Edvo-Kit # 123

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Nucleic Acid Testing for COVID-19

Experiment Objective:

SARS-CoV-2 is a novel coronavirus that has caused a world-wide outbreak of respiratory disease beginning in 2019. In this simulated medical test, we will use electrophoresis to detect the presence of the SARS-CoV-2 virus in samples from patients with symptoms of COVID-19.

See page 3 for storage instructions.

Version #123.200824

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	Refrigerator	❑
B	Negative Control	Refrigerator	❑
C	Positive Control	Refrigerator	❑
D	Patient 1 Sample	Refrigerator	❑
E	Patient 2 Sample	Refrigerator	❑
F	Patient 3 Sample	Refrigerator	❑

Experiment #123 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 all components below 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

Background Information

Each year, experts estimate that coronaviruses cause 15-30% of all common cold cases. These symptoms are generally mild and include fever and sore throat. Sometimes a novel strain of the virus emerges that causes severe respiratory distress (for example SARS in 2003 and MERS in 2012). SARS-CoV-2 is a novel coronavirus that has caused a worldwide outbreak of respiratory disease. The first cases of COVID-19 were diagnosed in December 2019 and traced to the emergence of the SARS-CoV-2 virus. According to the World Health Organization, COVID-19 spread worldwide in a very short period of time. Public health officials are currently working on strategies to identify infected individuals and to prevent the further spread of the virus.

IMPORTANT DEFINITIONS:

SARS-CoV-2, or Severe Acute Respiratory Syndrome Coronavirus 2: The name of the novel coronavirus responsible for the current pandemic.

COVID-19, or Coronavirus Disease 2019: This is the disease caused by SARS-CoV-2, characterized by fever, cough, and shortness of breath.

Outbreak: A rapid increase in the number of cases of a disease at a specific time and place

Pandemic: An outbreak of a disease in many different geographic areas that affects a significant proportion of the population.

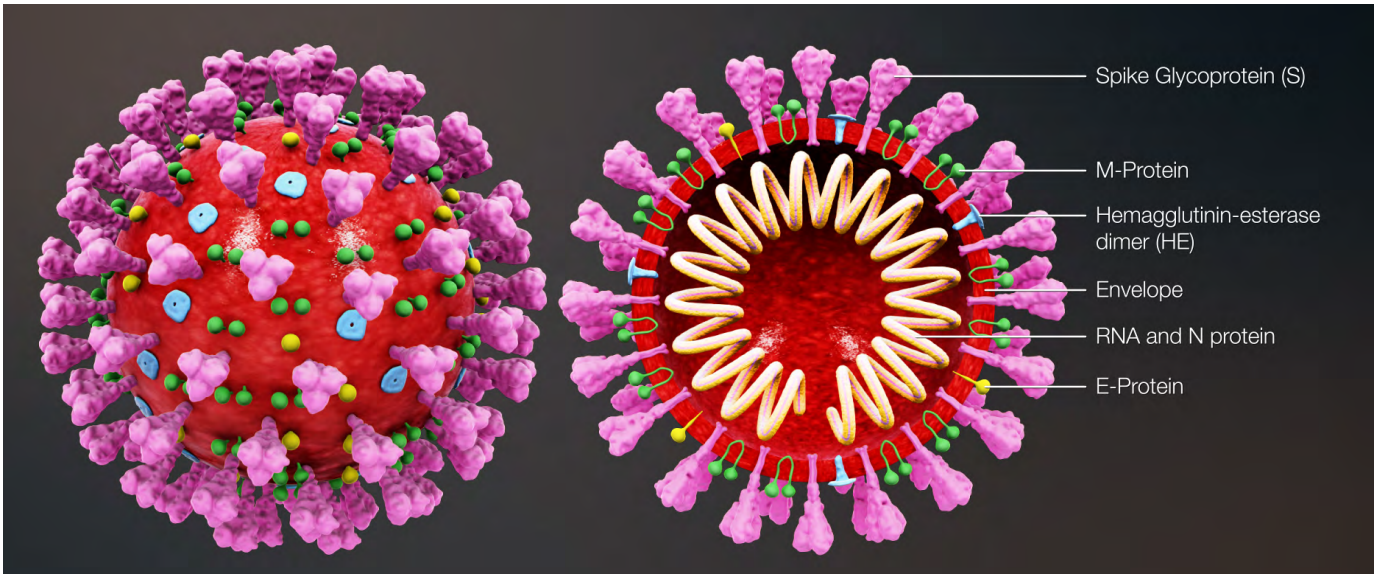


Figure 1: Coronavirus structure.

Coronaviruses have a single-stranded RNA genome wrapped in a helical capsid. A host-derived membrane envelope surrounds the capsid. The envelope is studded with proteins that help the virus infect cells. By electron microscopy, the envelope proteins create a hazy halo around the virus particle. Scientists described them with the Latin word *corona*, which means "crown" or "halo" (Figure 1).

Like all coronaviruses, SARS-CoV-2 has four main structural proteins. Monomers of the nucleocapsid protein (N) link together to form a helical capsid which wraps around and protects the RNA genome. Embedded in the membrane are several viral proteins: the spike (S), the envelope (E) and the membrane (M) proteins. The S protein binds with human cell surface proteins, allowing the virus to inject its genetic material into its host cells. The M protein coordinates interactions between the other viral proteins and the host cell factors, turning cells into virus factories. As a viroporin, the E protein binds to itself to form channels that facilitate viral release.

Luckily, with proper precautions, we can prevent the spread of COVID-19. Coronaviruses like SARS-CoV-2 transmit from person-to-person through liquid droplets that come out when you cough or sneeze. Soap, hand sanitizer and other disinfectants kill coronaviruses, so frequent washing of hands can limit its spread. Touching your face with contaminated hands can introduce the virus to your mucus membranes, so it is important to keep hands away from your eyes, nose and mouth. We can wear cloth masks to cover the mouth and nose, which prevent our respiratory droplets from spreading via cough or sneeze. Furthermore, we can take actions like social distancing to reduce the likelihood of infecting those around us, which can decrease the spread of the disease.

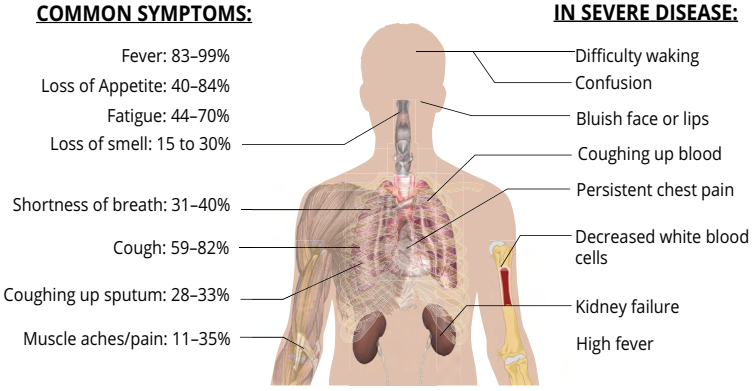


Figure 2: Symptoms of Coronavirus Disease.

Testing for SARS-CoV-2

Symptoms of COVID-19 may include fever, cough, and shortness of breath. In severe cases, patients may have pneumonia, respiratory distress, and/or kidney failure (Figure 2). Sadly, this infection can be fatal. Treatment for COVID-19 includes rest, fluids, and over-the-counter cold medications. The continued development of vaccines and antiviral medications give clinicians the necessary tools to combat infection. If you are exhibiting symptoms of COVID-19, seek medical attention from your doctor to be tested for the virus.

There are two types of diagnostic tests to confirm COVID-19 infection – Reverse Transcription PCR (RT-PCR) and Enzyme-Linked Immunosorbent Assay (ELISA). RT-PCR tests are currently in use by public health laboratories around the world for the presence of the viral genome, signifying active infection. Because RT-PCR is extremely sensitive and can detect minute amounts of the virus, it is an ideal assay to detect active SARS-CoV-2 infections. A positive test does not mean that a patient will become seriously ill; however, these diagnoses are important as they allow epidemiologists to trace and limit the spread of COVID-19.

The virus responsible for COVID-19, SARS-CoV-2, does not integrate itself into the human genome during infection (like HIV). Once the patient's immune system has cleared the infection, no viral nucleic acid remains in the body which makes the RT-PCR test ineffective. However, the antibodies generated to fight off the infection remain in the body after the patient has healed. The ELISA is used to identify the presence of these antibodies in patients, signifying that a person had been previously infected by the virus. However, since the body takes several days to produce these antibodies, the ELISA cannot detect infected people before clinical symptoms arise.

The RT-PCR Test for SARS-CoV-2

In 1984, Dr. Kary Mullis revolutionized the field of molecular biology when he devised a simple and elegant method to copy specific pieces of DNA. Mullis recognized that he could replicate DNA in vitro using short, synthetic DNA oligonucleotides (known as primers) and DNA Polymerase I in a process similar to DNA replication in a cell's nucleus. Furthermore, because researchers can customize the primers to target a specific gene, this method would allow for the rapid amplification of a selected DNA sequence. For the development of this technique, known today as the Polymerase Chain Reaction (or PCR), Mullis was awarded the Nobel Prize in Chemistry in 1993.

To perform PCR, purified double-stranded DNA is mixed with primers, a thermostable DNA polymerase (*Taq*) and nucleotides (Figure 3). Then, the mixture is heated to 94°C to denature the DNA duplex (i.e., unzip it into single strands). Next, the

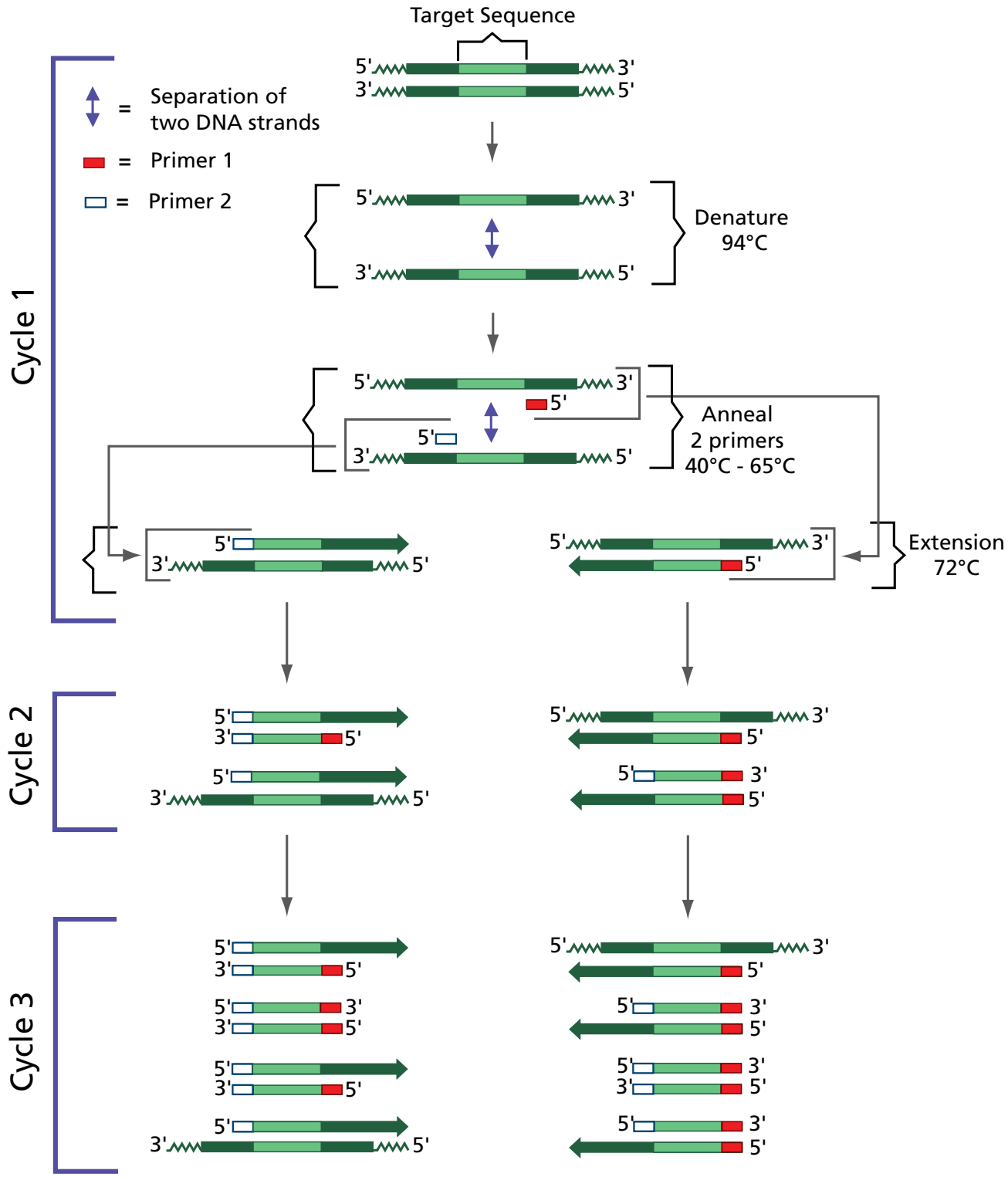


Figure 3:
DNA Amplification by the Polymerase Chain Reaction

sample is then cooled to 45°C-60°C, allowing the primers to base pair with the target DNA sequence (called "annealing"). Lastly, the temperature is raised to 72°C, the optimal temperature at which *Taq* polymerase will extend the primer to synthesize a new strand of DNA. Each "PCR cycle" (denaturation, annealing, extension) doubles the amount of the target DNA in less than five minutes (summarized in Figure 3). In order to produce enough DNA for analysis, twenty to forty cycles may be required. To simplify this process, a specialized machine, called a "thermal cycler" or a "PCR machine", was created to rapidly heat and cool the samples. Because of its ease of use and its ability to rapidly amplify DNA, PCR has become indispensable in medical diagnostic laboratories for the detection of pathogens.

Unfortunately, since *Taq* polymerase is a DNA-dependent DNA polymerase, it cannot use the RNA genome of SARS-CoV2 as a template. In order to detect COVID-19 using PCR, Reverse Transcriptase (RT) is used to synthesize complementary DNA (cDNA) copies of the RNA genome. A small amount of the cDNA is mixed with *Taq* polymerase, dNTPs and primers for amplification by PCR. Because RT-PCR is extremely sensitive and can detect very low levels of the virus, it is considered the "gold standard" for SARS-CoV-2 detection. However, since RT-PCR tests are performed in a medical diagnostic laboratory, it may take several days to get the results, even though the actual test takes a few hours.

Commonly used RT-PCR tests combine three primer sets in one PCR test, which is known as multiplex PCR. The first two sets of primers target regions in the SARS-CoV-2 N protein. As an internal control, the third set of primers amplify the human housekeeping gene RNase P (RP). When combined in a PCR sample, the three sets of primers produce DNA fragments of different lengths depending upon whether the SARS-CoV-2 virus is present in a patient sample.

In order to analyze this sample, scientists may use a technique called agarose gel electrophoresis to separate DNA fragments according to size. In electrophoresis, mixtures of amplified DNA molecules are added into depressions (or "wells") within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode (Figure 4A).

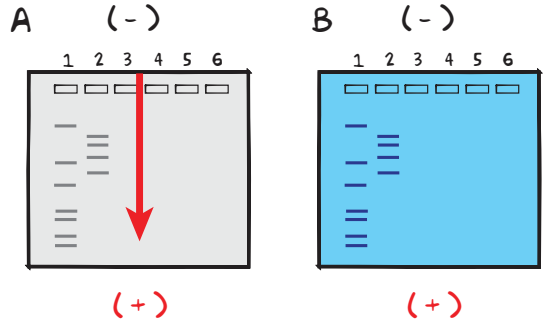


Figure 4:
Overview of Agarose Gel Electrophoresis

At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be the bands can be visualized using a stain that sticks to DNA (Figure 4B).

In this simulated medical test, we will use electrophoresis to analyze samples from three patients who have symptoms of COVID-19. Samples were collected using a nasopharyngeal swab, the DNA was extracted, and then analyzed using RT-PCR. A diagnosis is made after performing electrophoresis on the samples. For the patients that have been infected with the virus, the test will detect both the viral genome and the internal control, resulting in three bands on the gel (two from the SARS-CoV N gene and one from the human control gene). In contrast, a patient who was not infected with SARS-CoV-2 will only have one band on the gel from the internal control.

Figures:
Figure 1: Picture of a coronavirus: https://en.wikipedia.org/wiki/Coronavirus#/media/File:3D_medical_animation_coronavirus_structure.jpg / CC BY-SA (https://creativecommons.org/licenses/by-sa/4.0)
Figure 2: https://en.wikipedia.org/wiki/Coronavirus_disease_2019#/media/File:Symptoms_of_coronavirus_disease_2019_3.0.svg

Experiment Overview

EXPERIMENT OBJECTIVE:

SARS-CoV-2 is a novel coronavirus that has caused a worldwide outbreak of respiratory disease beginning in 2019. In this simulated medical test, we will use RT-PCR to detect the presence of the SARS-CoV-2 virus in blood sample from three patients with symptoms of COVID-19

LABORATORY SAFETY

Wear gloves and safety goggles

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

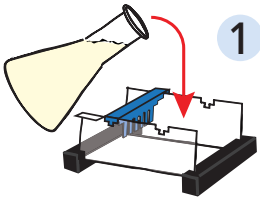
After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

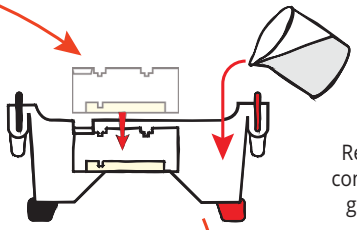
Experiment Overview

MODULE I: Agarose Gel Electrophoresis

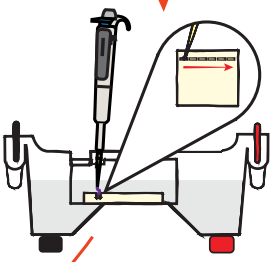
Time required: See Table C



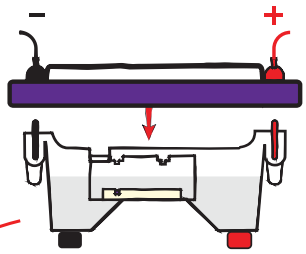
1 Prepare agarose gel in casting tray.



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



3 Load each sample in consecutive wells



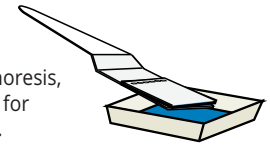
4 Attach safety cover & connect leads to power source to conduct electrophoresis.

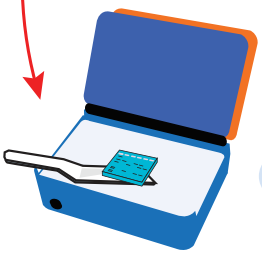
Quick Reference for EDVO-Kit #123			
Size of gel casting tray	*Groups per gel*	*Placement of comb*	*Wells required per group*
7 x 7 cm	1 group	1st set of notches	6
10 x 7 cm	1 group	1st set of notches	6
14 x 7 cm	2 groups	1st and 3rd sets of notches	6

MODULE II: Staining Agarose Gels Using FlashBlue™

Time required: 30 min.

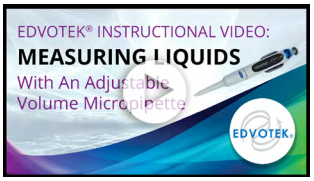
5 After electrophoresis, transfer gel for staining.

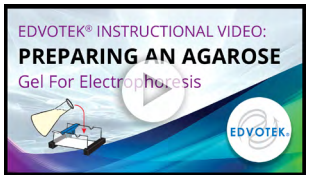


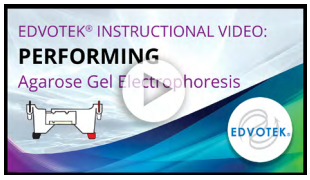


6 Analysis on white light source.

Related EDVOTEK® Instructional Videos

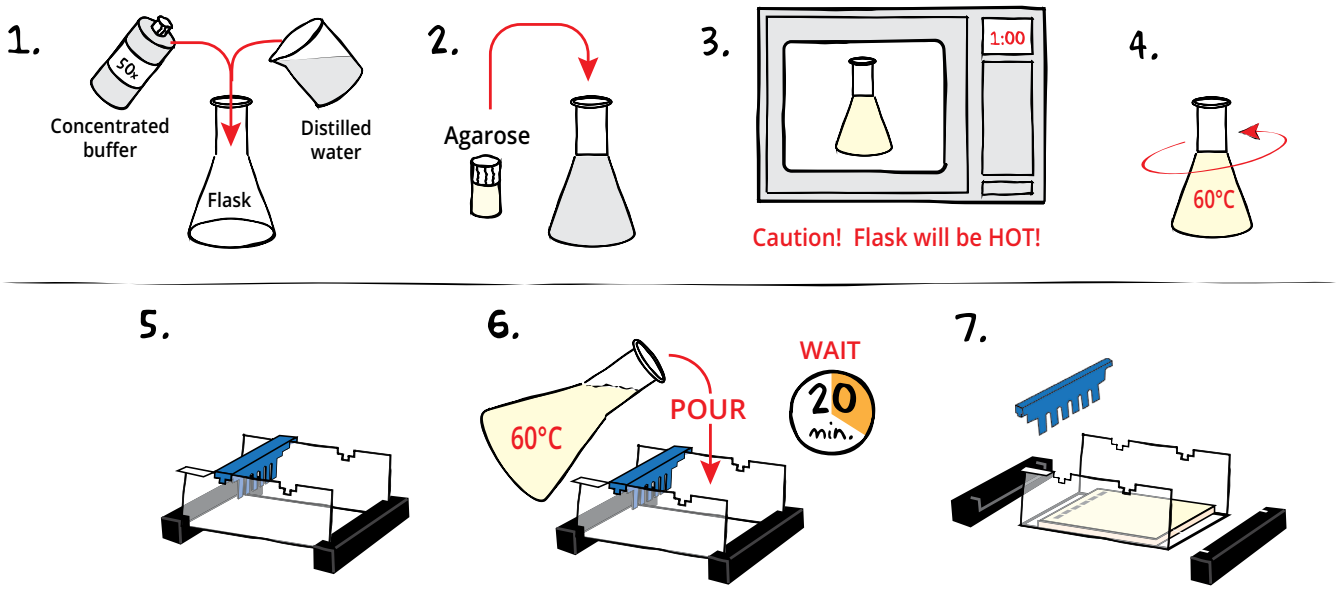






www.youtube.com/edvotekinc

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

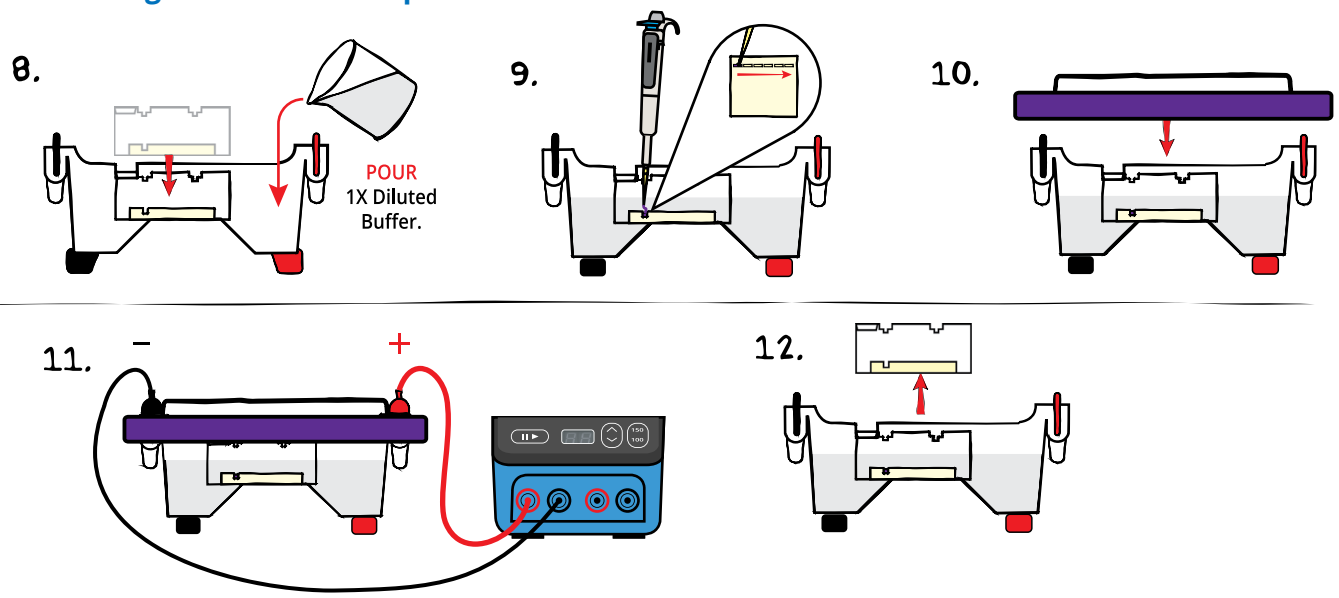
REMINDER:
This experiment requires 0.8% agarose gels cast with 6 wells.

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	Tube	Sample
1	Tube A	DNA Standard Marker
2	Tube B	Negative Control
3	Tube C	Positive Control
4	Tube D	Patient 1 Sample
5	Tube E	Patient 2 Sample
6	Tube F	Patient 3 Sample

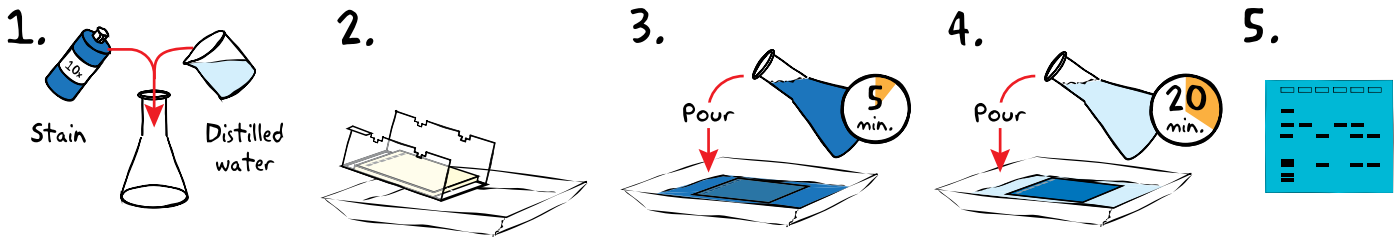
Table B — **1x Electrophoresis Buffer (Chamber Buffer)**

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

Module II: 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 de-staining.
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.
6. **ESTIMATE** the base pair length of each fragment by comparing the distance each fragment traveled from the well to the bottom of the gel to the distance that each Standard DNA Marker fragment migrated. For a more accurate size calculation you can use a standard curve (see Appendix C).
7. Fill out the table below.

Wear gloves and safety goggles

Sample	Number of Fragments	Size of each Fragment
DNA Standard Marker	7	6751, 3652, 2827, 1568, 1118, 825, 630
Negative Control		
Positive Control		
Patient 1		
Patient 2		
Patient 3		

Study Questions

1. Name and describe the SARS-CoV-2 viral proteins.

2. What tests are used to identify COVID-19? What are the advantages and disadvantages?

3. What is Reverse Transcriptase and how does it work? Why is it important for detecting SARS-CoV-2 in patient samples?

4. A patient was sick with COVID-19 symptoms but didn't make it to the doctor's office until after the symptoms had subsided. Which test would you use to test the patient and why?

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

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.

Quick Reference for EDVO-Kit #123

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

FOR MODULE I
Each group will need:

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

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

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.

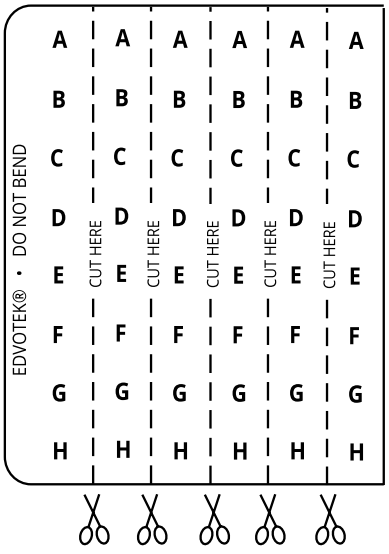
NOTE:
This kit is compatible with SYBR® Safe Stain (Cat #608, not included). Instructions for preparing gels and visualizing results can be found in Appendix C.

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

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.

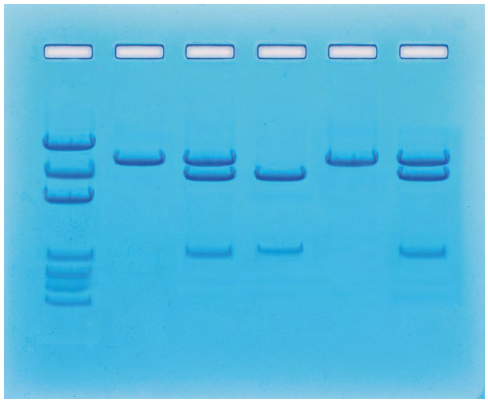
PHOTODOCUMENTATION OF DNA (OPTIONAL)

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



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

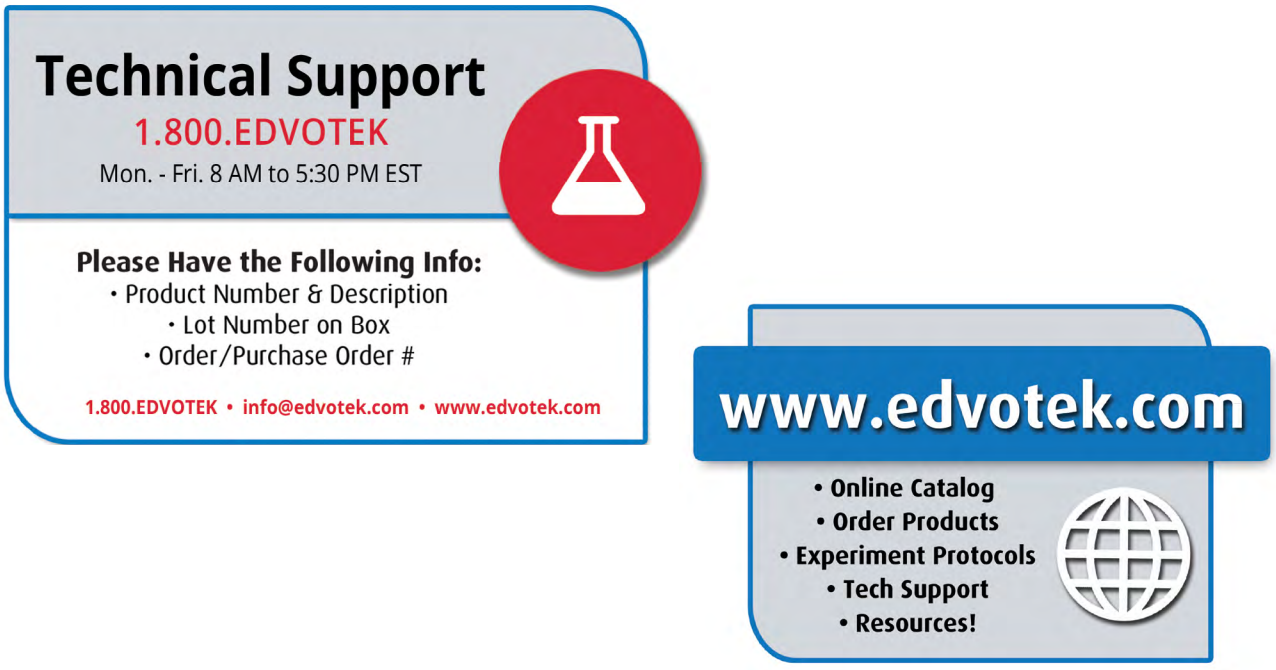
Lane	Tube	Sample	Result	Molecular Weights (MW)
1	A	DNA Standard Markers	---------	6751, 3652, 2827, 1568, 1118, 825, 630
2	B	Negative Control	Negative (human control only)	4282
3	C	Positive Control	Positive (human control and viral proteins)	4282, 3000, 1282
4	D	Patient #1	Indeterminant: test again	3000, 1282
5	E	Patient #2	Negative for SARS-CoV-2	4282
6	F	Patient #3	Positive for SARS-CoV-2	4282, 3000, 1282

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
D 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)

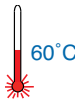
Batch Agarose Gels (0.8%)

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 11).

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).

Figure 5:
Measure distance migrated from the lower edge of the well to the lower edge of each band.

1. Measure and Record Migration Distances

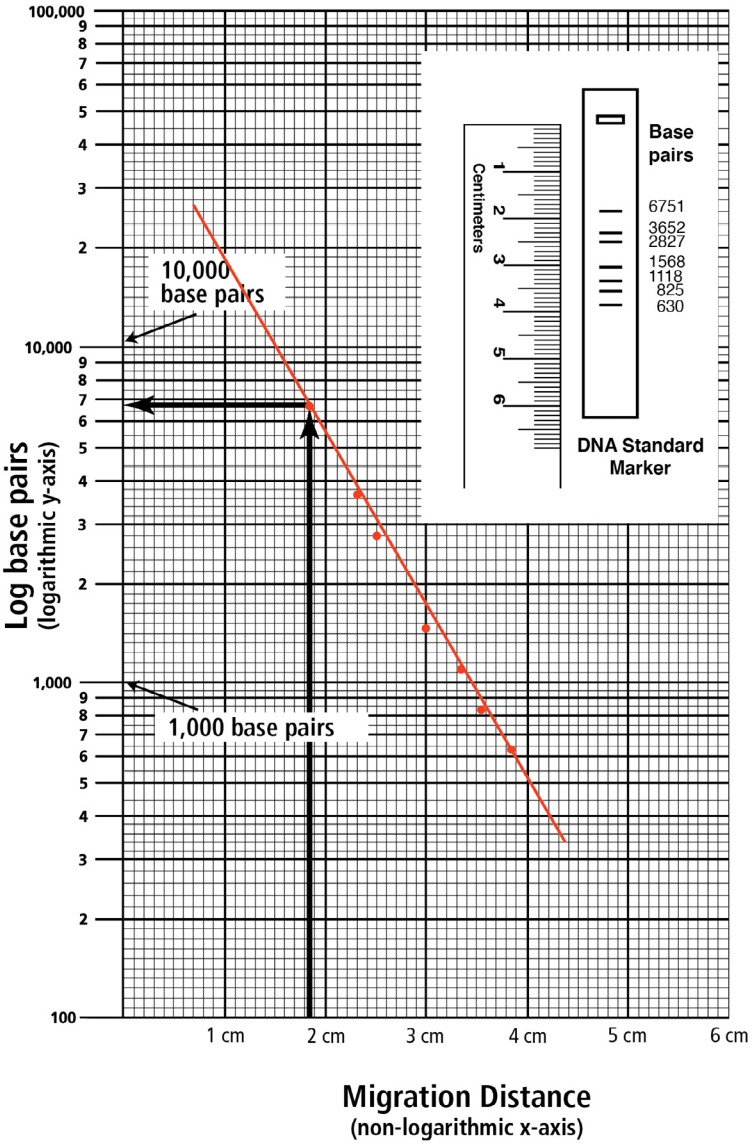
Measure the distance traveled by each Standard DNA Fragment from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters (to the nearest millimeter) in your notebook. Repeat this for each DNA fragment in the standard.

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 $\log_{10}$ 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 6: Semilog graph example



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 6 for an example).

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

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

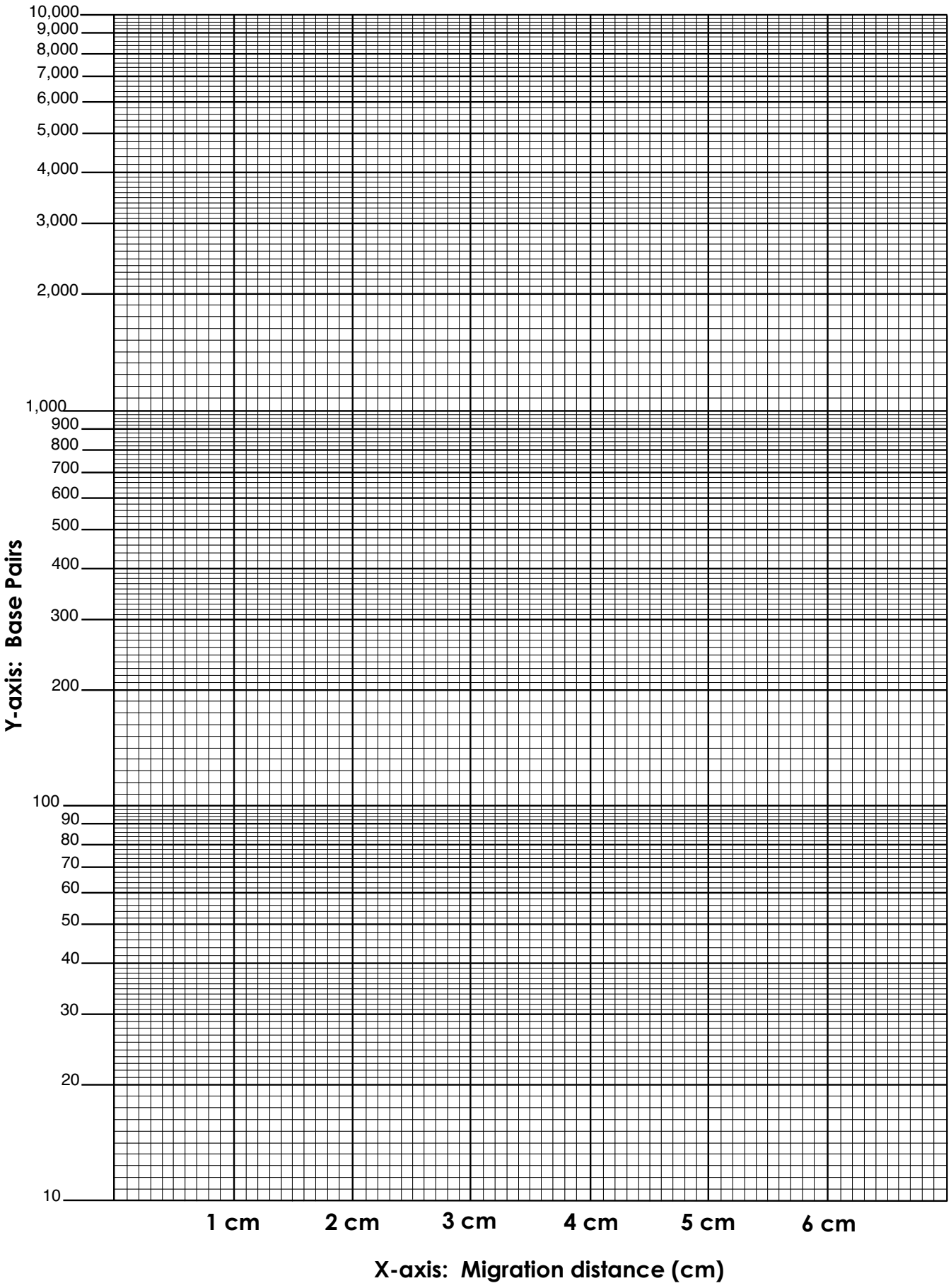
3. Determine the length of each unknown fragment.

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

c. Repeat for each fragment in your unknown sample.

Appendix C


10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
Y-axis: Base Pairs
1 cm
2 cm
3 cm
4 cm
5 cm
6 cm
X-axis: Migration distance (cm)

Appendix D
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

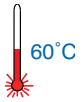
This experiment requires one 0.8% agarose gel for each student group. Instructors can choose whether to prepare the gels in advance (METHOD A) or have the students prepare their own (METHOD B). Allow approximately 30-40 minutes for this procedure.

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 11), followed by the **VISUALIZATION** procedures and exercise on page 27. **NO ADDITIONAL STAINING IS NECESSARY.**

Appendix D
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

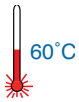
** Recommended gel volume for the EDGE™ Integrated Electrophoresis System.*

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 11), followed by the **VISUALIZATION** procedures and exercise on page 27. **NO ADDITIONAL STAINING IS NECESSARY.**

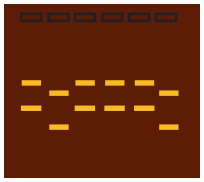
Appendix D
Using SYBR® Safe DNA Stain (OPTIONAL)

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.

2.



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

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. **VISUALIZE** results.
3. **ESTIMATE** the base pair length of each fragment by comparing the distance each fragment traveled from the well to the bottom of the gel to the distance that each Standard DNA Marker fragment migrated. For a more accurate size calculation you can use a standard curve (see Appendix C).
4. Fill out the table below.

Sample	Number of Fragments	Size of each Fragment
DNA Standard Marker	7	6751, 3652, 2827, 1568, 1118, 825, 630
Negative Control		
Positive Control		
Patient 1		
Patient 2		
Patient 3		

NOTE: After visualizing the results, turn the unit **OFF**. **REMOVE** and **DISPOSE** of the gel. **CLEAN** the transilluminator surfaces with distilled water.