

**LAB MANUAL**

**Glencoe Science**

# Forensics and Biotechnology



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**Glencoe**

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# To the Student

In the real world, biology is often used to solve problems—sometimes even to solve mysteries. Biologists may examine problems in order to improve human life, such as: “How can a rice crop be made more disease resistant?” and “How can a genetic disease in humans be detected?” Biology can also be used to solve mysteries by answering questions like: “When and how did a person die?” and “To whom does the blood or other biological evidence at a crime scene belong?” Biologists work in the fields of forensics and biotechnology to find the answers to these and many other questions.

In *Glencoe’s Forensics and Biotechnology Lab Manual*, you will be presented with in-depth investigations that deal with DNA, collecting and analyzing data, or interpreting evidence found at a crime or accident scene. You will use your knowledge of scientific inquiry and your problem-solving skills as you learn current biotechnology techniques and forensics procedures. You will then apply these techniques and procedures to real-world scenarios.

Each lab begins with *The Problem*, a section that describes either a biotechnology issue or the discovery of a crime or accident. Information in the *Background* section will help you understand the science involved in the problem or case. The *Procedure* section provides step-by-step instructions for learning a technique or procedure or for solving the problem presented. Finally, the *Analyze and Conclude* section allows you to interpret your data and demonstrate your problem-solving skills and understanding of the scientific processes involved.

# Laboratory and Safety Guidelines

## Emergencies

- Inform the teacher immediately of *any* mishap—fire, injury, glassware breakage, chemical spills, and so forth.
- Know the location of the fire extinguisher, safety shower, eyewash, fire blanket, and first-aid kit. Know how to use this equipment.
- If chemicals come into contact with your eyes or skin, flush with large quantities of water and notify your teacher immediately.

## Preventing Accidents

- Do NOT wear clothing that is loose enough to catch on anything. Do NOT wear sandals or open-toed shoes. Remove loose jewelry—chains or bracelets—while doing lab work.
- Wear protective safety gloves, goggles, and aprons as instructed.
- Always wear safety goggles (not glasses) in the laboratory.
- Wear goggles throughout the entire activity, cleanup, and handwashing.
- Keep your hands away from your face while working in the laboratory.
- Remove synthetic fingernails before working in the lab (these are highly flammable).
- Do NOT use hair spray, mousse, or other flammable hair products just before or during laboratory work where an open flame is used (they can ignite easily).
- Tie back long hair and loose clothing to keep them away from flames and equipment.
- Eating, drinking, chewing gum, applying makeup, and smoking are prohibited in the laboratory.
- Do NOT inhale vapors or taste, touch, or smell any chemical or substance unless instructed to do so by your teacher.

## Working in the Laboratory

- Study all instructions before you begin a laboratory or field activity. Ask questions if you do not understand any part of the activity.
- Work **ONLY** on activities assigned by your teacher. **NEVER** work alone in the laboratory.
- Do NOT substitute other chemicals/substances for those listed in your activity.
- Do NOT begin any activity until directed to do so by your teacher.
- Do NOT handle any equipment without specific permission.
- Remain in your own work area unless given permission by your teacher to leave it.
- Do NOT point heated containers—test tubes, flasks, and so forth—at yourself or anyone else.
- Do NOT take any materials or chemicals out of the classroom.
- Stay out of storage areas unless you are instructed to be there and are supervised by your teacher.

## Laboratory Cleanup

- Keep work, lab, and balance areas clean, limiting the amount of easily ignitable materials.
- Turn off all burners, water faucets, probeware, and calculators before leaving the lab.
- Carefully dispose of waste materials as instructed by your teacher.
- With your goggles on, wash your hands thoroughly with soap and warm water after each activity.

# Safety Symbols

SAFETY SYMBOLS		HAZARD	EXAMPLES	PRECAUTION	REMEDY
DISPOSAL		Special disposal procedures need to be followed.	certain chemicals, living organisms	Do not dispose of these materials in the sink or trash can.	Dispose of wastes as directed by your teacher.
BIOLOGICAL		Organisms or other biological materials that might be harmful to humans	bacteria, fungi, blood, unpreserved tissues, plant materials	Avoid skin contact with these materials. Wear mask or gloves.	Notify your teacher if you suspect contact with material. Wash hands thoroughly.
EXTREME TEMPERATURE		Objects that can burn skin by being too cold or too hot	boiling liquids, hot plates, dry ice, liquid nitrogen	Use proper protection when handling.	Go to your teacher for first aid.
SHARP OBJECT		Use of tools or glassware that can easily puncture or slice skin	razor blades, pins, scalpels, pointed tools, dissecting probes, broken glass	Practice common-sense behavior and follow guidelines for use of the tool.	Go to your teacher for first aid.
FUME		Possible danger to respiratory tract from fumes	ammonia, acetone, nail polish remover, heated sulfur, moth balls	Make sure there is good ventilation. Never smell fumes directly. Wear a mask.	Leave foul area and notify your teacher immediately.
ELECTRICAL		Possible danger from electrical shock or burn	improper grounding, liquid spills, short circuits, exposed wires	Double-check setup with teacher. Check condition of wires and apparatus.	Do not attempt to fix electrical problems. Notify your teacher immediately.
IRRITANT		Substances that can irritate the skin or mucous membranes of the respiratory tract	pollen, moth balls, steel wool, fiberglass, potassium permanganate	Wear dust mask and gloves. Practice extra care when handling these materials.	Go to your teacher for first aid.
CHEMICAL		Chemicals that can react with and destroy tissue and other materials	bleaches such as hydrogen peroxide; acids such as sulfuric acid, hydrochloric acid; bases such as ammonia, sodium hydroxide	Wear goggles, gloves, and an apron.	Immediately flush the affected area with water and notify your teacher.
TOXIC		Substance may be poisonous if touched, inhaled, or swallowed.	mercury, many metal compounds, iodine, poinsettia plant parts	Follow your teacher's instructions.	Always wash hands thoroughly after use. Go to your teacher for first aid.
OPEN FLAME		Open flame may ignite flammable chemicals, loose clothing, or hair.	alcohol, kerosene, potassium permanganate, hair, clothing	Tie back hair. Avoid wearing loose clothing. Avoid open flames when using flammable chemicals. Be aware of locations of fire safety equipment.	Notify your teacher immediately. Use fire safety equipment if applicable.
	<b>Eye Safety</b> Proper eye protection should be worn at all times by anyone performing or observing science activities.		<b>Clothing Protection</b> This symbol appears when substances could stain or burn clothing.		<b>Animal Safety</b> This symbol appears when safety of animals and students must be ensured.
					<b>Radioactivity</b> This symbol appears when radioactive materials are used.

# Lab 1

## Where did they drown?

### The Problem

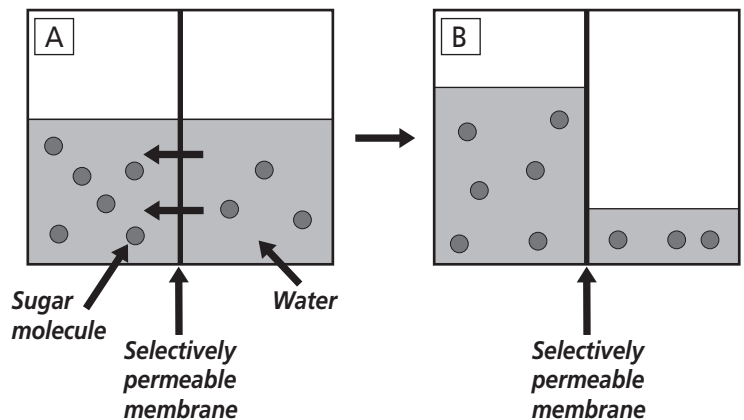
The Coast Guard discovered two bodies, a man and a woman, in the salt water of the San Francisco Bay. Both victims apparently drowned; their lungs were filled with water, and a frothy mixture of water, air, and mucus was found in their mouths and airways. Your job as the coroner will be to determine where the victims drowned and whether the victims died of accidental drowning or were victims of murder. To help you in your determination, you have taken blood samples from both victims. You must interpret the findings from these blood samples to solve the mystery.

### Background

Our bodies contain many compartments of liquid water, such as blood, tissues, and fluids between tissues. This water is composed of many substances, including salts, sugars, and proteins which have dissolved in the water. The concentration of any given substance is the amount of that substance per unit volume of water. Cells, such as those found in the walls of blood vessels and tissues, separate the various compartments of water. The membranes of these cells control which molecules can move between the compartments by allowing some molecules to pass through while limiting others. This is known as selective permeability.

**Diffusion** How do you know which way substances will move through a membrane? Generally, substances move from an area of high concentration to an area of low concentration. This movement is called diffusion. Diffusion occurs in solids, liquids, and gases. For example, if you cut an onion at the back of your classroom, people at the front of the room will eventually be able to smell it because molecules from the onion are transmitted (diffused) from an area of high concentration (the back of the room) to an area of low concentration (the front of the room). Diffusion continues until the concentration of molecules from the onion in the air is equal in all areas of the room.

All substances, including water, can diffuse. However, the diffusion of water across a selectively permeable membrane has a different name, *osmosis*. Suppose you have two solutions of sugar of different concentrations (high and low) in a clear box. A membrane that is permeable to water but not to sugar separates the two solutions. High concentration is on the left side, and low concentration is on the right side, as shown in Part A of **Figure 1**. The solution on the left has a higher sugar concentration relative to the one on the right and is said to be *hypertonic* to the one on the right. The solution on the right has a lower sugar concentration compared to the one on the left and is said to be *hypotonic* to the solution on the left.



**Figure 1**

## Lab

## 1

Where did they drown? *continued*

The more sugar that is dissolved in water, the less concentrated the water becomes; in other words, pure water is 100% water, and the concentration of water decreases as you add sugar. Therefore, the concentration of water on the left side is less than that on the right side. As a result, water will diffuse from the right to the left until the concentrations of water on both sides of the membrane are equal, as shown in Part B of **Figure 1**. At that time, the concentrations of sugar on both sides of the membrane will also be equal, or *isotonic*. Solutions in your body behave the same way.

**Diffusion in the Lungs** Your lungs form a compartment of air separated from a compartment of water (your blood) by cells that make up the air sacs called alveoli. When you breathe, gases diffuse from one compartment to another. Oxygen diffuses from the air into the blood, and carbon dioxide from the blood diffuses into the air. When a person drowns, the lungs fill with freshwater or salt water, depending on the type of water in which he or she

drowned. The blood and lungs become two water-filled compartments (similar to **Figure 1**) in which water can move across the membranes separating the blood and the lungs. Salts in the compartments do not move across the membranes.

In this lab, you will simulate what happens in the human body when a person drowns. You will use sugar solutions to represent the solutions of water, salt, and other substances found in the lungs and blood. Solutions in beakers represent the blood; dialysis tubes, which are selectively permeable membranes, represent the alveoli of the lungs; and solutions in the dialysis tubes represent water in the lungs. You will first experiment with several beakers and dialysis tubes containing different concentrations of sugar representing hypertonic, hypotonic, and isotonic solutions. These will help you understand the movement of water that occurs with the differing solutions. Finally, two solution combinations will represent what happens when a person drowns in freshwater and in salt water.

**Everyday Materials**

- string (optional)
- tissues
- marker

**Lab Materials**

- sucrose solutions (1%, 5%, 10%, 20%, 40%)
- 250-mL beakers (7)
- 2.5 cm × 30 cm pieces of water-soaked dialysis tubing (7)
- distilled water
- 250-mL graduated cylinder
- 25-mL graduated cylinder
- balance that is sensitive to at least 0.1 g

**Safety**

- Never eat or drink anything in the lab.

**Procedure**

1. Label the beakers A through G.
2. Take each section of dialysis tubing and tie one end using the tube itself or string. Be careful not to tear the bag.
3. Fill each dialysis tube with 25 mL of sucrose solution, according to the table on the next

page. The tubes should be about  $\frac{1}{3}$  full. Fill each beaker with 150 mL of sucrose solution according to the table.

4. Once you fill a dialysis bag, squeeze the air out and tie the remaining end a few centimeters above the top of the liquid without tearing the bag. Rinse the bag with distilled water, blot it dry with a tissue, and weigh it on the balance. Record the mass in the table on the next page (initial bag mass) and place the bag in the appropriate beaker. Repeat this procedure for each bag.
5. Allow each bag to stay in the beaker for 30 minutes.

## Lab

## 1

**Where did they drown?** *continued*

6. After 30 minutes, remove each bag, rinse with distilled water, blot dry, and determine its mass. Record the mass in the table (final bag mass). Measure the amount of liquid that remains in the beaker after the bag is removed. Discard the bags and empty the beakers.
7. Calculate the change in each bag's mass (final mass – initial mass) and the percent change  $\left(\frac{\text{mass change}}{\text{initial mass}} \times 100\right)$ . Record the values in the table below and use the data to answer the questions.

**Table 1**

Beaker	Bag Sol	Beaker Sol	Initial Bag Mass	Final Bag Mass	Mass Change	%Change
A	10%	1%				
B	10%	5%				
C	10%	10%				
D	10%	20%				
E	10%	40%				
F	1%	10%				
G	40%	10%				

**Conclude and Apply**

1. What happened to the mass of Bags A and B during the experiment?

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2. Were the concentrations of the solutions in Beakers A and B more than or less than the concentrations of the solutions inside the bags? Would you classify the solutions in the beakers as hypertonic, hypotonic, or isotonic relative to the solution inside the bag? (Refer to the *Background* section for more information.)

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3. Explain the changes observed in Bags A and B in terms of the concentrations of solutions inside and outside the bags and the movement of water.

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4. What happened to the mass of Bag C?

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## Lab

## 1

**Where did they drown?** *continued*

5. How did the solution in Beaker C compare to the solution inside the bag? Would you classify it as hypertonic, hypotonic, or isotonic relative to the solution inside the bag?

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6. Explain any changes observed in Bag C in terms of the concentrations of solutions inside and outside the bag and the movement of water.

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7. What happened to the masses of Bags D and E during the experiment?

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8. Were the concentrations of the solutions in Beakers D and E more than or less than the concentrations of the solutions inside the bags? Would you classify the solutions in the beakers as hypertonic, hypotonic, or isotonic relative to the solution inside the bag? (Refer to the *Background* section for more information.)

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## Lab

## 1

**Where did they drown?** *continued*

9. Explain the changes observed in Bags D and E in terms of the concentrations of solutions inside and outside the bags and the movement of water.

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10. Beaker F represents a person who drowned in freshwater. The bag represents the lungs, and the solution in the beaker represents the blood. The 1% sucrose inside the bag approximates the total salt concentration in freshwater, while the 10% sucrose in the beaker approximates the total salt concentration in the blood. What happened to the mass of the bag? Did water move out of the bag or into the bag? What happened to the concentration of sucrose in the beaker? Explain.

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11. Beaker G represents a person who drowned in salt water. The 40% sucrose inside the bag approximates the total salt concentration in salt water, while the 10% sucrose in the beaker approximates the total salt concentration in the blood. What happened to the mass of the bag? Did water move out of the bag or into the bag? What happened to the concentration of sucrose in the beaker? Explain.

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## Lab

## 1

**Where did they drown?** *continued***Analyze and Conclude**

12. The following table contains the concentrations (in millimoles per liter) of various substances in the blood of the two drowning victims. Just as the term *dozen* refers to a specific number of things (12), the term *mole* refers to a specific number of particles ( $6.02 \times 10^{23}$ ). A millimole is  $1/1000^{\text{th}}$  of a mole. When concentration is given in millimoles (or moles) per liter, higher numbers indicate more particles dissolved in the water—in the table below, higher concentrations of sodium, potassium, or chloride.

**Table 2**

Concentration (mmole/L)			
Substance	Man	Woman	Normal Values
Sodium	200	100	145
Potassium	10	2	5
Chloride	125	75	100

Where do you think each victim drowned? Explain your answer.

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13. Should you look for murderers or did the victims drown accidentally? Explain your answer.

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# Lab 2

## How can you extract DNA from cells?

### The Problem

The Biotech Foods Company is developing an improved strain of bananas through genetic engineering. In order to obtain DNA that they can examine and use, scientists working for the company must extract the DNA from banana cells in a way that does not destroy the DNA molecules. Conditions that can damage or destroy DNA molecules include exposure to heat, acids, and ultraviolet radiation. In this lab, you will follow a simple procedure for extracting DNA.

**DNA Extraction** Procedures for changing an organism's DNA through genetic engineering require DNA molecules. Therefore, the first step in most genetic engineering procedures is DNA extraction. First, the lipids and proteins that make up the cell's plasma membrane are dissolved, and the bonds between them are broken. Second, the lipids and proteins form complexes that are filtered out of solution, leaving the DNA behind in the liquid that passes through the filter. Third, the DNA molecules are made visible by adding alcohol to this liquid.

#### Everyday Materials

- blender
- banana (1)
- 5-oz plastic cups (2)
- color-free shampoo
- table salt
- plastic teaspoon
- #2 cone coffee filter

#### Lab Materials

- sealed (rubber stopper) test tube containing cold alcohol (95% ethanol or 91% isopropyl alcohol)
- pipette or dropper
- 270 mL distilled water

### Safety

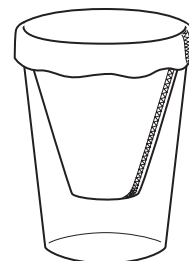


- Always wear safety goggles and a lab apron.
- Be careful when handling glass test tubes because the glass is fragile.
- Never eat or drink anything in the lab.
- Be sure to keep your hands away from your eyes and face in the science lab.
- Wash your hands thoroughly after each lab activity.

### Procedure

1. Add 1 banana and 250 mL distilled water to the blender, replace the lid on the blender, and blend for 20 seconds. Do not put any objects into the blender while it is in operation.
2. To a 5-oz cup, add 1 teaspoon shampoo, 2 pinches salt, and 20 mL distilled water. To avoid foaming, stir slowly with a spoon until the salt and shampoo dissolve.

3. Add 3 heaping teaspoons of the banana mixture to the shampoo and salt solution. Stir gently for 10 minutes.
4. Place a #2 cone coffee filter inside the second 5-oz plastic cup. Fold the filter over the edge of the cup so that it does not touch the bottom.
5. Pour the solution from Step 3 into the filter and let it drain for several minutes.
6. Fill the pipette or dropper with filtrate and transfer it to the cold alcohol in the test tube.
7. Let the solution sit undisturbed for 3 minutes. Do not shake or stir the test tube. Observe as the banana DNA precipitates out. It will have the appearance of white, stringy mucus.



## Lab

## 2

**How can you extract DNA from cells?***continued***Cleanup and Disposal**

1. Rinse, dry, and put away the test tube.
2. Dispose of all other leftover materials as instructed.

**Data and Observations**

In the space below, draw a sketch of the DNA strands.

**Conclude and Apply**

1. The purpose of the table salt in Step 2 of the *Procedure* was to enable the DNA strands to come together. What purpose do you think the shampoo serves?

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2. Suppose you substituted distilled water for alcohol in the cold test tube in Step 6 of the *Procedure*. How do you think this would affect the outcome?

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3. Could you extract DNA from cooked food to use in a genetic engineering procedure? Why or why not?

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**Analyze and Conclude**

4. Explain why DNA extraction is the first step for many procedures in genetic engineering.

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**Lab  
3**

## How can you transform cells with new DNA?

### The Problem

Researchers are working to develop gene therapy for human genetic disorders, such as cystic fibrosis and diabetes mellitus. The goal of gene therapy is to give functional genes to people with nonfunctional genes. Researchers have genetically engineered animals, such as lab mice, as well as plants. Such engineering provides genes for resistance to pests or diseases.

### Background

**DNA Transformation** The basic concept behind genetic engineering is removing a functional DNA fragment, or gene, from one organism and combining it with the DNA of another organism. When a cell takes up new DNA, the process is called transformation. Plasmids often are used in this process.

**Plasmids** Plasmids are small, circular pieces of DNA. They are found naturally in some species of bacteria and have two features that make them useful for DNA transfer.

One feature that makes plasmids useful is their ability to direct their own replication. When a plasmid enters a bacterial cell, it instructs the cell to make copies of plasmid DNA. The replication of plasmid DNA is independent of the replication of the bacterial host's DNA.

The second feature that makes plasmids useful is a genetic marker that allows bacteria carrying the plasmid to be distinguished from those that are not. Genes for antibiotic resistance are commonly used as genetic markers. After transformation, the bacteria are cultured with the antibiotic. Only those cells that have been transformed survive, because only they carry the gene for antibiotic resistance.

**Preparing Bacterial Cells** Bacterial cells will not normally take up plasmid DNA. Preparing cells to take up plasmid DNA involves incubating the bacteria in a buffer solution. Even when bacterial cells are prepared in such a manner, many of them will not take up plasmid DNA. Because plasmid DNA contains the Lux gene and a gene conferring resistance to antibiotics, only cells that take up plasmid DNA will tend to grow in the presence of an antibiotic. In this way, you can select for the cells that take up plasmid DNA.

In this lab, you will gain hands-on experience with genetic engineering methods. You will use these methods to transform nonglowing bacteria into glowing bacteria, due to a gene that makes them glow in the dark. This will allow you to observe the new phenotype of the genetically engineered bacteria. You will work with a plasmid that contains both a gene for antibiotic resistance and a gene called the Lux gene. The Lux gene causes successfully transformed cells to glow in the dark. In the instructions that follow, your teacher will provide you with a supply of *E. coli* cells that have been treated with a buffer solution to enable them to take up plasmid DNA. You will grow these cells on agar treated with ampicillin, an antibiotic.

## Lab

## 3

## How can you transform cells with new DNA? *continued*

### Everyday Materials

- plastic container of ice
- marking pen
- straws (2)

### Lab Materials

- 10- $\mu$ L aliquot plasmid DNA (1)
- 100- $\mu$ L aliquots competent *E. coli* cells (2)
- microcentrifuge tubes (2)
- incubator
- 100- $\mu$ L aliquots Luria broth (2)
- plates of culture medium with ampicillin (2)
- micropipettes (2)
- protective gloves

### Safety



- Always wear gloves, goggles, and an apron.
- Handle the micropipets carefully. The glass is fragile.
- Although the bacteria should be harmless, wash your hands thoroughly after handling the bacterial cultures and after you complete the lab.
- Be sure to keep your hands away from your eyes and face in the science lab.
- Follow your teacher's instructions for disposal of lab materials.

### Procedure

#### Part A: Preparing the Plasmid Culture

1. In a microcentrifuge tube, mix the 10- $\mu$ L aliquot of plasmid DNA with one of the 100  $\mu$ L aliquots of competent *E. coli* cells.
2. Incubate the microcentrifuge tube on ice for 15 minutes.
3. Remove the test tube from the ice and incubate it in the incubator at 42°C for 2 minutes.
4. Add 100  $\mu$ L of Luria broth to the microcentrifuge tube and mix.
5. Incubate at 37°C for 10 minutes (or hold the tube in a clenched fist for the same amount of time).



6. Label an agar plate containing ampicillin with your initials and the letters *LUX*, for Lux gene.
7. Pipette the contents of the tube onto the agar.
8. Fold a straw into a triangular shape and use it as a spreader to evenly distribute the bacteria over the surface of the agar, as shown in the figure below.
9. Discard the straw as directed by your teacher and thoroughly wash your hands.

#### Part B: Preparing the Control Culture

10. Add the other 100- $\mu$ L aliquot of competent *E. coli* cells to the second microcentrifuge tube.
11. Incubate the microcentrifuge tube on ice for 15 minutes.
12. Remove the microcentrifuge tube from the ice and incubate it in the incubator at 42°C for 2 minutes.
13. Add 100  $\mu$ L of Luria broth to the microcentrifuge tube and mix.
14. Incubate at 37°C for 10 minutes.
15. Label the second agar plate with your initials and the letters *CON*, for control.
16. With the clean micropipette, deposit the contents of the microcentrifuge tube onto the agar. Make sure you do not use the same micropipette that you used in Part A.
17. Fold the other straw into a triangular shape and use it to spread the bacteria over the surface of the agar. Do not use the same straw that you used in Part A.
18. Write a hypothesis about what you think will happen to the bacteria on the two agar plates.

**Hypothesis:** \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

## Lab

## 3

**How can you transform cells  
with new DNA?** *continued***Part C: Observing Bacterial Growth and Phenotypes**

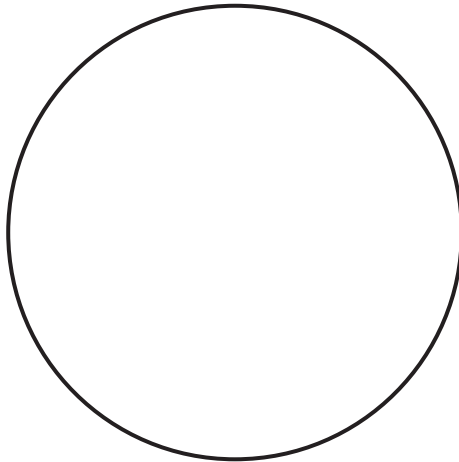
19. Incubate both plates overnight at 35°C, and then the next day at room temperature.
20. The following day, inspect the plates for bacterial growth. Record what you observe under *Data and Observations*.

**Cleanup and Disposal**

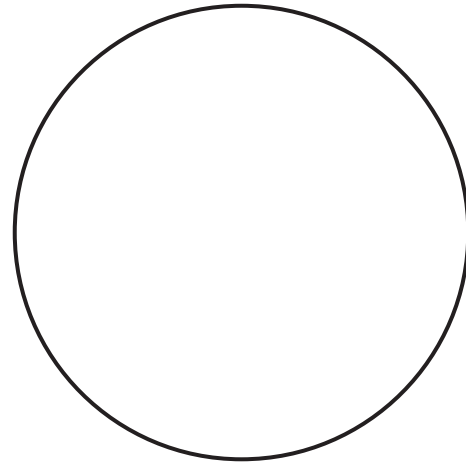
1. Wipe down your work surface as instructed by your teacher. Wash and dry all containers and equipment and return them to their proper places.
2. Dispose of any leftover solutions as instructed.

**Data and Observations**

On the drawings below, sketch any bacterial growth that you observed on the two plates.



E. coli with plasmid DNA



E. coli without plasmid DNA

**Conclude and Apply**

1. Describe how the two cultures appeared to differ after incubating for a day at room temperature. Explain any differences you observed.

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2. What was the purpose of the control culture in this procedure?

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## Lab

**3****How can you transform cells  
with new DNA?** *continued*

3. What was the reason for culturing the bacteria on agar plates containing ampicillin?

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**Analyze and Conclude**

4. Some people have diabetes mellitus because of an inherited genetic defect in the cells of the pancreas that normally produce insulin. Explain how genetic engineering might be used to help people with this disease.

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Lab  
4

# How can you transcribe and translate a gene?

## The Problem

Scientists have identified many of the tens of thousands of genes in the human genome. One application of this knowledge is the production of human proteins by genetically engineered bacteria. Insulin and some other vital human proteins cannot be artificially synthesized outside of living cells. However, genes for human proteins can be inserted into living bacteria and large quantities of these human proteins can be produced for medical use.

Bacteria are able to “read” human genes and synthesize human proteins because the same genetic code is found in all organisms. In this lab, you will use the genetic code to simulate the synthesis of protein segments.

## Background

**Genetic Code** The genetic code is the relationship between the sequence of nucleotide bases in DNA molecules and the sequence of amino acids in proteins. There are four different bases found in DNA and 20 different amino acids found in proteins. Groups of three bases, called codons, code for individual amino acids.

**Transcription** Cells read genes and produce the correct strings of amino acids that form proteins using the information in the genetic code. This is a two-step process. The first step, transcription, involves copying the DNA sequence of the gene into messenger RNA (mRNA). Like DNA, RNA contains four nucleotide bases. However, in RNA, the base uracil (U) replaces the base thymine (T).

Before the information in DNA can be decoded, a small portion of the DNA double helix must be uncoiled. A strand of mRNA is then synthesized that is complementary to one strand of the DNA. The mRNA molecule carries the information for making a protein from the cell’s nucleus to the cytoplasm, where protein synthesis takes place.

**Translation** The second step in protein production is translation. Recall that groups of three bases are called codons, and codons code for

individual amino acids, as shown in the table on the next page. The table is based on the mRNA sequence, so the base thymine (T) is replaced with the base uracil (U).

As you can see from the table, some amino acids have more than one codon, but each codon codes for just one amino acid. The codon AUG, which codes for the amino acid methionine, is a signal to start protein synthesis. The three codons UAA, UAG, and UGA do not code for any amino acids. Instead, each is a signal to end protein synthesis.

In the cytoplasm, the mRNA binds to a ribosome, where the message is translated into a sequence of amino acids. The amino acids that are incorporated into the protein are carried by relatively small RNA molecules known as transfer RNA, or tRNA. At one end of each tRNA molecule is a specific sequence of three nucleotides, complementary to the mRNA codon and called an anticodon, that can bind to the mRNA. At the other end of the tRNA molecule is the amino acid encoded by the anticodon. Only the information contained up to the stop codon (UAA, UAG, or UGA) in the mRNA molecule is used to produce the amino acid sequence. Starting with the start codon, the ribosome reads sets of three nucleotides at a time until a stop codon is reached.

## Lab

## 4

How can you transcribe and translate a gene? *continued*

	U	C	A	G	
U	UUU=Phe	UCU=Ser	UAU=Tyr	UGU=Cys	U
	UUC=Phe	UCC=Ser	UAC=Tyr	UGC=Cys	C
	UUA=Leu	UCA=Ser	UAA=stop	UGA=stop	A
	UUG=Leu	UCG=Ser	UAG=stop	UGG=Trp	G
C	CUU=Leu	CCU=Pro	CAU=His	CGU=Arg	U
	CUC=Leu	CCC=Pro	CAC=His	CGC=Arg	C
	CUA=Leu	CCA=Pro	CAA=Gln	CGA=Arg	A
	CUG=Leu	CCG=Pro	CAG=Gln	CGG=Arg	G
A	AUU=Ile	ACU=Thr	AAU=Asn	AGU=Ser	U
	AUC=Ile	ACC=Thr	AAC=Asn	AGC=Ser	C
	AUA=Ile	ACA=Thr	AAA=Lys	AGA=Arg	A
	AUG=Met	ACG=Thr	AAG=Lys	AGG=Arg	G
G	GUU=Val	GCU=Ala	GAU=Asp	GGU=Gly	U
	GUC=Val	GCC=Ala	GAC=Asp	GGC=Gly	C
	GUA=Val	GCA=Ala	GAA=Glu	GGA=Gly	A
	GUG=Val	GCG=Ala	GAG=Glu	GGG=Gly	G

**Key****RNA Bases:**

A = adenine, C = cytosine,  
G = guanine, U = uracil

**Amino Acids:**

Phe = phenylalanine,  
Leu = leucine,  
Ile = isoleucine,  
Met = methionine,  
Val = valine,  
Ser = serine,  
Pro = proline,  
Thr = threonine,  
Ala = alanine,  
Tyr = tyrosine,  
His = histidine,  
Gln = glutamine,  
Asn = asparagine,  
Lys = lysine,  
Asp = aspartic acid,  
Glu = glutamic acid,  
Cys = cysteine,  
Trp = tryptophan,  
Arg = arginine,  
Gly = glycine

**Procedure****Part A: Transcribing and Translating Mystery DNA Sequences**

1. You have been given two mystery DNA sequences, shown under *Data and Observations*. One of them codes for some of the amino acids in a known human protein. Transcribe and translate the mystery DNA sequences to identify the amino acid sequence encoded in each, following steps 2–4. Record your work under *Data and Observations*.
2. First, transcribe each DNA sequence by writing the corresponding sequence of complementary bases in the mRNA molecule.
3. Then, translate each mRNA sequence into a complementary sequence of tRNA anticodons.
4. Finally, use the genetic code to translate the mRNA sequence into the correct sequence of amino acids.

**Part B: Translating and Transcribing DNA Sequences for Known Hemoglobins**

5. Repeat steps 2–4 to determine the sequence of the first nine amino acids encoded in the DNA for normal hemoglobin (normal Hb). Record your results under *Data and Observations*.
6. A point mutation in the seventh codon for normal hemoglobin produces sickle-cell hemoglobin (Hb S). A change in a single nucleotide base results in the amino acid valine in this position. Record the sequence of the first nine amino acids in hemoglobin S under *Data and Observations*.
7. There are many other known hemoglobin variants, including hemoglobin C (Hb C), which has lysine instead of glutamic acid in position seven, and hemoglobin G (Hb G), which has glycine instead of glutamic acid in position eight. Record the sequences of the first nine amino acids in hemoglobins C and G under *Data and Observations*.

## Lab

## 4

How can you transcribe and translate a gene? *continued***Data and Observations****Mystery DNA sequence #1**

TAC	CAT	GTG	GAA	TGA	GGA	CTC	CCT	TTC
1	2	3	4	5	6	7	8	9

**mRNA #1**

1	2	3	4	5	6	7	8	9

**tRNA #1**

1	2	3	4	5	6	7	8	9

**Amino acid sequence #1**

1	2	3	4	5	6	7	8	9

**Mystery DNA sequence #2**

TAC	CAC	GAC	TGC	GGT	CCG	CTC	GTG	TTC
1	2	3	4	5	6	7	8	9

**mRNA #2**

1	2	3	4	5	6	7	8	9

**tRNA #2**

1	2	3	4	5	6	7	8	9

**Amino acid sequence #2**

1	2	3	4	5	6	7	8	9

**DNA sequence normal hb**

TAC	CAT	GTG	GAA	TGA	GGA	CTC	CTC	TTC
1	2	3	4	5	6	7	8	9

**mRNA normal hb**

1	2	3	4	5	6	7	8	9

**tRNA normal hb**

1	2	3	4	5	6	7	8	9

**Amino acid sequence normal hb**

1	2	3	4	5	6	7	8	9

## Lab

## 4

**How can you transcribe and translate a gene?** *continued*

Amino acid sequence Hb S

1	2	3	4	5	6	7	8	9

Amino acid sequence Hb C

1	2	3	4	5	6	7	8	9

Amino acid sequence Hb G

1	2	3	4	5	6	7	8	9

**Analyze and Conclude**

1. Compare the sequences of amino acids for the two mystery DNA samples with the sequences of amino acids in the four known hemoglobin molecules. Which mystery amino acid sequence is a match for a hemoglobin molecule? Which hemoglobin molecule is it?

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2. Identify the point mutations that would change the DNA sequence for normal hemoglobin into a DNA sequence for hemoglobins S, C, and G. Remember: a point mutation is a change in a single nucleotide base.

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3. If the final segment, CTC CTC TTC, of the normal hemoglobin DNA sequence underwent a point mutation to become CTC CTT TTC, would it affect the resulting protein? Explain your answer.

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4. Predict how a mutation in the seventh codon of normal hemoglobin, changing it from CTC to ATC, would affect the protein being synthesized.

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5. Explain the significance of a genetic code that is consistent between organisms to biotechnology.

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# Lab 5

## How can you diagnose genotypes using DNA?

### The Problem

Sickle-cell anemia is a disease caused by a mutation in the gene that codes for  $\beta$ -globin (beta-globin), one of the two types of polypeptide chains that make up the hemoglobin molecule. The two  $\beta$ -globin alleles that are relevant to sickle-cell anemia are abbreviated A (normal allele) and S (sickle-cell allele). In general, only people who are homozygous for the mutation (SS) have the disease. People who are heterozygous (AS) are called carriers. They have one normal and one sickle-cell gene and usually do not have symptoms of sickle-cell anemia. However, if two heterozygotes marry, their children can have the disease. Therefore, it is important to be able to diagnose suspected carriers. In this lab, you will learn how to diagnose sickle-cell genotypes using a biotechnology method called DNA restriction enzyme analysis.

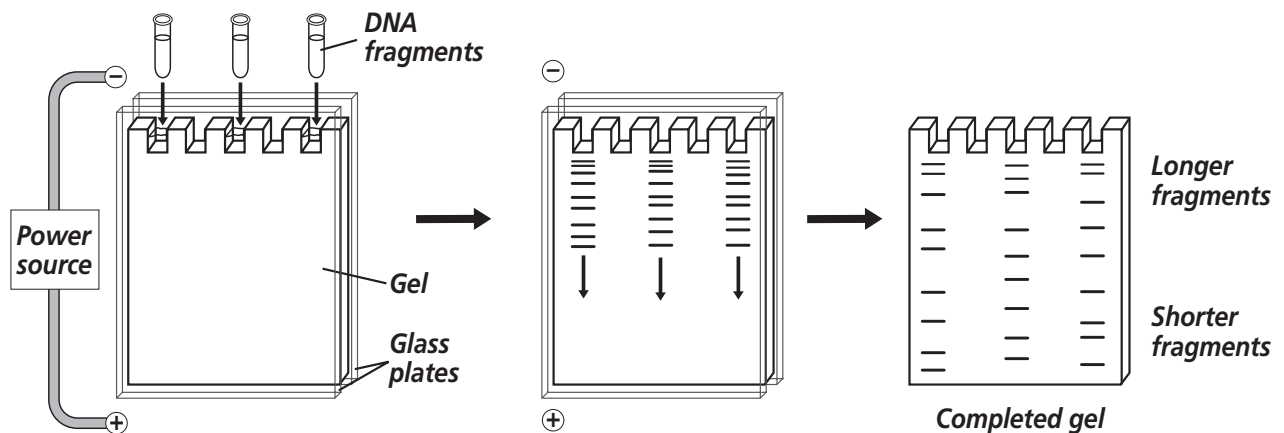
### Background

**Cutting DNA into Fragments** DNA molecules are too large to be analyzed without cutting them into smaller fragments. This can be done with restriction enzymes. There are more than 100 known restriction enzymes, and each cuts DNA at one specific nucleotide sequence.

**Separating DNA Fragments** To analyze DNA fragments, they must be separated. This can be done with gel electrophoresis. In this procedure, a mixture of DNA fragments is deposited at one end of a porous gel that is similar to a slice of gelatin. An

electric current produces negative and positive ends in the gel, and this bipolarity causes the DNA fragments to move across the gel. Because DNA is negatively charged, it moves toward the positive end. However, smaller fragments move faster and travel farther across the gel in a given length of time. Based on their size, the DNA fragments create a pattern of bands on the gel, as shown in **Figure 1**. These bands can be compared with other, known samples of DNA. Matching them with known samples allows researchers to identify the size of the DNA fragments.

**Figure 1**



## Lab

## 5

## How can you diagnose genotypes using DNA? *continued*

**Diagnosis of Sickle-cell Genotypes** A restriction enzyme called Mst II recognizes and cuts DNA at the nucleotide sequence CCTGAGG. This sequence appears in the gene that codes for normal  $\beta$ -globin. However, the mutation that causes sickle-cell anemia changes the A to T in this sequence. The new nucleotide sequence, CCTGTGG, not only causes sickle-cell anemia, but also loss of the recognition site for the restriction enzyme Mst II, which means the enzyme will not cut the DNA at this site.

As a result, DNA fragments cut from people who are homozygous for the mutation (SS) will be longer than those cut from people who are homozygous for the normal allele (AA). While DNA fragments from an SS individual will be longer than those from an AA individual, an AS individual will have both long and short fragments. These fragments can be separated with electrophoresis and identified by their characteristic banding patterns when compared with known samples.

### Everyday Materials

- clear glass plate

### Lab Materials

- test tubes containing simulated DNA fragments (7)
- gel cassette for electrophoresis
- P-20 micropipette with 7 tips
- 150-mL 1% TAE buffer
- gel electrophoresis box
- protective gloves

### Safety



- Always wear gloves and goggles.
- Be sure to keep your hands away from your eyes and face in the science lab.
- Use power cords with caution.
- Wear an apron to protect your clothes from the dye in the samples.

### Procedure

1. From your teacher, obtain four “DNA” samples for one family and also one each of the three known “DNA” samples. The code for the seven tubes is: M = mother, F = father, T = teenager, I = infant, N = known normal, C = known carrier, and S = known sickle-cell patient.
2. Under *Data and Observations*, record in the key whether you have Family #1 or Family #2.
3. Obtain a cassette of gel and place it in the electrophoresis box. The wells should be facing up at the end with the black (negative) electrode.
4. Add enough 1% TAE buffer to the gel box so that the gel is covered by about 1 or 2 mm of buffer. Place the lid on the box.
5. Using a P-20 micropipette set to 8  $\mu$ L, load each of the seven DNA samples into a separate well. Care should be taken not to damage the wells with the micropipette tip. Take turns loading with the other students in your group. Use a new micropipette tip for each sample.
6. Use the key under *Data and Observations* to indicate which sample you put in each lane of the gel.
7. Plug in the electrical leads, set at 120 V, and turn on the power. Run the gel for 10 minutes.
8. Turn off the power, unplug the electrical leads, and open the gel box. Remove the gel and carefully slide it onto a clear glass plate. Pour off the extra buffer.
9. Place the dish on white paper for better contrast. Observe the pattern of bands in the gel. Color this pattern in the drawing of the gel under *Data and Observations*.

## Lab

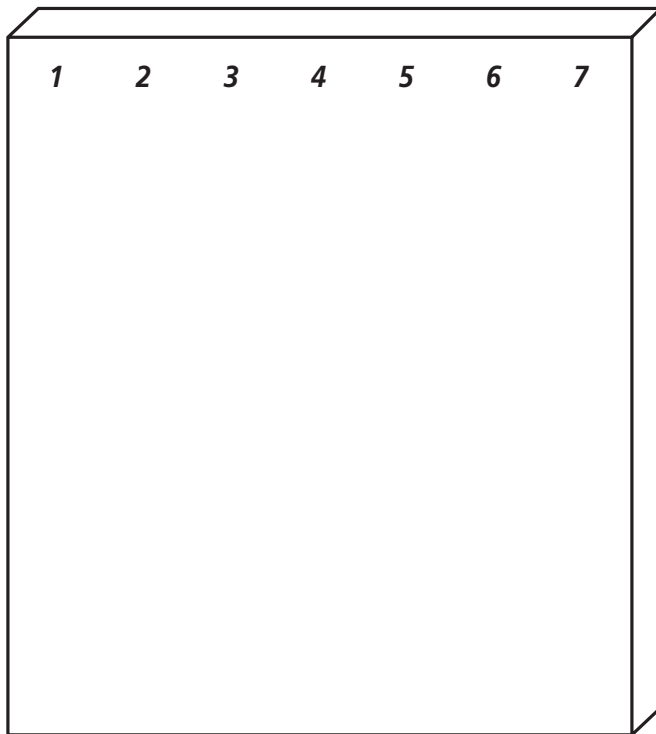
## 5

## How can you diagnose genotypes using DNA? *continued*

### Cleanup and Disposal

1. Dispose of the gel and buffer as instructed by your teacher.
2. Return all equipment to its proper place.

### Data and Observations



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Key: Family # \_\_\_\_\_

1 = \_\_\_\_\_ 3 = \_\_\_\_\_ 5 = \_\_\_\_\_ 7 = \_\_\_\_\_

2 = \_\_\_\_\_ 4 = \_\_\_\_\_ 6 = \_\_\_\_\_

### Conclude and Apply

1. What properties of the two dyes used in the lab make them suitable for the simulation of DNA restriction analysis?

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## Lab

## 5

**How can you diagnose genotypes using DNA?** *continued*

2. Based on your restriction analysis, what are the genotypes of each family member?

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3. Make a Punnett square for the parents' genotypes. Use it to determine the chance of the parents having a child with sickle-cell anemia.

**Analyze and Conclude**

4. If the Mst II enzyme recognized and cut DNA at a different nucleotide sequence, could it still be used to diagnose sickle-cell genotypes? Explain your answer.

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**Lab  
6**

# How can genetically engineered plants be multiplied?

## The Problem

Dr. Ramirez has used genetic engineering in an attempt to make African violets more resistant to a fungal disease. First, she isolated DNA from a bacterial species that resists the fungus. Then, using a particle gun, a device used to insert new DNA into cells such as plant cells and bacteria, she “shot” the bacterial DNA into the cells of tiny pieces of African violet leaves. Now that the transfer of DNA is complete, she must grow new plants from the tiny pieces of modified leaf tissue. How can this be done?

The answer is micropropagation. In this careful procedure, which is explained in the following paragraphs, tiny pieces of plant tissue are disinfected and then cultured in a sterile medium until they start to produce new shoots. In this lab, you will use micropropagation to produce new shoots from tiny pieces of African violet leaves.

## Background

**DNA Transfer** New genetic traits can be incorporated into an organism by directly transferring DNA from another organism. DNA can be transferred to plants by “shooting” them with a particle gun or by infecting plant tissues with a bacterium that then incorporates part of its DNA into the DNA of the host plant. In both methods, the DNA is transferred to a tiny fragment of plant tissue or a small mass of plant cells. Once the DNA has been transferred, the only way to regenerate new plants from such small pieces of tissue or clumps of cells is with tissue culture using micropropagation methods.

**Micropropagation** Micropropagation differs from all other plant propagation techniques in requiring aseptic conditions, conditions that are sterile or free of contamination by microorganisms, in order to be successful. The growing medium promotes the growth of bacteria and fungi spores, which are commonly found on surfaces and in the air. If the plant tissue cultures are contaminated by these organisms, they will grow rampantly and destroy or infect the plant tissues in the same culture.

**Maintaining Aseptic Conditions** To maintain aseptic conditions during the micropropagation process, it is necessary to keep all dust particles (which may contain bacteria) and spores of fungi away from the work area. This can be done in various ways. In large labs, the work usually is performed under a hood that filters these contaminants out of the work area. A simpler, but still relatively effective, way to reduce the chance of contamination is to spray the work area, hands, instruments, and all other objects in the work area with a 70% ethanol solution before the work begins.

To cut down on air movement that might transfer contaminants into the work area once it has been disinfected with ethanol, the work can be done inside a new large, clear plastic bag, which should be relatively sterile, or inside an aquarium or cardboard box that has been lined with aluminum foil and sprayed with ethanol. It is important to keep traffic around the work area to a minimum to reduce air movement and the potential for contamination.

## Lab

## 6

## How can genetically engineered plants be multiplied? *continued*

### Everyday Materials

- prepackaged African violet medium
- table sugar
- fresh African violet leaves (2)
- 0.1% detergent solution
- 10% bleach solution
- sterile razor blade
- scissors
- marking pen

### Lab Materials

- protective gloves
- 70% ethanol in spray bottle
- distilled water
- 250-mL beaker
- stirring rod
- pH meter or paper
- dropper
- NaOH and HCl (as needed to adjust pH)
- 250-mL bottle
- agar powder
- sterile petri dishes (5)
- 250-mL or larger glass jar with screw-cap lid
- 1 L sterile water
- sterile forceps
- parafilm

### Safety



- Always wear gloves, goggles, and a lab apron.
- Use caution when handling the razor blade and scissors.
- Make sure the ethanol is never near an open flame.
- Be careful to avoid burns when handling hot water or working with a hot plate.
- Avoid splashing bleach solution onto your clothes because it will remove the color.
- Be sure to keep your hands away from your eyes and face in the science lab.
- Never eat or drink anything in the lab.
- Wash your hands thoroughly after each lab activity.

### Procedure

#### Part A: Creating an Aseptic Environment

1. Put on a pair of protective gloves. Spray them with 70% ethanol before starting the procedure, and then respray them whenever you touch any nonsterile surface or material throughout the remainder of the procedure.
2. Spray your work surface with 70% ethanol. Also, spray all containers before placing them on the work surface as you carry out the procedure.

#### Part B: Preparing the Culture Medium

3. Place about 50 mL of distilled water, 0.58 g of prepackaged African violet medium, and 3.75 g of table sugar into a 250-mL beaker.
4. Add distilled water to bring the solution up to 100 mL. Stir until the sugar and African violet medium are dissolved.
5. Check the pH using a pH meter or paper. Add a few drops of NaOH or HCl as supplied by your teacher as needed to bring the pH between 5.6 and 5.8.
6. Add more distilled water to bring the solution up to 125 mL.
7. Pour the solution into a 250-mL bottle and add 1.0 g of agar powder.
8. Keeping the cap loose on the bottle, sterilize it by placing it in boiling water for 30 minutes.
9. Remove the bottle from the hot water bath and gently swirl the medium to mix the agar until it is completely dissolved.
10. After the medium cools to about 50°C, pour about 25 mL into each of four sterile petri dishes. Store the dishes in their sleeves in the refrigerator until you need them in Part D of the *Procedure*.

## Lab

## 6

**How can genetically engineered plants be multiplied?** *continued***Part C: Disinfecting the Leaves**

11. Put the African violet leaves in the screw-cap jar and half-fill the jar with 0.1% detergent solution. Cap the jar tightly and gently agitate it for 3 minutes.
12. Pour off the detergent solution and rinse the leaves and jar with cool tap water.
13. Repeat step 11, substituting 10% bleach solution for detergent solution and gently agitate it for 10 minutes.
14. Pour off the bleach solution into the sink while keeping the lid loosely in place over the jar. Be careful not to pour out the leaves along with the bleach solution. The leaves should now be sterile. From this point on, prevent them from becoming contaminated by using only sterile water and sterile tools on them.
15. Spray the outside of the jar with 70% ethanol and place it on your sterile work surface.
16. Remove the lid from the jar and pour sterile water over the leaves until the jar is about half-full. Replace the lid and gently shake the jar for 2 minutes. Carefully pour off the rinse water into the sink without touching the leaves or removing them from the jar.
17. Repeat step 16 three times for a total of four sterile water rinses.

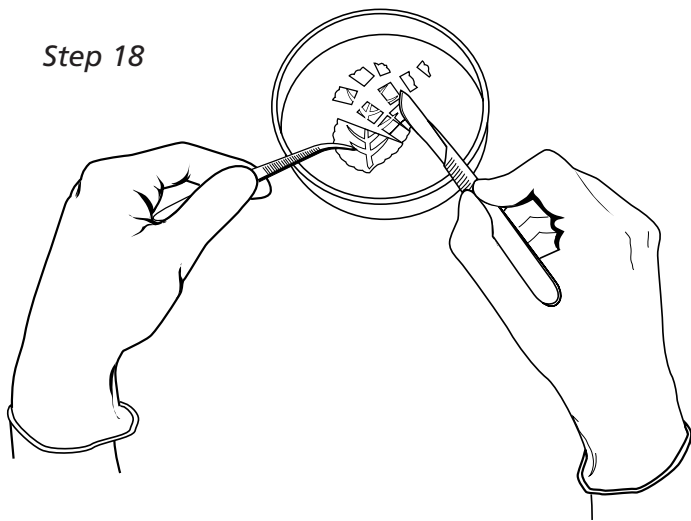
**Part D: Preparing Leaf Tissue Cultures**

18. Using the forceps, place the leaves on the remaining empty sterile petri dish. Hold the leaves with the forceps while you use the razor blade to cut them into squares about 1.5 cm on a side.
19. Place two or three strips of leaf into a dish of medium. Gently press the pieces against the medium with the forceps. Replace the cover on the dish and wrap it with a piece of parafilm, stretching the film to seal it. You and your partner should each prepare two dishes in this way. Label your two dishes with your initials and number them *one* and *two*.

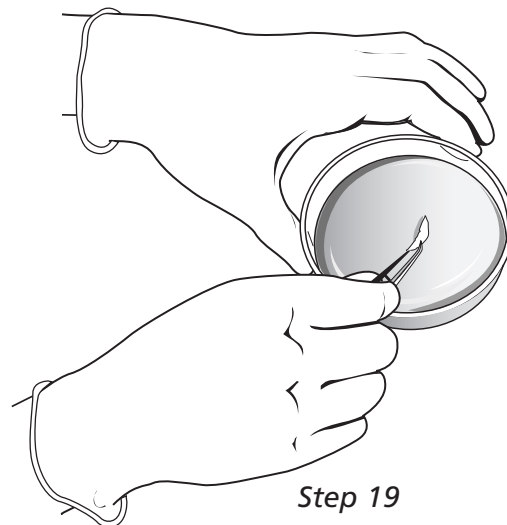
**Part E: Growing and Monitoring the Cultures**

20. Place both dishes under lights in the place provided by your teacher.
21. Check the dishes after a few days for evidence of growth. Any dishes with fuzzy or slimy growth visible on them have been contaminated by fungi or bacteria and should be discarded as directed by your teacher.
22. Continue to check the dishes once a week for at least five weeks and record your observations each time.

Step 18



Step 19



## Lab

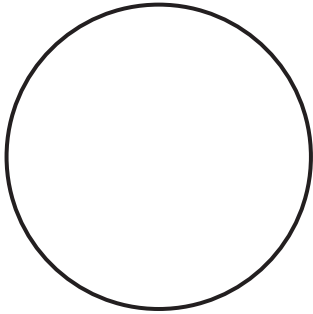
## 6

**How can genetically engineered plants be multiplied?** *continued***Cleanup and Disposal**

1. Rinse and dry all containers and equipment and return them to their proper place.
2. Dispose of any leftover solutions or culture medium according to your teacher's instructions.

**Data and Observations**

Each week when you observe your petri dishes, record the date and dish number and draw a sketch of what you see, using a circle like the one shown below to represent the perimeter of the petri dish.



Date: \_\_\_\_\_

Dish  
Number: \_\_\_\_\_**Conclude and Apply**

1. Why is it crucial to maintain aseptic conditions when carrying out micropropagation of plants? What is likely to happen if aseptic conditions are not maintained?

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2. Explain the role that plant micropropagation plays in genetic engineering.

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3. How is it possible to regenerate a new plant, with all its different tissues and organs, from just a tiny piece of leaf tissue?

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**Analyze and Conclude**

4. Micropropagation is used by plant breeders and growers as well as researchers. What do you think its commercial advantages are over conventional methods of plant propagation, such as stem cuttings?

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**Lab  
7****When did she die?**

Your medical examiner team has been given the following case to review. It is your job to determine whether the victim died accidentally and what the time of death was. Study the details and complete the medical examiner's report that follows.

**The Problem**

The victim was found in her home at 10:00 A.M. on Saturday morning by her sister, with whom she was supposed to go jogging. The sister promptly called the police who then notified you, the medical examiner. You noted the following:

- The victim was lying facedown at the bottom of the stairs, facing away from the stairs. The sister indicated the victim was dressed in the clothes she had worn to dinner the night before.
- The victim had no pulse.
- The body was cold to the touch, but the internal temperature, which was measured at 10:30 A.M., was 27°C (room temperature was 20°C).
- Her neck was apparently fractured, and she appeared to have sustained head injuries.
- There were purplish marks on the front of her shoulders and neck; the marks did not change color when touched.
- Her entire body was stiff.
- The victim's eyes were open and cloudy with a thin film.

**Additional Police Notes** The victim had eaten dinner with her sister at 5:00 P.M. the night before (Friday evening). At dinner, they had agreed to meet at the victim's townhouse at 10:00 A.M. Saturday morning to go jogging. The sister returned to her own home at 11:00 P.M., but she was not sure when the victim returned to her townhouse after dinner. Neighbors did not recall seeing the victim return to her townhouse.

When you performed an autopsy on the victim later that day, you noted that she died of a broken neck and subsequent asphyxiation. The victim was 5 feet 8 inches tall and weighed 130 pounds, her stomach was empty, and her small intestine was full. Your job is to provide police with the time of death.

## Lab

## 7

**When did she die?** *continued***Background**

When a body is discovered, one of the first things that a medical examiner must do is determine the time of death. The medical examiner uses several indicators to help establish the time of death, including body temperature, rigor mortis, discoloration (livor mortis or lividity), and the appearance of the eyes.

**Body Temperature** When a person dies, the body immediately begins to cool. On average, the body temperature drops at a rate of  $0.75^{\circ}\text{C}$  per hour for the first 12 hours. After 12 hours, the rate of cooling slows by about one half (approximately  $0.4^{\circ}\text{C}$  per hour) until the body reaches ambient temperature, the temperature of the environment. The rate of cooling is also affected by the following factors:

- *Air temperature* – A body will cool faster on a cold winter night than on a warm summer night.
- *Body fat* – Fat tends to insulate the body, so the more fat a person has, the slower the body cools after death.
- *Clothing* – Clothing also insulates the body, so heavy clothing will slow the rate of cooling.
- *Water* – A body in water cools much faster than one in air. Therefore, it is difficult to use body temperature to estimate the time of death for a victim found in the water.

**Rigor Mortis** At the time of death, the body's muscles are relaxed. However, within 1–2 hours, the muscles begin to stiffen as their stores of adenosine triphosphate (ATP) become exhausted. This stiffening is known as rigor mortis. Rigor mortis begins with the muscles of the face, jaws, and neck, proceeds down the body through the upper arms and torso, and ends with the legs. This process is complete within 8–12 hours after death. As the muscles begin to break down, they begin to relax in the same order as they stiffened. By 24–48 hours after death, the body is totally relaxed again.

**Livor Mortis (Lividity)** Within 1–2 hours after death, the blood settles into the lowest parts of the body (parts that are closest to or resting on the ground) due to gravity. The red blood cells settle out and break down into the tissues, leaving purplish marks that later become yellow (due to the breakdown of hemoglobin). The color (lividity) becomes fixed in the tissue within 6–8 hours after death. If a body is moved after this time, then the position of the purplish marks may not agree with the position in which the body is found. Finally, if skin appears discolored, but turns white when touched, then lividity has not been fixed and death probably occurred more than 2 hours, but less than 10 hours ago.

**Appearance of the Eyes** If the eyes remain open at the time of death, then a thin film will appear on them as they begin to dry out. As the blood cells within the body break down, they release potassium. Potassium enters the eyes and causes them to appear cloudy. This process takes approximately 2–3 hours after death; however, if the eyes remain closed after death, then the process takes much longer (approximately 24 hours).

**Stomach Contents** After you eat, the process of digestion takes place. Digestion begins in the stomach. It takes about 4–6 hours for the stomach to empty its contents into the small intestine. Finally, it takes approximately 12 hours for the food to leave the small intestine. As a rule of thumb:

- Undigested stomach contents—death occurred 0–2 hours after the meal
- Stomach empty—death occurred 4–6 hours after the meal
- Small intestines empty—death occurred 12 hours or more after the meal



## Lab

**7****When did she die?** *continued*

6. Plot the data for temperature versus time after death on graph paper.
7. Based on the body temperature of the victim, how long has it been since the victim died?
- \_\_\_\_\_
8. Estimate the time of death using body temperature.
- \_\_\_\_\_
9. Based on the observations of rigor mortis, how long has the victim been dead?
- \_\_\_\_\_
10. Where was lividity observed on the body? Was it fixed? How long ago did she die? Was the victim found in the position that she died or was her body moved? Explain your answer.
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_
11. Based on the appearance of her eyes, how long ago did she die? Explain your answer.
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_
12. Based on the examination of her digestive system, how long after a meal did the victim die? Explain.
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_

**Analyze and Conclude**

13. Based on all of the evidence available, estimate the time of death for the victim. Explain your answer.
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_
14. Was the victim's death an accident? Explain your answer.
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_

Lab  
8

## A Sweet Season

**The Problem**

The boys' basketball team has been practicing long hours and playing exhausting games all season. Their hard work has paid off. They will be playing in the state championship in two weeks. However, two of the starting players, Jorge and Kyle, have reported to the team doctor. Both boys (age 17) have been complaining about fatigue and muscle weakness and are worried about being able to play their best in the championship.

Listed below are the symptoms the players shared with the doctor.

*Jorge:* sleepy in classes; muscle weakness; increased appetite; drinks plenty of fluids; occasional dizzy spells after heavy exertion; increased urination (not excessive)

*Kyle:* sleepy in classes, especially after lunch; muscle weakness; increased appetite, almost excessive; drinks plenty of fluids, but always feels thirsty; frequent dizzy spells after long practices or heavy exertion; urinates frequently; 15 pound weight loss

Both boys had pre-season physical exams and were healthy. However, both boys have a family history of diabetes, a disease in which the body does not effectively respond to the levels of glucose, a very important type of sugar, in the blood. The result is dangerously high concentrations of glucose in the blood. Jorge's father developed diabetes at age 50 and is currently managing it by dieting. Kyle's grandfather had diabetes all of his life. He had to inject insulin daily, developed complications from diabetes, and died at age 55.

Because the symptoms and histories indicate that one or both boys may have diabetes, a glucose tolerance test is ordered to diagnose the disease.

**Glucose Tolerance Test** On the day of the test, each boy does not eat breakfast. During the test, each boy drinks a concentrated glucose solution (50–100 g glucose) and has blood drawn every 30 minutes for 2.5 hours. The blood samples are spun in a centrifuge to separate plasma from blood cells. A portion of each sample is sent to an outside lab, where the insulin and glucose contents in each plasma sample will be measured.

You are a technician in the lab that will measure the blood glucose by using a glucose test strip. The strip contains an absorbent pad that contains two enzymes, *glucose oxidase* and *peroxidase*, and a color indicator. When a blood sample is applied to the pad, glucose oxidase converts glucose to gluconic acid and hydrogen peroxide. Peroxidase then reacts the hydrogen peroxide with the color indicator, which varies in color from light green to brown depending upon the amount of glucose in the sample. Light green indicates lower glucose concentrations, while dark brown indicates higher concentrations. You will compare the color of the strip to a color scale on the bottle to determine the glucose concentration in the sample. You will then graph and analyze the data to make a preliminary diagnosis.

## Lab

## 8

A Sweet Season, *continued*

## Everyday Materials

- tissues
- watch with a second hand display or stopwatch
- ruler
- graph paper

## Lab Materials

- labeled tubes (12) containing simulated plasma samples (5 mL) from each boy (before, 0.5, 1, 1.5, 2, 2.5 h)
- glucose test strips (12)
- copy of the test strip color chart (if you do not have a copy, see the description in the *Procedure*)

## Safety



- Never taste or drink anything in the lab.
- Be sure to keep your hands away from your eyes and face in the science lab.
- Wash your hands thoroughly after each lab activity.

## Procedure

1. To measure glucose in each sample with the test strips, do the following:
  - a. Take a strip and hold it by the end opposite the test area.
  - b. Dip the strip into the tube containing the sample and remove it immediately (draw the edge of the strip against the rim of the test tube to remove any excess fluid).
  - c. Start the stopwatch and compare the color of the strip to the color scale exactly 30 seconds after wetting the strip (ignore any color changes that occur after 30 seconds).
  - d. Note the reading on the data table.
  - e. Repeat the procedure with the next sample and continue until you have tested all of the samples.

Plasma Glucose Concentration (mg/dL)		
Time (h)	Jorge	Kyle
Before (0)		
0.5		
1.0		
1.5		
2.0		
2.5		

Test Strip Color Scale	
Color	Glucose (mg/dL)
Aqua	<100
Light Green	100
Green	250
Olive	500
Light Brown	1000
Dark Brown	≥ 2000

2. On graph paper, plot the data for each boy on the same graph. Plot the plasma glucose concentration on the  $y$ -axis and time on the  $x$ -axis.

Use your data to answer the following questions. You may also wish to have your textbook and some additional information about diabetes available.

## Conclude and Apply

1. Was the blood glucose concentration the same in each boy prior to the test (i.e. fasting blood glucose)? If not, whose blood glucose concentration was higher?

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## Lab

## 8

**A Sweet Season, *continued***

2. Describe the changes in blood glucose that occur over time in each boy.

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3. How does glucose that you eat get into your bloodstream? In your answer, describe the organs that it must pass through.

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4. Does the pattern of the changes in blood glucose that you observed in the boys make sense in light of your answer to question 3? Explain why or why not.

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5. Whose blood glucose concentration is more stable (i.e. controlled), Jorge's or Kyle's?

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**Analyze and Conclude**

6. Based on the information that you have so far, do either of the boys have diabetes? If so, which boy and how did you make that conclusion?

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7. Obtain a copy of the insulin report from your teacher and look over the data. Describe how insulin levels change with time during the test for each boy.

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8. How do the changes in plasma insulin concentration correlate with the changes in blood glucose?

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## Lab

**8****A Sweet Season, *continued***

9. Based on the information that you now have, do either of the boys have diabetes? Support your conclusion.

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10. Based on your data, what does insulin do to blood glucose levels?

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11. Which boy might be better able to withstand a long period without food? Why?

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12. If either of the boys has diabetes, explain how the disease accounts for his symptoms. If neither of the boys has diabetes, then what might cause their symptoms? (You will need to obtain information about diabetes.)

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13. What treatment might you recommend for each boy? Why?

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# Lab 9

## Use Blood Types to Help Solve a Crime

### The Problem

Some school property was vandalized, and a few drops of blood were found at the scene of the crime. The detectives investigating the incident think that the blood came from the perpetrator of the crime, who apparently was slightly injured during the vandalism. The detectives have gathered a group of suspects who were all on school property when the crime was committed. However, the only hard evidence is the blood found at the scene. The detectives want to rule out as many of the innocent suspects as possible on the basis of the blood evidence. In this lab, you will act as a lab technician, analyzing a sample of blood from a suspect to determine the blood type. Each one of your classmates will analyze a sample from a different suspect. Then, you and your classmates will compare the blood types of your samples with the type of blood found at the crime scene in order to narrow down the field of suspects.

### Background

**Using Blood Typing in Forensics** Blood typing is frequently used in forensic investigations. A very small quantity of blood can easily be tested for dozens of genetically controlled traits that have little, if any, environmental influences on their expression. This means that if you know a person's phenotype for these traits, you also know his or her genotype, or genetic identity.

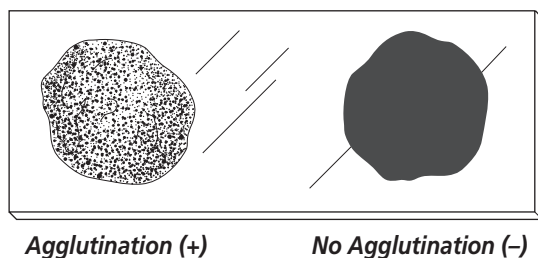
There are hundreds of known human blood-group systems in addition to the familiar ABO and Rh, or rhesus, blood groups. Each system is based on classes of antigenic molecules on the surface of the red blood cells. An antigenic molecule is recognized by the body's immune system as a foreign substance, to which it reacts by producing an antibody. Classification, or typing, of a person's blood to determine which molecular forms are present on the red blood cell depends on antigen-antibody reactions.

**Agglutination Reaction** If you take serum from a person of known blood type and add it to a

drop of blood to be typed, one of two things will happen. The serum may mix freely with the red cells with no noticeable change, or the mixture may agglutinate, that is, the red blood cells clump together, producing a readily observable change as shown in **Figure 1**.

**ABO Blood Type** There are two common antigenic substances in the ABO blood group, A and B. The O type is not associated with an antigen, which means it is not recognized as a foreign substance by anyone's immune system—even people who do not have O type blood. Antibodies to type A antigen normally are found in the serum of people without the A antigen (that is, in people with type B or type O blood). These anti-A antibodies cause type A cells to agglutinate if they are mixed together. If a blood sample is agglutinated only by anti-A antibodies and not by anti-B antibodies, then the sample is type A, as shown in **Table 1**. Agglutination reactions that identify blood types B, AB, and O also are shown in **Table 1**.

**Figure 1**



**Table 1**

ABO Blood Type	Anti-A Reaction (+/-)	Anti-B Reaction (+/-)
A	+	-
B	-	+
AB	+	+
O	-	-

## Lab

## 9

## Use Blood Types to Help Solve a Crime, *continued*

**Rhesus Blood Type** Rhesus blood types have only one common antigen associated with them. It is often referred to as antigen D. Blood from people with the D antigen (Rh<sup>+</sup>) is agglutinated by anti-D antibodies, which may be found in the blood of people without the antigen (Rh<sup>-</sup>). Agglutination reactions that identify Rhesus blood types are shown in Table 2.

Table 2

Rhesus Blood Type	Anti-D Reaction (+/-)
Rh <sup>+</sup>	+
Rh <sup>-</sup>	-

### Everyday Materials

- 10% bleach solution
- paper towels

### Lab Materials

- ABO/Rh blood-typing test kit (with artificial or aseptic blood samples)
- protective gloves

### Safety



- In this lab, you will work with artificial or aseptic blood samples obtained from your teacher. Handle and dispose of the samples as instructed by the testing kit or your teacher.
- Be sure to keep your hands away from your eyes and face in the science lab.
- Remember to wash your hands thoroughly before and after completing the lab procedure.

3. After waiting the time specified in the kit instructions, observe the samples and compare them with **Figure 1**. Record the results, positive or negative for each reaction, in columns 1–3 of **Table 3** under *Data and Observations*.
4. Refer to **Tables 1** and **2** to determine ABO and Rh blood types based on the agglutination reactions. Record the ABO and Rh blood types in column 4 of **Table 3**. Notify your teacher of your results.

### Procedure

1. Obtain an ABO/Rh blood-typing test kit from your teacher. Open the kit and assemble the contents for the procedure. Read the kit's instructions.
2. Obtain the blood sample from your teacher. Perform the ABO/Rh test as instructed by the kit. Pay close attention to the amount of time you need to wait for the samples to react.

### Cleanup and Disposal

1. Dispose of anything with blood on it immediately after use, according to your teacher's instructions.
2. Wipe down your work surface with the bleach solution and paper towels.

## Lab

## 9

## Use Blood Types to Help Solve a Crime, *continued*

### Data and Observations

Table 3

1 Anti-A Reaction (+/-)	2 Anti-B Reaction (+/-)	3 Anti-D Reaction (+/-)	4 ABO/Rh Blood Type

### Conclude and Apply

1. Your teacher will tell you the number of suspects of each blood type. The type of blood found at the crime scene is A<sup>+</sup>. Based on this information, how many suspects can be ruled out as possible perpetrators of the crime?

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2. Explain why blood types can be used only to rule out potential suspects and not to prove conclusively who committed a crime.

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3. If you could test for other blood-group types in addition to ABO and Rh, how might this affect the investigation?

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## Lab

## 9

**Use Blood Types to Help  
Solve a Crime, *continued*****Analyze and Conclude**

4. All of the ABO/Rh blood types are relatively common in the United States. The most common, O<sup>+</sup>, occurs in 38 percent of people; the least common, AB<sup>-</sup>, occurs in 1 percent of people. A<sup>+</sup>, which was found at the crime scene, occurs in 34 percent of people. Some blood-group systems do not show as much variation, and only a small percentage of people do not have the same blood type. How useful would a less variable blood-group system be for forensic analysis?

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**Lab  
10**

# The Missing Restaurant Owner

## The Problem

The following is a description of a fictitious murder case. The victim is a restaurant owner who was last known to be at a bus stop approximately 800 meters from his home. He vanished and was never seen again.

Witnesses testified that they heard yelling from the office of the victim's restaurant about the time the victim usually left work for home. Using this testimony, police established the identity of the man the victim was arguing with, and they questioned him as a suspect. The suspect had scratches on his face, which he claimed were from a fight he had the previous evening, and soil particles in his ring and bracelet. The suspect had no explanation for the soil particles or for the reports of yelling from the restaurant office. After arresting the suspect, the police searched his car, the restaurant office, and the surrounding countryside and gathered the following evidence:

## List of Evidence

### From the suspect's car (trunk):

- Bloodstained watch with the clasp missing (identified as the victim's)
- Strands of hair

### From the restaurant office:

- Clasp matching the watch from the suspect's trunk
- Blood samples from the floor

### Countryside:

- Bloodstained clothes wrapped in trash bags similar to those used by the restaurant; samples of hair from one of the suspect's dogs and thread from the suspect's sweater found on the clothes
- Knotted electrical cord with hairs that matched those found in the suspect's trunk

The hairs found in the car and on the electrical cord matched samples of the victim's hair taken from his home. Neither the murder weapon nor the body have been found. Your job is to evaluate the forensics evidence and come to a decision about the guilt or innocence of the suspect.

## Lab

## 10

The Missing Restaurant Owner, *continued*

## Part 1

**Blood Type Analysis** This case is peculiar because there is no body, only hair samples and bloodstains. Whose blood was found? The first thing to do is type the blood samples and then try to identify them.

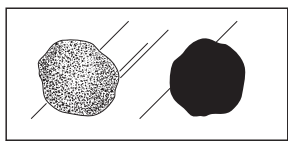
Three genes ( $i^A$ ,  $i^B$ ,  $i$ ) determine human blood type. Two genes ( $i^A$  and  $i^B$ ) code for two proteins, A and B, that are found on the surface of red blood cells. The  $i$  gene does not code for a protein. Because you inherit one gene from your mother and one from your father, there are several possible genotypes and phenotypes (i.e. blood types). If you have one copy of  $i^A$  ( $i^A i^A$  or  $i^A i$ ), then your red blood cells will have the A protein (blood type A). If you have one copy of  $i^B$  ( $i^B i^B$  or  $i^B i$ ), then your cells will

express the B protein (type B). If you have a copy of  $i^A$  and  $i^B$  ( $i^A i^B$ ), then your cells will express both proteins (type AB). If you only have  $i$  genes ( $ii$ ), then your blood cells will express no protein (type O).

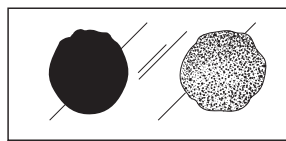
Blood typing is a fast, inexpensive, and easy procedure. To determine blood type, two drops or samples of blood are placed side by side on a glass slide. To one side, a drop of an antibody (anti-A) raised against the A protein is added, while to the other side, a drop of an antibody (anti-B) raised against the B protein is added. If A protein is present on the blood cells, then anti-A will cause them to clump together; likewise anti-B will cause the cells to clump if B protein is present. If no clumping is observed, then neither protein is present.

## Procedure

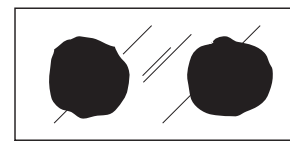
1. Blood type slides from several of the pieces of bloodstained evidence are shown below (anti-A is on the left side and anti-B is on the right side of each slide). The blood-type slide from the suspect is also shown. Because police did not have a blood type for the missing restaurant owner, blood samples were drawn from his parents and are shown as well. Analyze the slides and determine all of the blood types.



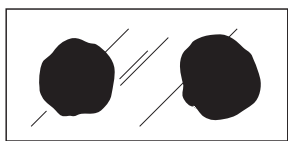
Father



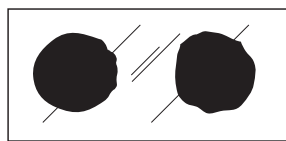
Mother



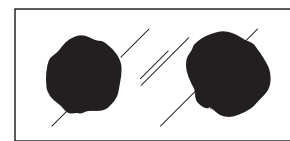
Suspect



Restaurant office



Watch



Clothing

## Blood Types:

Father \_\_\_\_\_

Mother \_\_\_\_\_

Suspect \_\_\_\_\_

Clothing \_\_\_\_\_

Restaurant office \_\_\_\_\_

Watch \_\_\_\_\_

## Analyze and Conclude

2. Is it possible that the blood found in the office and on the clothing was the missing restaurant owner's blood? What was his blood type? Use your knowledge of genetic crosses (i.e., Punnett squares) to determine the victim's possible blood types from the information that you have. Show your work in the space below.

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## Part 2

**DNA-Typing Analysis** The blood-typing evidence did not clearly reveal whether the bloodstains belonged to the missing victim. So, investigators turned to a newer technique called DNA fingerprinting or DNA typing. The DNA sequence of human genes (DNA that codes for proteins) follows fairly regular patterns; however, the DNA sequences between genes (i.e. non-coding DNA) differ greatly among individuals. Scientists understand how to identify and analyze these special sequences, so they can tell if two DNA samples probably came from the same person. Since DNA is inherited from each parent, an individual shares patterns within these sequences with his or her parents. Therefore, scientists can also tell whether or not two samples came from individuals who are related.

To conduct a DNA typing experiment, traces of DNA are collected from an individual or crime scene. Then, a technique called polymerase chain reaction (PCR) is used to make copies of the DNA to increase the amount. PCR enables analyzable DNA to be obtained from extremely small samples. The DNA is then cut into fragments using specific enzymes called restriction enzymes.

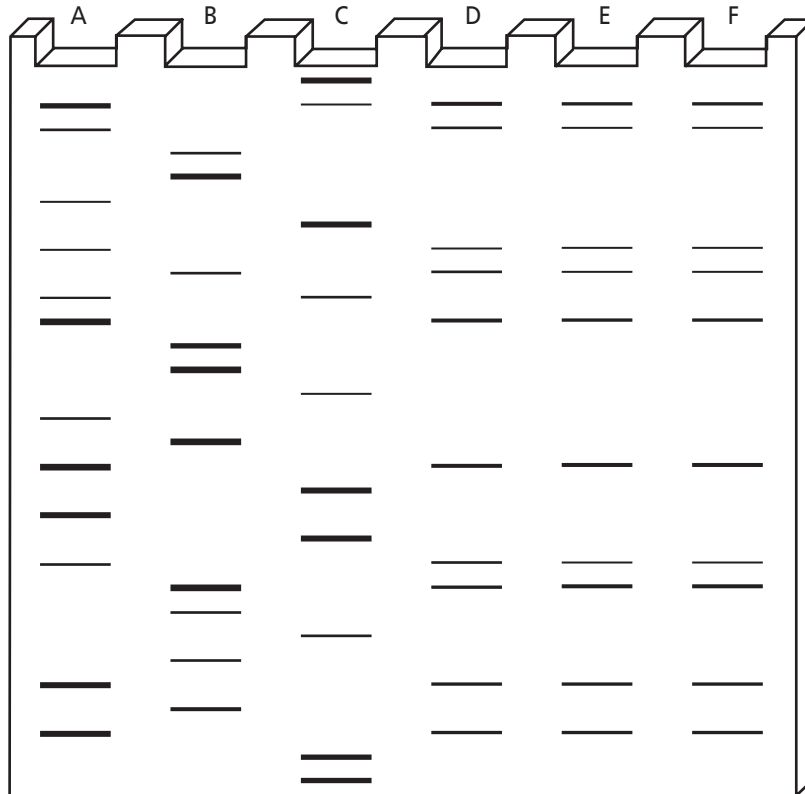
The fragments, which are of different sizes, are separated using a technique called gel electrophoresis. The pattern of separated DNA fragments are transferred from the gel to a piece of filter paper and mixed with radioactive segments of DNA that correspond to an individual's unique sequences. After exposing the filter paper to the radioactive compounds, it is dried and exposed to X-ray film; the fragments containing an individual's unique sequences will show up as dark bands on the film. Now you have a DNA fingerprint. In practice, many DNA samples are loaded on the gel for comparison.

DNA typing compares the bands from a known sample to those of suspects. The bands from two different samples will match only if the same individual donated both samples. Even though an individual's pattern of bands is unique, children will share bands with both parents.

To verify the identity of the victim, DNA typing was done using samples of DNA from his mother (A), father (B), the suspect (C), the watch (D), the restaurant office (E), and the clothing (F). Read the gels on the next page from top to bottom and compare the columns.

## Lab

## 10

The Missing Restaurant Owner, *continued*

3. Was the victim genetically related to the mother and father tested in columns A and B? Explain your answer.

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4. Given that there was no body or murder weapon found, do you think that the evidence indicates beyond a reasonable doubt that the suspect murdered the victim? Explain your answer.

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### Analyze and Conclude

5. Form groups of four students. Two students should act as prosecutors while the other two act as defense attorneys. Each pair should prepare an argument explaining why the suspect is guilty (prosecution) or innocent (defense). Use the lines below to list ideas you will use in your argument.

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