Edvo-Kit # 957

Edvo-Kit #957

Blinded by the Light: UV Rays and DNA Damage

Experiment Objective:

In this experiment students directly observe the effects of UV light on DNA. Students will run a time series test comparing UV exposed plasmid samples and examine their results using electrophoresis. Students may also test the ability of different sunscreens to prevent DNA damage.

See page 3 for storage instructions.

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SAMPLE LITERATURE
Please refer to included weblink for correct version

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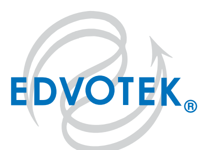

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Experiment Components

Components		**Storage**	**Check (✓)**
A	DNA Standard Marker	-20 °C	❑
B	Plasmid DNA Substrate	-20 °C	❑
•	SYBR® Safe Stain	-20 °C	❑

REAGENTS & SUPPLIES

Store all components below at room temperature.

Component		**Check (✓)**
•	UltraSpec-Agarose™	❑
•	Electrophoresis Buffer (50X)	❑
•	10X Gel Loading Solution	❑
•	Microtest Tubes	❑

This experiment is designed for 10 lab groups.

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.

Experiment Requirements

- Horizontal Gel Electrophoresis Apparatus
- D.C. Power Supply
- Automatic Micropipettes and tips
- Microwave, hot plate or burner
- Water bath (65 °C)
- UV Transilluminator (300 nm wavelength or shorter)
- UV Photodocumentation System (optional)
- Gloves and UV safety goggles
- Pipet Pump or bulbs
- 250 mL flasks
- Hot gloves
- Marking pens
- Distilled or Deionized Water
- Ice
- Sunscreen (optional)
- Plastic wrap (optional)

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Background Information

In songs, paintings, weather reports, and vacation photos sunshine is celebrated - and for good reasons. It's the foundation of most food webs, keeps our planet comfortably warm, and helps us and other animals see. It also has clear health benefits. Sunshine helps us produce vitamin D, fall asleep easily, and feel calm and happy. However, sunshine is also a leading carcinogen - a substance that causes cancer in living tissues. (Other top carcinogens include radon, asbestos, and formaldehyde which do not have the same positive reputation in our culture, to say the least!) Scientists study the interactions between the sun and our cells in order to understand how exposure leads to cancer and how to best avoid this risk while still enjoying the benefits. In this experiment you will use molecular biology to examine the relationship between UV light and DNA damage.

THE SUNSHINE YOU CAN'T SEE: UV

Every hour about around 430 quintillion (430,000,000,000,000,000,000) joules of solar energy hit the earth. This is a lot of energy. In fact, it's very close to the amount of energy that the global human population uses in a whole year! Energy from the sun (sunshine) is a mixture - or spectrum - of different light types. These types can be roughly grouped into three categories: infrared energy which we feel as heat, visible light which is what allows us to see, and ultraviolet light which we can't feel or see but which packs a large energy punch (Figure 1).

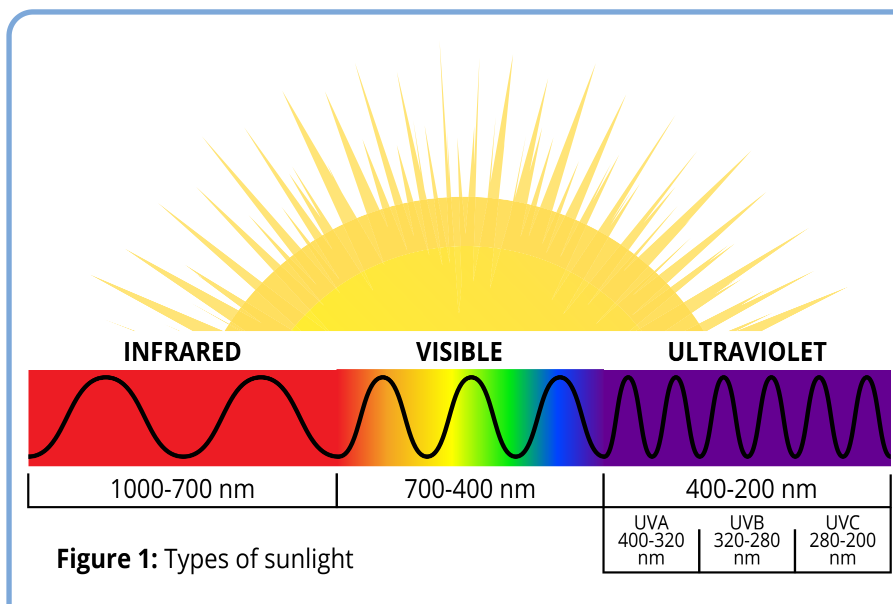

Figure 1: Types of sunlight

Of the three subtypes of sunlight, ultraviolet, or UV, light possesses the largest cancer risks because its short wavelengths have the most energy (Box 1). UV light was first discovered in 1801 when the German physicist Johann Ritter observed that silver chloride paper changed color after it was exposed to invisible rays just beyond violet light. He hypothesized that was because the "beyond violet" or "ultraviolet" light bombarded the silver chloride molecules with energy and caused them to break down into their component parts.

At its most energetic UV light can cause electrons to break away from their atoms. This process, known as ionization, changes the chemical properties of an atom and often causes old chemical bonds to break and new ones to form. Ionizing UV light is called UVC. However, UVC is all but absent on Earth because our atmosphere absorbs and reflects higher energy wavelengths like gamma rays, x-rays, and UVC rays.

BOX 1:
In physics you may have learned that light is both a wave and a particle which means that it can be described as a packet of energy (e.g. a photon) or as a time varying electric and magnetic field (e.g. an electromagnetic wave.) These two sides of light have the following relationship:
high energy = short wavelengths
low energy = long wavelengths.

Instead, the UV light reaching the Earth's surface is mostly in the form of UVA and UVB. These two types of ultraviolet light have slightly longer wavelengths and thus less energy. They can't remove electrons. However,

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they can still cause reactions by temporarily moving electrons to higher energy states. For example fluorescence occurs when UVA or UVB light causes electrons in certain materials to become excited and then, gradually return to a lower energy level. During the latter process, the material emits part of the absorbed light as visible light. This is why a yellow safety vest appears so bright in sunlight and also why black lights can make images pop out of specially printed paper.

The energy in UVA and UVB also affects cells. In mammalian cells, both UV light types trigger the synthesis of vitamin D. Less helpfully, both UV types also cause Vitamin A and collagen in cells to degrade. This causes skin cells to lose their elasticity and is a major reason for photoaging - the premature wrinkling and sagging of the skin due to repeated sun (or artificial tanning) exposure. Finally, UVB light can also affect cells by damaging their genetic code. This can lead to serious health consequences like cell death, mutations, and cancer.

THE DARK SIDE OF THE SUN: DNA DAMAGE

DNA is a self-replicating thread-like chain of nucleotides that contain the genetic instructions for life (Figure 2). When this molecule is exposed to the high energy packaged in UVB rays multiple things can happen. Around 99.9% of the time what happens is .... nothing. The molecular structure of DNA is highly stable and adept at handling UV radiation. In these cases, DNA molecules absorb the UV energy, convert it to heat, and then release the heat back into the environment.

This catch-and-release process is fast, but still dangerous because it temporarily puts a DNA molecule into a higher and more reactive energy state. When DNA is reactive new bonds can form and key structural bonds can break. For example, following UVB exposure, DNA nucleotides that usually bond horizontally with other nucleotides on the opposite strand can instead bind with the nucleotides above or below them. This creates a new structure in the DNA called a pyrimidine dimer (Figure 2B). Less frequently, strand breaks can occur. These happen when the bonds between the phosphate group and the sugar group of two neighboring nucleotides disconnect. Single strand breaks or nicks are when only one side of a DNA's double helix is damaged. In certain situations, these breaks actually help DNA molecules unwind themselves for copying which is why cells sometimes intentionally create them and why they can often recover from them. Much more damaging and difficult to recover from are double strand breaks (Figure 2C). These are when breaks form on both sides of the DNA helix close to each other.

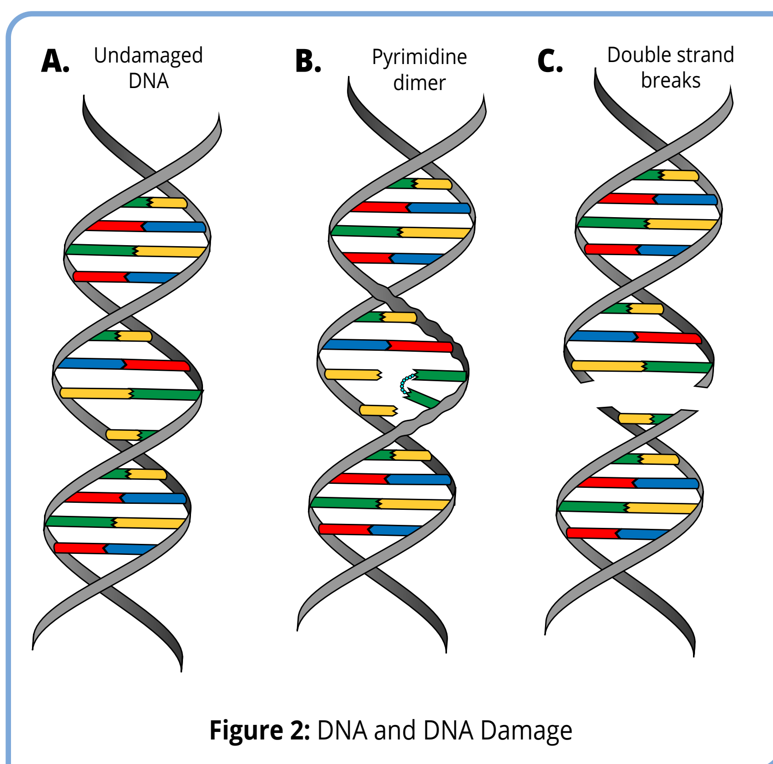

Figure 2: DNA and DNA Damage

New bonds and breaks both change the shape of DNA molecules and interfere with one of their primary functions – being accurately copied again and again and again. Before a cell divides, the DNA within that cell must be copied so that each daughter cell has the genetic instructions it needs to function. During this process DNA unwinds and its two strands separate. Next, an enzyme called DNA polymerase travels along each single strand adding complementary nucleotides and eventually creating two double stranded molecules that are the exact copy of each other. A similar process happens when DNA is used as the template to create mRNA during transcription - the first step in protein synthesis. However, breaks and extra bonds derail these processes either by stopping polymerase in its tracks or by causing this enzyme to make copy errors.

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Copy errors are changes in the nucleotide sequence of a DNA strand. When these changes occur in protein coding sections they can result in abnormal proteins and when these abnormal proteins are involved in cell growth, DNA repair, or tumor suppression the error may eventually lead to cancer. Cancerous cells grow and divide continuously (even when they are unneeded, old or damaged) and create daughter cells that do the same. If left unchecked, the resulting mass of cancerous cells - called malignant tumors - can invade surrounding tissues, spread to other parts of the body, and can eventually be fatal. In the case of UV light exposure, the major cancers are basal cell carcinoma, squamous cell carcinoma, and melanoma.

A SUNNIER OUTLOOK: PREVENTION AND REPAIR

Luckily, most cases of UV exposure do not lead to cancer. Instead, cells have evolved to prevent, repair, or isolate damaged DNA. Defenses start with the resilient structure of DNA and its careful packaging within cells. Additional protection is also provided when cells produce pigments that act like chemical bodyguards for DNA (Box 2). When breaks, bonds, or copy errors do occur DNA can identify these abnormalities and quickly repair them. In fact, cells are constantly running repair operations that either reverse the chemical changes created by UV energy or that remove and replace DNA regions flagged as potentially damaged and dangerous.

BOX 2:
Similar to how Earth's atmosphere blocks the highest energy light waves from hitting the surface, our skin shields the rest of our bodies from UV radiation. One way the skin does this is by producing melanins (compounds that absorb UV energy) when the body is exposed to high UV levels. So that tan you see? Think of it as your body's missile defense system and as a signal that your cells have been exposed to damaging sun levels.

However, in some cases too much repair is needed. When this happens a cell may begin a process of apoptosis or programmed cell death. This is particularly common in multicellular organisms where replacing a single cell is less energy intensive than repairing lots of damaged DNA and much less risky! If you've ever had a sunburn then you've experienced apoptosis first hand. During a sunburn many damaged cells in your skin die. The death of so many cells triggers inflammation which causes the skin to become red, hot, itchy and sensitive. However, these unconformable symptoms are also a powerful defense against cancer because they allow the body to get rid of cells that are now mostly a liability.

As your dermatologist (or mom) may have told you, we can also make smart choices that minimize the risk of sunburns and DNA damage. These include knowing when and how long to stay out in the sun, wearing protective clothes and eyewear, and applying sunscreen.

Sunscreens have been in the human survival arsenal for thousands of years. Early Philippine and Malaysian sailors used a paste made from plants, rice, and spices to protect their faces while the ancient Greeks tried olive oil. One of the first modern sunscreens was a sticky red concoction called RVP (Red Veterinary Petrolatum) which was used by US soldiers in the South Pacific during World War II. Shortly afterwards, in 1946, an Austrian chemist - Dr. Franz Greiter - developed a more agreeable and effective version after experiencing a particularly bad sunburn while mountaineering in the Alps. Greiter was also the first to describe sunscreen in terms of SPF (Sun Protection Factor), his had a SPF of around 2!

Today's sunscreens work by combining chemicals that either absorb or reflect UV radiation. For example, some sunscreens contain inorganic compounds like zinc oxide and titanium dioxide that stay on top of the skin and scatter UV radiation. While others contain organic compounds like oxybenzone and octinoxate that soak into the skin and absorb the energy in UV light much like the natural pigment melanin. While sunscreens have come a long way, scientists are still working to make these mixtures safer, water resistant, longer lasting, more effective, and safer for the environment.

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USING BIOTECHNOLOGY TO STUDY UV LIGHT AND DNA DAMAGE

A red sunburn or the development of a skin tan might be one way to identify that UV light is affecting someone's DNA but it's not an ideal tool to study this relationship or to test new protections. Instead scientists use biotechnologies like Polymerase Chain Reaction (PCR), electrophoresis, and fluorescent proteins to help them observe the effects that this invisible light can have on cells and molecules.

Scientists can use the Polymerase Chain Reaction - a type of artificial DNA replication carried out in a test tube - to compare DNA solutions that have been exposed to different UV conditions (Figure 3A). Because damaged DNA cannot be copied by the enzyme polymerase, a PCR sample that has undergone extensive damage will not amplify well. Consequently, by comparing the amount of amplified DNA in different samples an researcher can determine which UV conditions had the strongest damaging effect.

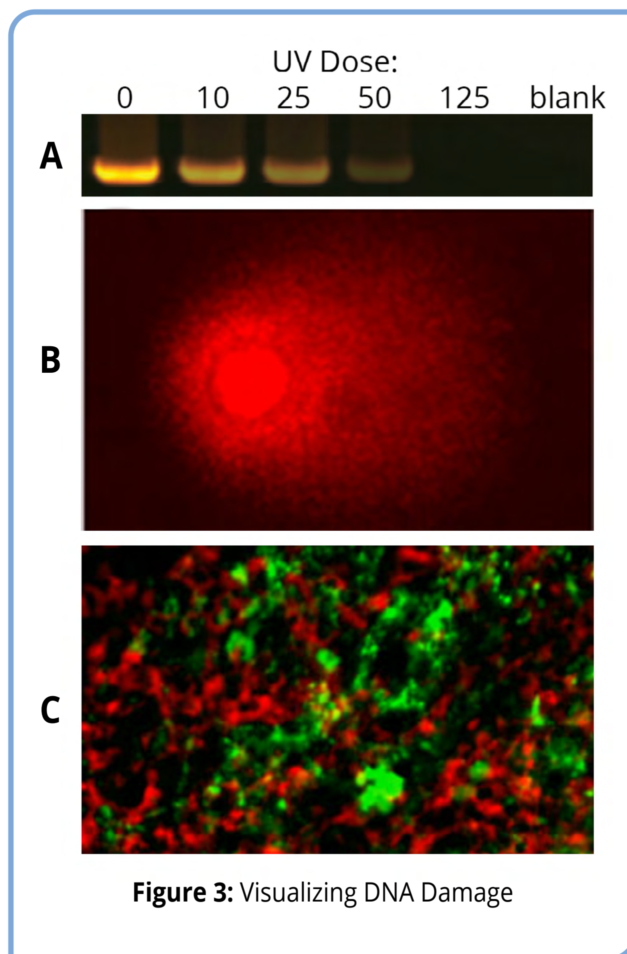

Figure 3: Visualizing DNA Damage

Another popular method is the Comet Assay (Figure 3B). In this method cells are mixed with agarose, lysed (broken open), run through electrophoresis, and then stained. The current provided by the electrophoresis causes DNA in the ruptured cells and nuclei to migrate. Because undamaged DNA is large and stays clumped together it moves slowly through the agarose while smaller strands of damaged DNA travel fast. This results in a comet like pattern where each cell has a central clump of intact DNA and a tail of damaged DNA. The bigger the tail, the more damage has occurred within a cell.

In a more recent, and yet unnamed approach, scientists create circular pieces of DNA containing different types of DNA damage (pyramide dimers, single strand breaks etc.) as well as a gene for a colorful fluorescent protein. They then insert this DNA into different living cells and look at these cells under a fluorescent microscope (Figure 3C). When a cell is able to repair the inserted DNA the colored protein gets created and causes that cell to fluoresce. Using this method, scientists have discovered that cells often specialize in certain types of DNA repair.

YOUR EXPERIMENT

In this lab, you will create a time series of UV exposed DNA samples and then use agarose gel electrophoresis to observe and compare the damage. In addition, your class may compare samples that have been protected by different types of sunscreen. We suggest you also include a sample of unexposed DNA as a control.

In order to identify DNA damage using agarose gel electrophoresis, you will use a particular type of DNA known as plasmid DNA. While often depicted as a circle, most plasmid DNA samples are made up of molecules in many different forms (Figure 4a). The most common form of DNA in most undamaged plasmid samples is supercoiled. In this configuration, the DNA strand is not only twisted but also wound around itself or around other nearby strands which creates a very condensed shape. This shape can become circular when one strand of a plasmid is damaged or “nicked”. This is because this type of break releases the coiling tension. Supercoiled plasmids can also become linear DNA when two strands are damaged - i.e. when a double-strand break occurs. Finally, some lab

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procedures can cause the two strands of a plasmid to separate. This creates circular and single-stranded molecules of DNA.

When run on an agarose gel these different shapes form distinct bands (Figure 4b). Circular single-stranded DNA is small and travels fast. When present, they form a band near the bottom of the gel. Compact, supercoiled plasmids also travel quickly through the agarose. In most samples, these form the most concentrated and so brightest band. Above this band are the bands created by the linear and nicked plasmid DNA molecules. The order of these two bands depends on the size of the plasmid - large plasmids will have the linear DNA band on top while smaller plasmids will have the nicked plasmid band on top. Sometimes, larger faint bands or even a smear of DNA may also appear high on a gel. These are made by plasmids that have twisted around each other the form large plasmid complexes.

In this experiment, your undamaged DNA controls should form a banding pattern very similar to the idealized bands shown in Figure 4b. In contrast, your experimental samples may have only linear and nicked DNA – or in some cases large plasmid complexes. This is because UV exposure will primarily cause strand breaks that reduce the number of single-stranded circular and double-stranded supercoiled DNA in the sample.

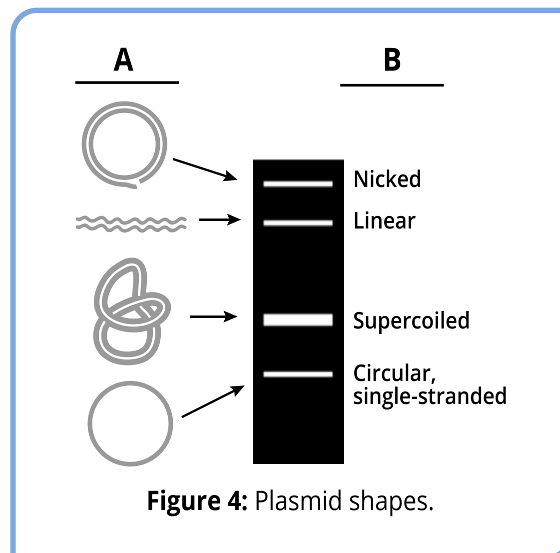

Figure 4: Plasmid shapes.

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Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment students directly observe the effects of UV light on DNA. Students will run a time series test comparing UV exposed plasmid samples and examine their results using electrophoresis. Students may also test the ability of different sunscreens to prevent DNA damage.

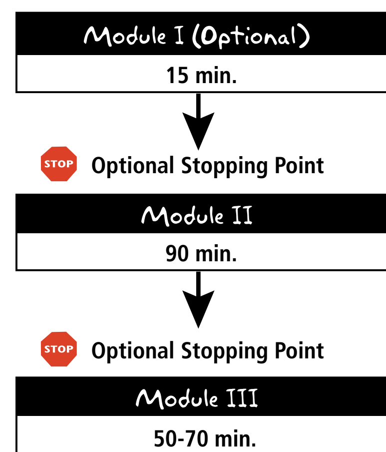
LABORATORY SAFETY:

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

- Wear gloves and goggles while working in the laboratory.
- Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after working 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.

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Module I: Preparing to Test Sunscreens (Optional)

1. As a class, **BRAINSTORM** different types of sunscreens. **DETERMINE** which groups will test sunscreens and which groups will test unprotected DNA. **DECIDE** who will bring in sunscreen samples.

2. As a class, **COLLECT** 1-5 sunscreen samples. ***NOTE: Only a small amount of sunscreen is needed. Each experiment requires <1 mL or a pea-sized sample.***

3. If your group is testing a sunscreen, **RECORD** the SPF and the active ingredients of this product in your lab notebook or below.

Sunscreen Name	SPF	Active Ingredient(s)

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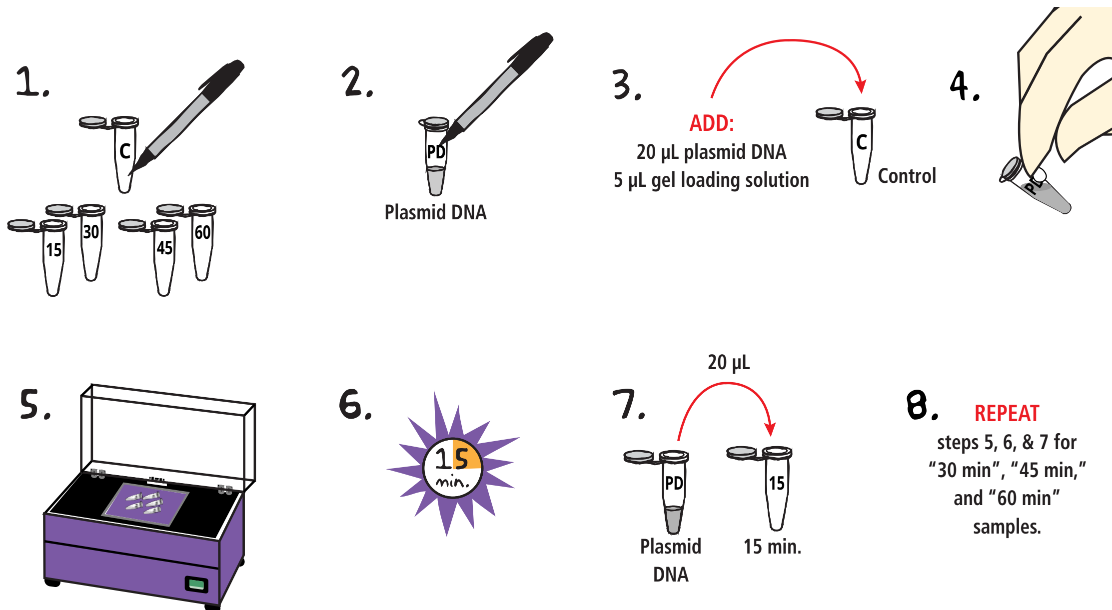
Module II: DNA Damage by UV Exposure



1. **LABEL** five microcentrifuge tubes with your group ID and with "Control", "15 min", "30 min", "45 min", and "60 min".
2. **LABEL** your Plasmid DNA (PD) tube with your group ID.
3. **TRANSFER** 20 µL of plasmid DNA to the "Control" tube and **ADD** 5 µL of 10x gel loading solution. **KEEP** this tube on ice or in the refrigerator.
4. If you are testing a sunscreen, **APPLY** a pea sized amount to the outside of the PD tube by hand. ***NOTE: Ensure that the entire tube is covered in a thin layer of sunscreen.***
5. As a class, **PLACE** all PD tubes directly on the short wave UV Transilluminator (UV light). **LAY** the tubes on their sides to maximize exposure.
6. **TURN ON** the UV light and begin timing. After 15 minutes, **REMOVE** the tubes.
7. **TRANSFER** 20 µL of plasmid DNA to a tube labeled "15 min" and **ADD** 5 µL of 10x gel loading solution. **KEEP** this tube on ice or in the refrigerator.
8. **REPEAT** steps 5, 6, and 7 three more times for the "30 min", "45 min", and "60 min" samples.

IMPORTANT: UV light can cause damage to skin and eyes. Wear UV safety goggles and avoid direct exposure to skin. As a safety measure, place a box over the UV light source when exposing the DNAs to UV light.

OPTIONAL STOPPING POINT: After the addition of 10x gel loading solution, samples are ready for electrophoresis. The samples may be stored in the freezer for electrophoresis at a later time.

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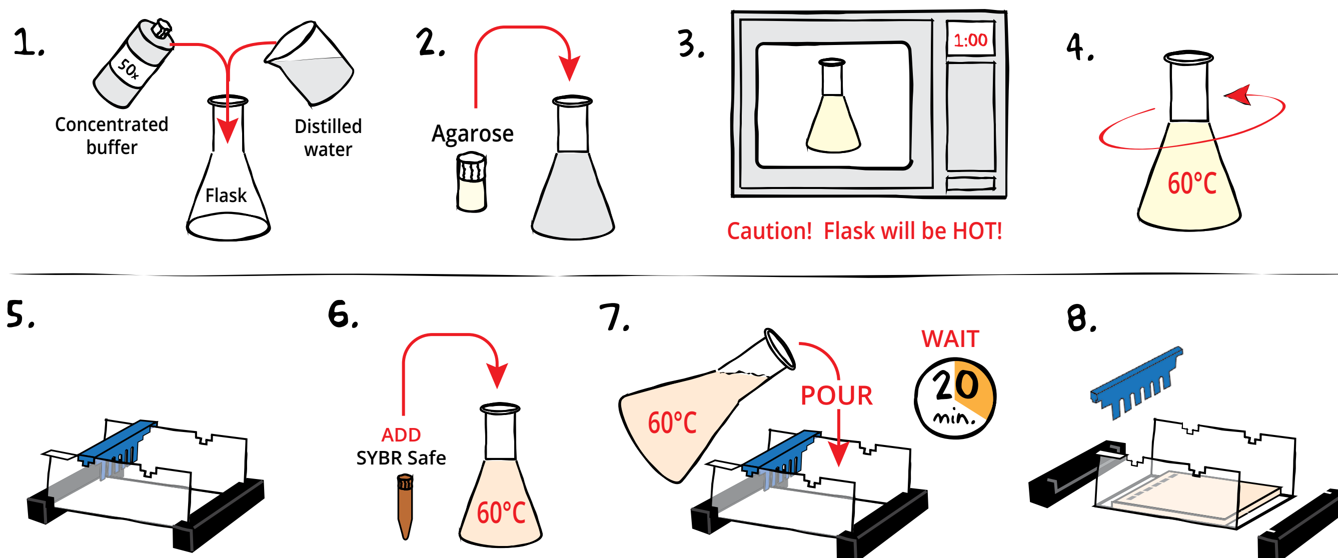
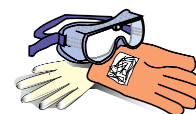
Module III: Separation of DNA Fragments by Electrophoresis



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

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

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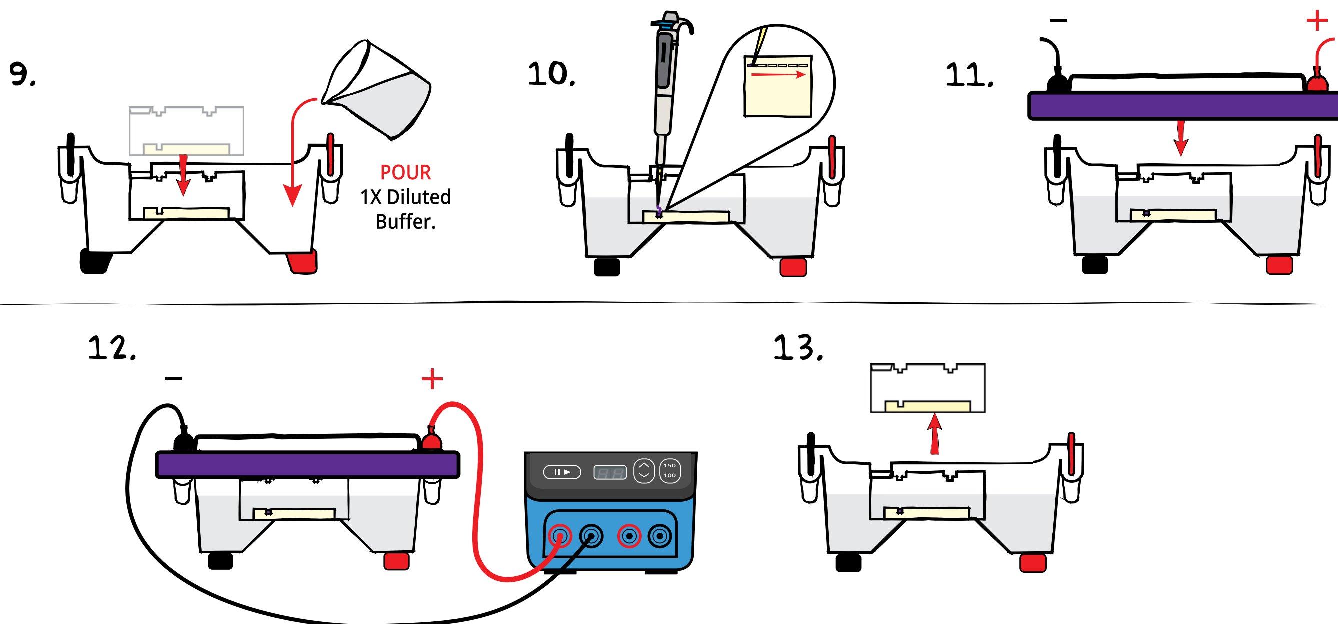
Module III: Separation of DNA Fragments by Electrophoresis, continued



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 sample into the well in the order indicated by Table 1.
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 1: Gel Loading		
Lane	**Tube Label**	**Sample Description**
1	DNA Standard Marker	DNA Standard Marker
2	Control	Control Plasmid DNA
3	15 min.	Plasmid DNA exposed to UV for 15 min.
4	30 min.	Plasmid DNA exposed to UV for 30 min.
5	45 min.	Plasmid DNA exposed to UV for 45 min.
6	60 min.	Plasmid DNA exposed to UV for 60 min.

OPTIONAL STOPPING POINT:

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.

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

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Module III: Separation of DNA Fragments by Electrophoresis, continued

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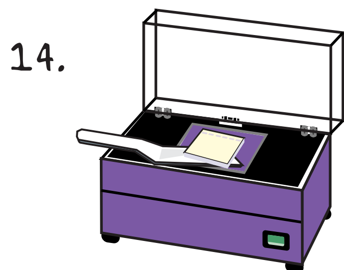

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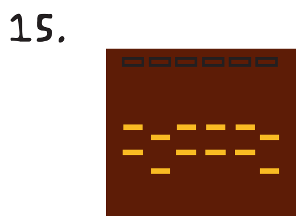
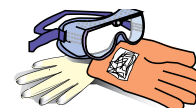


16.

VISUALIZING THE SYBR® GEL

14. **SLIDE** gel off the casting tray onto the viewing surface of the transilluminator.
15. **TURN** the unit on. DNA should appear as bright green bands on a dark background. **PHOTOGRAPH** results.
16. **TURN** the unit off. **REMOVE** and **DISPOSE** of the gel and **CLEAN** the transilluminator surfaces with distilled water.



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

ANALYZE THE RESULTS

1. **OBSERVE** your agarose gel then **ANSWER** the following questions either below or in your lab book.
    - At what UV exposure time did you observe a significant change in the plasmid samples' banding patterns?
    - Which bands changed?
    - What type(shape) of plasmids does each band consist of?
    - What can you infer about DNA damage from the band changes that you observed?
2. If your class tested different sunscreens, **COMPARE** your results with that of your classmates. **ANSWER** the following questions below or in your lab book.
    - At what UV exposure times did each group observe signs of DNA damage?
    - Did your classmates observe similar or different banding patterns in their control samples as compared to your control sample? What about in their damaged DNA samples as compare to your damaged DNA samples?
    - Did your class observe any differences between samples protected by sunscreen versus those not protected?
    - Did your class observe any differences between samples protect by different types of sunscreen?

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Study Questions

1. How does UV light damage DNA? How does UV light cause aging?

2. Name two health benefits of sunlight exposure?

3. After a long day at the beach, you wake up the next morning with a sunburn. Describe what is happening at a molecular level.

4. Choose a biotechnology (PCR, Comet Assay, transformation with fluorescently tagged plasmid DNA, plasmid DNA electrophoresis, etc.) that is used to help scientists observed DNA damage. Describe how scientists use this method to distinguish between damaged and undamaged DNA.

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Instructor's Guide

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION:

This section outlines the recommended prelab preparations and approximate time requirement to complete each prelab activity.

Preparation For:	What to do:	When:	Time Required:
Module II: DNA Damage by UV Exposure	Bring in different sunscreens or assign students this task. (OPTIONAL)	Anytime before Module II.	-----
	Aliquot plasmid DNA and gel loading solution.	Day of or 2-3 day before experiment, store plasmid DNA in the fridge.	10 min.
	Check and prepare UV transilluminator.	Anytime before Module II.	10 min.
Module III: Seperation of DNA by Agarose Gel Electrophoresis	Prepare diluted electrophoresis buffer.	Up to one week before performing the experiment.	45 min.
	Prepare molten agarose and pour gels (OPTIONAL) or aliquot agarose, buffer, and SYBR® Safe.		
	Aliquot DNA Standard Marker.		

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Pre-Lab Preparations - Module I

Preparing to Test Sunscreens (Optional):

Adding sunscreens are a great way to create a more open ended experiment and to discuss the health benefits of this invention. In our testing we found that most sunscreens – even those with low SPFs – protect plasmid DNA from damage. **Consequently, we recommend that several student groups carry out the experiment without any sunscreen on their plasmid DNA tubes.** This way the class will still be able to observe DNA damage on agarose gels.

Pre-Lab Preparations - Module II

DNA Damage by UV Exposure:

1. Prepare the Plasmid DNA tubes by aliquoting 120 µL of the Plasmid DNA Substrate into ten microtest tubes. (These are the smallest tubes - their thin walls help maximize UV exposure.)

2. Label 10 microcentrifuge tubes "10x Gel Load" and aliquot 35 µL of the 10x Gel Loading solution to each tube.

3. Also provide each group with five empty microcentrifuge tubes.

4. Make sure your transilluminator has a proper UV wavelength. Plasmid DNA will only be damaged by UV light with a wavelength close to or shorter than 300 nm.

5. If you are using sunscreen, tape a piece of saran wrap to the display plate of the transilluminator. This will not affect the intensity of UV light that reaches the tubes but will help with clean up!

FOR MODULE II
Each group receives:

- Tube of Plasmid DNA
- Tube of 10x Gel Load
- 5 empty microcentrifuge tubes
- Sunscreen sample (optional)

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Pre-Lab Preparations - Module III

Prepare SYBR® Safe Stain:

1. Prepare 1x Electrophoresis Buffer by combining 20 µL of 50X Concentrated Buffer with 980 µL of distilled water.
2. Add 450 µL of the 1X buffer from step 1 to the tube of SYBR® Safe and mix by tapping the tube several times. The SYBR® Safe Stain is now ready to be used during agarose gel preparation.

Preparation of Agarose Gels:

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

FOR MODULE III
Each Group should receive:
- 50X concentrated buffer
- Distilled Water
- UltraSpec-Agarose™ Powder
- Standard DNA Marker (30 µL)
- Diluted SYBR® Safe

- **Individual Gel Preparation**
  Each student group can be responsible for casting its own individual gel prior to conducting the experiment (see Module III in the Student's Experimental Procedure). Students will need 50X electrophoresis buffer, distilled water, agarose powder, and *diluted* SYBR® Safe Stain (see Table A on page 12 for volumes).

- **Batch Gel Preparation**
  To save time, a larger quantity of agarose solution can be prepared for sharing by the class. See Appendix B for instructions.

- **Preparing Gels in Advance**
  Gels may be prepared ahead and stored for later use. Solidified gels can be stored for up to 1 week in the refrigerator in water-tight bags with a small amount of buffer to prevent drying. We recommend adding 2 mL of buffer to the bag; excess buffer can lead to diffusion of SYBR® Safe out of the gels. Gels with SYBR® Safe must be stored in the dark away from light.

  Do not store gels at -20° C as freezing will destroy them.

  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.

Additional Materials:

Aliquot 30 µL of DNA Standard Marker into labeled microcentrifuge tubes and distribute one tube per gel.

Visualization and Photodocumentation:

Gels are ready to visualize immediately after electrophoresis is completed. If you 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.

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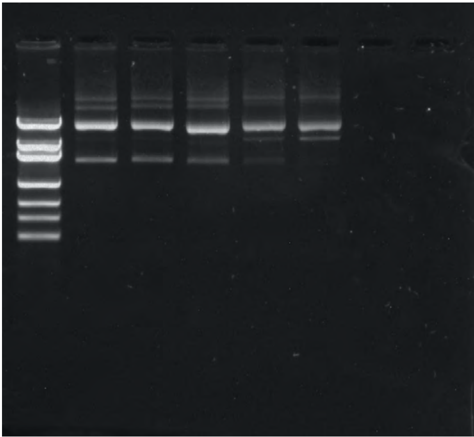
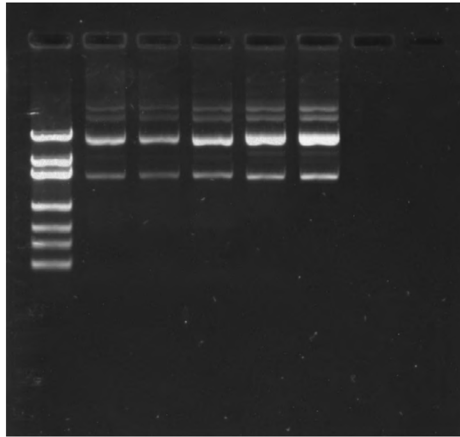
Experiment Results and Analysis

Time Series of Plasmid DNA Exposed to UV Radiation



Time Series of Plasmid DNA with SPF 4 Sunscreen Protection Exposed to UV Radiation



Time Series of Plasmid DNA with SPF 30 Sunscreen Protection Exposed to UV Radiation

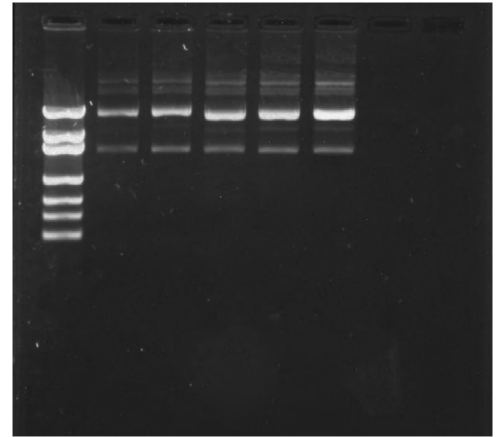


TABLE 1: Gel Loading

Lane	Tube Label	Sample Description
1	DNA Standard Marker	DNA Standard Marker
2	Control	Control Plasmid DNA
3	15 min.	Plasmid DNA exposed to UV for 15 min.
4	30 min.	Plasmid DNA exposed to UV for 30 min.
5	45 min.	Plasmid DNA exposed to UV for 45 min.
6	60 min.	Plasmid DNA exposed to UV for 60 min.

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

These results were generated using a transilluminator with a wavelength of 302/365 nm and using popular sunscreens with an SPF of 4 (Active Ingredients: Avobenzone 1.0%, Octocrylene 2.0%.) and an SPF of 30 (Active Ingredients: Avobenzone 2.7%, Homosalate 6.0%, Octisalate 4.5%, Octocrylene 4.5%.) They are representative. Your classes' results may differ. Similarly, the example answers below may differ from your classes' responses.

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

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

Appendices

A EDVOTEK® Troubleshooting Guide

B Bulk Preparation of Agarose Gels

Safety Data Sheets:

Now available for your convenient download on www.edvotek.com/safety-data-sheets

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Appendix A

EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
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 18.
Bands are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	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 from the wells to ensure adequate separation (if using a 7x7 cm tray).	Be sure to run the gel at least 3.5 cm before visualizing the DNA.
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. This experiment requires a 0.8% agarose gel.

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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		2940 mL	3000 mL (3 L)

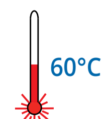
Batch Agarose Gels (0.8%)

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

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

Amount of Agarose	+	Concentrated Buffer (50x)	+	Distilled Water	Total Volume
3.6 g		9 mL		441 mL	450 mL

1. Use a 1 L flask or bottle to prepare the diluted gel buffer.
2. Pour 3.6 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 18) 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 and 45 mL for a 10 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.

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