Edvo-Kit #120

Ready-to-Load™ DNA Sequencing

Experiment Objective:

The objective of this experiment is to develop an understanding of DNA Sequencing.

See page 3 for storage instructions.
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Safety Data Sheets can be found on our website: [www.edvotek.com/safety-data-sheets](http://www.edvotek.com/safety-data-sheets)
Experiment Components

**READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS**

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

<table>
<thead>
<tr>
<th>Components (in QuickStrip™ format)</th>
<th>Check (✓)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A or E DNA Sequenced &quot;A&quot;</td>
<td></td>
</tr>
<tr>
<td>B or F DNA Sequenced &quot;C&quot;</td>
<td></td>
</tr>
<tr>
<td>C or G DNA Sequenced &quot;G&quot;</td>
<td></td>
</tr>
<tr>
<td>D or H DNA Sequenced &quot;T&quot;</td>
<td></td>
</tr>
</tbody>
</table>

**REAGENTS & SUPPLIES**

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

**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

Experiment #120 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.

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.
DNA SEQUENCING

DNA sequencing was developed during the 1970’s from research groups in the United States and England. Since its early days, these methods have been refined and automated.

There are two basic approaches to DNA sequence analysis. One involves a set of organic chemical reactions while the other uses an enzymatic process. The chemical method is tedious and labor-intensive, whereas the enzymatic approach, which is often called the dideoxy method or Sanger sequencing, is quite fast. The Sanger sequencing method is based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication.

A specialized cloning vehicle constructed from an E. coli virus, called M13, facilitates rapid DNA sequence analysis. This virus contains a polylinker, which is a short region of DNA, about 57 base pairs, containing several unique restriction sites. Segments of DNA to be sequenced are inserted into the polylinker region using standard cloning procedures (Figure 1).

The M13 virus contains a single-stranded circular genome with about 7200 nucleotides. The virus will infect E. coli strain JM101 cells which contain a fertility factor. These cells are F+, and male. The virus infects by attachment to the sex pilus. Shortly after infection, the viral DNA will become double-stranded. It is this form which serves as a template for production of single-stranded DNA progeny. The DNA associates with the viral proteins to form mature virus and the virus exits the cell by budding; the cell is not lysed. For cloning and sequencing purposes, double-stranded DNA to be sequenced is inserted into the M13 polylinker region of the DNA intermediate, and then transformed into competent JM101 E. coli cells. The transformed cells will begin to produce progeny virus.

To sequence DNA which has been inserted into the polylinker region of M13, single-stranded DNA is prepared from viral plaques. In this experiment, a short 17-base synthetic single-stranded DNA is allowed to hybridize (form a base pairing) with a unique site in M13 adjacent to the polylinker.

This 17-base oligonucleotide will serve as a primer for DNA synthesis by the Klenow fragment of DNA polymerase I, which lacks the 5'-3' exonuclease activity (Figure 2).

For sequence analysis, four separate enzymatic reactions are performed, one for each nucleotide. Each reaction contains the Klenow fragment of DNA polymerase I, the single-stranded DNA template to which the 17 nucleotide synthetic DNA to be Sequenced

5' CTCGGTCGCTCATTACTGGCCGTCGTTTTAC 3'

DNA Sequencing Primer

3' TGACCGGCAGCAAAATG 5'
DNA primer has been hybridized, all four deoxyribonucleotide triphosphates (dATP, dGTP, dCTP, dTTP), 32P-dATP, and the appropriate buffer for in vitro DNA synthesis. The “G” reaction contains dideoxyGTP, the “C” reaction dideoxyCTP, the “A” reaction dideoxyATP, and the “T” reaction dideoxyTTP. Once a Dideoxynucleotide is incorporated into a single strand, DNA synthesis is completely terminated (Figure 3). The site of the Dideoxynucleotide incorporation allows one to determine the position of that base. The Dideoxynucleotide lacks a 3’-OH group on the ribose ring and it is impossible for the Klenow fragment of DNA polymerase I to add another nucleotide to the growing strand since a 3’-OH group is absolutely required.

Non-isotopic methods using fluorescent dyes and automated DNA sequencing machines have replaced the traditional isotopic methods. In spite of the detection differences, the basic biochemistry of the dideoxy sequencing method is essentially the same.

Since a particular reaction will contain millions of growing DNA strands, a “nested set” of fragments is obtained with each fragment is terminated at a different position corresponding to the random incorporation of the Dideoxynucleotide.

Figure 4 shows the “nested set” of fragments produced for a hypothetical sequence in the “G” reaction. The “G” reaction contains dATP, dCTP, dGTP, dTTP, the Klenow fragment of DNA polymerase, an appropriate solution for DNA synthesis, dATP and a small amount of dideoxyGTP.

As can be seen, ddGTP (dideoxyGTP) incorporation randomly and infrequently will produce a “nested set” of fragments which terminate with a ddGTP. The “nested set” is complimentary to the region being sequenced. Similar “nested sets” are produced in the separate “A”, “T”, and “C” reactions. For example, the “A” “nested set” would terminate with a ddATP.

It should be readily apparent that together the “G,A, T, C” “nested sets” contain radioactive 32P-la-labeled fragments ranging in size successively from 20 to 29 nucleotides for the hypothetical sequence in Figure 4.

The “G” reaction contains fragments of 20, 24, 28, and 29 nucleotides in length. The first seventeen of these nucleotides are contained in the synthetic DNA sequencing primer. The rest are added by DNA Polymerase I.
POLYACRYLAMIDE SEQUENCING GEL

Traditionally, reaction products from the A, C, G, and T reactions are applied to separate sample wells on a thin vertical polyacrylamide gel, which is usually longer in size (approximately 14 - 18 cm tall). Well #1 contains the “A” reaction; well #2 the “C” reaction; well #3 the “G” reaction; and well #4 the “T” reaction. The electrophoresis apparatus containing polyacrylamide gel is connected to a power supply with the positive electrode at the bottom and the negative electrode at the top. High voltage is applied (2000 volts, D.C.) to separate the radioactive 32P-labeled fragments which migrate from top to bottom. The unique sequencing polyacrylamide gels can resolve fragments which differ in size by a single nucleotide, based on size. The smaller fragments move fastest while the larger are slowest.

After electrophoretic separation is complete, autoradiography is performed. The polyacrylamide gel is placed into direct contact with a sheet of x-ray film. Since the DNA fragments are radioactively labeled with 32P, their position can be detected by a dark exposure band on the sheet of x-ray film. For a given sample well, the horizontal “bands” appear in vertical lanes from the top to the bottom of the x-ray film. Generally, a single electrophoretic run can contain 12 sets of “GATC” sequencing reactions since there are 48 sample wells on a typical gel. A single autoradiograph can yield 1000 nucleotides of novel sequence information. Automated sequence machines will yield several multiples of this information.

Figure 5

<table>
<thead>
<tr>
<th>Deduced Sequence</th>
<th>Fragment Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>19</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
</tr>
<tr>
<td>T</td>
<td>21</td>
</tr>
<tr>
<td>G</td>
<td>22</td>
</tr>
<tr>
<td>T</td>
<td>23</td>
</tr>
<tr>
<td>T</td>
<td>24</td>
</tr>
<tr>
<td>A</td>
<td>25</td>
</tr>
<tr>
<td>G</td>
<td>26</td>
</tr>
<tr>
<td>A</td>
<td>27</td>
</tr>
<tr>
<td>A</td>
<td>28</td>
</tr>
<tr>
<td>T</td>
<td>29</td>
</tr>
<tr>
<td>G</td>
<td>30</td>
</tr>
<tr>
<td>T</td>
<td>31</td>
</tr>
</tbody>
</table>
Experiment Overview

EXPERIMENT OBJECTIVE:

The objective of this experiment is to develop an understanding of DNA Sequencing.

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

1. Prepare agarose gel in casting tray.

2. Remove end blocks & 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 and conduct electrophoresis.

5. After electrophoresis, transfer gel for staining.

6. Analysis on white light source.

Gel pattern will vary depending upon experiment.
Module I: Agarose Gel Electrophoresis

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.

**CASTING THE AGAROSE GEL**

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

**Table A**

<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>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7 cm</td>
<td>0.6 ml</td>
<td>29.4 ml</td>
<td>0.23 g</td>
<td>30 ml</td>
</tr>
<tr>
<td>7 x 10 cm</td>
<td>1.0 ml</td>
<td>49.0 ml</td>
<td>0.39 g</td>
<td>50 ml</td>
</tr>
<tr>
<td>7 x 14 cm</td>
<td>1.2 ml</td>
<td>58.8 ml</td>
<td>0.46 g</td>
<td>60 ml</td>
</tr>
</tbody>
</table>

**IMPORTANT:**
This experiment requires 7x14 cm gels. Place well template (comb) in the first set of notches.

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
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 3.5 cm from the wells.

12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to instructions for **STAINING** the agarose gel.

### Running the Gel

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

---

**Table 1: Gel Loading**

<table>
<thead>
<tr>
<th>Lane</th>
<th>Tube A or E</th>
<th>DNA Sequenced “A”</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tube B or F</td>
<td>DNA Sequenced “C”</td>
</tr>
<tr>
<td>2</td>
<td>Tube C or G</td>
<td>DNA Sequenced “G”</td>
</tr>
<tr>
<td>3</td>
<td>Tube D or H</td>
<td>DNA Sequenced “T”</td>
</tr>
</tbody>
</table>

---

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

<table>
<thead>
<tr>
<th>EDVOTEK Model</th>
<th>Total Volume Required</th>
<th>5X Concentration Buffer ml</th>
<th>Distilled Water ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+ &amp; M12 (new)</td>
<td>300 ml</td>
<td>6 ml</td>
<td>294 ml</td>
</tr>
<tr>
<td>M12 (classic)</td>
<td>400 ml</td>
<td>8 ml</td>
<td>392 ml</td>
</tr>
<tr>
<td>M36</td>
<td>1000 ml</td>
<td>20 ml</td>
<td>980 ml</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Electrophoresis Model</th>
<th>M6+</th>
<th>M12 (new)</th>
<th>M12 (classic) &amp; M36</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volts</strong></td>
<td>Min. / Max.</td>
<td>Min. / Max.</td>
<td>Min. / Max.</td>
</tr>
<tr>
<td>150</td>
<td>15 / 20 min.</td>
<td>20 / 30 min.</td>
<td>25 / 35 min.</td>
</tr>
<tr>
<td>125</td>
<td>20 / 30 min.</td>
<td>30 / 35 min.</td>
<td>35 / 45 min.</td>
</tr>
<tr>
<td>75</td>
<td>35 / 45 min.</td>
<td>55 / 70 min.</td>
<td>60 / 90 min.</td>
</tr>
</tbody>
</table>
Module II-A: Staining Agarose Gels Using FlashBlue™

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

**ALTERNATIVE PROTOCOL:**

1. **DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH₂O.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.
Module II-B: Staining Agarose Gels Using InstaStain® Blue

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

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. How is sequenced DNA read on a DNA sequencing gel?

2. What is a dideoxynucleotide? How are these used in DNA sequencing?

3. If the concentration of a dideoxynucleotide is too low when mixed with the other four deoxynucleotides, how would that affect the sequencing results?

4. What would happen if the concentration of a dideoxynucleotide is too high?
# Instructor's Guide

## 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>
</tbody>
</table>

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Pre-Lab Preparations: Module I

AGAROSE GEL ELECTROPHORESIS

This experiment requires a 0.8% agarose gel per student group. For best results, we recommend each group should use a 7x14 cm gel with the well template (comb) in the first set of notches. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

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

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

Preparing Gels in Advance:
Gels may be prepared ahead and stored for later use. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks.

Do not freeze gels at -20°C as freezing will destroy the gels.

Gels that have been removed from their trays for storage should be “anchored” back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

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 D and E. Take care not to damage the protective overlay while separating the samples.

Each lab group will receive one set of tubes, either rows A-D or E-H.
- A & E contain DNA Sequenced "A"
- B & F contain DNA Sequenced "C"
- C & G contain DNA Sequenced "G"
- D & H contain DNA Sequenced "T"

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 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.

MODULE II: PHOTODOCUMENTATION OF DNA (OPTIONAL)

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

### Table: Lane, Tube, and DNA Sequencing

<table>
<thead>
<tr>
<th>Lane</th>
<th>Tube</th>
<th>DNA Sequenced</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A or E</td>
<td>“A”</td>
</tr>
<tr>
<td>2</td>
<td>B or F</td>
<td>“C”</td>
</tr>
<tr>
<td>3</td>
<td>C or G</td>
<td>“G”</td>
</tr>
<tr>
<td>4</td>
<td>D or H</td>
<td>“T”</td>
</tr>
</tbody>
</table>
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

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.</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.</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 3.5 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.</td>
<td>Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 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>
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**

<table>
<thead>
<tr>
<th>50x Conc. Buffer (ml)</th>
<th>Distilled Water (ml)</th>
<th>Total Volume Required (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>2,940</td>
<td>3,000 (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, 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**

<table>
<thead>
<tr>
<th>Amt of Agarose (g)</th>
<th>Concentrated Buffer (50X) (ml)</th>
<th>Distilled Water (ml)</th>
<th>Total Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>7.5</td>
<td>382.5</td>
<td>390</td>
</tr>
</tbody>
</table>