Edvo-Kit # 124

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DNA Screening for Smallpox

Experiment Objective:

The objective of this experiment is to develop an understanding of Smallpox and the causative agent of the disease. Students will analyze simulated PCR products to confirm or rule out the presence of the Smallpox virus.

See page 3 for storage instructions.

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EDVOTEK®

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Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Components (in QuickStrip™ format)		Check (✓)
Store QuickStrip™ samples in the refrigerator upon receipt.		
A	DNA Standard Marker	❑
B	Simulated Smallpox positive control marker	❑
C	Negative control	❑
D	Monkeypox control DNA sample	❑
E	Sample from Patient #1	❑
F	Sample from Patient #2	❑

Experiment #124 is designed for 8 groups.

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

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.

Background Information

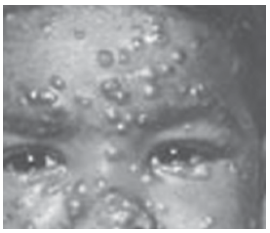


Figure 1: Smallpox victim

Smallpox is a serious, highly contagious disease characterized by severe skin eruptions. These eruptions leave pitted scars, known as "pocks", which is the basis of the word "pox". Throughout history, it is estimated that smallpox has killed over 100 million people and left another 200 million blind and/or permanently scarred. The first documented cases of smallpox occurred over 3000 years ago in China, Egypt, and India. The Egyptian pharaoh Ramses V died of smallpox in 1157 B.C. The plague of Antonine, approximately AD 180, was a smallpox epidemic that killed over 3 million people and coincided with the first stages of the decline of the Roman Empire. From 1512-1515, 3.5 million Aztecs in Mexico died from smallpox after being exposed to the disease by a single Spaniard accompanying the conquistador Hernando Cortez. In Europe in the late 18th century, smallpox killed over 400,000 people annually with an additional third of the survivors struck blind. Even as recently as the 1960s, the disease has infected over 10 million and killed over 2 million people worldwide annually.

Due to the high fatality and high communicability, many attempts have been made to use smallpox as a weapon. During the Middle Ages in Europe and Asia, bodies of smallpox victims were often catapulted over the walls of a city under siege in the attempts to start an epidemic in the attacking army. In the 16th century, it is believed that the Spanish conquistador Francisco Pizarro gave smallpox-infected clothing to native South Americans. In 1763, during the French-Indian War, Lord Jeffrey Amherst, the British commander-in-chief for America, distributed smallpox-laden blankets (from a local smallpox hospital) to enemy Indian tribes, killing one-third of their population. During the American Civil War, Dr. Luke Blackburn attempted to infect clothing with smallpox that he then sold to Union troops, although the success of this tactic was unclear, as infectious disease was already rampant among the soldiers. During World War II, the United States and Japan worked on weaponizing smallpox. In the late 1980s, the Soviet Union embarked on an ambitious project to place smallpox in intercontinental ballistic missiles, and reportedly produced hundreds of tons of the virus.

VARIOLA VIRUS

The virus that causes smallpox, known as variola, belongs to a family of viruses known as Poxviridae, recognizable in the microscope by their brick-shaped structures. Smallpox is divided into two subtypes: variola major, with a mortality rate of 20-40%, and variola minor, a less serious form of the disease, with a mortality rate of 1-2%. Chickenpox is a very common childhood virus resembling smallpox. It is caused by the Varicella-Zoster virus and is more superficial and has less serious skin eruptions. Monkeypox is native to Central and West Africa and is usually transmitted to humans by contact with primate blood or after suffering a bite. Monkeypox is moderately transmittable from human to human. Cowpox is not as serious and usually results from direct contact with the infected udder or teats of cattle and horses.

Smallpox and the other viruses carry their genetic material as DNA and replicate in the cytoplasm of infected cells. Variola virus has a genome size of 190 kilobases and consists of 200 genes. The virion is composed of over 100 proteins and carries its own RNA polymerase which transcribes the viral DNA. Virus assembly occurs in the cytoplasm, followed by host cell lysis with release of thousands of virus particles, resulting in extremely rapid multiplication of the virus during the incubation period.

Variola virions are quite stable in saliva aerosol droplets and usually enter the patient through the respiratory tract. Following a 10-15 day incubation period, symptoms begin to appear. Headache, severe back pain, high fever, prostration, and delirium are early symptoms. Following infection of internal organs, the virus re-enters the bloodstream and infects the skin (approximately 3 days after the initial onset of symptoms). This skin infection results in the characteristic smallpox rash which starts as small red dots that proceed to fluid-filled pustules. If the patient survives, the blisters turn to scabs that fall off, leaving a pitted scar. Corneal scarring usually results in blindness. The disease is most contagious when the rash is present. Smallpox is usually diagnosed based on the characteristic rash. Microscopic examination of

virus particles can also be used, as pox viruses have a unique shape. To conclusively diagnose smallpox and determine the exact strain, the polymerase chain reaction (PCR) can be used to demonstrate the unique DNA "fingerprint" of the virus strain.

Death from smallpox usually occurs during the initial rash. A different strain of Variola major, also known as hemorrhagic smallpox, produces internal bleeding and may cause death prior to rash formation. If a patient survives, he or she will be immune to smallpox for life. This immunity has been known for thousands of years. An early immunization technique known as *variolation* involved transferring infectious samples (pus or scabs) from a patient with mild smallpox to a healthy person via the nostrils or skin. In the 18th century rural Europe, it was known that milkmaids became immune to smallpox after contracting cowpox from the udders of cows. In 1796, Edward Jenner, an English country doctor, extracted pus from a cowpox-infected milkmaid and injected it into a healthy young boy. He then performed experiments to "challenge" the boy's skin by variolation with smallpox scabs, which verified that the boy was immune to smallpox. Jenner's work was the birth of the modern-day vaccination.

It is believed that immunity from vaccination diminishes after about ten years, although there is much disagreement over this subject. In 1952, the World Health Organization (WHO) initiated a worldwide vaccination campaign to eradicate smallpox. This program resulted in the steady decline of the virus with the last naturally occurring case in Somalia in 1977. On May 8, 1980, the WHO declared smallpox as officially eradicated from the world.

Despite eradication of natural smallpox, many experts fear that this disease could be reintroduced into the population intentionally by terrorists. As immunity is thought to diminish after ten years and mass vaccinations ceased in the mid 1970s, the current world population would likely be susceptible to the virus. Since the virus is very stable in aerosol droplets, it is possible that terrorists could employ some type of spraying device to attack an airport or other crowded building. Due to our highly mobile society and the high tranmissability of smallpox, one single case of smallpox may constitute a worldwide emergency.

POSSIBLE BIOTERRORISM SCENARIO

Following intelligence from overseas sources, the FBI issues a terrorist alert to the city of Northwest, with a population of 1.5 million. Local authorities are instructed to tighten security at large gatherings such as sporting events and music concerts. Health officials are directed to report any unusual medical cases that could represent bioterrorism.

Twenty days after the alert, a 27-year old Caucasian man appears at a local hospital emergency room with 106-degree fever and pustules concentrated on his face and extremities. At least one member of his family had a similar ailment. Twelve days earlier, the man had attended a football game with over 80,000 people present. In consideration of the terrorist alert, the man is immediately placed in isolation and tested for smallpox. A preliminary antibody-based test, however, is negative. One of the hospital physicians, who had been in Central Africa ten years before, diagnosed the patient's symptoms to be very similar to cases of monkeypox he had treated. The rest of the attending physicians concurred. In fact, the man's brother-in-law worked at the local zoo that had recently received several animals from a rainforest in the Democratic Republic of the Congo. To conclusively determine whether the man had smallpox, resulting from a bioattack, or monkeypox, which is much less contagious, fluid is taken from a large pustule on the patient's arm. This fluid is then immediately sent to the Centers for Disease Control (CDC) in Atlanta for PCR testing.

This experiment is a simulation of molecular biology tests used for the detection of the smallpox virus.

Experiment Overview

EXPERIMENT OBJECTIVE

The objective of this experiment is to develop an understanding of Smallpox and the causative agent of the disease. Students will analyze simulated PCR products to confirm or rule out the presence of the Smallpox virus.

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.

Wear gloves and safety goggles

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.

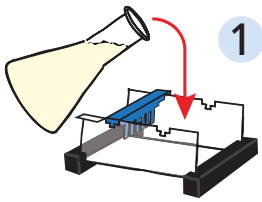
After the Experiment:

- 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.

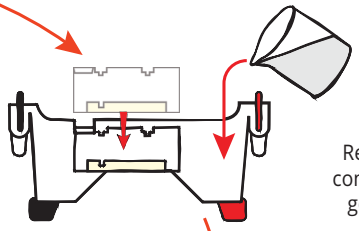
Experiment Overview

MODULE I: Agarose Gel Electrophoresis

Time required: See Table C

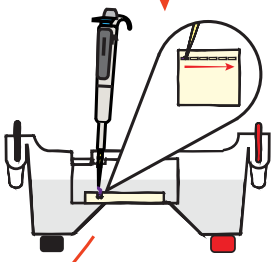


1 Prepare agarose gel in casting tray.

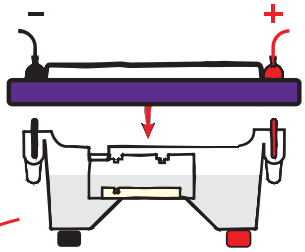


2 Remove end caps & comb, then submerge gel under buffer in electrophoresis chamber.

Quick Reference for EDVO-Kit #124			
Size of gel casting tray	*Groups per gel*	*Placement of comb*	*Wells required per group*
7 x 7 cm	1 group	1st set of notches	6
10 x 7 cm	1 group	1st set of notches	6
14 x 7 cm	2 groups	1st and 3rd sets of notches	6



3 Load each sample in consecutive wells

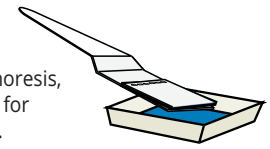


4 Attach safety cover & connect leads to power source to conduct electrophoresis.

MODULE II: Staining Agarose Gels Using FlashBlue™

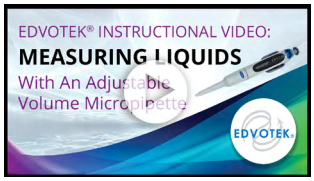
Time required: 30 min.

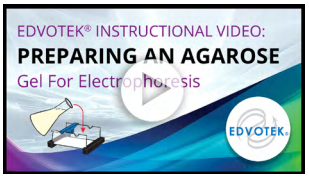
5 After electrophoresis, transfer gel for staining.

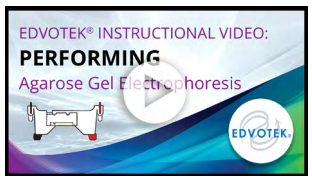


6 Analysis on white light source.

Related EDVOTEK® Instructional Videos

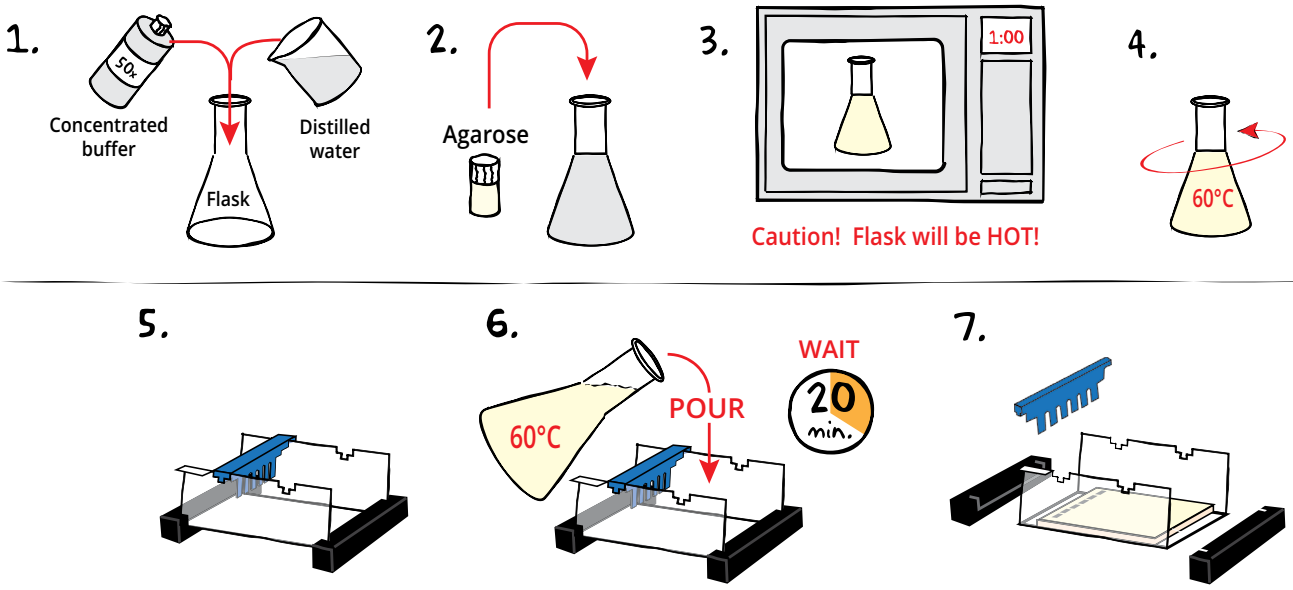






www.youtube.com/edvotekinc

Module I: Agarose Gel Electrophoresis



CASTING THE AGAROSE GEL

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

Wear gloves and safety goggles

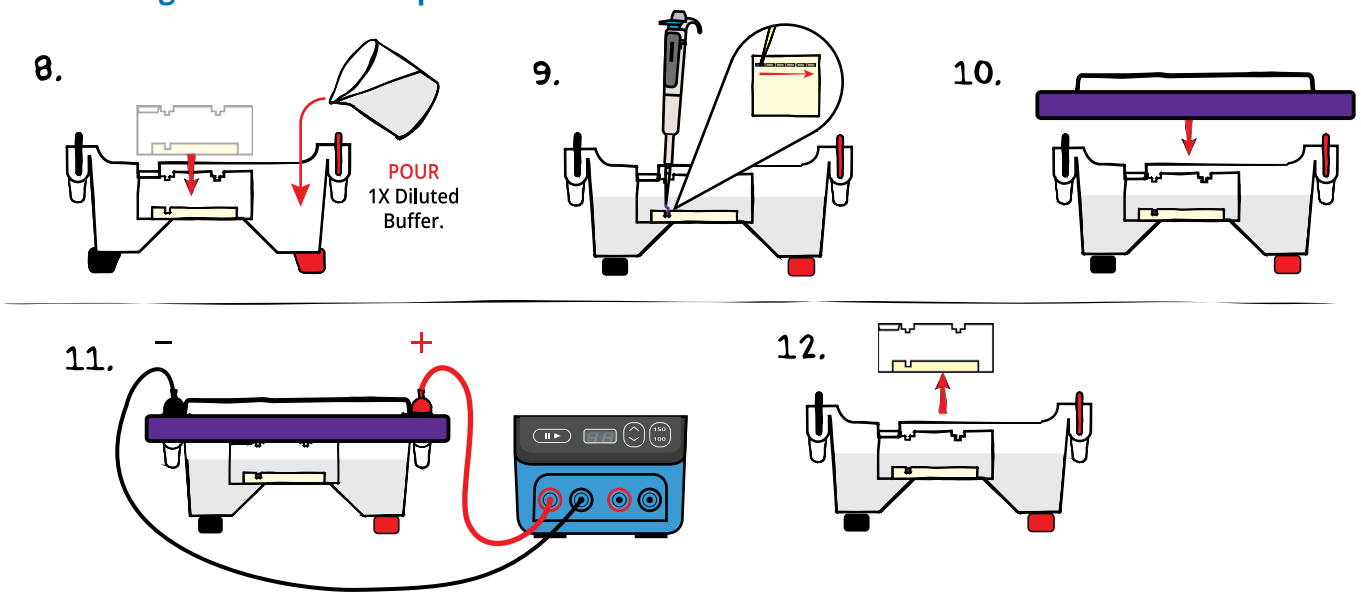
REMINDER:
This experiment requires 0.8% agarose gels cast with 6 wells.

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

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

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

Module I: Agarose Gel Electrophoresis



RUNNING THE GEL

8. **PLACE** the gel (still on the tray*) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35 µL) into the well in the order indicated by Table 1, at right.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

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

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

TABLE 1: GEL LOADING

Lane 1	Tube A	DNA Standard Marker
2	Tube B	Simulated Smallpox positive control marker
3	Tube C	Negative control
4	Tube D	Monkeypox control DNA sample
5	Tube E	Sample from Patient #1
6	Tube F	Sample from Patient #2

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

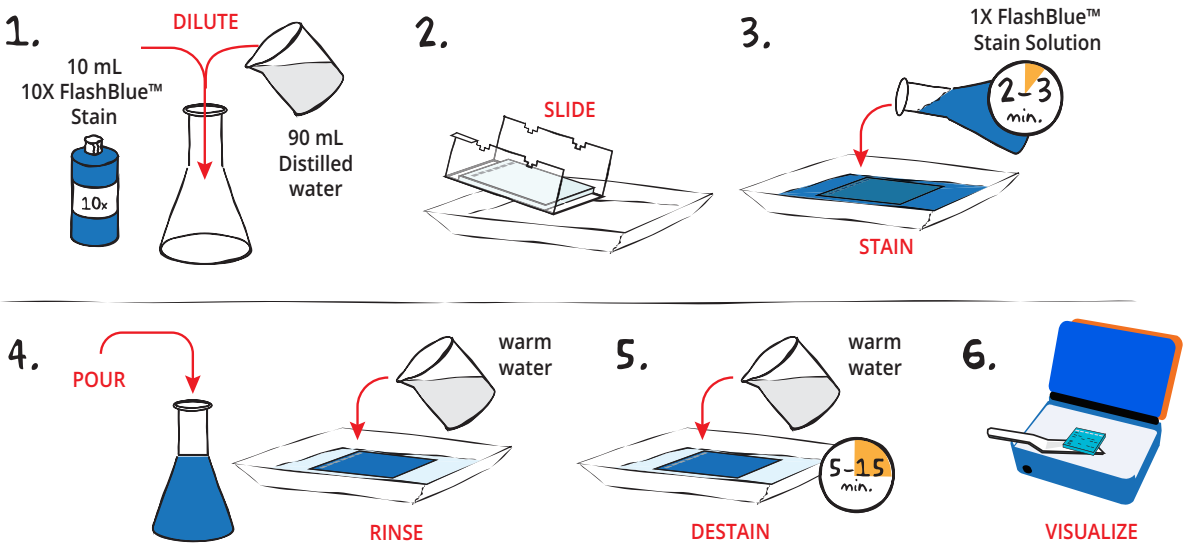
EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

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

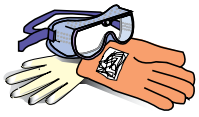
	Electrophoresis Model: EDGE™	M12 & M36
Volts	Min/Max (minutes)	Min/Max (minutes)
150	10/20	20/35
125	N/A	30/45
100	15/25	40/60

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

Module II: Staining Agarose Gels Using FlashBlue™



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



Wear gloves and safety goggles

ALTERNATIVE FLASHBLUE™ STAINING PROTOCOL:

1. **DILUTE** 1 mL of 10X FlashBlue™ stain with 149 mL distilled water.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.
4. Carefully **REMOVE** the gel from the staining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Module III: 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 log10 of molecule's length. To illustrate this, we ran a sample that contains bands of known lengths called a "standard". We will measure the distance that each of these bands traveled to create a graph, known as a "standard curve", which can then be used to extrapolate the size of unknown molecule(s).

Figure 2:
Measure distance migrated from the lower edge of the well to the lower edge of each band.

1. Measure and Record Migration Distances

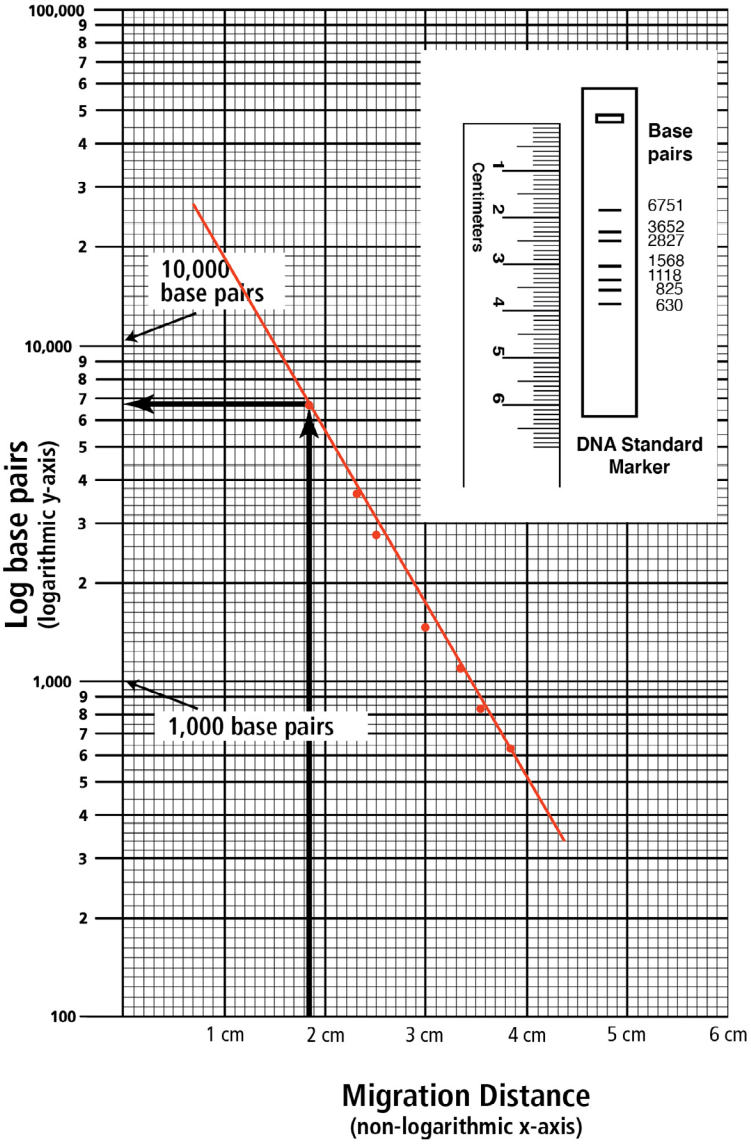
Measure the distance traveled by each Standard DNA Fragment from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters (to the nearest millimeter) in your notebook. Repeat this for each DNA fragment in the standard.

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

2. Generate a Standard Curve

Because migration rate is inversely proportional to the log10 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: Semilog graph example



Module III: 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 3 for an example).

Quick Reference:
DNA Standard fragment sizes - length is expressed in base pairs.

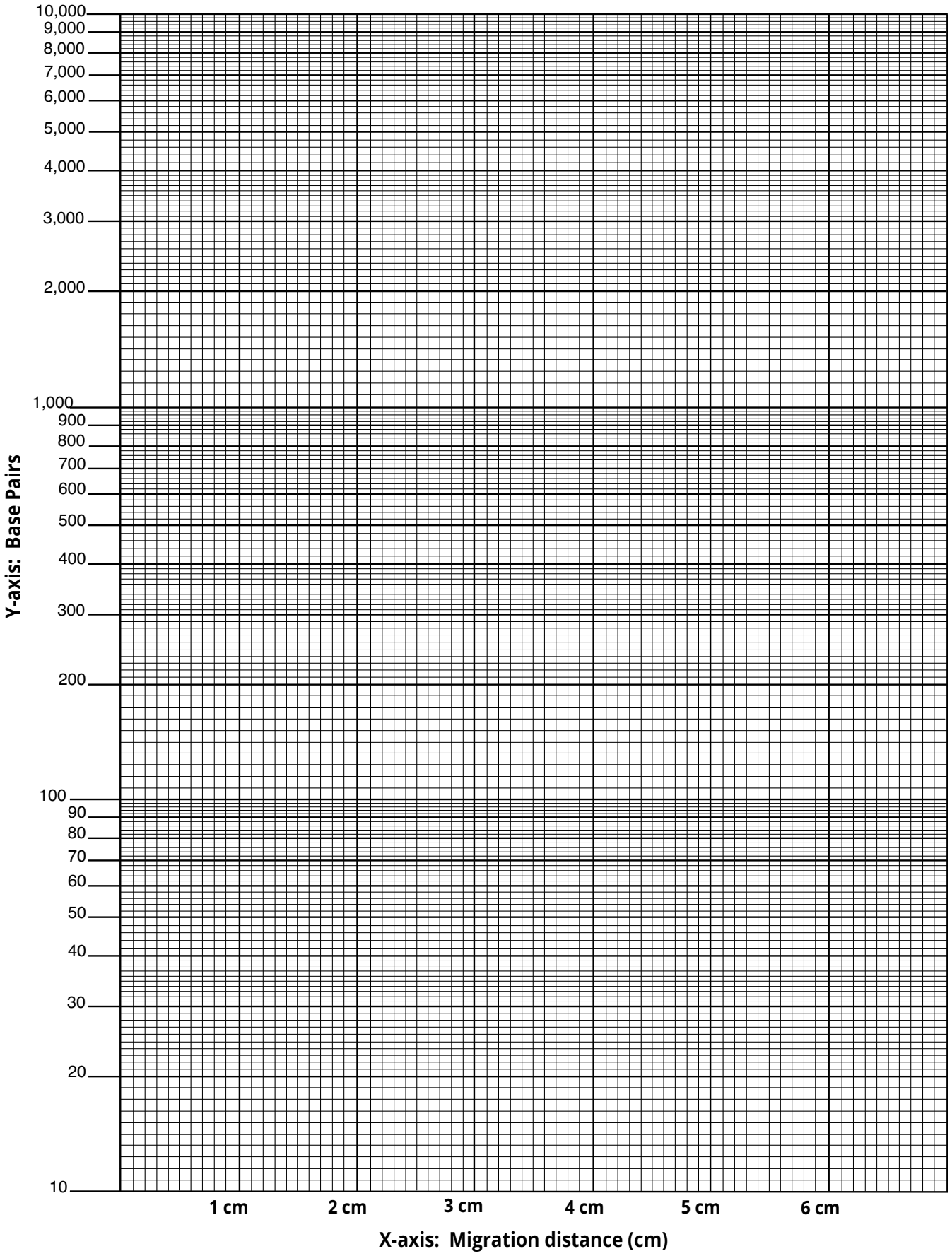
6751, 3652, 2827, 1568, 1118, 825, 630

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

c. Repeat for each fragment in your unknown sample.



Study Questions

1. Why is smallpox such a serious concern?

2. Describe the usual mechanism of smallpox transmission. What are some of the ways in which smallpox was used as a biological weapon?

3. List the symptoms associated with smallpox infection.

4. How was immunity to smallpox first discovered?

5. What are the differences between smallpox, chickenpox, and monkeypox?

Instructor's Guide

ADVANCE PREPARATION:

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

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.

FOR MODULE I
Each group will need:

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

Quick Reference for EDVO-Kit #124

Size of gel casting tray	*Groups per gel*	*Placement of comb*	*Wells required per group*
7 x 7 cm	1 group	1st set of notches	6
10 x 7 cm	1 group	1st set of notches	6
14 x 7 cm	2 groups	1st and 3rd sets of notches	6

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.

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.

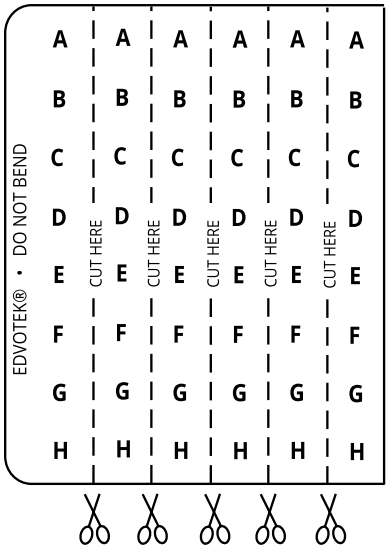
Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

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.***



Carefully cut between each set of tubes

Pre-Lab Preparations: Module II

STAINING AGAROSE GELS USING FLASHBLUE™

FOR MODULE II
Each group will need:

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

FlashBlue™ stain is optimized to shorten the time required for both staining and de-staining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to "equilibrate" in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (**Cat. #552**) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

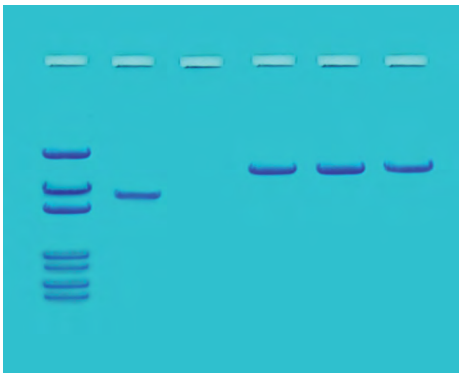
PHOTODOCUMENTATION OF DNA (OPTIONAL)

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

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

If students are unfamiliar with using micropipettes, we recommended performing **Cat. #S-44, Micropipetting Basics** or **Cat. #S-43, DNA DuraGel™** prior to conducting this experiment.

Experiment Results and Analysis



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

Lane	Tube	Sample	Molecular Weights (in bp)
1	A	DNA Standard Markers	6751, 3652, 2827, 1568 1118, 825, 630
2	B	Simulated Smallpox Positive Control	3000
3	C	Negative Control	No Bands
4	D	Monkeypox Control DNA Sample	4282
5	E	Sample from Patient PCR #1	4282
6	F	Sample from Patient PCR #2	4282

Conclusion: The patient does not have smallpox. It appears that the patient is infected with monkeypox.

- Samples E and F are duplicate PCR experiments that yield the same results.
- Sample C is the negative control using a bacterial plasmid. Since the plasmid does not contain viral sequences (specifically, the smallpox virus) no PCR amplification occurred.

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

Appendices

A EDVOTEK® Troubleshooting Guide

B Bulk Preparation of Electrophoresis Buffer and Agarose Gels

C Using SYBR® Safe Stain (OPTIONAL)

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
Bands are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	The gel was not stained properly.	Repeat staining protocol.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
After staining the gel, the DNA bands are faint.	The gel was not stained for a sufficient period of time.	Repeat staining protocol.
	The background of gel is too dark after staining with FlashBlue™.	Destain the gel for 5-10 minutes in distilled water.
DNA bands were not resolved.	Tracking dye should migrate at least 3 cm from the wells to ensure adequate separation.	Be sure to run the gel at least 3 cm before staining and visualizing the DNA (approximately 15-20 minutes at 150 V).
DNA bands fade when gels are kept at 4 °C.	DNA stained with FlashBlue™ may fade with time.	Re-stain the gel with FlashBlue™.
There is no separation between DNA bands, even though the tracking dye ran the appropriate distance.	The wrong percent gel was used for electrophoretic separation.	Be sure to prepare the correct percent agarose gel. For reference, the Ready-to-Load™ DNA samples should be analyzed using a 0.8% agarose gel.
There's not enough sample in my QuickStrip™.	The QuickStrip™ has dried out.	Add 40 µL water, gently pipet up and down to mix before loading.

Visit www.edvotek.com for additional troubleshooting suggestions.

Appendix B

Bulk Preparation of Electrophoresis Buffer and Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

Table D

Bulk Preparation of Electrophoresis Buffer			
50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 mL		2,940 mL	3000 mL (3 L)

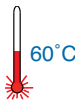
Batch Agarose Gels (0.8%)

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

Table E

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

1. Use a 500 mL flask to prepare the diluted gel buffer.
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60 °C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. Measure 30 mL for a 7 x 7 cm tray, 45 mL for a 10 x 7 cm tray, and 60 mL for a 14 x 7 cm tray. ***For this experiment, 7 x 7 cm gels are recommended.***
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks. Do not freeze gels.

NOTE:
The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

PROCEED to Loading and Running the Gel (page 9).

Appendix C
Using SYBR® Safe DNA Stain (OPTIONAL)

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

PREPARING SYBR® SAFE STAIN

Instructors:
1. Prepare 1x Electrophoresis Buffer by combining 10 µL of 50X Concentrated Buffer with 490 µL of distilled water.

2. Add 20 µL of the SYBR® Safe to the tube of 1X buffer from Step 1 and mix by tapping the tube several times. The diluted SYBR® Safe Stain is now ready to be used during agarose gel preparation.

AGAROSE GEL PREPARATION

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

Table E

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

Instructor Preparation (METHOD A):
For quantity (batch) preparation of agarose gels, see Table E.

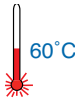
1. Use a 500 mL flask to prepare the diluted gel buffer.

2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.

3. With a marking pen, indicate the level of solution volume on the outside of the flask.

4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.

5. Cool the agarose solution to 60 °C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Add the entire tube of ***diluted*** **SYBR® Safe** stain to the cooled agarose and mix well.

7. Dispense the required volume of cooled agarose solution for casting each gel. Measure 30 mL for a 7 x 7 cm tray, 45 mL for a 10 x 7 cm tray, and 60 mL for a 14 x 7 cm tray. ***For this experiment, 7 x 7 cm gels are recommended.***

8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Solidified gels can be stored in the refrigerator for up to 2 weeks. Place 1-2 mL of electrophoresis buffer in a sealable bag with the gels to prevent them from drying out. Excessive buffer will cause SYBR® Safe to diffuse out of the gels. Do not freeze gels.

PROCEED to Loading and Running the Gel (Steps 8-12 on page 9), followed by the VISUALIZATION procedures on page 25.
NO ADDITIONAL STAINING IS NECESSARY.

Appendix C
Using SYBR® Safe DNA Stain (OPTIONAL)

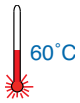
AGAROSE GEL PREPARATION, CONTINUED

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

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

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

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

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A.2).
2. **MIX** agarose powder with 1X buffer in a 250 mL flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60 °C with careful swirling to promote even dissipation of heat. 

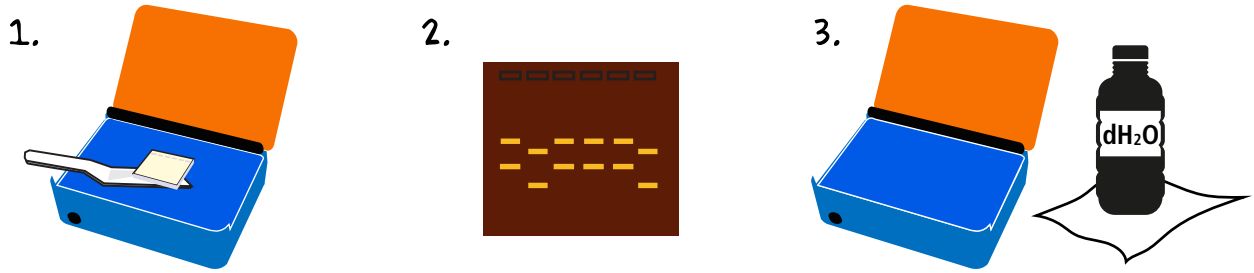
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. Before casting the gel, **ADD** ***diluted SYBR® Safe*** to the cooled agarose and swirl to mix (see Table A.2).
7. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
8. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

PROCEED to Loading and Running the Gel (Steps 8-12 on page 9), followed by the VISUALIZATION procedures on page 25.
NO ADDITIONAL STAINING IS NECESSARY.

Appendix C
Using SYBR® Safe DNA Stain (OPTIONAL)

VISUALIZING THE SYBR® GEL

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



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

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