Biology

Laboratory Manual

Twelfth Edition

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Mc Graw Hill Education

BIOLOGY LABORATORY MANUAL, TWELFTH EDITION

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Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

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Contents

Preface

We have designed this laboratory manual for an introductory biology course with a broad survey of basic laboratory techniques. The experiments and procedures are simple, safe, easy to perform, and especially appropriate for large classes. Few experiments require more than one class meeting to complete the procedure. Each exercise includes many photographs and illustrations, traditional topics, and experiments that help students learn about life. Procedures within each exercise are numerous and discrete so that an exercise can be tailored to the needs of the students, the style of the instructor, and the facilities available.

TO THE STUDENT

We hope this manual is an interesting guide to many areas of biology. As you read about these areas, you'll probably spend equal amounts of time observing and experimenting. Don't hesitate to go beyond the observations that we've outlined—your future success as a scientist and an informed citizen depends on your ability to seek and notice things that others may overlook. Now is the time to develop this ability with a mixture of hard work and relaxed observation. Have fun, and learning will come easily. Also, remember that this manual is designed with your instructors in mind as well. Go to them often with questions—their experience is a valuable tool that you should use as you work.

TO THE INSTRUCTOR

This manual's straightforward approach emphasizes experiments and activities that optimize students' investment of time and your investment of supplies, equipment, and preparation. Simple, safe, and straightforward experiments are most effective if you interpret the work in depth. Most experiments can be done easily by a student in 2 to 3 hours. Terminology, structures, photographs, and concepts are limited to those that the student can readily observe and understand. In each exercise we have included a few activities requiring a greater investment of effort if resources are available, but omitting them will not detract from the objectives.

This manual functions best with an instructor's guidance and is not an autotutorial system. We've tried to guide students from observations to conclusions, to help students make their own discoveries, and to make the transition from observation to understanding biological principles. But discussions and interactions between student and instructor are major components of a successful laboratory experience. Be sure to examine the "Questions for Further Study and Inquiry" in each exercise. We hope they will help you expand students' perceptions that each exercise has broad application to their world.

DIGITAL INTEGRATION

As educators, we recognize that today's students are digital learners. Virtually every exercise of this manual is accompanied by tailor-made digital resources, including assignable questions and a variety of high-definition videos, PowerPoint images, and other resources that demonstrate basic techniques, emphasize biological principles, test for understanding, and engage students as they learn biology in the laboratory.

Digital resources are available to instructors at **connect.mheducation.com.** Instructors will want to assign these resources to help students know what they'll be doing, what principles they'll be investigating, and what concepts they'll need to understand before coming to lab.

WHAT'S NEW IN THIS EDITION

Throughout the manual, we have expanded and improved several of the most popular and effective features of previous editions, including

- **Learning Objectives** have been updated to provide an overview of what students will do and learn in the exercise.
- **Procedures** and **Doing Biology Yourself** require students to *do* biology as they apply skills they've learned to develop and study hypotheses about biology.
- **Questions** throughout each exercise encourage students to pause and think about their data and what they've learned in lab.
- **Questions for Further Study and Inquiry** at the end of each exercise help students apply what they've learned to broader topics and issues in biology.
- **Writing to Learn Biology** encourages students to develop their ideas about what they learned in lab.

- **Caution** and **Safety First** icons make students aware of safety issues associated with the procedures they'll use in lab.
- Boxed readings titled **Inquiry-Based Learning** encourage students to apply what they've learned to independently answer questions about intriguing biological topics.
- Updated health-related exercises help students better understand topics such as blood pressure, atherosclerosis, and their risk of cardiovascular disease.
- Several illustrations have been replaced with photographs to provide more realistic images to support the Exercise content.
- Approximately 60 illustrations and photos have been revised.
- Questions within procedures now include lines on which students can write their answers.
- An assignable, updated library of videos and Connect questions helps students prepare for lab and understand the instruments and techniques that will be important for their investigations. Instructors may assign these videos before class time to help ensure that students arrive prepared for lab.

Exercise-Specific Changes

- Exercise 1—Additional explanation provided for both mean and standard deviation
- Exercise 2—Mass, volume, and median are further defined; new illustration in figure 2.3 on measuring the volume of liquid; figure 2.4b has explanatory labels added
- Exercise 3—Additional questions have been added to Procedure 3.6 Using a dissecting microscope
- Exercise 4—Several illustrations have better labels; a new photo is supplied for figure 4.6a *Elodea* cells; figure 4.13 has been redrawn to more directly correlate to the associated photo; a new question is added to Questions for Further Study and Inquiry to compare plant and animal cells
- Exercise 6—Qualitative tests are defined; a new photo has been added to figure 6.2 to explain Benedict's test
- Exercise 7—Clarifying edits made to introductory material
- Exercise 9—Explanations of hypotonic, hypertonic, and isotonic are expanded
- Exercise 10—Steps of Procedures 10.1 and 10.2 are clarified; a new question on experimental design has been added to Questions for Further Study and Inquiry
- Exercise 13—Figure 13.2 caption is expanded
- Exercise 14—Explanation of the structure of chromatids is expanded
- Exercise 15—Labels for figure 15.2 have been added for paternal versus maternal chromosomes; description of the structure of replicated versus nonreplicated chromosomes has been clarified; figure 15.6 is new; figure 15.7 is revised to clarify the state and number of chromosomes in first polar bodies and second polar bodies, and corpus albicans has been labeled and added as a defined term in the text
- Exercise 16—Global prevalence of genetically transformed crops has been updated to 2017 statistics
- Exercise 17—Figure 17.4 has a panel of 3 new photos on sickle cell anemia; figure 17.6 contains improved photos of hairlines
- Exercise 18—Definition of evolution is revised to be more concise; questions about Hardy-Weinberg genetics are expanded for clarity; a new question about the effect of natural selection on sickle cell anemia has been added to Questions for Further Study and Inquiry
- Exercise 19—Figure 19.2 has been revised to better illustrate lineages of human evolution; the term "diastema" has been added and defined; figure 19.4 is relabeled for clarity
- Exercise 20—Procedure 20.4 is expanded to help students design and implement experimental controls.
- Exercise 22—Formula for population growth is revised; data for Figure 22.5 are updated to reflect 2018 predictions; question 6 is expanded to include 2018 population values and growth rates
- Exercise 23—Question 1 is revised to emphasize hypothesis testing; table 23.3 is reorganized to accept handwritten student data
- Exercise 24—Organization of domains and kingdoms is updated to current taxonomy; table 24.1, prokaryotic versus eukaryotic characteristics, is modified for precision; figure 24.2, structure of a bacterial cell, is revised and contains a new photo; explanation of binary fission is expanded to include protein FtsZ and its role in cell separation
- Exercise 25—Explanations of Archaeplastida and the term "protist" are clarified; in table 25.2 the list of chlorophylls diagnostic to each type of algae is updated; figure 25.4 is relabeled to clarify sexual versus asexual reproductive paths; figure 25.8 contains a new photo of Volvox colonies

- Exercise 26—Photomicrograph and illustration of African sleeping sickness blood cells and parasites are revised to clarify their relationship
- Exercise 27—Explanations of fungal sporangiophores and sporangia are expanded; figure 27.13 is modified to better show the diagnostic reproductive structure, ascus; Questions for Further Study and Inquiry has a new question to explain the benefit of fungi to other organisms
- Exercise 31—A learning objective is added on understanding flower structure and function; the explanation of sporogenesis is expanded; a Question for Further Study and Inquiry has been added to help students understand flower parts
- Exercise 32—A new question is added to Questions for Further Study and Inquiry on common leaf morphologies
- Exercise 35—The definition of bioassay is revised
- Exercise 36—Introductions to terms animals, multicellular, ancient, and primitive have been clarified; description of intracellular versus extracellular digestion in poriferans has been clarified
- Exercise 37—Taxonomic hierarchy of the classes and subphyla of flatworms is updated; the groups Neodermata and Turbellaria have been redefined and updated; taxonomy of tapeworms is updated
- Exercise 39—Taxonomy of major arthropod classes has been updated and reorganized to include Chelicerata, Crustacea, Myriapoda, and Hexapoda; table 39.3 has been relabeled to reflect updated arthropod taxonomy
- Exercise 40—The taxonomy of pre-vertebrate groups has been updated; class Actinopterygidii has replaced Osteichthyes; figure 40.21 of amphibian transitional stages is revised
- Exercise 41—Figure 41.2 has revised labeling; figure 41.3 is relabeled to distinguish flat cuboidal and columnar cells more clearly; figure 41.4 is relabeled to show Bowman's capsule more clearly; figure 41.5 is relabeled to more clearly distinguish columnar cells; figure 41.7 has been replaced to better show stratified squamous epithelium; types of connective tissue have been separated into connective tissue proper and special connective tissue
- Exercise 42—Descriptions of the appendicular skeleton and the axial skeleton are added; the number of skull, spine, and rib cage bones has been updated to conventional values; figure 42.2 is new; Figure 42.4 has been replaced with improved images of normal and osteoporotic bone; revisions to Questions for Further Study and Inquiry
- Exercise 43—A new learning objective is added to distinguish between isotonic and isometric contractions; explanations of muscle load, muscle tone, and muscle tension are expanded; figure 43.2 is relabeled to clearly distinguish between flexion and extension; Procedure 43.1 concerning flexion and extension of the forearm has been modified for clarity
- Exercise 44—Descriptions of negative pressure and its role in breathing have been expanded; procedures to distinguish the role of intercostal muscles and breathing are expanded and clarified; Procedure 44.1 has been modified for more consistent chest expansion measurements; typical values for tidal, expiratory, inspiratory, and residual volumes have been provided; directions for measuring breathing rate in Procedure 44.7 are clarified
- Exercise 46—Figure 46.1 has been modified to illustrate fovea centralis; Procedure 46.3 has been modified to accommodate lab partners
- Exercise 47—A new Question 2 has been added; Question 3 has been expanded to provide more examples and practice with terms such as cranial, caudal, lateral, distal, etc.; directions for the skinning and abdominal incision during rat dissection are expanded
- Exercise 48—Descriptions of the thyroid gland and diaphragm are expanded; explanatory questions about the lung structure and heart musculature are expanded
- Exercise 49—Figure 49.4 has been revised and enlarged to better show the structure and cross section of a kidney
- Exercise 50—Distinction has been enhanced between the animal and vegetal poles
- Exercise 51—Directions are enhanced for Procedure 51.1 to examine kinesis in pill bugs; directions are enhanced for Procedure 51.2 to study agonistic behavior in fighting fish, to encourage better creativity by the students in experimental design; a new question has been added to Questions for Further Study and Inquiry
- Appendix II has been updated to include upcoming changes to how a basic unit of the metric system is defined

Teaching and Learning Tools

McGraw-Hill Connect® Biology

McGraw-Hill Connect Biology provides online presentation, assignment, and assessment solutions. It connects your students with the tools and resources they'll need to succeed at **connect.mheducation.com**.

With Connect Biology, you can deliver assignments and quizzes online. A robust set of questions and activities is presented and aligned with this lab manual's learning outcomes. Pre-lab worksheets and Investigation worksheets are also included within Connect. As an instructor, you can edit existing questions and write entirely new questions. Track students' performance—by question, by assignment, or in relation to the class overall—with detailed grade reports. Integrate grade reports easily with Learning Management Systems (LMS), such as Blackboard—and much more.

McGraw-Hill Create™

With **McGraw-Hill Create**, you can easily rearrange exercises, combine material from other content sources, and quickly upload content you have written, such as your course syllabus or teaching notes. Find the content you need in Create by searching through thousands of leading McGraw-Hill textbooks. Arrange your book to fit your teaching style. Create even allows you to personalize your book's appearance by selecting the cover and adding your name, school, and course information. Order a Create book and you'll receive a complimentary print review copy in 3–5 business days or a complimentary electronic review copy (eComp) via e-mail in minutes. Go to **create.mheducation.com** today and register to experience how McGraw-Hill Create empowers you to teach *your* students *your* way.

Laboratory Resource Guide

The *Laboratory Resource Guide* is essential for instructors and laboratory assistants and is available free to adopters of the Laboratory Manual within Connect under the Instructor Resources tab.

Welcome to the Biology Laboratory

Welcome to the biology laboratory! Although reading your textbook and attending lectures are important ways of learning about biology, nothing can replace the importance of the laboratory. In lab you'll get hands-on experience with what you've heard and read about biology—for example, you'll observe organisms, do experiments, test ideas, collect data, and make conclusions about what you've learned. You'll do biology.

You'll enjoy the exercises in this manual—they're interesting and informative and can be completed within the time limits of your laboratory period. We've provided questions to test your understanding of what you've done; in some of the exercises, we've also asked you to devise your own experiments to answer questions that you've posed. To make these exercises most useful and enjoyable, follow these guidelines noted in the next sections.

THE IMPORTANCE OF COMING TO CLASS

Biology labs are designed to help you experience biology firsthand. To do well in your biology course, you'll need to attend class and pay attention. To appreciate the importance of class attendance as it relates to making a good grade in your biology course, examine figure 1, which is a graph showing how students' grades in an introductory biology

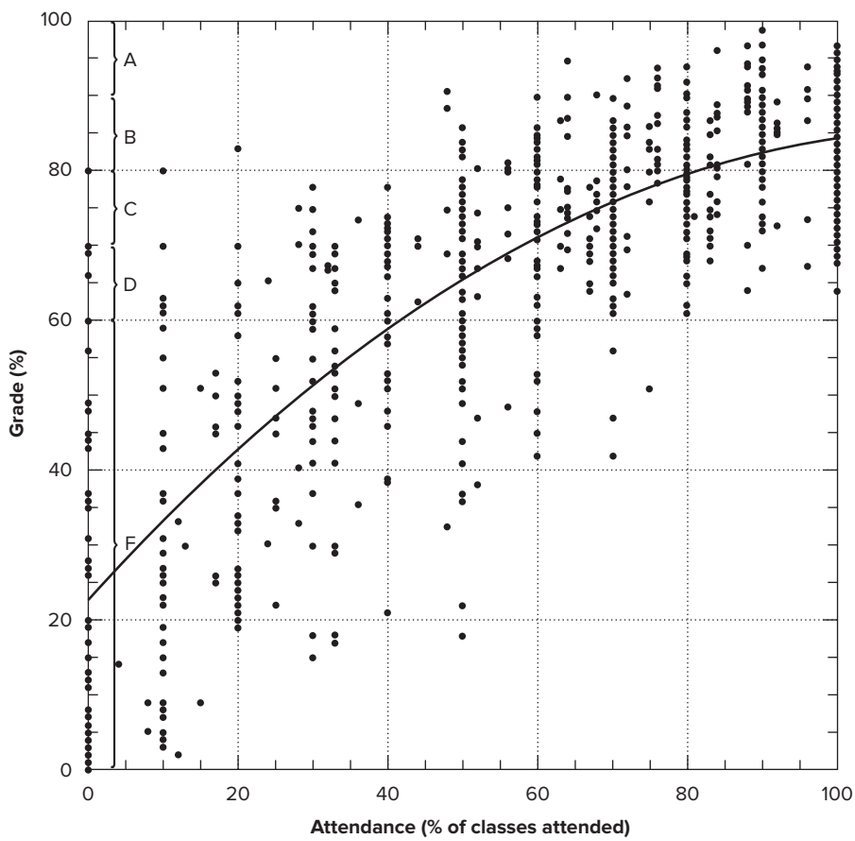

Figure 1 Relationship of students' grades in an introductory biology course to their rates of class attendance.

course correlate to their rates of class attendance. Data are from a general biology class at the University of Minnesota. On page xii, write an analysis of the data shown in figure 1. What do these data mean?

BEFORE COMING TO LAB

Watch the lab video. Videos are provided for several of the labs in this manual. Be sure to watch any assigned video associated with the lab you will be completing. These videos will help you know more about what you will be doing, what principles you will be investigating, and what concepts you need to understand before coming to lab.

Read the exercise before coming to lab. This will give you a general idea about what you're going to do, as well as why you're going to do it. Knowing this will not only save time, it will also help you finish the experiments and make you aware of any safety-related issues associated with the lab.

Review any of the lab safety concerns. Before doing any procedures, you'll encounter a section of each exercise titled "SAFETY FIRST" that is marked with its icon:

This icon will warn you of safety concerns (e.g., solvents, acids, bases, hotplates) associated with the work. If you have questions about these safety issues, contact your lab instructor before starting the lab work.

Notify your instructor if you are pregnant, are colorblind, are taking immunosuppressive drugs, have allergies, or have any other conditions that may require precautionary measures. Also, before coming to lab, cover any cuts or scrapes with a sterile, waterproof bandage.

WHEN IN LAB

1. Know what you are going to do. Read and understand the lab before coming to lab.
2. Don't start the exercise until you've discussed the exercise with your laboratory instructor. She or he will give you specific instructions about the lab and tell you how the exercise may have been modified.
3. Work carefully and thoughtfully, and stay focused as you work. You'll be able to finish each exercise within the allotted time if you are well prepared and stay on task.
4. Discuss your observations, results, and conclusions with your instructor and lab partners. Perhaps their comments and ideas will help you better understand what you've observed.
5. Always follow instructions and follow safety guidelines presented by your instructor.
6. If you have questions, ask your instructor.

SAFETY IN THE LABORATORY

Laboratory accidents can affect individuals, classes, or the entire campus. To avoid such accidents, the exercises in this manual were designed with safety as a top priority. You'll be warned about any potentially hazardous situations or chemicals with this image:

When you see this image, pay special attention to the instructions.

The laboratory safety rules listed in table 1 will help make lab a safe place for everyone to learn biology. Remember, it is much easier to prevent an accident than to deal with its consequences.

Read the laboratory safety rules listed in table 1. If you do not understand them, or if you have questions, ask your instructor for an explanation. Then complete table 1 and sign the statement at the bottom of page xii.

BEFORE YOU LEAVE LAB

Put away all equipment and glassware, and wipe clean your work area.

AFTER EACH LABORATORY

Soon after each lab, review what you did. What questions did you answer? What data did you gather? What conclusions did you make?

Also note any questions that remain. Try to answer these questions by using your textbook or visiting the library. If you can't answer the questions, discuss them with your instructor. Welcome to the biology laboratory!

Table 1

Laboratory Safety Rules

Rule	Why is this rule important? What could happen if this rule is not followed?
Behave responsibly. No horseplay or fooling around while in lab.	
Do not bring any food or beverages into lab, and do not eat, drink, smoke, chew gum, chew tobacco, or apply cosmetics when in lab. Never taste anything in lab. Do not put anything in lab into your mouth. Avoid touching your face, chewing on pens, and other similar behaviors while in lab. Always wear shoes in lab.	
Unless you are told otherwise by your instructor, assume that all chemicals and solutions in lab are poisonous, and act accordingly. Never pipette by mouth. Always use a mechanical pipetting device (e.g., a suction bulb) to pipette solutions. Clean up all spills immediately, and report all spills to your instructor.	
Wear safety goggles when working with chemicals. Carefully read the labels on bottles and know the chemical you are dealing with. Do not use chemicals from an unlabeled container, and do not return excess chemicals back to their container. Report all spills to your instructor immediately.	
Unless your instructor tells you to do otherwise, do not pour any solutions down the drain. Dispose of all materials as per instructions from your instructor.	
If you have long hair, tie it back. Don't wear dangling jewelry. If you are using open flames, roll up loose sleeves. Wear contact lenses at your own risk; contacts hold substances against the eye and make it difficult to wash your eyes thoroughly.	
Treat living organisms with care and respect.	
Your instructor will tell you the locations of lab safety equipment, including fire extinguishers, fire blanket, eyewash stations, and emergency showers. Familiarize yourself with the location and operation of this equipment.	
If anything is splashed into your eyes, wash your eyes thoroughly and immediately. Tell your lab instructor what happened.	
Notify your instructor of any allergies to latex, chemicals, stings, or other substances.	
If you break any glassware, do not pick up the pieces of broken glass with your hands. Instead, use a broom and dustpan to gather the broken glass. Ask your instructor how to dispose of the glass.	
Unless told by your instructor to do otherwise, work only during regular, assigned hours when the instructor is present. Do not conduct any unauthorized experiments; for example, do not mix any chemicals without your instructor's approval.	
Do not leave any experiments unattended unless you are authorized by your instructor to do so. If you leave your work area, slide your chair under the lab table. Keep walkways and desktops clean and clear by putting books, backpacks, and so on along the edge of the room, in the hall, in a locker, or in an adjacent room. Keep your work area as clean and uncluttered as possible.	
Don't touch or put anything on the surface of hotplates unless told to do so. Many types of hotplates have no visible sign that they are hot. Assume they are hot.	
Know how to use the equipment in lab. Most of the equipment is expensive; you may be required to pay all or part of its replacement cost. Keep water and solutions away from equipment and electrical outlets. Report malfunctioning equipment to your instructor. Leave equipment in the same place and condition that you found it. If you have any questions about or problems with equipment, contact your instructor.	
Know what to do and whom to contact if there is an emergency. Know the fastest way to get out of the lab. Immediately report all injuries—no matter how minor—to your instructor. Seek medical attention immediately if needed. If any injury appears to be life-threatening, call 911 immediately.	
At the end of each lab, clean your work area, wash your hands thoroughly with soap, slide your chair under the lab table, and return all equipment and supplies to their original locations. Do not remove any chemicals or equipment from the lab.	

Name _____

Lab Section _____

Your lab instructor may require that you submit this page at the end of today's lab.

1. In the space below, write an analysis of the data shown in figure 1.

After completing table 1, read and sign this statement:

2. I have read and I understand and agree to abide by the laboratory safety rules described in this exercise and discussed by my instructor. I know the locations of the safety equipment and materials. If I violate any of the laboratory safety rules, my instructor will lower my grade and/or remove me from the lab.

_____

Signature

_____

Name (printed)

_____

Date

Separating Organic Compounds

Column Chromatography, Paper Chromatography, and Gel Electrophoresis

EXERCISE

7

Learning Objectives

By the end of this exercise you should be able to:

1. Explain how column chromatography, paper chromatography, and gel electrophoresis are used to separate compounds from mixtures.
2. Use column chromatography, paper chromatography, and gel electrophoresis to separate organic compounds from mixtures.

Please visit connect.mheducation.com to review online resources tailored to this lab.

Cells are a mixture of the types of organic compounds that you studied in Exercise 6 ("Biologically Important Molecules"), including carbohydrates, proteins, lipids, and nucleic acids. Biologists characterize and study these compounds to understand how organisms function. This requires that biologists separate the compounds, such as amino acids and nucleotides, from mixtures.

Biologists often use **chromatography** to separate mixtures. In this procedure, the mixture is dissolved in a fluid that moves through a matrix made of materials such as beads, paper, or a gel. During the process, the different parts of the mixture move at different speeds, causing them to separate. In today's exercise you will use column chromatography, paper chromatography, and gel electrophoresis to separate compounds from mixtures. The procedures are simple and model how these techniques are used by biologists in their research.

COLUMN CHROMATOGRAPHY

Column chromatography often separates molecules according to their size and shape. The procedure is simple and involves placing a sample onto a matrix that is a column of beads having tiny pores. Molecules can move through the column of beads in two ways: a fast route between the beads or a slower route through the tiny pores of the beads. Molecules too big to fit into the beads' pores move through the column quickly, whereas smaller molecules enter the beads' pores and move through the column more slowly (fig. 7.1). Movement of the molecules is analogous to going through or walking around a maze: It takes more time to walk through a maze than to walk around it.

The apparatus used for column chromatography is shown in figure 7.2 and consists of a chromatography column, a matrix, and a buffer.

- The **chromatography column** is a tube having a frit and a spout at its bottom. The frit is a membrane or porous disk that supports and keeps the matrix in the column but allows water and solutes to pass.
- The **matrix** is the material in the column that fractionates, or separates, the chemicals mixed in the sample. The matrix consists of beads having tiny pores and internal channels. The size of the beads' pores determines the matrix's **fractionation range,** which is the range of molecular weights the matrix can separate. These molecular weights are measured in units called daltons; 1 dalton $\approx$ 1 g mole^{-1}. Different kinds of matrices have different fractionation ranges. In today's exercise you'll use a matrix having a fractionation range of 1000 to 5000 daltons. As they move through the matrix, small molecules spend much time in the maze of channels and pores in the matrix. Large molecules do not.
- The **buffer** helps control the pH of the sample (see Exercise 5). A buffer is a solution with a known pH that resists changes in pH if other chemicals are added. The pH of a buffer remains relatively constant. This is important because the shapes of molecules such as proteins often vary according to their pH. The buffer carries the sample through the matrix, which separates the chemicals mixed in the sample.

Column chromatography can also separate compounds having the same molecular weight but different shapes. Compact, spherical molecules penetrate the pores and channels of the matrix more readily than do rod-shaped molecules. Thus, spherical molecules move through a column more slowly than do rod-shaped molecules.

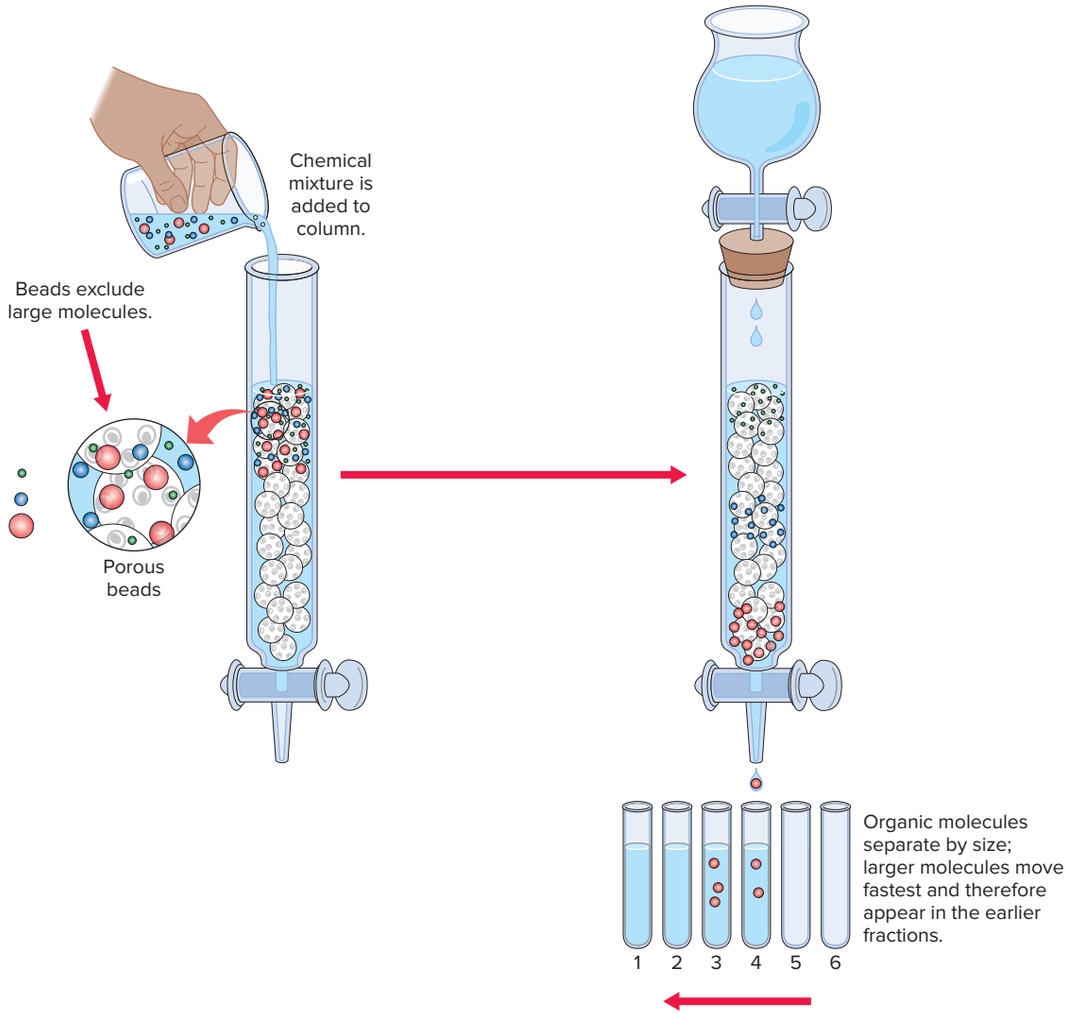

Figure 7.1 Separation of organic molecules by column chromatography. As the solution flows through the column, the smaller molecules are slowed down as they pass through the pores of the beads. Medium-sized molecules will pass through a bead with pores less frequently, and the largest molecules will quickly flow around all the beads. The exiting fluid is collected in fractions. The first fractions collected will contain the largest molecules.

During column chromatography, the buffer containing the sample mixture of chemicals moves through the column and is collected sequentially in test tubes from the bottom of the column. Biologists then assay the content of the tubes to determine which tubes contain the compounds in which they are interested.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Question 1

In today's exercise you will isolate colored compounds from mixtures. However, it is important to note that most biological samples are colorless. How would you determine the contents of the test tubes if all of the samples were transparent?

Procedure 7.1 Separate compounds by column chromatography

1. Label nine microtubes 1–9.
2. Obtain an apparatus for column chromatography and carefully remove all of the buffer from above the beads with a transfer pipet. Do not remove any of the matrix.
3. Obtain a sample to be separated. The sample is a mixture of Orange G (molecular weight = 452 g $mole^{-1}$) and a rodlike polymer of glucose stained blue and having a molecular weight of about 2,000,000 g $mole^{-1}$.

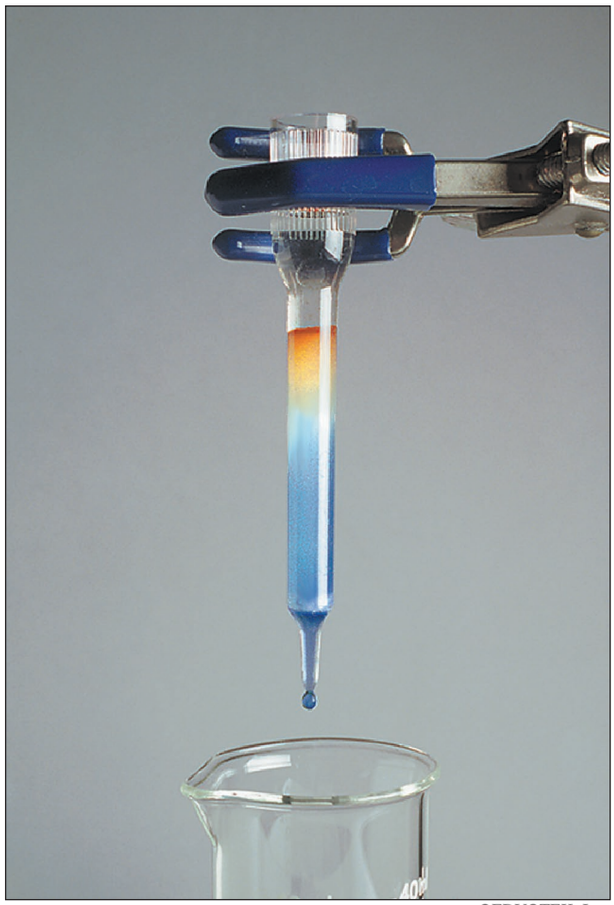

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Figure 7.2 Apparatus for column chromatography. A fraction is being collected, drop by drop, in the beaker. Smaller fractions would be collected in test tubes.

4. Use a transfer pipet to slowly load 0.2 mL of the sample onto the top of the beads. Drip the sample down the inside walls of the column.
5. Place a beaker under the column.
6. Slowly open the valve. This will cause the sample to enter the beads. Close the valve after the sample has completely entered the beads (i.e., when the top of the beads is exposed to air).
7. Use a transfer pipet to slowly cover the beads with buffer. Add buffer until the reservoir is almost full.
8. Hold microtube 1 under the column and open the valve until you have collected about 1.0 mL of liquid.
9. Repeat step 8 for tubes 2–9. The sample will separate in the column.
10. Identify the tubes containing (1) the most orange dye and (2) the most blue dye that eluted from the column.
11. Refill the reservoir with buffer and cover the reservoir with Parafilm.

Question 2

a. Was the color separation distinctive? Would you expect a longer column to more clearly separate the compounds? Why or why not?

b. Suppose your sample had consisted of a mixture of compounds having molecular weights of 50,000, 100,000, and 1,000,000 g mole^{-1}. What type of results would you predict? Explain your answer.

PAPER CHROMATOGRAPHY

Biologists often analyze the amino acid content of samples to determine protein sequences and enzyme structures. Amino acids can be separated by partitioning them between the stationary and mobile phases of paper chromatography. The **stationary phase** is the paper fibers, and the **mobile phase** is an organic solvent that moves along the paper.

Separation by paper chromatography begins by applying a liquid sample to a small spot on an origin line at one end of a piece of chromatography paper. The edge of the paper is then placed in a solvent. As the solvent moves up the paper, any sample molecules that are soluble in the solvent will move with the solvent. However, some molecules move faster than others based on their solubility in the mobile phase and their attraction to the stationary phase. These competing factors are different for different molecular structures, so each type of molecule moves at a different speed and occurs at a different position on the finished chromatogram.

Amino acids in solution have no color but react readily with molecules of ninhydrin to form a colored product. A completed chromatogram is sprayed with a ninhydrin solution and heated to detect the amino acids. The distance of these spots from the origin is measured and used to quantify the movement of a sample. The resulting R_f value (retardation factor) characterizes a known molecule in a known solvent under known conditions and is calculated as follows:

$$R_f = \frac{\textbf{Distance moved by sample}}{\textbf{Distance from origin to solvent front}}$$

Procedure 7.2 Separate amino acids and identify unknowns by paper chromatography

1. Obtain a piece of chromatography paper 15 cm square. Avoid touching the paper with your fingers. Use gloves, tissue, or some other means to handle the paper because oils from your skin will alter the migration of the molecules on the paper.

Table 7.1

Chromatography Data for Determining Amino Acid Unknowns

Tick Mark Number	Amino Acid or Sample Number	Distance to Solvent Front	Distance Traveled by Sample	R_f	Identity of Unknown
1					
2					
3					
4					
5					

2. Lay the paper on a clean paper towel. Then use a pencil to draw a light line 2 cm from the bottom edge of the paper.

3. Draw five tick marks at 2.5 cm intervals from the left end of the line. Lightly label the marks 1–5 below the line.

4. Locate the five solutions available for the chromatography procedure. Three of the solutions are known amino acids. One solution is an unknown. The last solution is a plant extract or another unknown.

5. Use a wooden or glass applicator stick to "spot" one of the solutions on mark #1. To do this, dip the stick in the solution and touch it to the paper to apply a small drop (2–3 mm in diameter). Let the spot dry; then make three to five more applications on the same spot. Dry between each application. Record in table 7.1 the name of the solution next to the appropriate mark number.

6. Repeat step 5 for each of the other solutions.

7. Staple or paper clip the edges of the paper to form a cylinder with the spots on the outside and at the bottom.

8. Obtain a quart jar containing the chromatography solvent. The solvent should be 1 cm or less deep. The solvent consists of butanol, acetic acid, and water (2:1:1).

9. Place the cylinder upright in the jar (fig. 7.3). *The solvent must be below the pencil line and marks*. Close the lid to seal the jar.

10. Keep the jar out of direct light and heat. Allow the solvent to move up the paper for 2 hours (h) but not all the way to the top.

11. Open the jar and remove the chromatogram. Unclip and flatten the paper. Dry it with a fan or hair dryer. Work under a fume hood if possible to avoid breathing the solvent vapors.

12. Spray the chromatogram with ninhydrin. Carefully dry the chromatogram with warm air.

13. Circle with a pencil each of the spots. Measure the distance each of the spots has traveled and calculate the R_f for each spot. Record the values in table 7.1.

14. Determine the contents of the unknown solutions by comparing R_f values. Record the results in table 7.1.

GEL ELECTROPHORESIS

Gel electrophoresis separates molecules according to their charge, shape, and size (fig. 7.4). Buffered samples (mixtures of organic chemicals) are loaded into a Jello-like gel, after which an electrical current is placed across the gel. This current moves the charged molecules toward either the cathode or anode of the electrophoresis apparatus. The speed, direction, and distance that each molecule moves are related to its charge, shape, and size.

The apparatus for gel electrophoresis is shown in figure 7.5 and consists of an electrophoresis chamber, gel, buffer, samples, and a power supply.

- The gel is made by dissolving agarose powder (a derivative of agar) in hot buffer. When the solution cools, it solidifies into a gel having many pores that function as a molecular sieve. The gel is submerged in a buffer-filled chamber containing electrodes.
- The buffer conducts electricity and helps control the pH. The pH affects the stability and charge of the samples.
- The samples are mixtures of chemicals loaded into wells in the gel. These samples move in the gel during electrophoresis. Samples are often mixed with glycerol

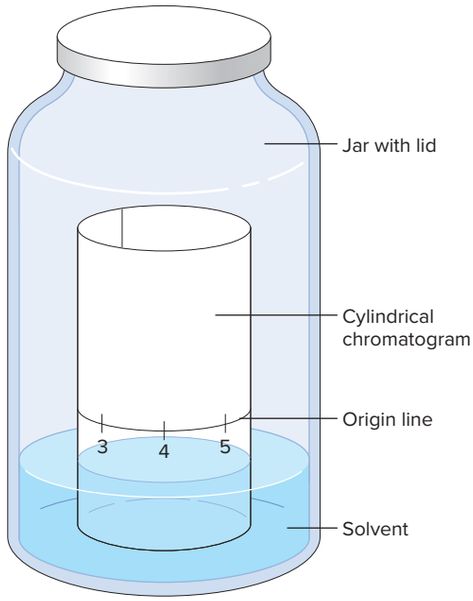

Figure 7.3 Apparatus for paper chromatography. Numbers on the chromatogram indicate the positions of multiple samples applied to the chromatogram. The samples will move up the chromatogram along with the solvent.

or sucrose to make them denser than the buffer so that they will not mix with the buffer.

- The power supply provides a direct current across the gel. Charged molecules respond to the current by moving from the sample wells into the gel. Negatively charged molecules move through the gel toward the positive electrode (anode), whereas positively charged molecules move through the gel toward the negative electrode (cathode). The greater the voltage, the faster the molecules move.

The sieve properties of the gel affect the rate of movement of a sample through the gel. Small molecules move more easily through the pores than do larger molecules. Consequently, small, compact (e.g., spherical) molecules move faster than do large, rodlike molecules. If molecules have similar shapes and molecular weights, the particles having the greatest charge move fastest and, therefore, the farthest.

Procedure 7.3 Separate organic molecules by gel electrophoresis

1. Obtain an electrophoresis chamber. Cover the ends of the bed as shown in figure 7.6 and demonstrated by your instructor.
2. Place a six-tooth comb in or near the middle set of notches of the gel-cast bed. There should be a small space between the bottom of the teeth and the bed.

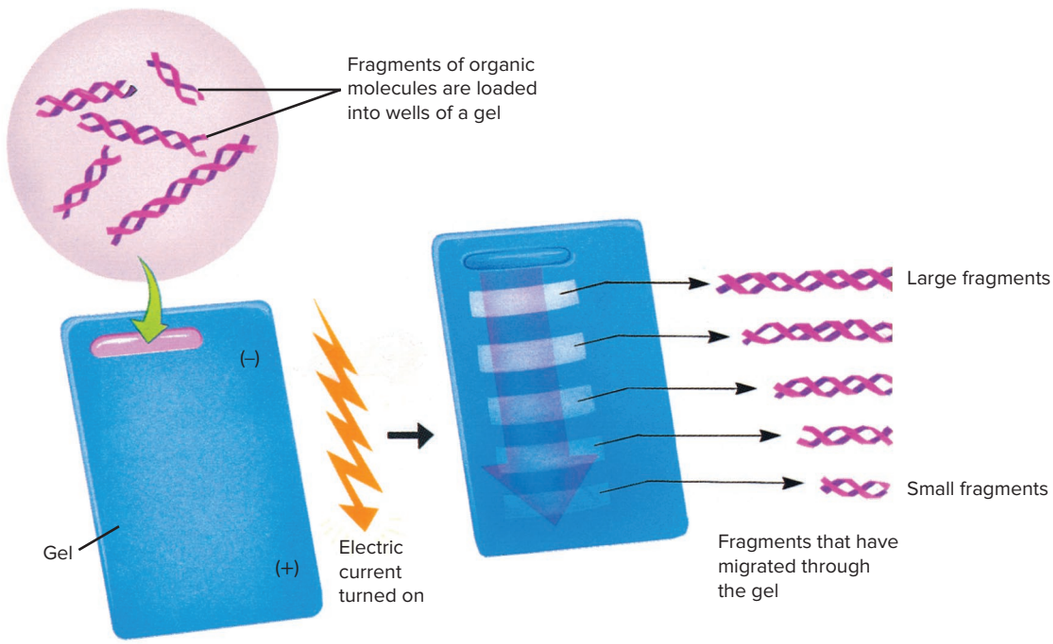

Figure 7.4 Gel electrophoresis. This process separates DNA fragments, protein fragments, and other organic compounds by causing them to move through an electrically charged gel. Because DNA molecules are negatively charged, the electrical field will push the molecules toward the positive electrode. The fragments also move according to their size and shape, and some fragments move slowly and some move quickly. When their migration is complete, the fragments can be stained and visualized easily. In the example shown here, the DNA fragments were separated by size.

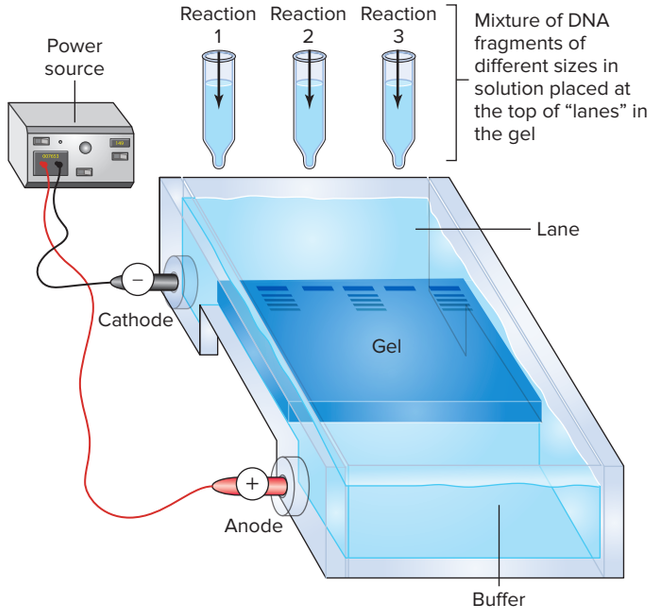

Figure 7.5 Apparatus for gel electrophoresis. The power supply produces an electrical gradient between the + and − poles and across the gel.

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Figure 7.6 Cover the ends of the removable gel bed with rubber end-caps or tape.

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Figure 7.7 Place comb near the center set of notches of the gel bed. Prepare the agarose solution and pour the gel.

3. Mix a 0.8% (weight by volume) mixture of agarose powder in a sufficient volume of buffer to fill the gel chamber. Heat the mixture until the agarose dissolves.
4. When the hot agarose solution has cooled to 50°C, pour the agarose solution into the gel-cast bed (fig. 7.7).
5. After the gel has solidified, gently remove the comb by pulling it straight up (fig. 7.8). Use of a plastic spatula may help prevent tearing the gel. Use the sketch in figure 7.9 to label the wells formed in the gel by the comb.
6. Submerge the gel under the buffer in the electrophoresis chamber.
7. You will study six samples:

   Sample 1: Bromophenol blue (molecular weight = 670 g mole^{-1})

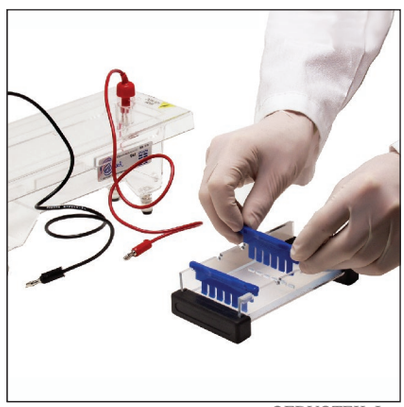

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Figure 7.8 After the gel solidifies, gently remove the rubber end-caps (or tape) and pull the combs straight up from the gel.

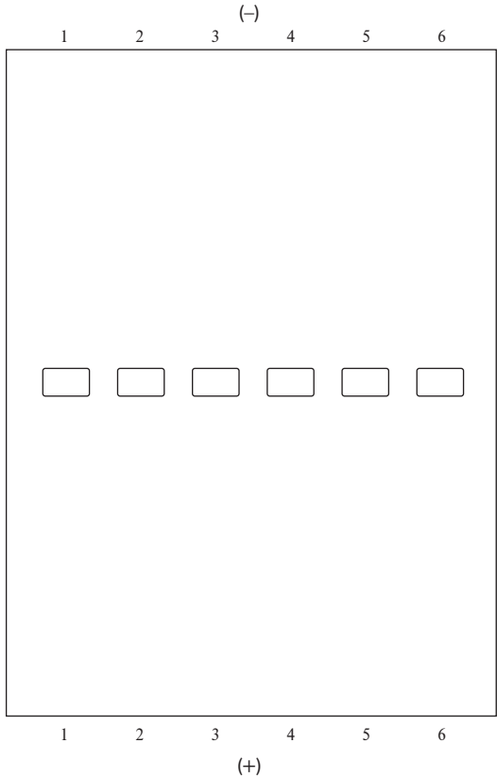

Figure 7.9 Sketch of the wells formed in the gel by the comb as viewed from above.

Sample 2: Methylene blue (molecular weight = 320 g mole^{-1})

Sample 3: Orange G (molecular weight = 452 g mole^{-1})

Sample 4: Xylene cyanol (molecular weight = 555 g mole^{-1})

Samples 5 and 6: Unknowns

Use a micropipettor or a simple pipet and bulb to load the samples into the wells of the gel. If you use a micropipettor, your instructor will demonstrate its use. If you use a simple pipet and bulb, gently squeeze the pipet bulb to draw Sample 1 into the pipet. Be sure that the sample is in the lower part of the pipet. If the sample becomes lodged in the bulb, tap the pipet until the sample moves into the lower part.

8. To eliminate excess air, hold the pipet above the sample tube and slowly squeeze the bulb until the sample is near the pipet's opening.

9. Place the pipet tip into the electrophoresis buffer so it is barely inside sample well 1 (fig. 7.10). Do not touch the bottom of the sample well. Maintain pressure on the pipet bulb to avoid pulling buffer into the pipet.

10. Slowly inject the sample into the sample well. Stop squeezing the pipet when the well is full. Do not release the pressure on the bulb. Remove the pipet from the well.

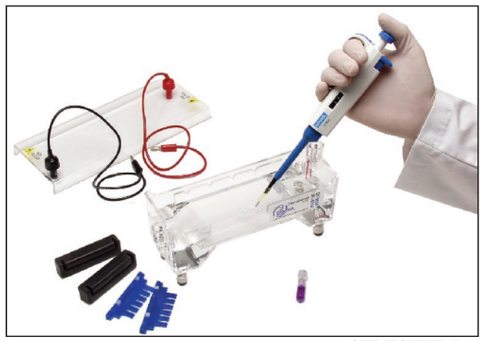

Figure 7.10 Submerge the gel in the buffer-filled electrophoresis chamber and load the samples into the wells of the gel.

11. Thoroughly rinse the pipet with distilled water.

12. Load the remaining five samples into the gel by repeating steps 6–10 (fig. 7.10). Load Sample 2 into the second well, Sample 3 into the third well, etc.

13. Carefully snap on the cover of the electrophoresis chamber (fig. 7.11). The red plug in the cover should be placed on the terminal indicated by the red dot. The black plug in the cover should be placed on the terminal indicated by the black dot.

14. Insert the plug of the black wire into the black (negative) input of the power supply. Insert the plug of the red wire into the red (positive) input of the power supply.

15. Turn on the power and set the voltage at 90 V. You'll soon see bubbles forming on the electrodes. Examine the gel every 10 min.

16. After 30 min, turn off the power and disconnect the leads from the power source. Gently remove the cover from the chamber and sketch your results in figure 7.9.

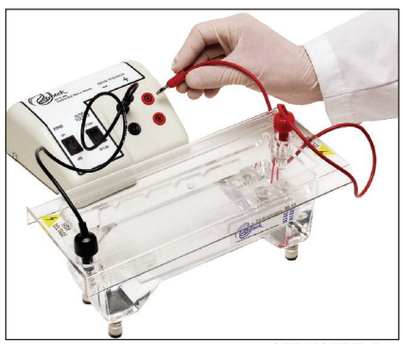

Figure 7.11 Attach the safety cover, connect the power source, and run the electrophoresis.

Question 3

a. Bromophenol blue, Orange G, and xylene cyanol each has a negative charge at neutral pH, whereas methylene blue has a positive charge at neutral pH. How does this information relate to your results?

b. Did Orange G, bromophenol blue, and xylene cyanol move the same distance in the gel? Why or why not?

c. What compounds do you suspect are in Samples 5 and 6? Explain your answer.

INTERPRETING A DNA-SEQUENCING GEL

Examine figure 7.12, which includes a photograph of a gel used to determine the order, or sequence, of nucleotides in a strand of DNA. To prepare the sample for

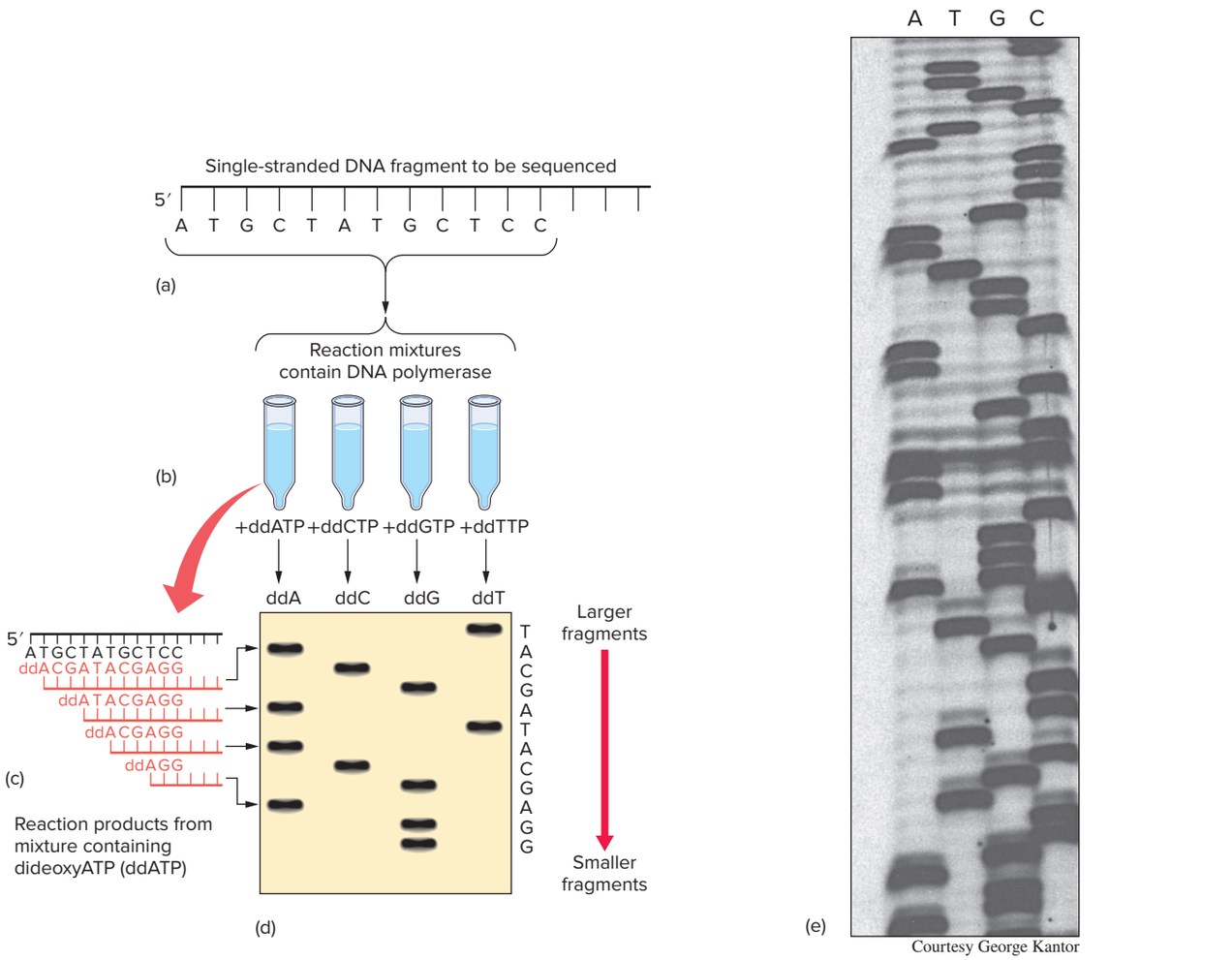

Figure 7.12 Determining the sequence of nucleotides in DNA. (*a*) Treating DNA with sodium hydroxide (NaOH) denatures double-stranded DNA into single-stranded DNA. One of the single strands of DNA to be sequenced is placed in each of four tubes. (*b*) The enzyme DNA polymerase is added to each tube along with a specific nucleotide-terminator. As polymerase replicates the DNA, the terminators are incorporated and will terminate various lengths of fragments of DNA. For example, the terminator ddATP will halt the reaction wherever adenosine occurs. The terminator ddATP (dideoxy adenosine triphosphate) will terminate a growing strand because it lacks a 3′ hydroxyl group and therefore cannot bond with the next deoxynucleotide. (*c*) Each tube will contain a sample of all possible replicated fragment lengths corresponding to the positions of that specific nucleotide. The sequences in red are the complement strands. (*d*) During electrophoresis, the fragments migrate at different rates according to their length. (*e*) The lanes of the resulting gel are labeled according to their base: A, adenine; T, thymine; G, guanine; and C, cytosine. This technique is usually referred to as "Sanger" sequencing in honor of Fred Sanger, a Nobel laureate who, in 1977, first sequenced a piece of DNA.

electrophoresis, samples of the DNA being investigated were put into each of four tubes and induced to replicate. Also, into the first tube, an adenine-terminator was added in addition to all the other nucleotides. As the complementary strand was being constructed, the terminators were occasionally incorporated wherever an adenine nucleotide was used. This random incorporation resulted in all possible lengths of DNA pieces that had an adenine on the end. The same process was conducted in the other tubes with thymine-, guanine-, and cytosine-terminators; one treatment for each of the four lanes in the gel. Electrophoresis separated the replicated pieces of DNA by size. Staining the gel revealed which lengths of the complementary DNA were terminated by which nucleotide-terminators. Examine figure 7.12*d*.

The gel consists of four "lanes," labeled A, T, G, and C, indicating either adenine-, thymine-, guanine-, or cytosine-terminated pieces of DNA. By "reading" down the gel, you can determine the sequence of nucleotides in the DNA. For example, the uppermost band of the gel is in the T (thymine) lane. Therefore, the first base of the piece of DNA is thymine. Similarly, the next bands are in the A, C, G, and A lanes. Thus, the first five bases of the complementary strand DNA are T-A-C-G-A. List the next seven nucleotides of the DNA as indicated by the gel. Also list the sequence of the first 12 nucleotides in the original DNA being investigated.

Question 4

a. How did the sequence of nucleotides revealed on the gel differ from the sequence of the original strand of DNA?

b. Assume that the gel shown in Figure 7.12*d* is from blood collected at a murder scene. This blood does not match that of the victim. You have collected DNA from five people suspected of murder. Gels comparable to the one shown in Figure 7.12*d* read as follows for each of the suspects:

Suspect #1: T-A-C-G-A-T-A-C-G-A-C
Suspect #2: T-A-C-G-A-T-A-C-G-A-C
Suspect #3: T-A-C-G-A-C-A-C-G-C-G
Suspect #4: T-A-C-G-A-T-G-C-G-A-C
Suspect #5: T-A-C-G-A-T-C-C-G-T-C

What do you conclude from this evidence?

INQUIRY-BASED LEARNING I

Is there always room for improvement in laboratory techniques?

Carefully planned and refined procedures are critical for laboratory techniques such as paper chromatography. The sensitivity of these techniques depends on a variety of factors, including the many parameters associated with timing, chemicals, measurements, and temperatures. In procedure 7.2 you were given a rather standardized protocol, but it can always be improved for specific experiments. For example, how would you modify the paper chromatography procedure to better resolve two amino acids having approximately the same R_f values? What parameter(s) of the experimental design might be tweaked to increase the technique's resolving power? We suggest that you begin your investigation in the following way:

a. List the parameters involved in paper chromatography. Think carefully; many factors are involved.

b. Choose one or two parameters that you can test for their impact on the chromatography results. Why did you choose these?

c. Choose two amino acids for experimentation. Why did you choose these two?

d. Choose your treatment levels for each parameter, and then do your experiment.

e. What did you conclude?

INQUIRY-BASED LEARNING II

What's the best column length for effective column chromatography?

Observation: Column chromatography is a common means of separating molecules according to their size and shape. The movement of molecules through a column is affected by several factors, including the column's matrix and the column's length.

Question: How does the length of a column affect the separation of molecules via column chromatography?

a. Establish a working lab group and obtain Inquiry-Based Learning Worksheet 7 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 7 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Study and Inquiry

1. How are column chromatography, paper chromatography, and gel electrophoresis different? How are they similar?

2. How would the results of electrophoresis vary if the voltage was increased? If the agarose was made more dense? Or if the migration was allowed to run twice as long?

3. How could knowing the nucleotide base sequence of a piece of DNA be important to a biologist?

4. How could knowing the nucleotide base sequence of a piece of DNA be important to someone trying to solve a crime?

5. How could knowing the nucleotide base sequence of a piece of DNA be important for someone studying a hereditary disease?

6. How could knowing the nucleotide base sequence of a piece of DNA be important for someone wanting to improve the yield of a crop such as corn?

WRITING TO LEARN BIOLOGY

Which of the methods discussed in this exercise would best quantify the relative amounts of the molecules being separated? Why?