Edvo-Kit # 205

Edvo-Kit #205

Analysis of DNA Methylation Using Restriction Enzymes

Experiment Objective:

In this experiment, students will explore the effects of DNA methylation on the activity of restriction enzymes.

See page 3 for storage instructions.

Version 205.210628

EDVOTEK®

Table of Contents

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

Experiment Components

	Component	Storage	Check (✓)
A	DNA Sample 1	-20° C Freezer	❑
B	DNA Sample 2	-20° C Freezer	❑
C	EdvoQuick™ DNA Ladder	-20° C Freezer	❑
D	Restriction Digest Reaction Buffer	-20° C Freezer	❑
E	*Dpn*I Restriction Enzyme	-20° C Freezer	❑
F	*Dpn*II Restriction Enzyme	-20° C Freezer	❑
G	Restriction Enzyme Dilution Buffer	-20° C Freezer	❑

Experiment #205 contains biologicals for 6 groups to perform 6 sets of restriction digests, and electrophoresis reagents for 6 gels.

REAGENTS & SUPPLIES

Store all components below at room temperature.

- UltraSpec-Agarose™ ❑
- Electrophoresis Buffer (50x) ❑
- 10x Gel Loading Solution ❑
- SYBR® Safe DNA Stain ❑
- Microcentrifuge Tubes ❑

Requirements *(not included with this kit)*

- Horizontal Gel Electrophoresis Apparatus
- D.C. Power Supply
- Automatic Micropipets (5-50 µL) and tips
- Water bath (37 °C)
- Microwave, hot plate or burner
- UV Transilluminator or Blue Light visualization system (**Cat. #557** recommended)
- UV safety goggles
- Disposable vinyl or latex laboratory gloves
- Hot Gloves
- Safety goggles
- Laboratory glassware
- Marking pens
- Ice buckets and ice
- Distilled or deionized water

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Background Information

The DNA within our cells contains distinct units of genetic information called genes. These genes act like blueprints, directing the cell to build proteins essential to an organism's growth, development, and metabolism. However, expression of a gene in the wrong tissue or at the wrong time can be harmful to the organism. As such, gene expression must be restricted to the proper place and time.

In general, gene expression is regulated by a DNA sequence known as a promoter, which acts like a genetic "on/off" switch. The promoter is located just before ("upstream" of) the protein coding sequence of a gene. In order to activate gene expression, transcription factors bind to the promoter, which recruits RNA polymerase to the gene (Figure 1). Gene transcription occurs only when the correct set of factors is available to bind to that promoter.

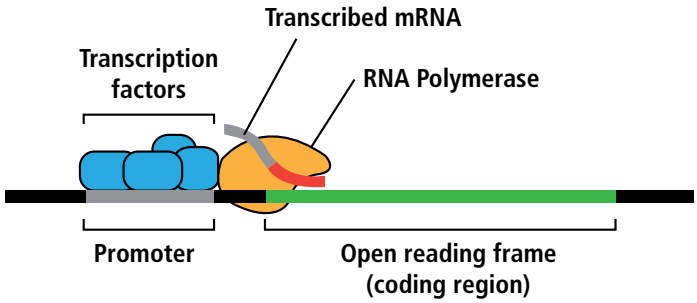


Figure 1: Activation of Transcription

While studying the genome, molecular biologists discovered that gene expression could also be regulated by chemically modifying the DNA itself. These stable, heritable changes to the DNA structure (rather than to the sequence) that affect gene transcription are known as epigenetic changes. DNA methylation is a common epigenetic tool that is essential for normal development. In this process, nucleotides are modified by the enzyme DNA methylase, which transfers a methyl group from the molecule S-adenosyl-L-methionine (SAM) to a cytosine or adenine nucleotide located within a specific palindromic stretch of DNA. When this modification occurs in a gene's promoter, the gene is locked in the "off" position through many consecutive cell divisions (Figure 2).

DNA methylation plays an important role in long-term gene regulation by ensuring that specific gene products are not being created at the wrong place and time. In fact, many diseases have been linked to methylation defects. For example, methylation defects are common in several types of cancer. In healthy tissues, DNA methylation regulates the expression of genes that are important for early embryonic development. If genes involved in cell growth and proliferation are hypomethylated (under methylated), they can cause uncontrolled cell growth in an adult. Conversely, the promoters of genes that slow cell division or repair DNA could be hypermethylated (over methylated), turning off these genes that suppress cancer progression.

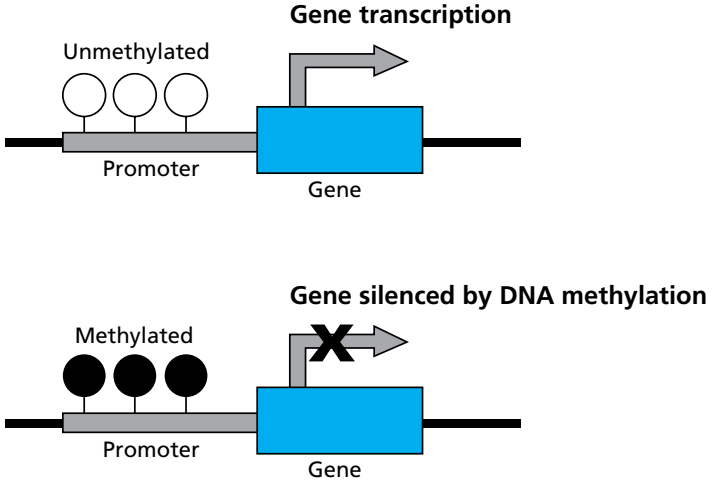


Figure 2: Promoter Methylation Affects Gene Expression

In mammals, certain genes are methylated differently depending on whether they came from the mother or the father. This means that only one

copy of the gene is turned on in the offspring. This phenomenon, known as genomic imprinting, occurs during gamete formation. The imprinted genes remained turned off through an individual's life. However, the methylation is "reset" in the egg or sperm to ensure proper gene expression in the next generation (Figure 3). Because many imprinted genes have only one active copy with no backup they are particularly sensitive to pathologies are also under greater selective pressure.

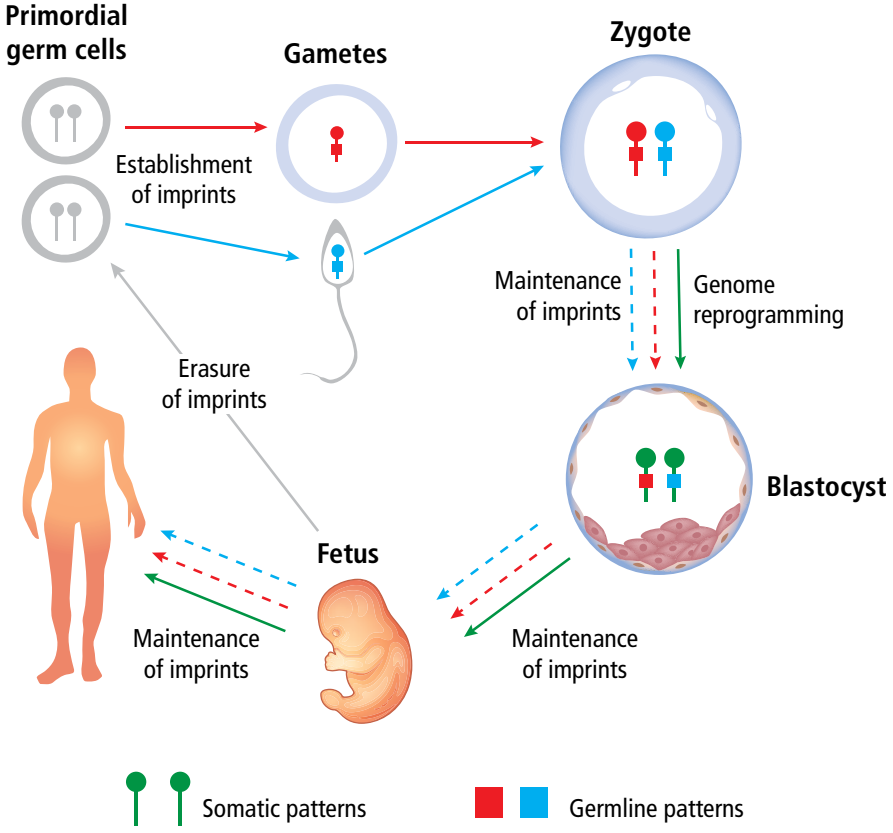


Figure 3: Normal Genomic Imprinting

Genomic imprinting influences the phenotype of the offspring without changing the DNA sequence. Research has shown that a specific gene may have a different influence on an individual depending on which parent contributed the gene. For example, a small region of human chromosome 15 has several imprinted genes that are important for normal development. Some of these genes are paternally imprinted, while others are maternally imprinted. Deletion of this region of the chromosome produces different effects, depending upon which parent contributes the abnormal chromosome (Figure 4). Patients with Prader-Willi syndrome inherited the abnormal chromosome from the father. These individuals will exhibit low muscle tone, cognitive impairment, and an uncontrollable feeling of hunger that leads to obesity. In contrast, individuals with Angelman's syndrome, who inherit the abnormal chromosome from the mother, exhibit epilepsy, jerky movements, and a perpetually smiling face.

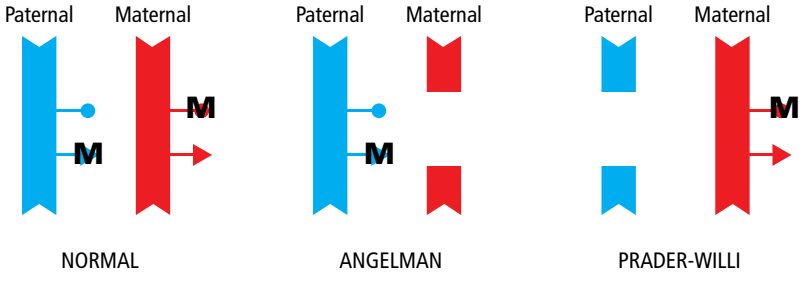


Figure 4: Effects of Imprinting on Human Development

ANALYSIS OF DNA METHYLATION

In the research laboratory, DNA methylation is commonly evaluated using mass spectrometry, an analytical technique that examines the chemical composition of a sample. In the classroom laboratory, we can use special enzymes to determine whether DNA is methylated. These enzymes, called restriction endonucleases, act like molecular scissors that cut double-stranded DNA at specific sequences. Most restriction enzymes recognize specific palindromic stretches of DNA, generally 4-8 base pairs in length. However, the presence of methyl groups in these recognition sites also affects restriction enzyme cleavage (Figure 5). For example, both *Dpn*I and *Dpn*II cleave DNA with the sequence GATC. However, *Dpn*I cleaves only when its recognition site is methylated, whereas *Dpn*II is blocked by methylation. When digested with these enzymes, methylated and unmethylated DNA will produce restriction fragments that are distinct from one another.

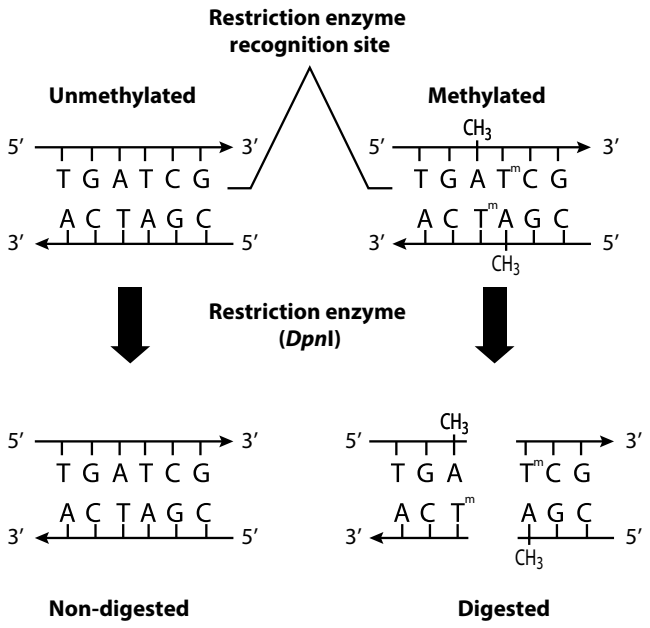


Figure 5: Methylation Affects Restriction Enzyme Digests

To analyze the digested DNA, scientists use a technique called agarose gel electrophoresis to separate DNA fragments according to size. The mixture of DNA molecules is added into depressions (or "wells") within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode (Figure 6A).

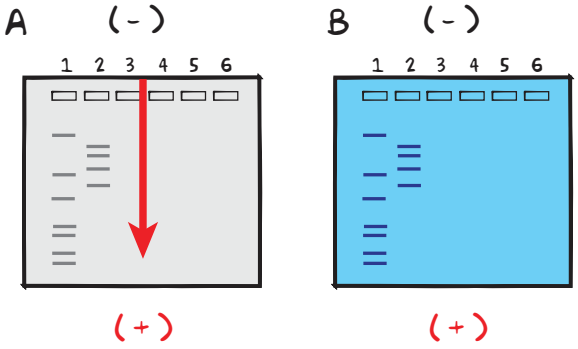


Figure 6: Overview of Agarose Gel Electrophoresis

At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA (Figure 6B).

In this experiment, students explore the effects of DNA methylation on restriction enzyme activity. First, DNA will be digested with the restriction enzymes *Dpn*I and *Dpn*II. The restriction fragments are then analyzed using agarose gel electrophoresis. After visualizing the gel, students determine which sample is methylated.

Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, students will explore the effects of DNA methylation on the activity of restriction enzymes.

LABORATORY SAFETY

Wear gloves and safety goggles

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.

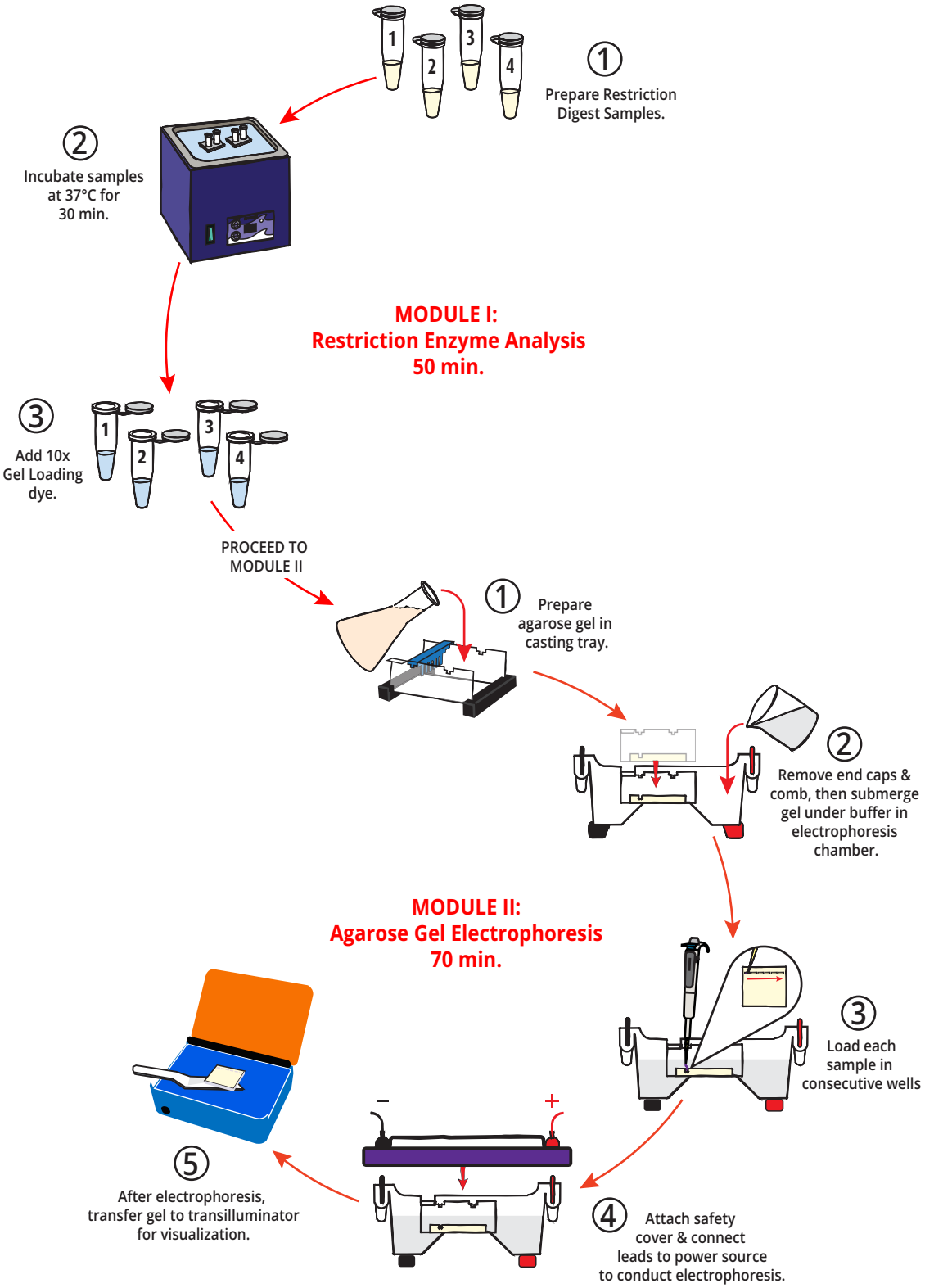
During the Experiment:

- Record your observations.

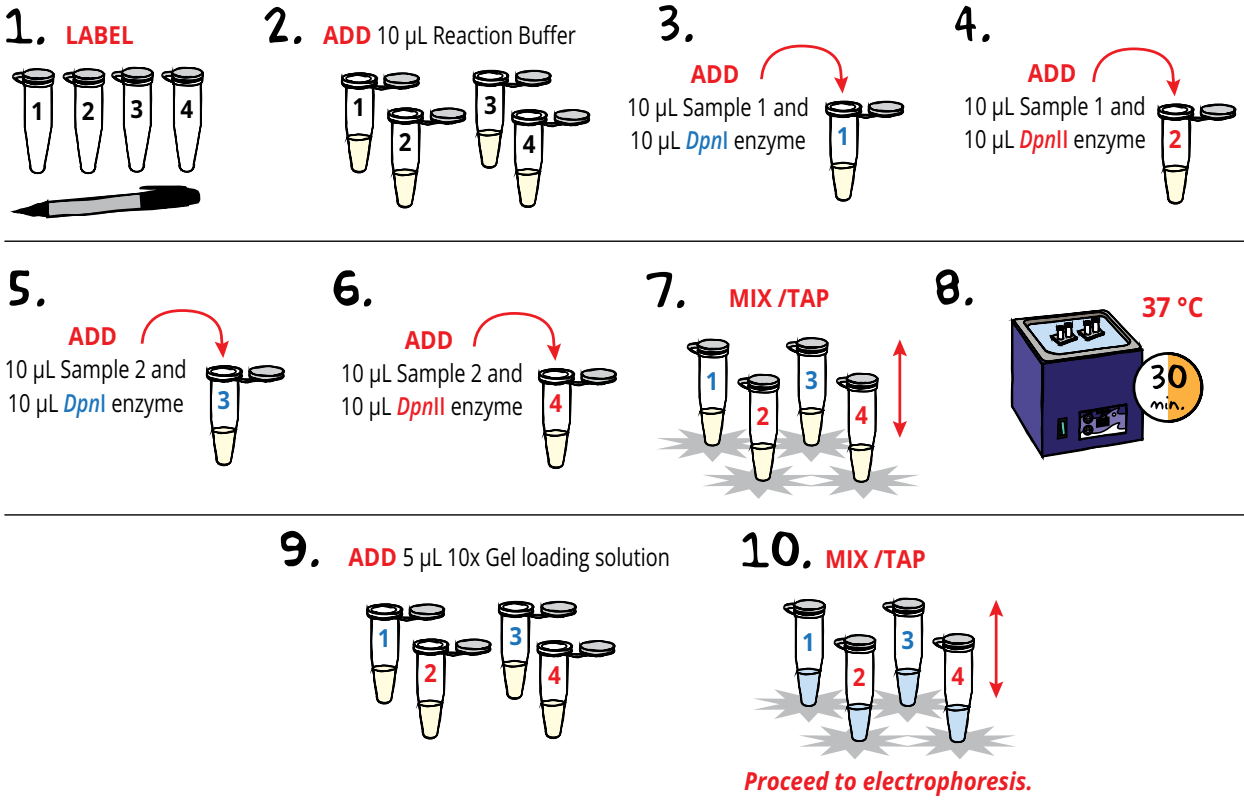
After the Experiment:

- 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.

Experiment Overview, continued



Module I: Restriction Enzyme Analysis



1. **LABEL** four microcentrifuge tubes with your student group and the number 1, 2, 3, or 4.
2. **ADD** 10 µL of Reaction Buffer (RB) to each of the four microcentrifuge tubes.
3. **ADD** 10 µL of DNA Sample 1 (DS1) and 10 µL of *DpnI* enzyme to Reaction Tube 1.
4. **ADD** 10 µL of DNA Sample 1 (DS1) and 10 µL of *DpnII* enzyme to Reaction Tube 2.
5. **ADD** 10 µL of DNA Sample 2 (DS2) and 10 µL of *DpnI* enzyme to Reaction Tube 3.
6. **ADD** 10 µL of DNA Sample 2 (DS2) and 10 µL of *DpnII* enzyme to Reaction Tube 4.
7. **MIX** the restriction digestion reactions by gently tapping the tubes.
8. **INCUBATE** the samples at 37 °C for 30 minutes.
9. After the incubation, **ADD** 5 µL of 10x gel loading solution to each reaction tube.
10. **CAP** the tubes and **MIX** by tapping the tube or vortexing vigorously. **PROCEED** to electrophoresis with the digested DNA samples.

IMPORTANT:

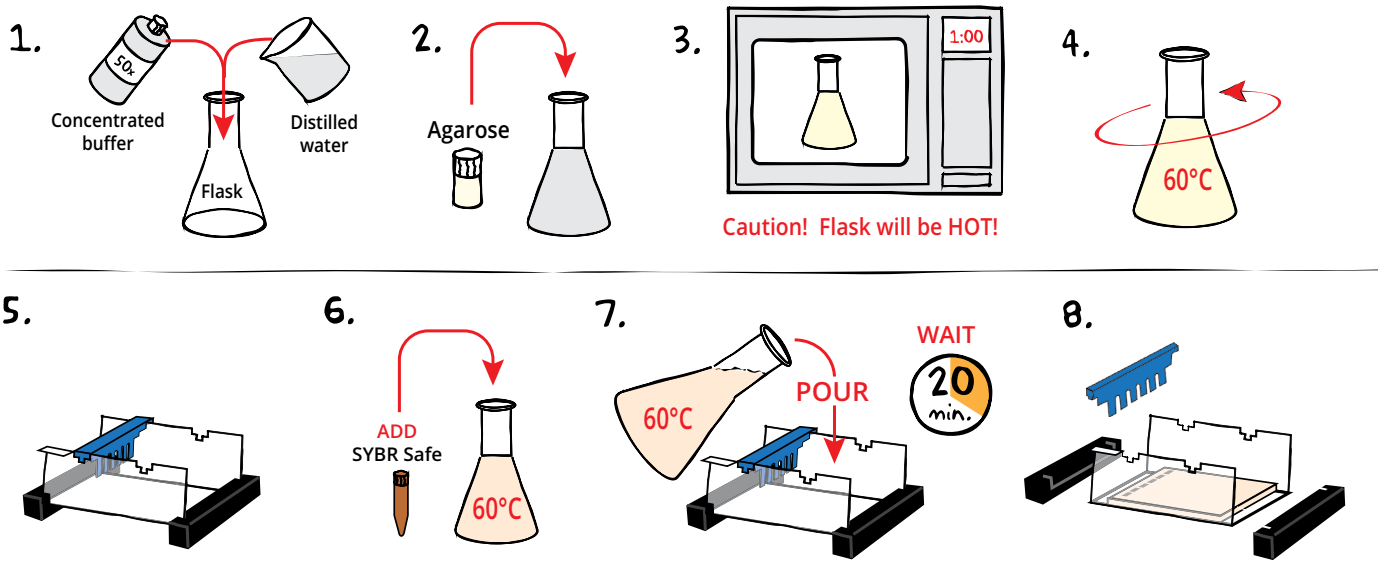
To prevent contamination, be sure to use a fresh pipet tip before going into the enzyme, DNA, and buffer stocks. Keep the enzymes on ice when not in use.

OPTIONAL STOPPING POINT:
The restriction digest samples can be stored at -20 °C for electrophoresis at a later time.

TABLE 1: Summary of Restriction Enzyme Digestion Reactions

Reaction Tube	Reaction Buffer	DNA Sample 1	DNA Sample 2	*Dpn* I Enzyme	*Dpn* II Enzyme	Final Reaction Volume
1	10 µL	10 µL	----	10 µL	----	30 µL
2	10 µL	10 µL	----	----	10 µL	30 µL
3	10 µL	----	10 µL	10 µL	----	30 µL
4	10 µL	----	10 µL	----	10 µL	30 µL

Module II: 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.

Wear gloves and safety goggles

IMPORTANT:
10 x 7 cm gels are recommended.

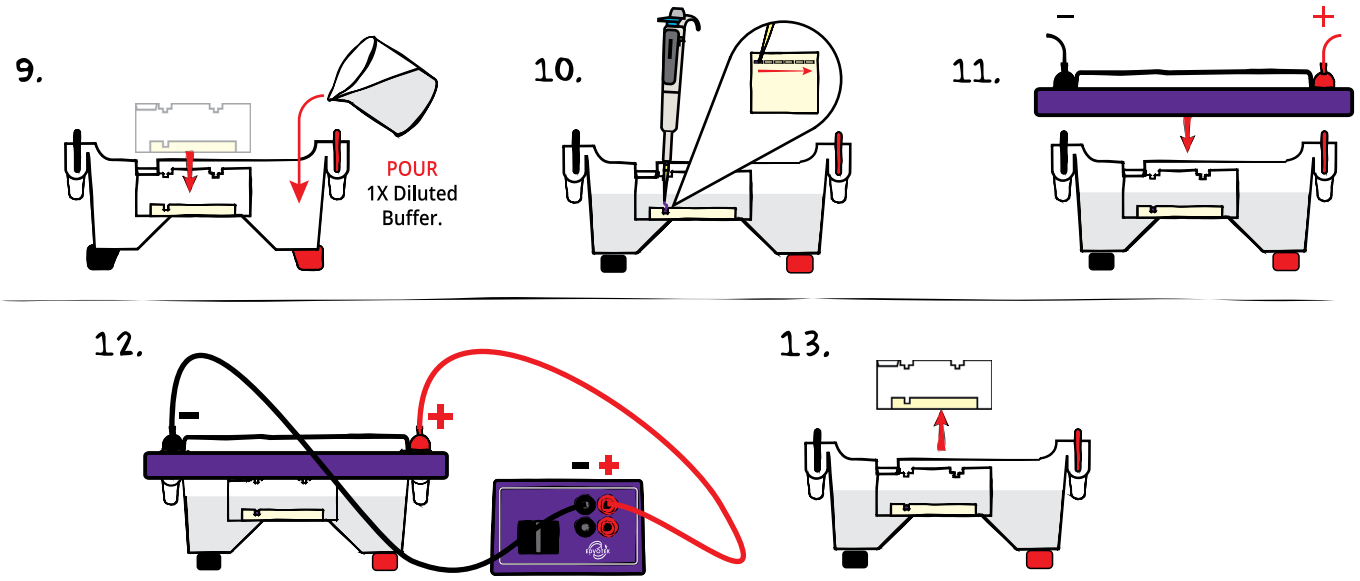
1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 mL flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask 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.

Table A — **Individual 1.5% 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	0.5 mL	24.5 mL	0.375 g	25 mL	25 µL
10 x 7 cm*	0.8 mL	39.2 mL	0.6 g	40 mL	40 µL
14 x 7 cm	1.0 mL	49 mL	0.75 g	50 mL	50 µL

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

Module II: Agarose Gel 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** the entire sample volume (35 µL) into the well in the order indicated by Table 2.
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 2: Gel Loading

Lane 1	EdvoQuick™ DNA Ladder
2	Reaction Tube 1 - DNA Sample 1 + *Dpn* I
3	Reaction Tube 2 - DNA Sample 1 + *Dpn* II
4	Reaction Tube 3 - DNA Sample 2 + *Dpn* I
5	Reaction Tube 4 - DNA Sample 2 + *Dpn* II

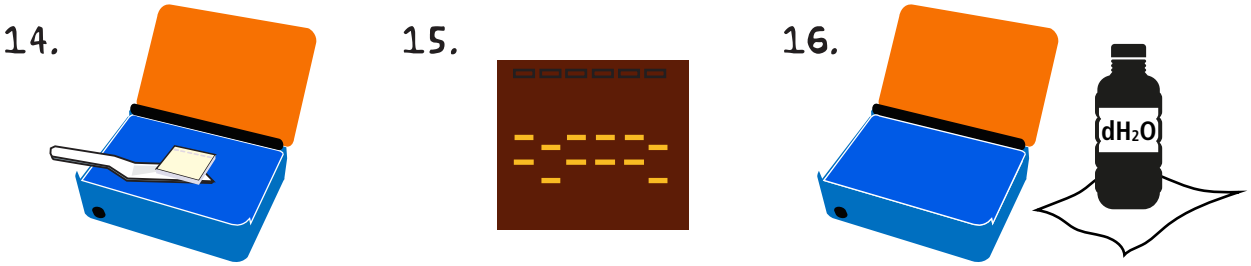
Table B — **1x Electrophoresis Buffer (Chamber Buffer)**

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 (1.5% - 7 x 10 cm Agarose Gel)**

	Electrophoresis Model: EDGE™	M12 & M36
Volts	Min./Max. Run Time	Min./Max. Run Time
150	15/30 minutes	20/40 minutes
125	N/A	30/50 minutes
100	30/40 minutes	30/60 minutes

Module II: Agarose Gel Electrophoresis, continued



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.

Study Questions

1. How is gene expression regulated? Why is it important to regulate gene expression?

2. What is DNA methylation and how does it affect gene expression?

3. Describe how DNA methylation can contribute to cancer.

4. After analyzing your results, which DNA sample is methylated? Which sample is unmethylated? Explain how you came to your answer.

Instructor's Guide

ADVANCE PREPARATION:

Preparation For:	What To Do:	When:	Time Required:
Module I: Restriction Enzyme Analysis	Prepare and aliquot reagents	One day to 30 minutes before performing the experiment.	20 min.
	Equilibrate water bath	One to two hours before the experiment.	10 min.
	Prepare and aliquot restriction enzymes	30 minutes before use.	30 min.
Module II: Agarose Gel Electrophoresis	Prepare diluted electrophoresis buffer & SYBR® Safe Stain	Up to one day before performing the experiment.	10 min.
	Prepare molten agarose and pour gels	Up to one day before performing the experiment.	45 min.

Module I: Pre-Lab Preparations

1. Dispense 50 µL Restriction Enzyme Reaction Buffer (D) into 6 microcentrifuge tubes "RB". Cap the tubes.
2. Dispense 22 µL of the DNA Sample 1 (A) into 6 microcentrifuge tubes "DS1". Cap the tubes.
3. Dispense 22 µL of the DNA Sample 2 (B) into 6 microcentrifuge tubes "DS2". Cap the tubes.
4. Dispense 22 µL of 10x Gel Loading Solution into 6 microcentrifuge tubes. Cap the tubes.
5. Prepared reagents can be stored at room temperature if prepared on the day of the lab. If prepared in advance, store the reagents in the refrigerator (4 °C).

DAY OF THE LAB

Equilibrate a 37 °C water bath.

Preparation of *Dpn*I and *Dpn*II Restriction Enzymes

Prepare restriction digests within 30 minutes of diluting the restriction enzymes.

1. Within 30 minutes of starting the Module I experiment, remove the restriction enzymes from the freezer and place them on ice. Gently tap the tubes on the tabletop or centrifuge them to collect the enzymes at the bottom of the tube.
2. Dilute the restriction enzymes in the Restriction Enzyme Dilution Buffer (G):

   a. Add 150 µL of ice cold dilution buffer to the tube of *Dpn*I (E).
   b. Add 150 µL of ice cold dilution buffer to the tube of *Dpn*II (F).

   Be sure to mix the diluted enzymes well as the concentrated enzyme solutions contain glycerol and are very viscous. Change tips between samples to avoid cross-contamination. At this point, the enzymes can no longer be stored. They must be used as soon as possible. Keep the tubes on ice until use.
3. Aliquot the following reagents into labeled 0.5 mL microcentrifuge tubes. Store on ice until use.

   a. 22 µL diluted *Dpn*I
   b. 22 µL diluted *Dpn*II

TABLE 3: Summary of Reagent Preparation

Component	Number of tubes	Label	Volume per tube
Reaction Buffer	6	RB	50 µL
DNA Sample 1	6	DS1	22 µL
DNA Sample 2	6	DS2	22 µL
Dpn I	6	*Dpn* I	22 µL
Dpn II	6	*Dpn* II	22 µL
10x Gel Loading Buffer	6	10x	22 µL

FOR MODULE I
Each Group Requires:

- 1 tube of Reaction Buffer "RB"
- 1 tube of DNA Sample 1 "DS1"
- 1 tube of DNA Sample 2 "DS2"
- 1 tube of DpnI (on ice) "*Dpn*I"
- 1 tube of DpnII (on ice) "*Dpn*II"
- 10x Gel Loading Buffer "10x"
- 4 microcentrifuge tubes
- Micropipet with tips

Module II: Pre-Lab Preparations

AGAROSE GEL ELECTROPHORESIS

This experiment requires a 1.5% agarose gel per student group. 10 x 7 cm gels are recommended. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 min. for this procedure.

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 300 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 Part 1 in the Student's Experimental Procedure. Students will need 50x concentrated buffer, distilled water, agarose powder, and *diluted* SYBR® Safe Stain.

FOR MODULE II
Each Group Requires:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™ Powder
- Diluted SYBR® Safe Stain
- DNA Samples
- DNA Standard Markers

Batch Gel Preparation

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. See Appendix B.

Preparing Gels in Advancex

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 DNA Samples & DNA Standard Marker

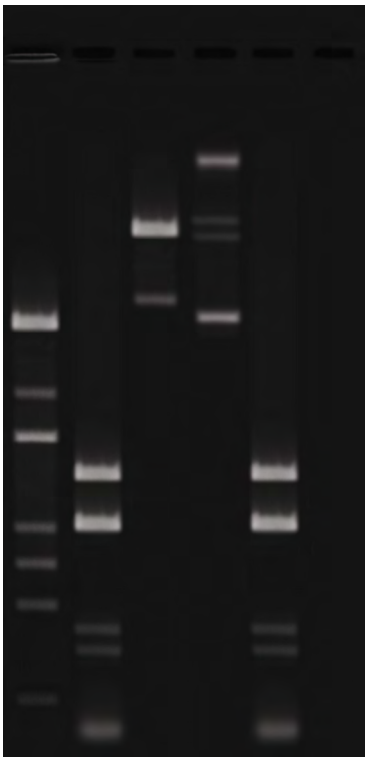
The DNA Standard Marker for this experiment is provided in bulk in a screw-top microcentrifuge tube. Students can share the tube, or they can be pre-aliquoted into individual tubes (not included).

Each group will use the four DNA Digestions that they created in Module I as their DNA samples.

Visualizing SYBR® Safe-Stained Gels

SYBR® Safe is a DNA stain that fluoresces when bound to double-stranded DNA, allowing us to visualize our samples. This DNA stain is compatible with both UV and blue-light transilluminators. For best results, we recommend the TruBlu™ 2 Blue/White Light Transilluminator (Cat #557).

Experiment Results and Analysis



The molecular weight of the plasmid DNA being analyzed in this experiment is 3000 bp. Digestion of the DNA samples with *Dpn*I or *Dpn*II will produce 16 fragments of varying sizes (940, 680, 341, 283, 258, 105, 91, 78, 75, 46, 36, 18, 17, 12, 11, and 8). Smaller DNA fragments may not be visible since they will only bind a small amount of the stain. Fragments that are close in size will not be resolved as individual bands.

Includes EDVOTEK's All-NEW EdvoQuick™ DNA Ladder

- Better separation
- Easier band measurements
- No unused bands

EdvoQuick™ DNA ladder sizes:
2640, 1400, 1100, 700, 600, 400, 200

Several unexpected bands may be visible in the lanes containing undigested plasmid DNA because plasmids can exist in different conformations. First, the supercoiled plasmid DNA is tightly wound in a compact secondary structure, which makes the DNA appear smaller than its molecular weight when analyzed by electrophoresis. In contrast, the DNA backbone of some plasmids may be nicked during purification. Due to the conformational change, this DNA will appear larger than its molecular weight when analyzed by electrophoresis. If the DNA backbone is linearized, it runs at the actual size of 3000 bp. This will create two distinct bands when analyzed by electrophoresis. All of these isoforms are digested by restriction enzymes, so they will produce the same series of DNA fragments when analyzed by electrophoresis.

Lane	Sample	Results	Conclusion
1	EdvoQuick™ DNA Ladder	-----	-----
2	Reaction tube 1 - DNA Sample 1 + *Dpn*I	DNA is digested	DNA is methylated
3	Reaction tube 2 - DNA Sample 1 + *Dpn*II	DNA is not digested	DNA is methylated
4	Reaction tube 3 - DNA Sample 2 + *Dpn*I	DNA is not digested	DNA is not methylated
5	Reaction tube 4 - DNA Sample 2 + *Dpn*II	DNA is digested	DNA is not methylated

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

Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
The DNA did not digest	The restriction enzymes were not active.	Be sure that the restriction enzymes were diluted in the correct buffer.
		For optimal activity, prepare the enzymes within 30 minutes of use.
There are bands on my gels that can't be explained by the restriction digests.	Some bands may represent partially digested DNA.	The sample was not digested at the right temperature.
		The sample was not digested for the appropriate amount of time.
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 16.
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.	Ensure that diluted SYBR® Safe was added to the gel.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
DNA bands were not resolved.	Tracking dye should migrate at least 5 cm (if using a 10x7 cm tray) from the wells to ensure adequate separation.	Be sure to run the gel at least 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. For reference, the DNA samples should be analyzed using a 1.5% agarose gel.

Appendix B
Bulk Preparation of Agarose Gels

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

Bulk Electrophoresis Buffer

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

Table D — Bulk Preparation of Electrophoresis Buffer

50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 mL		2940 mL	3000 mL (3 L)

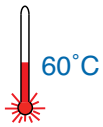
Batch Agarose Gels (1.5%)

For quantity (batch) preparation of 1.5% agarose gels, see Table E.

Table E — Batch Prep of 1.5% UltraSpec-Agarose™

Amount of Agarose	+	Concentrated Buffer (50x)	+	Distilled Water	Total Volume
4.5 g		6 mL		294 mL	300 mL

1. Use a 500 mL flask to prepare the diluted gel buffer.
2. Pour 4.5 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 16) to the cooled agarose and mix well.
7. Dispense the required volume of cooled agarose solution for casting each gel. Measure 25 mL for a 7 x 7 cm tray, 40 mL for a 10 x 7 cm tray, and 50 mL for a 14 x 7 cm tray. ***NOTE: For this experiment, 10 x 7 cm gels are recommended.***
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. 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.