Edvo-Kit #117

Detection of Mad Cow Disease

Experiment Objective:

The objective of this experiment is to educate students about Bovine Spongiform Encephalopathy (BSE), better known as Mad Cow disease.

See page 3 for storage instructions.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment Components</td>
<td>3</td>
</tr>
<tr>
<td>Experiment Requirements</td>
<td>3</td>
</tr>
<tr>
<td>Background Information</td>
<td>4</td>
</tr>
<tr>
<td>Experiment Procedures</td>
<td></td>
</tr>
<tr>
<td>Experiment Overview</td>
<td>6</td>
</tr>
<tr>
<td>Module I: Agarose Gel Electrophoresis</td>
<td>8</td>
</tr>
<tr>
<td>Module II: Staining Agarose Gels</td>
<td>10</td>
</tr>
<tr>
<td>Study Questions</td>
<td>12</td>
</tr>
<tr>
<td>Instructor’s Guidelines</td>
<td>13</td>
</tr>
<tr>
<td>Pre-Lab Preparations</td>
<td>14</td>
</tr>
<tr>
<td>Experiment Results and Analysis</td>
<td>16</td>
</tr>
<tr>
<td>Study Questions and Answers</td>
<td>17</td>
</tr>
<tr>
<td>Appendices</td>
<td>18</td>
</tr>
</tbody>
</table>

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.

Components (in QuickStrip™ format)  Check (√)
A  Standard DNA Marker
B  Positive bovine protein control
C  Negative bovine protein control
D  Feed sample from mill #1
E  Feed sample from mill #2
F  Feed sample from mill #3

REAGENTS & SUPPLIES

• UltraSpec-Agarose™
• Electrophoresis Buffer (50x)
• 10x 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

Includes EDVOTEK’s All-NEW DNA Standard Marker

• Better separation
• Easier band measurements
• No unused bands

NEW DNA Standard ladder sizes: 6751, 3652, 2827, 1568, 1118, 825, 630

Experiment #117 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.
Bovine Spongiform Encephalopathy (BSE), better known as Mad Cow disease, is a fatal condition characterized by vacuoles (empty spaces) which form within the cytoplasm of neurons. These vacuoles result in a sponge-like appearance of the brain of the infected animal. Epidemics of BSE has led to the slaughter of tens of thousands of cattle.

Strong evidence exists that transmission of BSE does not involve nucleic acid vectors (as in a virus) but a proteinaceous particle known as a prion. The prion is an altered form of an endogenous membrane protein and appears to self-replicate, following infection, by distorting the shape of its native counterpart (Figure 1). BSE appears initially in tonsils and other lymphoid organs and then spreads to the nervous system, where it causes apoptosis (programmed cell death) of neurons. Infected cows become lethargic and later exhibit erratic behavior (hence the origin of “mad cow disease”).

In addition to cattle, prions also cause neurodegenerative disease in sheep, deer, mink, and humans. A rare, devastating human disorder known as Creutzfeldt-Jacob disease (CJD) typically strikes humans over the age of 45; patients are almost never under 30. In 1996, however, several cases appeared in the United Kingdom in patients under the age of 25. The brain pathology of these patients was different from classical CJD; this disease was termed new variant CJD, or “nvCJD”. Analysis revealed that the prion infectious agent from these patients was identical to the prion from BSE-infected cows, suggesting that the patients became infected by eating contaminated beef. Other studies suggested that prion infections could indeed cross species barriers by expressing the sheep prion gene in mice, which resulted in mouse spongiform encephalopathy. One theory of the origin of BSE in the U.K. is that cattle feed may have contained ruminants of infected sheep, resulting in species crossover of the sheep prion to cattle. Additionally, BSE-infected cow carcasses were believed to be used to prepare feed, resulting in widespread propagation of the disease.

Human deaths from nvCJD have been reported in the UK and France which are believed to be due to species crossover of BSE to humans. Widespread panic has devastated the beef industry in Europe and caused fear of a BSE outbreak in the United States. One method of preventing domestic cattle infection is by prohibiting the use of cow or sheep parts in cattle feed; the U.S. Food and Drug Administration (FDA) banned this practice in 1997.

To enforce this ban, inspectors test for the presence of bovine protein in cattle feed. One method of testing uses the polymerase...
chain reaction (PCR) on feedstuffs. PCR is a powerful technique universally used to amplify DNA at very specific sequences. PCR uses a heat-stable enzyme known as Taq DNA polymerase. The reaction mixture contains the polymerase and two synthetic oligonucleotides, known as “primers” which flank the sequence(s) to be amplified, known as the “template”. In the case of cattle feed compliance, the template would be feedstuffs from various feed mills and the primers would be complimentary to a bovine-specific gene.

In the first step of a PCR reaction (Figure 2, next page), the template complimentary DNA strands are melted/separated from each other at 94°C, at which temperature the Taq DNA polymerase remains stable. In the second step, known as annealing, the sample is cooled to allow hybridization of the primers to the two strands of the target sequence(s). In the third step, known as extension, the temperature is raised to 72°C and the Taq polymerase adds nucleotides to the primers to complete synthesis of the new complementary strands. The three steps - denaturation, annealing, and extension - constitute one PCR “cycle”. This process is typically repeated for 20-30 cycles, amplifying the target sequence exponentially (Figure 2, bottom). PCR is performed in a thermal cycler, which is programmed to rapidly heat, cool and maintain samples at designated temperatures for varying amounts of time.

In this experiment, a hypothetical scenario involves the U.S. Federal Drug Administration (FDA) laboratory, which has obtained cattle feed samples from different feed mills. Using bovine specific primers, PCR has been performed on each of these samples. Students submit the samples to agarose gel electrophoresis to determine if any of the cattle feed samples contain bovine proteins, which could propagate mad cow disease. The presence of an amplified product indicates the presence of bovine products in the cattle feed, in violation of the federal statute.
Experiment Overview

EXPERIMENT OBJECTIVE:

The objective of this experiment is to educate students about Bovine Spongiform Encephalopathy (BSE), better known as Mad Cow disease.

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

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>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>0.23 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:**
If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at www.edvotek.com
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 and proceed to instructions for STAINING the agarose gel.

Table 1: Gel Loading

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard DNA Marker</td>
</tr>
<tr>
<td>2</td>
<td>Positive bovine protein control</td>
</tr>
<tr>
<td>3</td>
<td>Negative bovine protein control</td>
</tr>
<tr>
<td>4</td>
<td>Feed sample from mill #1</td>
</tr>
<tr>
<td>5</td>
<td>Feed sample from mill #2</td>
</tr>
<tr>
<td>6</td>
<td>Feed sample from mill #3</td>
</tr>
</tbody>
</table>

Table B: 1x Electrophoresis Buffer (Chamber Buffer)

<table>
<thead>
<tr>
<th>Model #</th>
<th>Total Volume Required</th>
<th>Dilution</th>
<th>Distilled Water</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>Voltage</th>
<th>M6+</th>
<th>M12 (new)</th>
<th>M12 (classic) &amp; M36</th>
</tr>
</thead>
<tbody>
<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. What is a prion? How might a prion-based disease be transmitted?
2. What is bovine spongiform encephalopathy? What are some characteristics of this condition?
3. What is Creutzfeld-Jacob disease? How is it contracted?
## 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>
Pre-Lab Preparations: Module I

AGAROSE GEL ELECTROPHORESIS

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

Individual Gel Preparation:

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

Batch Gel Preparation:

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. 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, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the protective overlay while separating the samples.

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

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

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

If students are unfamiliar with using micropipets, we recommended performing Cat. #5-44, Micropipetting Basics or Cat. #5-43, DNA DuraGel™ prior to conducting this advanced level experiment.

FOR MODULE I
Each Student Group should receive:
• 50x concentrated buffer
• Distilled Water
• UltraSpec-Agarose™
• QuickStrip™ Samples

Carefully cut between each set of tubes
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

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

#### Includes EDVOTEK’s All-NEW DNA Standard Marker
- Better separation
- Easier band measurements
- No unused bands

**NEW DNA Standard ladder sizes:**
- 6751, 3652, 2827, 1568, 1118, 825, 630

<table>
<thead>
<tr>
<th>Lane</th>
<th>Tube</th>
<th>Sample</th>
<th>Molecular Weights (in bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>DNA Standard Markers</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Positive Bovine Protein Control</td>
<td>4282</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Negative Bovine Protein Control</td>
<td>No Bands</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>Feed Sample from Mill #1</td>
<td>4282</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>Feed Sample from Mill #2</td>
<td>No Bands</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>Feed Sample from Mill #3</td>
<td>4282</td>
</tr>
</tbody>
</table>

The idealized schematic shows feed sample from mill #1 and mill #3 are positive for bovine protein. Feed sample from mill #2 shows negative for bovine protein.
Please refer to the kit insert for the Answers to Study Questions
Appendices

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

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 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>Table D</th>
<th>Bulk Preparation of Electrophoresis Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>50x Conc. Buffer + Distilled Water</td>
<td>Total Volume Required</td>
</tr>
<tr>
<td>60 ml</td>
<td>2,940 ml</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.

<table>
<thead>
<tr>
<th>Table E</th>
<th>Batch Prep of 0.8% UltraSpec-Agarose™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amt of Agarose (g) + Concentrated Buffer (50X) + Distilled Water (ml)</td>
<td>Total Volume (ml)</td>
</tr>
<tr>
<td>3.0</td>
<td>7.5</td>
</tr>
</tbody>
</table>
Appendix C
Data Analysis Using a Standard Curve

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

1. **Measure and Record Migration Distances**

Measure the distance traveled by each Standard DNA Fragment from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters (to the nearest millimeter) in your notebook. Repeat this for each DNA fragment in the standard.

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

2. **Generate a Standard Curve.**

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

![Figure 3: Measure distance migrated from the lower edge of the well to the lower edge of each band.](image)

![Figure 4: Semilog graph example](image)
Appendix C

Data Analysis Using a Standard Curve

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

c. Repeat for each fragment in your unknown sample.