</th>
<th>Placement of comb</th>
<th>Wells required per group</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7 cm</td>
<td>1 group</td>
<td>1st set of notches</td>
<td>6</td>
</tr>
<tr>
<td>10 x 7 cm</td>
<td>1 group</td>
<td>1st set of notches</td>
<td>6</td>
</tr>
<tr>
<td>14 x 7 cm</td>
<td>2 groups</td>
<td>1st and 3rd sets of notches</td>
<td>6</td>
</tr>
</tbody>
</table>

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. 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 foil overlay. Each well contains pre-aliquoted sample.

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 foil 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. Puncture the foil overlay of the QuickStrip™ with a pipet tip to aspirate the sample. Do not remove the foil as samples can spill.

FOR MODULE III
Each group will need:
- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- QuickStrip™ Samples

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.
Pre-Lab Preparations: Module II

STAINING AGAROSE GELS USING FLASHBLUE™

FlashBlue™ stain is optimized to shorten the time required for both staining and destaining 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.

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. #5-44, Micropipetting Basics or Cat. #5-43, DNA Duragel™ prior to conducting this experiment.
## Experiment Results and Analysis

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

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

* 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 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**

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bands are not visible on the gel.</td>
<td>The gel was not prepared properly.</td>
<td>Ensure that the electrophoresis buffer was correctly diluted.</td>
</tr>
<tr>
<td></td>
<td>The gel was not stained properly.</td>
<td>Repeat staining protocol.</td>
</tr>
<tr>
<td></td>
<td>Malfunctioning electrophoresis unit or power source.</td>
<td>Contact the manufacturer of the electrophoresis unit or power source.</td>
</tr>
<tr>
<td>After staining the gel, the DNA bands are faint.</td>
<td>The gel was not stained for a sufficient period of time.</td>
<td>Repeat staining protocol.</td>
</tr>
<tr>
<td></td>
<td>The background of gel is too dark after staining with FlashBlue™.</td>
<td>Destain the gel for 5-10 minutes in distilled water.</td>
</tr>
<tr>
<td>DNA bands were not resolved.</td>
<td>Tracking dye should migrate at least 3 cm from the wells to ensure adequate separation.</td>
<td>Be sure to run the gel at least 3 cm before staining and visualizing the DNA (approximately 15-20 minutes at 150 V).</td>
</tr>
<tr>
<td>DNA bands fade when gels are kept at 4 °C.</td>
<td>DNA stained with FlashBlue™ may fade with time.</td>
<td>Re-stain the gel with FlashBlue™.</td>
</tr>
<tr>
<td>There is no separation between DNA bands, even though the tracking dye ran the appropriate distance.</td>
<td>The wrong percent gel was used for electrophoretic separation.</td>
<td>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.</td>
</tr>
<tr>
<td>There's not enough sample in my QuickStrip™.</td>
<td>The QuickStrip™ has dried out.</td>
<td>Add 40 µL water, gently pipet up and down to mix before loading.</td>
</tr>
</tbody>
</table>

Visit [www.edvotek.com](http://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>
<thead>
<tr>
<th>50x Conc. Buffer</th>
<th>Distilled Water</th>
<th>Total Volume Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 mL</td>
<td>2,940 mL</td>
<td>3000 mL (3 L)</td>
</tr>
</tbody>
</table>

**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, 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.

PROCEED to Loading and Running the Gel (page 10).
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.

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 10), followed by the VISUALIZATION procedures on page 23.
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.

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 10), followed by the VISUALIZATION procedures on page 23.

**NO ADDITIONAL STAINING IS NECESSARY.**

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**Table A.2**

<table>
<thead>
<tr>
<th>Size of Gel Casting Tray</th>
<th>Concentrated Buffer (50X)</th>
<th>Distilled Water</th>
<th>Amount of Agarose</th>
<th>Total Volume</th>
<th>Diluted SYBR® (Step 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7 cm</td>
<td>0.6 mL</td>
<td>29.4 mL</td>
<td>0.24 g</td>
<td>30 mL</td>
<td>30 µL</td>
</tr>
<tr>
<td>10 x 7 cm*</td>
<td>0.9 mL</td>
<td>44.1 mL</td>
<td>0.36 g</td>
<td>45 mL</td>
<td>45 µL</td>
</tr>
<tr>
<td>14 x 7 cm</td>
<td>1.2 mL</td>
<td>58.8 mL</td>
<td>0.48 g</td>
<td>60 mL</td>
<td>60 µL</td>
</tr>
</tbody>
</table>

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