EDVOTEK®

THE **BIOTECHNOLOGY**
EDUCATION COMPANY®

Edvo-Kit #
100

Edvo-Kit #100

MyLab™ Electrophoresis Sampler Kit

Experiment Objective:

In this experiment, students will explore four unique experiments that utilize agarose gel electrophoresis to separate DNA samples. The DNA banding patterns will be analyzed within scenarios that examine forensic, medical, and scientific investigations.

See page 3 for storage instructions.

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Table of Contents

Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

COMPONENTS (in QuickStrip™ format)		Check (✓)
Store QuickStrip™ samples in the refrigerator upon receipt.		
A	Scenario 1 DNA Samples	❑
B	Scenario 2 DNA Samples	❑
C	Scenario 3 DNA Samples	❑
D	Scenario 4 DNA Samples	❑

REAGENTS & SUPPLIES

Store the following at room temperature.

- UltraSpec-Agarose™ ❑
- Electrophoresis Buffer (50x) ❑
- SYBR® Safe DNA Stain ❑
- Practice Loading Dye ❑
- FlashBlue™ DNA Stain ❑

Experiment #100 contains samples for 4 gel electrophoresis experiments.

STORAGE:

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.

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipettes with tips
- Balance
- Microwave, hot plate or burner for preparing agarose
- Pipet pump
- 250 mL flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Small plastic trays or large weigh boats (for gel staining/destaining)
- UV Transilluminator or Blue Light visualization system (use if staining with SYBR® Safe)
- UV safety goggles (use if staining with SYBR® Safe)
- White light visualization system (use if staining with FlashBlue™)
- Distilled or deionized water
- Laboratory journal

Background Information

PRINCIPLES OF GEL ELECTROPHORESIS

Gel electrophoresis is widely used to separate molecules based upon charge, size, and shape. It is particularly useful in separating charged biomolecules such as DNA, RNA, and proteins. This technique possesses great resolving power, yet is simple, straightforward, and safe to perform.

Agarose, a polysaccharide derived from seaweed, is the most common matrix used for DNA and RNA gel electrophoresis. To make an agarose gel, solid agarose powder is added to buffer and dissolved by boiling. The buffer regulates the pH of the solution throughout the electrophoresis process, which is important to the charge and stability of biological molecules. Once the solution has cooled to approximately 60°C a DNA stain will be gently mixed into the molten agarose solution. Finally, the gel solution is poured into a gel tray and a special comb is used to form depressions in the gel called "wells".

Once solidified, the gel is placed in a horizontal electrophoresis chamber and covered with buffer containing ions necessary to conduct an electrical current. The biomolecule samples are prepared for electrophoresis by mixing them with glycerol or sucrose, which makes them denser than the electrophoresis buffer. When loaded into the wells, the dense samples sink through the buffer and remain in the wells. The gel is now ready for electrophoresis.

Electrodes placed at each end of the electrophoresis chamber generate current when connected to a direct current power supply. Once the current is applied, the biomolecules in the sample are forced out of the well and into the gel matrix. Since DNA has a negatively charged phosphate backbone the current will push DNA strands towards the positive electrode.

At first glance, an agarose gel appears to be solid at room temperature. On the molecular level, the gel contains small channels that the biomolecules are forced through. These pores act as a molecular sieve that affects the rate at which a molecule can migrate through the gel. Factors such as charge, size, and shape, together with buffer conditions, gel concentrations, and voltage, can affect the mobility of molecules in a gel. For example, small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with different sizes travel at different speeds, they become separated and form discrete "bands" within the gel.

DNA samples are clear and colorless in solution, making it impossible to see the separated DNA fragments in the agarose gel. Fortunately, scientists have developed dyes and stains that can bind to DNA and allow it to be visualized. Some of the most sensitive dyes can bind to nucleic acids and will fluoresce under UV or specific wavelengths of blue light. In this experiment, you will use SYBR® Safe DNA stain to examine the results of the electrophoresis experiment. Alternatively, FlashBlue™ stain can be used if a blue light transilluminator is unavailable.

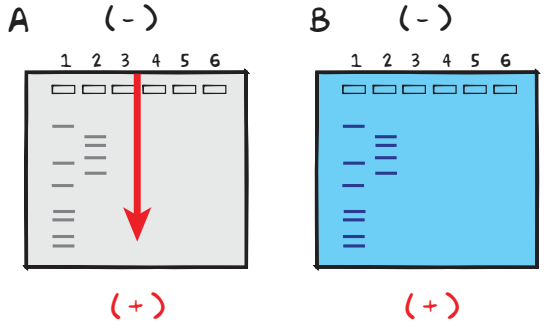


Figure 1:
Overview of Agarose Gel Electrophoresis

Background Information, continued

THEORY OF PCR

The polymerase chain reaction (PCR) is a DNA amplification technique that has revolutionized almost all aspects of biological research. PCR was invented in 1984 by Dr. Kary Mullis at the Cetus Corporation in California. The enormous utility of the PCR method is based on its ease of use and its ability to allow the amplification of small DNA fragments. For this ground breaking technology, Mullis was awarded the Nobel Prize in Chemistry in 1993.

To perform PCR, the template DNA and a molar excess of primers are mixed with the four “free" deoxynucleotides (dATP, dCTP, dGTP, and dTTP), and a thermostable DNA polymerase. Before performing PCR, template DNA is extracted from various biological sources. Because PCR is very sensitive, only a few copies of the gene are required. In order to amplify the specific DNA or target sequence, two primers (short, synthetic DNA molecules) are designed to correspond to the ends of the target sequence. The primers hybridize to the DNA template, and define the sequence to be copied. Starting from the primer, DNA Polymerase builds a new strand of DNA in the 5' –-> 3' direction, using the DNA template as a guide.

The most commonly used DNA polymerase is *Taq* DNA polymerase. This enzyme, originally purified from a bacterium that inhabits hot springs, is stable at very high temperatures. These components (template DNA, primers, the four deoxynucleotides, and *Taq* DNA polymerase) are mixed with a buffer that contains Mg^{+2}, an essential cofactor for *Taq* polymerase.

The PCR reaction mixture is subjected to sequential heating/cooling cycles at three different temperatures in a thermal cycler.

- In the first step, known as “denaturation", the mixture is heated to near boiling (94°C - 96°C) to “un-zip" (or melt) the target DNA. The high temperature disrupts the hydrogen bonds between the two complementary DNA strands and causes their separation.

- In the second step, known as “annealing", the reaction mixture is cooled to 45°C - 65°C, which allows the primers to base pair with the target DNA sequence.

- In the third step, known as “extension", the temperature is raised to 72°C. This is the optimal temperature at which *Taq* polymerase can add nucleotides to the hybridized primers to synthesize the new complementary strands.

These three steps - denaturation, annealing, and extension - constitute one PCR “cycle" (Figure 2, page 6). Each PCR cycle doubles the amount of the target DNA in less than five minutes. 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 and life sciences labs. For example, today’s research laboratories can quickly create copies of a specific region of DNA for cloning applications. Medical diagnostics use PCR to identify genetic mutations and infectious agents. In addition, because amplification by PCR requires very little starting material, it is ideal for forensic analysis of biological samples or determination of paternity.

Background Information, continued

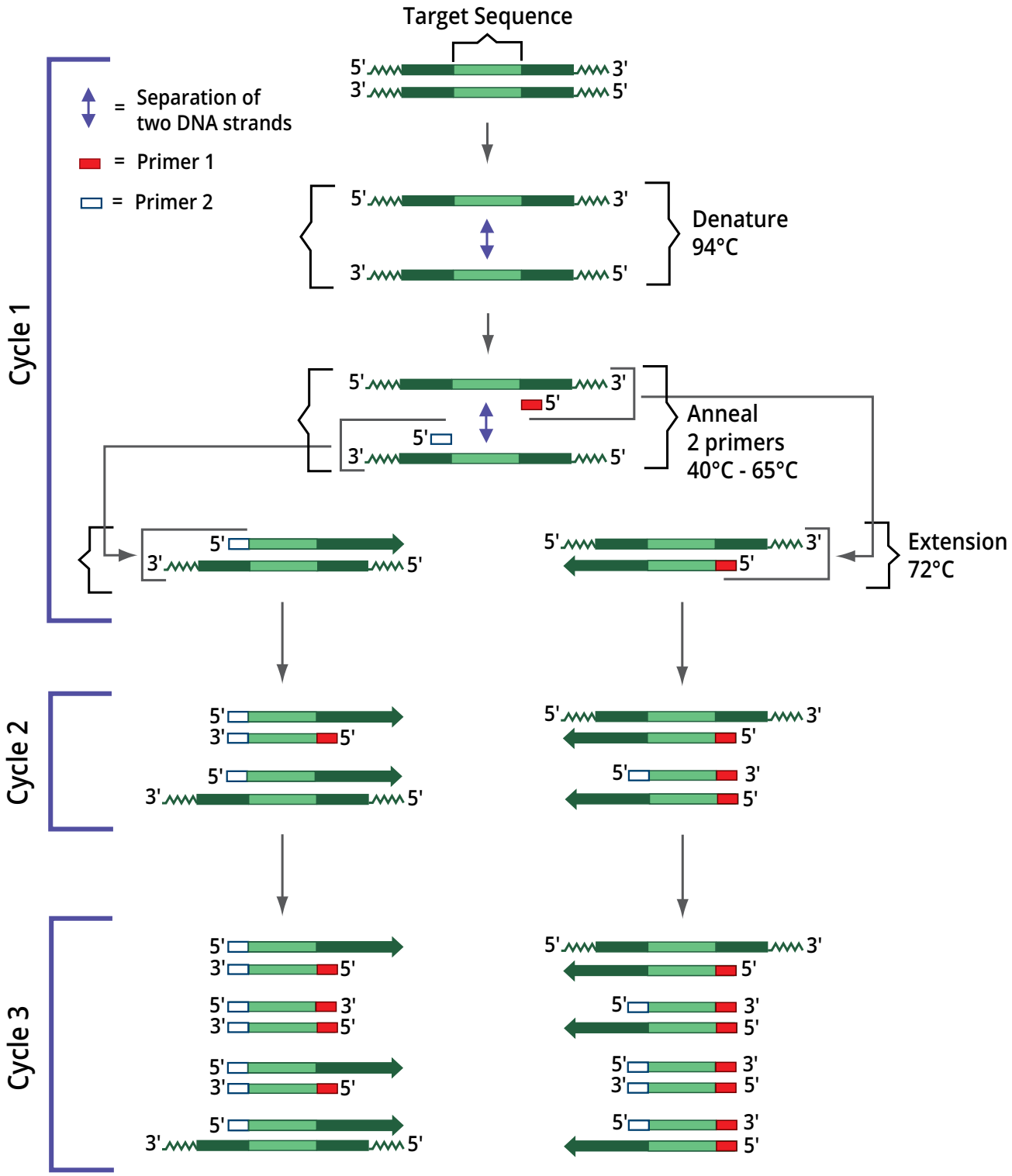


Figure 2:
DNA Amplification by the Polymerase Chain Reaction

Scenario 1: DNA Fingerprinting by PCR Amplification

Deoxyribonucleic acid (DNA) is present in every living cell. It is the genetic material that acts as the blueprint for protein synthesis. Polymorphic DNA refers to chromosomal regions that vary among individuals. By examining several of these regions within genomic DNA, one can determine a "DNA Fingerprint" for an individual. DNA polymorphisms are now widely used for determining paternity/maternity, kinship, identification of human remains, and to determine the genetic basis of various inherited diseases. The most widely used and far-reaching application has been to the field of criminal forensics. DNA from crime victims and offenders can now be definitively matched, affecting outcomes of criminal and civil trials.

DNA fingerprinting was first used as a forensic tool in the United Kingdom in 1984, following the pioneering work of Dr. Alex Jeffreys. Analysis by Jeffreys led to the apprehension of a murderer in the first DNA fingerprinting case in September 1987. The first U.S. conviction occurred on November 6, 1987 in Orlando, FL. Since then, DNA analysis has been used in thousands of convictions. Additionally, DNA fingerprinting has been used to exonerate hundreds of convicted felons, including multiple death row inmates.

In 1990, the Federal Bureau of Investigation (FBI) established the Combined DNA Index System (CODIS), a system that allows comparison of crime scene DNA to DNA profiles of convicted offenders. CODIS has now been used to solve dozens of cases where authorities had no suspect for the crime under investigation.

The first step in forensic DNA fingerprinting is the collection of blood or other tissue samples from the crime scene or victim (Figure 3). A blood sample, often present as a stain, is treated with a reagent mixture that contains detergent to rupture the cell membrane and obtain DNA for further analysis. When this technology was in its early stages, a method, called restriction fragment length polymorphism (RFLP) analysis, was used.

Additionally, RFLP analysis involves digesting the DNA with restriction enzymes, separation on an agarose gel, transferring the DNA to a membrane, and hybridizing the DNA on the membrane with probes to detect polymorphic regions. This procedure, known as a Southern Blot, requires relatively large amounts of DNA and takes several weeks to complete.

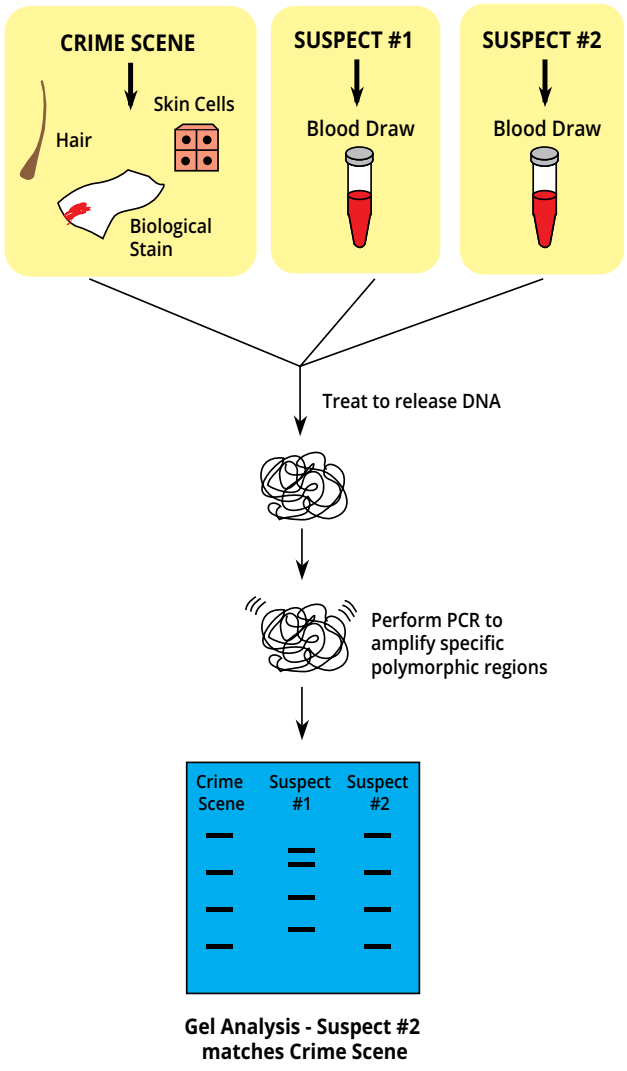


Figure 3: Comparison of a crime scene DNA fingerprint with those of two suspects.

Scenario 1, continued

More recently, the polymerase chain reaction (PCR) has been used in forensics to analyze DNA (See Figure 2, page 6). This technique requires about 500-fold less DNA than RFLP analysis and is less time-consuming.

Forensic scientists use PCR to amplify and examine polymorphic DNA regions (Figure 4). These are regions that vary in length from individual to individual and fall into two categories: 1) variable number of tandem repeats (VNTR) and 2) STR (short tandem repeats). A VNTR is a region that varies amongst individuals and is typically composed of 15 to 70 base pair sequences, repeated 5 to 100 times. An STR is similar to a VNTR except that the repeated unit is only 2 to 4 nucleotides in length. Due to the shorter length, STRs are easier to amplify and analyze, making them the preferred fingerprinting method. By examining several different VNTRs or STRs from the same individual, investigators obtain a unique DNA profile for that individual that is unlike that of any other person (except for identical twins).

In this experiment, you will analyze PCR results obtained from different suspects. The unique DNA fingerprints will be compared to a sample collected from a crime scene to determine which of the suspects was present during the crime.

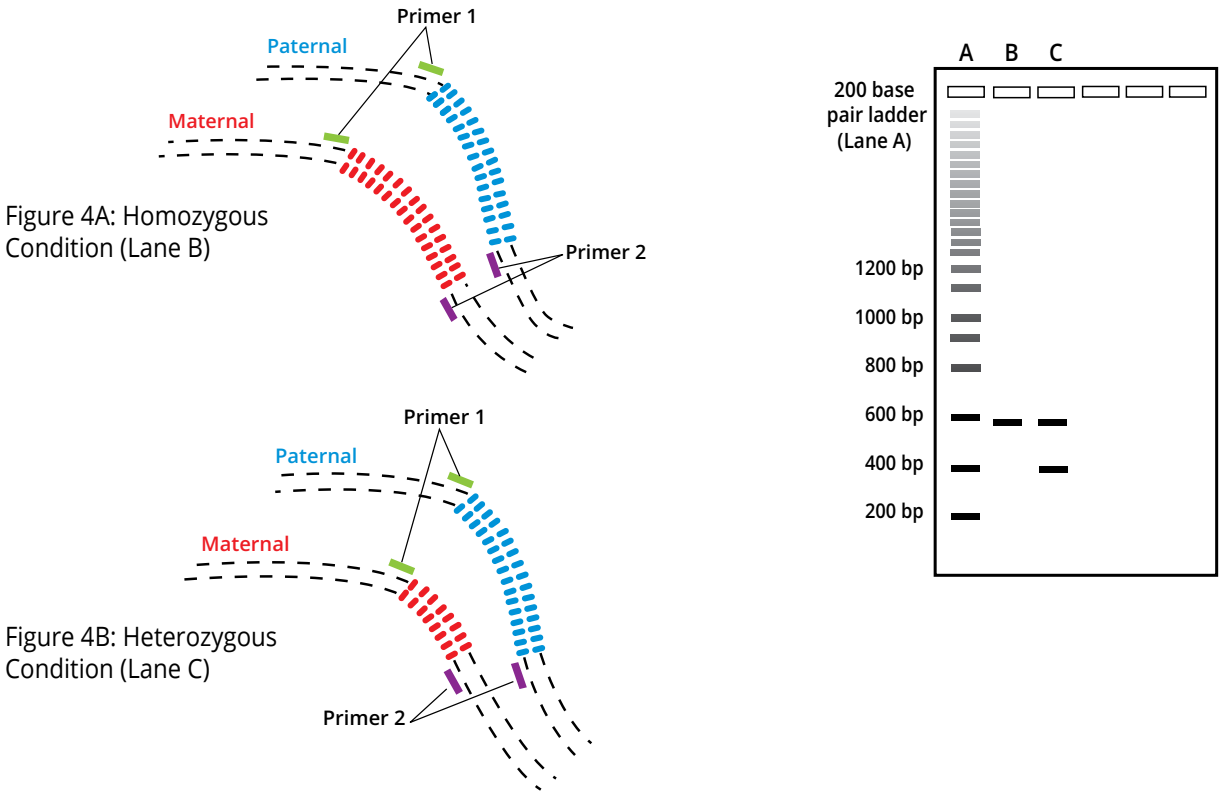


Figure 4: PCR Amplification of highly variable DNA Sequences.

Scenario 2: Nucleic Acid Testing for COVID-19

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.

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 5).

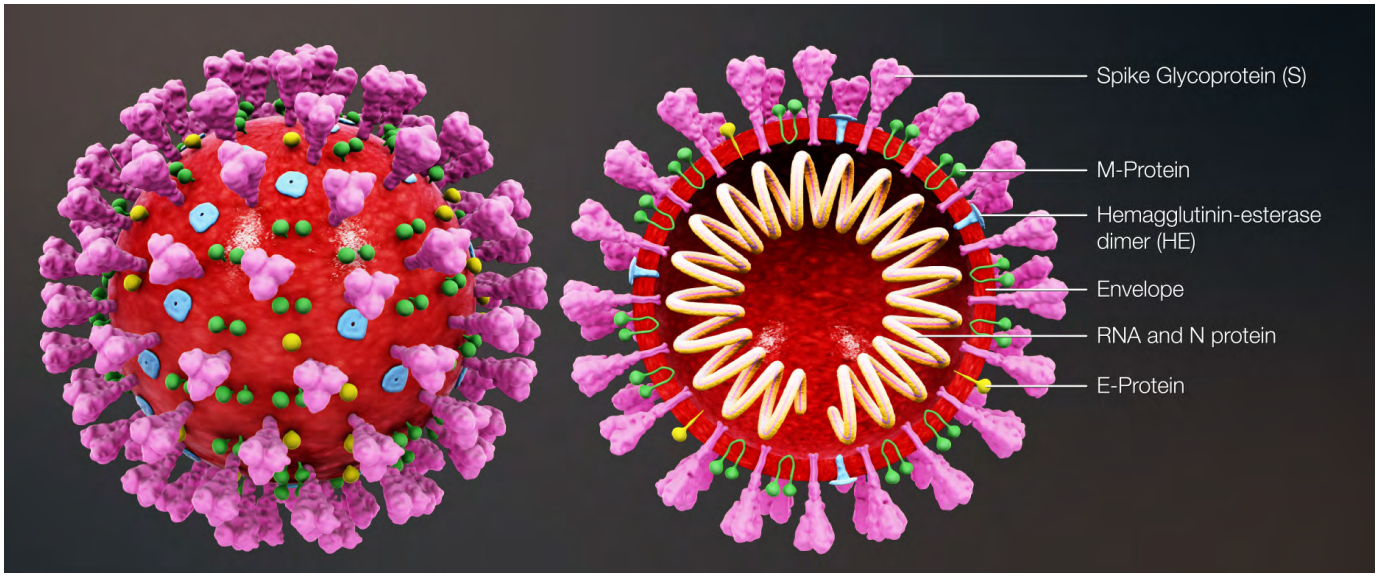


Figure 5: Coronavirus structure.

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.

Scenario 2, continued

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.

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 6). 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.

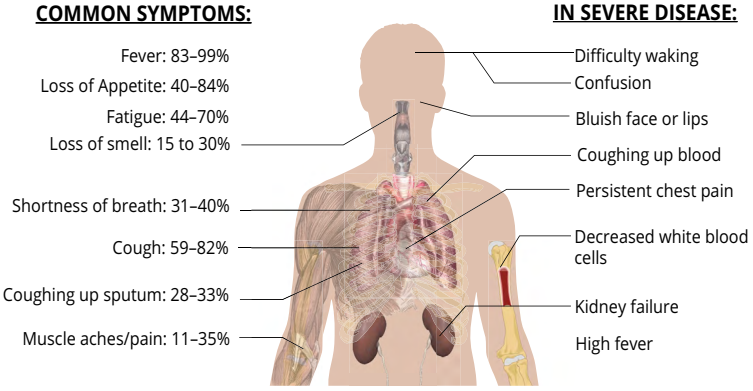


Figure 6: Symptoms of Coronavirus Disease.

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 RT-PCR Test for SARS-CoV-2

Like PCR (described on page 5) RT-PCR is extremely sensitive and accurate. 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 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 nucleic acid 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.

Scenario 3: Detection of Genetically Modified Organisms

WHAT ARE GENETICALLY MODIFIED ORGANISMS?

Over the past one hundred years, genetic research has greatly increased our understanding of the genome (the hereditary material of an organism encoded by its DNA) and its role as a blueprint for all processes within an organism. Variations in the DNA sequence, called mutations, can cause changes in the way an organism interacts with its environment. Most mutations result in negative effects for the organism; however, on occasion, a mutation grants an organism an advantage that promotes survival in its particular environment.

Humans have long recognized and taken advantage of genetic variation through traditional plant and animal husbandry techniques. For centuries, selective breeding and conventional hybridization have been used to increase crops' yields or give rise to other desirable qualities. For example, the corn we eat today was produced by artificial selection. Ages ago, farmers may have noticed that one plant was producing larger kernels, while another plant was producing more flavorful corn (Figure 7A). By crossing the two plants, these farmers encouraged those traits (observable characteristics) in the next generation. This allowed for the development of plants that yielded the best possible product—a plant with many ears of large, delicious kernels of corn (Figure 7B). In this way, over the last fifty years—a period during which the world population more than doubled, but farmland only increased by 10%—selective breeding and new agricultural technologies have allowed food yields to increase 25% per person!

Figure 7A:
Traditional varieties of corn.

Figure 7B:
Cultivated corn

While it used to take years of selective breeding to produce the genomic changes necessary to give rise to such desirable traits, the advent of biotechnology has accelerated this pace. The introduction of genetic engineering now allows scientists to directly manipulate a DNA sequence in order to generate desirable traits. These engineered genes, called transgenes, can be inserted, deleted or mutated in a matter of weeks using recombinant DNA technology. In order to be to be properly expressed *in vivo*, a transgene must include a promoter sequence, which recruits RNA polymerase to the transgene for transcription, and a terminator sequence, which signals RNA polymerase to end transcription (Figure 8). The promoter from the Cauliflower Mosaic Virus (CaMV) and the terminator from the *Agrobacterium tumefaciens* nopaline synthase gene (NOS) are commonly used by genetic engineers because they are recognized by transcriptional machinery of many different types of plants.

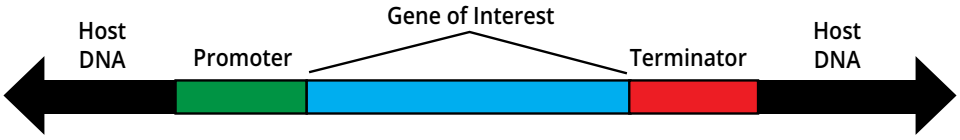


Figure 8: Features of a Transgene.

Scenario 3, continued

Current plant biotechnology promises to increase both the yield and nutritional value of many food crops. For example, the enzyme polygalacturonase (PG) digests pectin in the tomato's cell wall, making the fruit softer and more easily damaged during shipping. The Flavr Savr tomato has been engineered to "turn off" production of the PG enzyme, slowing the softening process. Therefore, the tomatoes are less fragile. "Bt-corn" expresses a naturally occurring pesticide that protects the plant from insects (Figure 9). This technology allows farmers to use less chemical pesticides, some of which are harmful to people and the environment. Another success story is that of "golden rice". Normally, rice, a staple food for much of the world population, does not provide β-carotene or vitamin A. Because vitamin A deficiency is a widespread problem in developing countries, rice has been modified to produce β-carotene, a precursor of vitamin A. Switching to cultivation of "golden rice" and other nutrient-supplemented crops in these areas represents major progress in combating malnutrition.

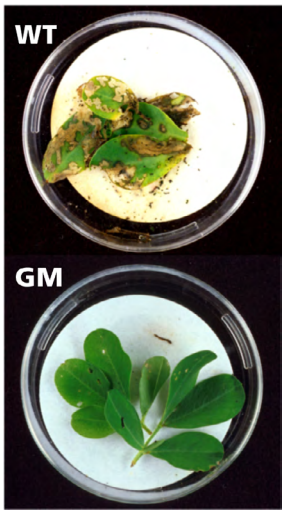


Figure 9:
Insect resistance of GM plants

In addition to boosting crop yields and enhancing nutrition, transgenic technology could be used to create allergy-free peanuts and low protein rice for people with kidney disease. Genetically modified foods may soon allow for the synthesis and delivery of various pharmaceutical products. In "pharming", transgenic plants can be used to make proteins with medicinal value like insulin or growth hormone. "Farmaceuticals" can be produced in many crops, including tobacco, carrots, tomatoes, soybeans and rice. By taking advantage of engineering DNA within the chloroplast, which maintains high protein expression levels and does not distribute its DNA via pollen, scientists may one day be able to generate great amounts of pharmaceuticals with little chance of non-target exposure to the product.

ETHICS OF GENETICALLY MODIFIED ORGANISMS

Tomatoes, soybeans and corn were among the first genetically modified food products approved by U.S. agencies in the 1990s. Since then, the safety, efficacy and benefits of GM foods have been debated at a global level. Many studies of GMOs and related technologies have been published in leading peer-reviewed science journals like Nature and Science. Proponents of GM technology cite studies showing improved quantity and quality of plants, decreasing costs for growers, and benefits for the environment. Critics of GM technology fear the spread of transgenes to other crops, increased allergens, and the creation of unanticipated dangers to people and the environment.

The responsibility of public health and policy concerning GMOs rests on the shoulders of the government and the biotechnology industry. The United States government has carefully monitored GM food production and created strict regulations to protect the health of Americans. There are several federal agencies in the United States that oversee food safety: the Federal Drug Administration (FDA) is responsible for the safety of human and animal food products; the U.S. Department of Agriculture (USDA) oversees the development of new plant varieties and their use in farming; and the Environmental Protection Agency (EPA) monitors pesticide levels in plants and determines what is acceptable for human consumption. To gain acceptance, the plant biotechnology industry must communicate its research and development of new GM food products effectively to these agencies.

Scenario 3, continued

USING PCR TO IDENTIFY GMOS

Over the past several years, some food companies have decided to remove GMOs from their foods. In order to determine whether the raw materials (corn, wheat, soy) have been genetically modified, DNA was extracted from the samples and analyzed using the Polymerase Chain Reaction (PCR). In this experiment, the primers were designed to differentiate between wild-type plants and those that have been genetically engineered. PCR can be used to determine whether a plant or food has been genetically modified using primers that target the 35S CaMV promoter and/or NOS terminator. As a positive control for DNA extraction, the plant chloroplast gene is also amplified.

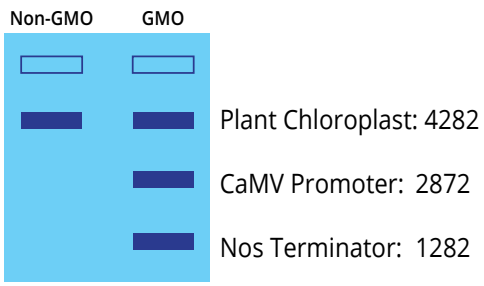


Figure 10: PCR Analysis of Non-GMO and GMO corn.

Scenario 4: DNA Paternity Testing

DNA fingerprinting (also called DNA typing) allows for the identification of the source of DNA samples. The method has become very important to provide evidence in paternity and criminal cases. In contrast to the more conventional methodologies, such as blood typing, which can only exclude an individual, DNA fingerprinting can provide positive identification with great accuracy.

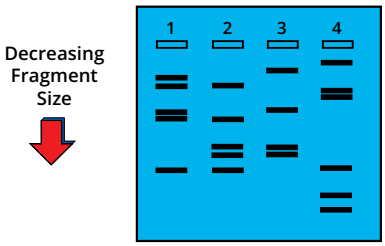


Figure 11: The child's DNA pattern (lane 2) contains DNA from the mother (lane 1) and the biological father (lane 3).

Paternity determination based on DNA analysis (genetic DNA fingerprinting) has become an important procedure for matching children with biological fathers and mothers. Examples of recent court cases that have utilized this procedure have included rape, incest, immigration, citizenship of children to the United States and matching of children with parents who were mismatched at birth due to hospital errors. This type of testing is also used during unrest, as in nations in civil war, where children are often separated from parents and subsequently reunited.

For paternity DNA fingerprinting, samples obtained from the mother, the child, and possible fathers are analyzed. A child's DNA is a composite of its parent DNAs. Therefore, comparison of DNA fragmentation patterns obtained from the mother and child will give a partial match. Bands in the child's DNA fingerprint that are not present in the mother's must be contributed by the father. Because of allelic differences, the DNA bands present in the child's fingerprint must be found in either the father's or mother's fingerprint.

The polymerase chain reaction (PCR) is routinely used to analyze DNA. This technique requires about 500-fold less DNA than other methods and is less time-consuming. In forensics and DNA paternity testing, PCR is used to amplify and examine highly variable (polymorphic) DNA regions. These are regions that vary in length from individual to individual and fall into two categories: 1) variable number of tandem repeats (VNTR) and 2) STR (short tandem repeats). A VNTR is a region that is variably composed of a 15-70 base pair sequence, typically repeated 5-100 times. An STR is similar to a VNTR except that the repeated unit is only 2-4 nucleotides in length. By examining several different VNTRs or STRs from the same individual, investigators obtain a unique DNA profile for that individual which is unlike that of any other person (except for identical twins).

In this simulation experiment, DNA was extracted from samples obtained from the mother, child and two possible fathers. The objective is to analyze and match the DNA fragment patterns after agarose gel electrophoresis and determine if Father 1 or Father 2 is the biological parent of the child.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA.

Figures:
Figure 5: 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 6: https://en.wikipedia.org/wiki/Coronavirus_disease_2019#/media/File:Symptoms_of_coronavirus_disease_2019_3.0.svg

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will explore four unique experiments that utilize agarose gel electrophoresis to separate DNA samples. The DNA banding patterns will be analyzed within scenarios that examine forensic, medical, and scientific investigations.

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.

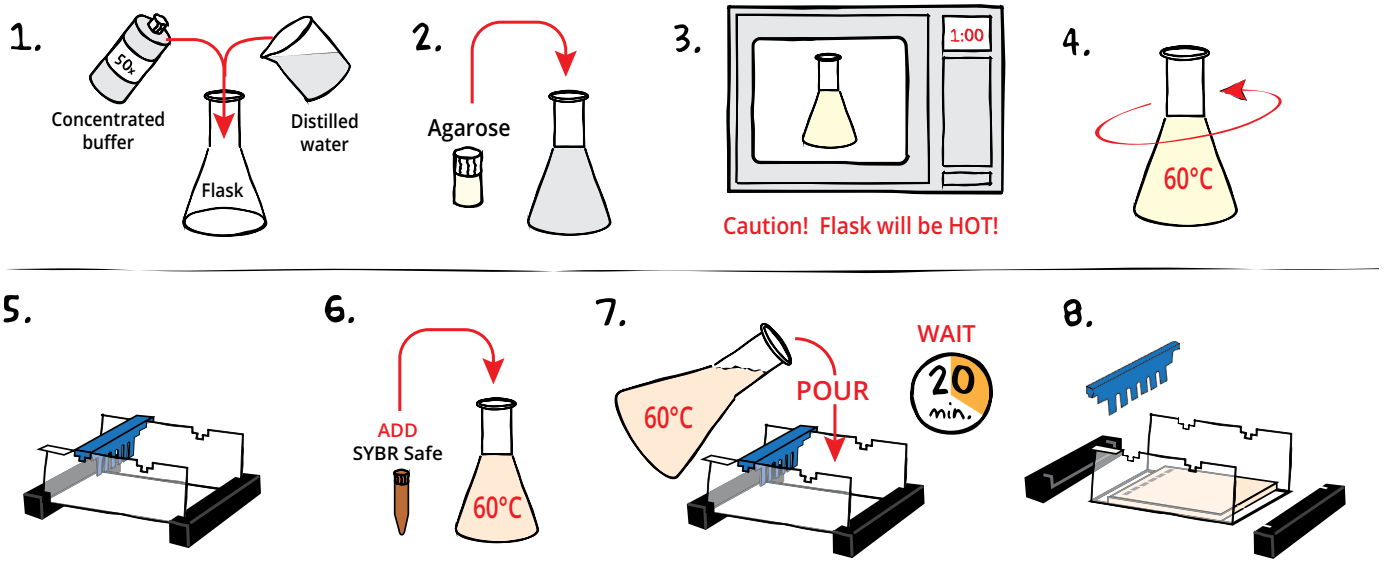
During the Experiment:

- 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.

Module I: Agarose Gel Electrophoresis



NOTE: If you are casting your own gels, review the following instructions. If you are using pre-cast gels, proceed to Step 8.

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 mL flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60°C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. Before casting the gel, **ADD** ***diluted SYBR® Safe*** to the molten agarose and swirl to mix (see Table A).
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.

Wear gloves and safety goggles

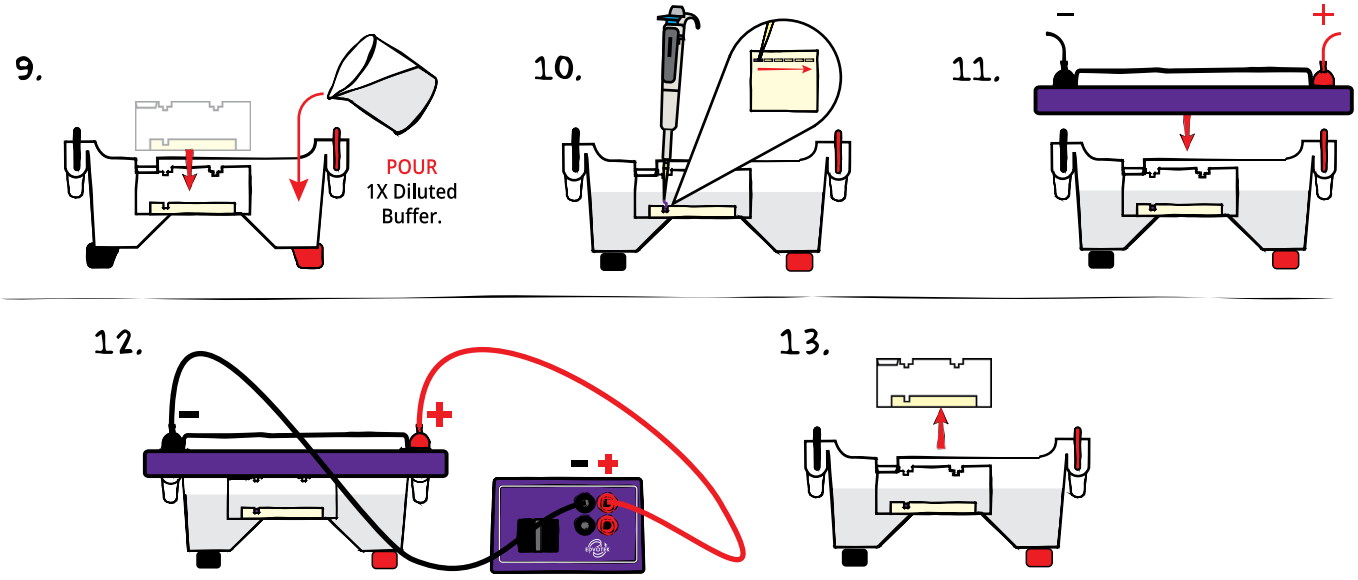
Reminder:
This experiment requires 0.8% agarose gels cast with 6 wells.

Table A — **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	0.23 g	30 mL	30 µL
10 x 7 cm*	1.0 mL	49.0 mL	0.39 g	50 mL	50 µL
14 x 7 cm	1.2 mL	58.8 mL	0.46 g	60 mL	60 µL

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

Module I: Gel Electrophoresis of Restriction Fragments, continued



9. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
10. **LOAD** 25 µL of each QuickStrip™ sample into the well in the order indicated by Table D.
11. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
12. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
13. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

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

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

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE™	300 mL	6 mL	294 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)**

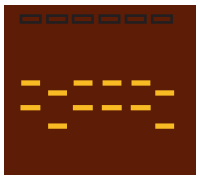
	Electrophoresis Model: EDGE™	M12 & M36
Volts	Min/Max (minutes)	Min/Max (minutes)
150	10/15	20/35
125	N/A	30/45
100	15/25	40/60

Table D: Gel Loading Table

TUBE	LANE	SCENARIO 1	SCENARIO 2	SCENARIO 3	SCENARIO 4
A	1	DNA Standard Markers	DNA Standard Markers	DNA Standard Markers	DNA Standard Markers
B	2	Crime Scene PCR reaction	Negative Control	GMO Negative Control	Mother DNA fragments
C	3	Suspect #1 PCR reaction	Positive Control	GMO Positive Control	Child DNA cut fragments
D	4	Suspect #2 PCR reaction	Patient #1	Corn Sample	Father 1 DNA cut fragments
E	5	Suspect #3 PCR reaction	Patient #2	Wheat Sample	Father 2 DNA cut fragments
F	6	---	Patient #3	Soy Sample	---

Module I: Gel Electrophoresis of Restriction Fragments, continued

14. 

15. 

16. 

VISUALIZING THE SYBR® GEL

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

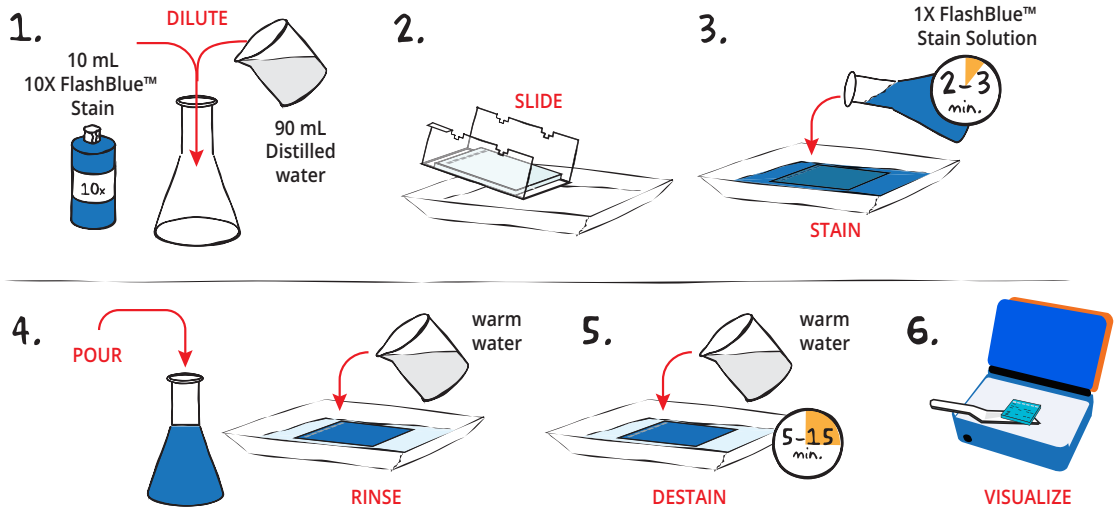
14. **SLIDE** gel off the casting tray onto the viewing surface of the transilluminator and turn the unit on. **ADJUST** the brightness to the desired level to maximize band visualization. DNA should appear as bright green bands on a dark background.

15. **PHOTOGRAPH** results.

16. **REMOVE** and **DISPOSE** of the gel and **CLEAN** the transilluminator surfaces with distilled water.

Module II: Staining Agarose Gels Using FlashBlue™ (OPTIONAL)

FlashBlue™ Stain is a simple and effective visible DNA stain that can be used as an alternative, or in addition to, UV-reactive DNA stains like SYBR® Safe. ***IF staining with both SYBR® Safe and FlashBlue™, you must examine and record the SYBR® Safe bands before beginning the FlashBlue™ Staining.***



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.

Study Questions

GENERAL QUESTIONS:

1. What factors influence how molecules are separated during agarose gel electrophoresis?

2. Explain migration according to charge during electrophoresis. What is the change of DNA and which electrode will DNA migrate towards?

3. Why is glycerol added to the sample solutions before they are loaded into the wells?

4. What would happen if distilled water were substituted for buffer in either the gel or the electrophoresis chamber?

SCENARIO 1:

1. What is polymorphic DNA? How is it used for identification purposes?

2. What is CODIS? How is it used to solve crimes?

3. What is an STR? A VNTR? Which (STR or VNTR) is predominantly now used in law enforcement? Why?

SCENARIO 2:

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

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

3. A patient was sick with COVID-19 symptoms but didn't make it to the doctor's office until after the symptoms had subsided. What results would you expect from a COVID RT-PCR test?

Study Questions, continued

SCENARIO 3:

1. What is artificial selection? Describe how farmers use artificial selection to improve crops.

2. What are some benefits of GM plants? What are some common concerns about GM plants?

3. Which Federal agencies are responsible for oversight on GM plants and foods?

4. What is PCR? How is PCR used to identify Genetically Modified Organisms?

5. Theoretically, you have extracted DNA from three different samples (corn, wheat, and soy) and analyzed them for genetic modifications using PCR. Knowing the results from your electrophoresis experiment, which samples have been genetically modified?

SCENARIO 4:

1. Why do different individuals, such as siblings, have different DNA sequences?

2. What is the function of PCR primers used in DNA paternity analysis?

3. Why is there more than one single locus used in an actual paternity DNA test?

Instructor's Guide

This experiment includes QuickStrip™ samples for four different scenarios. Each strip of samples contains a unique pattern of DNA that corresponds to the designated activity. The samples have already been mixed with a loading dye and are ready for use. Before beginning the experiment the students should read through the general and specific background information for the designated scenario.

LAB SETUP

Each scenario will require a 6-well agarose gel for analysis. The gels can be prepared ahead of time or, if time permits, students can mix and prepare the agarose gels prior to performing the experiment. There are two staining options included in the kit: SYBR® Safe and FlashBlue™. Only one stain should be used at a time. If the instructor chooses SYBR® Safe, it MUST be diluted and added to the molten agarose during gel casting. SYBR® Safe stained gels can be visualized using shortwave UV light or with a blue light transilluminator, such as the TruBlu™ 2 and EDGE™ electrophoresis system.

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 (if using FlashBlue™)	Prepare staining components	The class period or overnight after the class period	10 min.

MODULE I PREPARATIONS: AGAROSE GEL ELECTROPHORESIS

This experiment requires a 0.8% agarose gel for each scenario. 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.

Prepare SYBR® Safe Stain

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

2. Add 250 µL of the 1X buffer from step 1 to the tube of SYBR® Safe and mix by tapping the tube several times. The diluted SYBR® Safe Stain is now ready to be used during agarose gel preparation.

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, agarose powder, and diluted SYBR® Safe Stain.

Batch Gel Preparation

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. See Appendix B. If prepared in advance, any leftover agarose solution can be allowed to solidify in the flask, covered, and stored in the refrigerator until needed.

Preparing Gels in Advance

Gels may be prepared ahead and stored for later use. Solidified gels can be store 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 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.

Preparing the QuickStrips™

QuickStrip™ tubes consist of a microtiter block covered with a protective overlay. Each well contains pre-aliquoted DNA.

Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube.

The QuickStrip™ wells contain 30 µL of each sample and should be loaded into the agarose gel in the following manner:

Tube A	Lane 1	DNA Standard Markers
Tube B	Lane 2	Sample 1
Tube C	Lane 3	Sample 2
Tube D	Lane 4	Sample 3
Tube E	Lane 5	Sample 4
Tube F	Lane 6	Sample 5
Tube G	---	---
Tube H	---	---

NOTE: Scenarios 1 and 4 contain 5 lanes of samples.

MODULE II PREPARATIONS: STAINING WITH FLASHBLUE™ (OPTIONAL)

FOR MODULE II
Each Student Group should receive:
- 10 mL 10X concentrated FlashBlue™
- Small plastic tray or weigh boat
- Distilled or deionized water

FlashBlue™ can be used as an alternative or in addition to SYBR® Safe in this experiment. If only staining with FlashBlue™, you can omit SYBR® Safe from the gel preparation. However, FlashBlue™ is less sensitive than SYBR® Safe and will take a longer time to obtain results. Alternatively, gels can be visualized first with SYBR® Safe and then with FlashBlue™.

Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 15 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to develop in the destaining solution, resulting in dark blue DNA bands that contrast with a uniformly light blue background. A white light box (Cat. #552) is recommended for visualizing gels stained with FlashBlue™.

Wear gloves and safety goggles

- Stained gels may be stored in destaining liquid for several weeks if they are refrigerated, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels should be discarded in the garbage and destaining solutions should be disposed of down the drain.

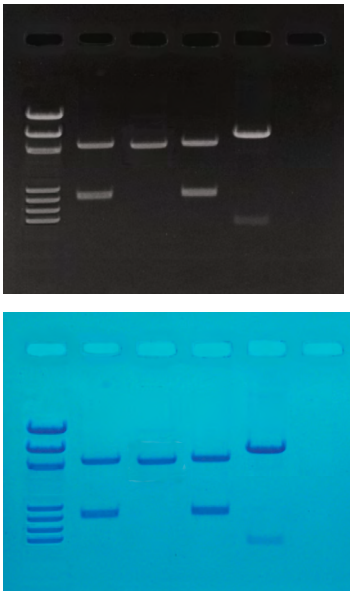
Photodocumentation of DNA (Optional)

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

Expected Results

Load and run the gel as described in the directions. Stain the finished product and observe the resulting bands. Results are shown for each scenario. The DNA standards in Lane 1 make it possible to measure the DNA bands obtained from the PCR reactions.

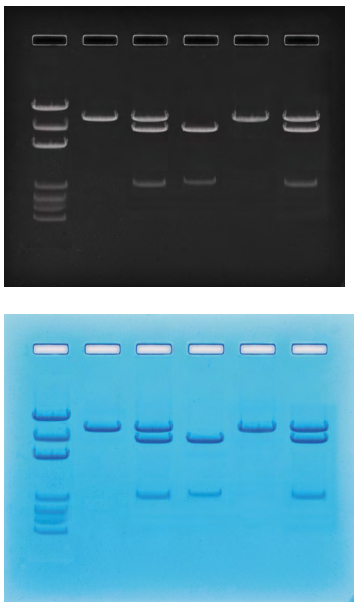
SCENARIO 1: DNA FINGERPRINTING BY PCR AMPLIFICATION



The results of this analysis indicates an identical pattern in Lanes 2 and 4. This is strong evidence that the crime scene DNA and Suspect 2 match. In criminal investigations, several known variable regions in DNA are analyzed to match crime scene and suspect DNAs.

Lane	Tube	Sample	Molecular Weights (in bp)
1	A	DNA Standard Markers	6751, 3652, 2827, 1568, 1118, 825, 630
2	B	Crime scene PCR reaction	3000, 1282
3	C	Suspect #1 PCR reaction	3000
4	D	Suspect #2 PCR reaction	3000, 1282
5	E	Suspect #3 PCR reaction	3652, 630

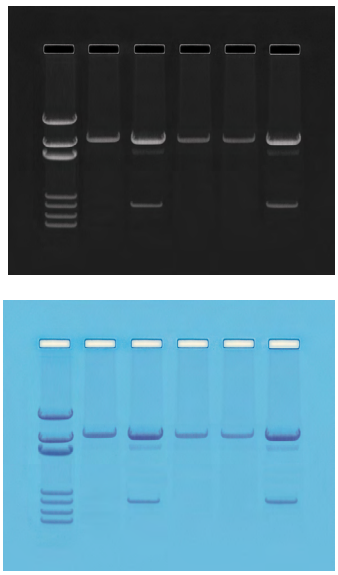
SCENARIO 2: NUCLEIC ACID TESTING FOR COVID-19



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

Expected Results, continued

SCENARIO 3: DNA FINGERPRINTING BY PCR AMPLIFICATION

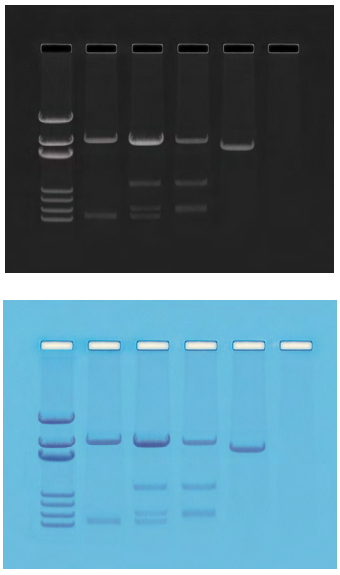


Lane	Tube	Sample	Result	Molecular Weights (in bp)
1	A	DNA Standard Markers	-------	6751, 3652, 2827, 1568, 1118, 825, 630
2	B	GMO Negative Control	GMO Negative	4282
3	C	GMO Positive Control	GMO Positive	4282, 2872, 1282
4	D	Corn Sample	GMO Negative	4282
5	E	Wheat Sample	GMO Negative	4282
6	F	Soy Sample	GMO Positive	4282, 2872, 1282

Plant Chloroplast: 4282
CaMV: 2872
Nos: 1282

SCENARIO 4: DNA PATERNITY TESTING

Parentage (meaning maternity and paternity) can be determined from a child's DNA profile. By comparing the DNA profile of a mother and her child it is possible to identify DNA fragments in the child which are absent from the mother. Therefore, these polymorphisms have been inherited from the biological father. In this case, the two bands in the child's DNA profile that are not explained by the mother's profile are found in father #1.



Lane	Tube	Sample	Molecular Weights (in bp)
1	A	DNA Standard Markers	6751, 3652, 2827, 1568, 1118, 825, 630
2	B	Mother DNA fragments	3652, 630
3	C	Child DNA fragments	3652, 1300, 700, 630
4	D	Father 1 DNA fragments	3652, 1300, 700
5	E	Father 2 DNA fragments	3000

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

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

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

Appendix A
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.
	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 properly.	Ensure that diluted SYBR® Safe was added to the gel.
	The gel was not stained for a sufficient period of time with FlashBlue™.	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 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 14x7 cm tray) from the wells to ensure adequate separation.	Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 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.
There is only a small amount of SYBR® Safe in my tube.	SYBR® Safe is a concentrate that is diluted before use.	Centrifuge or tap the tube to move the contents to the bottom of the tube. Dilute the SYBR® Safe before use as outlined on page 23.

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 E.

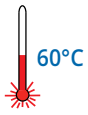
Table E: Bulk Preparation of Electrophoresis Buffer

50x Conc. Buffer	+	Distilled Water	Total Volume Required
30 mL		1470 mL	1500 mL (1.5 L)

Batch Agarose Gels (0.8%)

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

1. Use a 500 mL flask to prepare the diluted gel buffer.
2. Pour 2.0 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 (see page 23) 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, 50 mL for a 10 x 7 cm tray, and 60 mL for a 14 x 7 cm tray.
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.

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

Table F: Batch Prep of 0.8% UltraSpec-Agarose™

Amount of Agarose	+	Concentrated Buffer (50x)	+	Distilled Water	Total Volume
2.0 g		5.0 mL		245 mL	250 mL