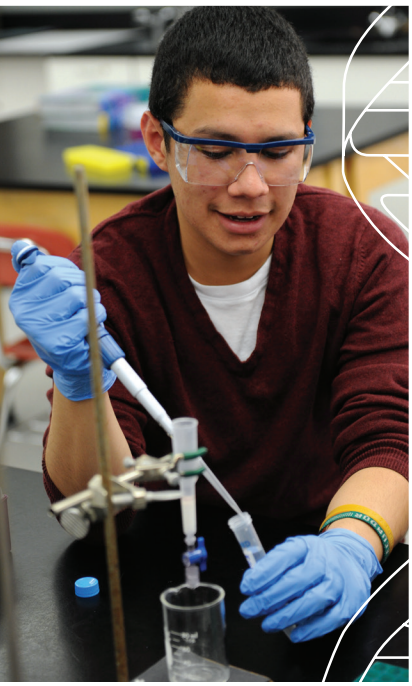
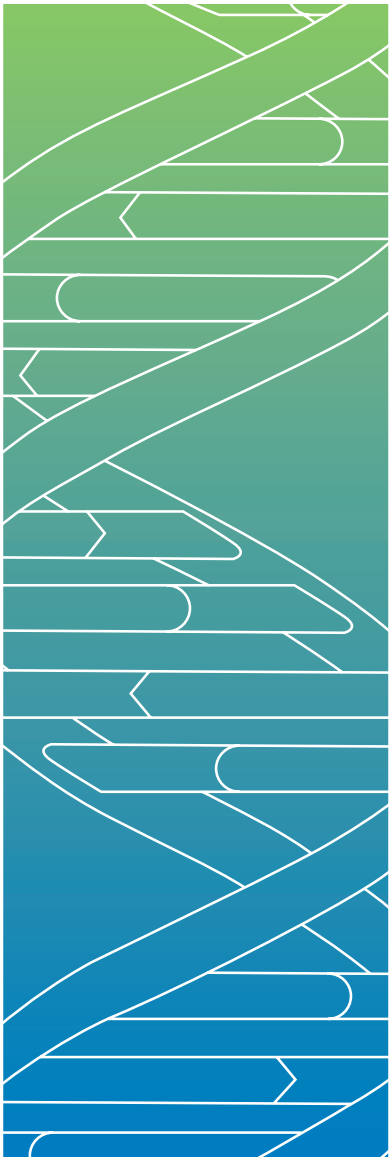
Amgen Biotech Experience

Scientific Discovery for the Classroom

Student Guide





INTRODUCTION TO BIOTECHNOLOGY

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ABOUT THE AMGEN BIOTECH EXPERIENCE

Genetic engineering is a branch of biotechnology that uses special procedures and techniques to change an organism's *DNA*. This ability has had a huge impact on the field of medicine, as genetically modified bacteria can make human *insulin* (the hormone responsible for regulating *glucose* levels in the blood) and other life-saving products. It's rare for high school students to have the chance to learn about and actually practice the procedures and techniques that are the foundation of the biotechnology industry—but in this program, you will have just that opportunity. As you work in the laboratory and carry out the very experiments that led to breakthroughs in biotechnology, you will gain hands-on experience with producing genetically modified bacteria.

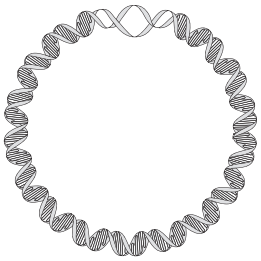
The procedures in this program were developed through a series of discoveries that led to important breakthroughs in biotechnology. Some of the pioneering scientists who made these discoveries received Nobel Prizes in Physiology or Medicine in 1978 and Chemistry in 1980 and 1993. (The Nobel Prize is the highest distinction awarded to scientists in these fields from around the world.) The work that you are about to do is based on this Nobel Prize-winning science—science that is significant and will continue to play an important role in the development of biotechnology and medicine. You will follow in the footsteps of the many scientists who have pushed and continue to push the boundaries of biotechnology. There are many advances still to be made—and students who decide to continue studying this field may contribute to those advances.

In science, the ability to keep track of what you are doing and communicate your work is extremely important. In order to demonstrate that you performed an experiment, either so that it can be duplicated and verified by others or if you want to apply for a patent—you need to have a very accurate record of what you've done. As you carry out this program, carefully record your notes, ideas, observations, results, and answers to questions in a science notebook, in pen. (For scientific purposes, it is important to keep a record—even of your mistakes.) If possible, use a separate bound composition notebook and organize the labs with a table of contents at the front. Since you will use a pen to write with, you'll need to cross out any mistakes you make—and it is good practice to simply "X" out the section you want to change (so that it can still be read) and to note

why you've done so. Following these best practices will make this program even better preparation for you!

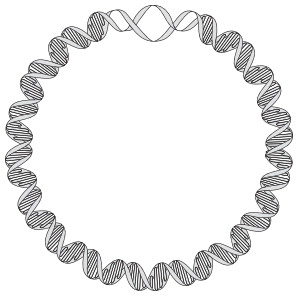
The Amgen Biotech Experience (formerly Amgen-Bruce Wallace Biotechnology Lab Program) had humble beginnings almost 25 years ago with visionary scientists and teachers who shared passion and energy for imparting their knowledge with students. Bruce Wallace, one of Amgen's first staff members, wanted all students to experience the joy of discovery and the excitement of having science at their fingertips. A desire for more robust science education at schools near Amgen's global headquarters led to involving area high school teachers and, later, a college professor, in developing curriculum and educator training in biotechnology. The program grew through word of mouth and teacher interest, and expanded over time to other states and countries.

Visit the ABE website at www.amgenbiotechexperience.com.



PROGRAM INTRODUCTION

AMGEN BIOTECH EXPERIENCE



WHAT IS GENETIC ENGINEERING?

CONSIDER: Preview the title, subtitles, and illustrations found on pages 7 through 14 and then list the topics that you think will be covered in this reading.

TREATING DISEASE WITH GENE CLONING

Until relatively recently, people with certain diseases had to rely on remedies that were expensive and sometimes difficult to obtain. Amazing as it might seem, many of these diseases are the result of the loss of a single protein function, either because the protein produced is defective or because it is not produced in normal amounts. (A *protein* is a large *biomolecule* that carries out essential functions in *cells*.) For example, individuals with *hemophilia*, a bleeding disorder in which blood fails to clot normally, make little or no clotting factor protein; a deficiency of human growth hormone can cause poor growth, delayed puberty, and muscle weakness in children, and fatigue, reduced muscle and bone mass, baldness, increased body fat, and memory loss in adults.

By providing the patient with a functional protein, the symptoms of these diseases can be alleviated. Before genetic engineering technology, these therapeutic proteins had to be extracted from natural sources, such as human blood or animal tissue, a process that was generally difficult, inefficient, and expensive. Pharmaceutical companies can use genetic engineering—or *gene cloning*, as it is often called—to make these proteins cost-effectively, in far greater quantities, without the impurities and viruses that can be transmitted from blood and tissue samples. Gene cloning involves inserting the human gene that encodes the protein into bacteria where the protein is made along with all the other bacterial proteins.

CONSIDER: What do you already know about cloning?

The ability to make enough of the proteins to treat diseases is the result of two key discoveries about bacteria made by scientists in the 1970s and '80s. The first discovery was that bacteria contain tiny circles of DNA, called *plasmids*, that sometimes contain genes that can make them resistant to antibiotics. The second discovery was that bacteria also contain proteins called *restriction enzymes* that can cut DNA at very specific places.

The findings made by basic research often lead to fundamental understandings about the nature of life. In some instances these findings can also lead to new technologies that can improve life. With the discovery of plasmids and restriction enzymes, a whole new era of genetic engineering was launched. Scientists now have the ability to generate products that can improve health in ways never before imagined.

One of the first pharmaceutical products produced using these tools was insulin, which is used to treat *diabetes*, a debilitating and sometimes fatal disease. To generate large quantities of human insulin, the sequences of DNA that contain the codes of human insulin are inserted into a plasmid that is introduced into the common intestinal bacterium *Escherichia coli* (*E. coli*), where the new protein is synthesized along with all the other bacterial proteins. The genetically modified bacteria are then grown in large batches, and the insulin is purified for use in the treatment of diabetes.

CONSIDER: Do you think that treating diabetes with insulin can be considered a cure? What is the difference between a treatment and a cure?

Why is the ability to produce large quantities of insulin so important, and how exactly is this done? In the following readings you will learn about diabetes: what it is and why insulin is in such demand.

DATELINE: AMERICA

MAY 2012

TEENAGE DIABETES ON THE RISE

The occurrence of type 2 diabetes in teenagers, once a disease found primarily in adults, has increased dramatically over the past 10 years. Nearly one in four teens between the ages of 12 and 19 is prediabetic (i.e., shows early signs of diabetes) or already has the disease. To make matters even worse, research suggests that the disease progresses more rapidly in children than in adults. In diabetes, the levels of glucose (a type of sugar) in the blood can become dangerously high, causing complications such as loss of vision, kidney failure, and nerve and blood vessel damage. The onset of diabetes early in life could mean serious health issues, such as heart disease, blindness, and amputation, for individuals in their 30s and 40s, far younger than such complications have been seen in the past.

Why this sudden rise in teenage diabetes? Although being overweight or obese can contribute significantly to developing diabetes, weight is not the only factor; 35 percent of teens of normal weight have glucose levels that are higher than normal, which is one indicator of being prediabetic. Factors such as lack of exercise in this age of increased computer and mobile device use may be part of the problem. While many prediabetics go on to develop full-blown type 2 diabetes, studies indicate that eating less fat and fewer calories and exercising a mere 20 minutes a day can reduce the risk of developing type 2 diabetes by 60 percent.

Diabetes can result from the body's inability to make sufficient insulin (type 1) or to effectively use the insulin that it does produce (type 2). Many patients with diabetes must take in insulin as an injection. More diabetes in the population will mean a greater demand for insulin.

What is it like for a teen to learn she has diabetes? Read the following story about one teenager's struggle with the disease.

JENNIFER'S STORY

Jennifer felt hungry all the time, but despite eating whatever she felt like whenever she wanted to, she was losing weight. She was also very thirsty and was constantly drinking water and then needing to pee. Initially, she just thought it was typical for a 15 year old; she was in a growth spurt and very active with soccer and track at school. Of course she was hungry and thirsty! She was also pleased by her weight loss, since she'd been a bit overweight for a while. Jennifer also felt unusually tired and draggy, especially in the afternoon. But again, who wouldn't be, since school started at the ungodly hour of 7:30? When she began to have trouble seeing the board in class, she thought, Drat! Do I really need glasses? But it was the cut on her leg that refused to heal and became infected that finally got her to talk to her parents and ultimately go to the doctor's office. There Jennifer was diagnosed with diabetes.

Jennifer had heard about a disease called diabetes but never gave it much thought. Now she really needed to pay attention. Diabetes is the result of too much of a sugar called glucose in the blood and not enough of it getting into cells, where it provides the energy to construct the biological molecules the body needs to survive. Jennifer learned that in order for glucose to get into cells, the body makes a hormone called insulin, which binds to the cells and enables glucose to enter them. For some reason, Jennifer's body no longer produced normal amounts of insulin, resulting in Jennifer having very high levels of glucose in her blood and not enough glucose getting into her cells. Jennifer hoped she could control her diabetes by eating more fruits and vegetables and getting more exercise. But although this change in habits helped some, it was not sufficient, and Jennifer had to begin injecting herself daily with insulin.

Jennifer now is very aware of what she eats, monitoring exactly how much sugar and other carbohydrates she ingests. She checks the level of her blood glucose several times a day by pricking her finger and testing her blood. She also injects her insulin faithfully. She knows that she can't cure her diabetes and that if the disease progresses further she could suffer very serious complications.

DIABETES TYPES 1 AND 2

DIABETES: TOO MUCH OF A GOOD THING

What is diabetes? Diabetes is the result of elevated levels of glucose in the blood. Glucose is a major source of energy and is used to construct biological molecules in the body. What you ate for breakfast or lunch today is rapidly being converted to glucose, which in turn will be used to generate energy, to synthesize new cells and tissues, and to carry out processes required to sustain life. The starch in your bread or potato is made up of long chains of glucose molecules (**Figure P.1a**). As food passes through your mouth, esophagus, and stomach (**Figure P.1b**), these chains are broken down to release glucose. The glucose is then absorbed through the intestinal wall and enters the bloodstream, where it is carried to all the cells in the body (**Figure P.1c**).

Figure P.1: How glucose gets to cells

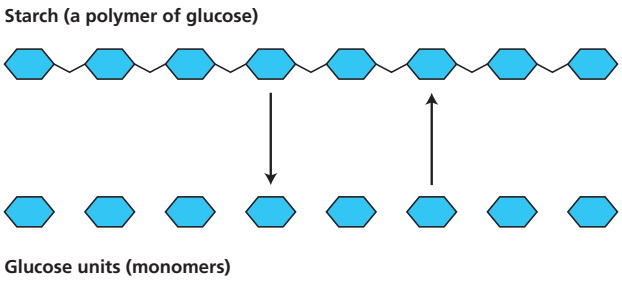


Figure P.1a: Starch is made up of subunits of glucose bonded together.

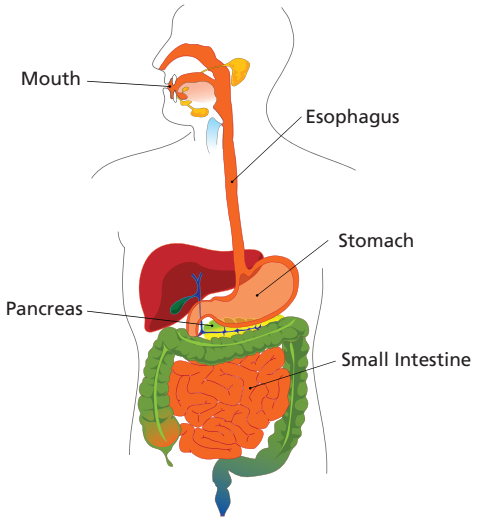


Figure P.1b: Nutrients such as starch are broken down into smaller molecules during digestion in the mouth, esophagus, and stomach.

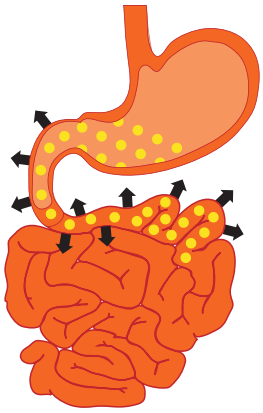
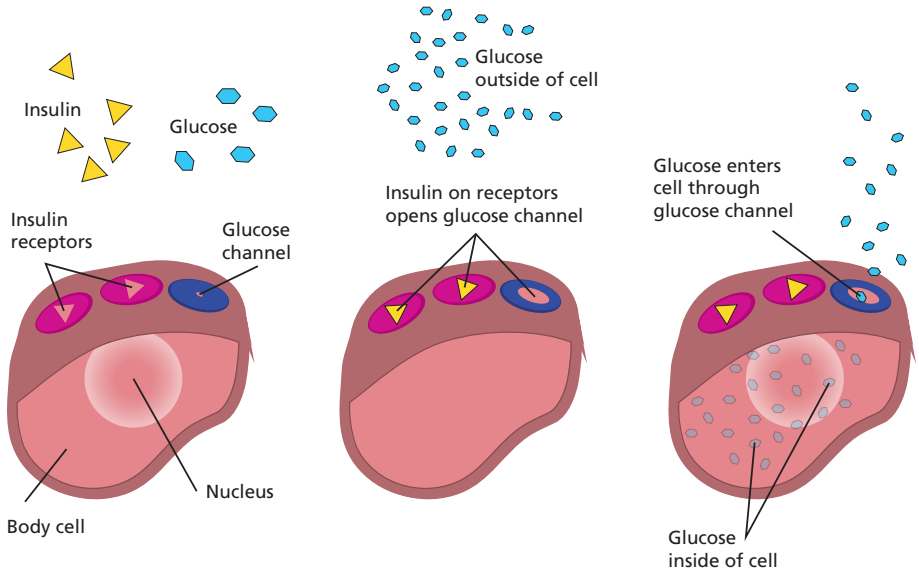


Figure P.1c: Glucose molecules pass through the small intestinal wall into the bloodstream, which delivers the glucose to cells in the body.

CROSSING THE CELLULAR DIVIDE

In order to get inside a cell, glucose must cross the cell membrane that separates the inside of the cell from its environment. Insulin, which is made by beta cells found in the pancreas (see **Figure P.1b**), binds to a special protein called a *receptor*, which causes an opening in the cell membrane and allows glucose to enter the cell (see **Figure P.2**). Without insulin, glucose cannot penetrate this cellular barrier.

Figure P.2: How glucose crosses the cell membrane



Insulin in the blood binds to specific receptors on the cell. This binding alters the conformation of the cell membrane, resulting in the formation of a glucose channel. Glucose in the blood can now enter the cell through these channels.

In both type 1 and type 2 diabetes, glucose is unable to enter the cells, resulting in elevated levels of glucose in the blood. In type 1 diabetes, the beta cells in the pancreas are unable to produce insulin. Without insulin to create glucose channels, the glucose remains in the blood (**Figure P.3a**). Type 2 diabetes is the result of a combination of two factors: (1) Cells become resistant to insulin, and the receptors can no longer bind the hormone (**Figure P.3b**). As the blood sugar levels rise, the beta cells pump out more and more insulin to no avail, since the cells cannot use it. (2) Eventually the beta cells are exhausted and can no longer produce insulin, and insulin levels in the blood drop while the sugar levels continue to increase.

Figure P.3: Reduced uptake of glucose by cells

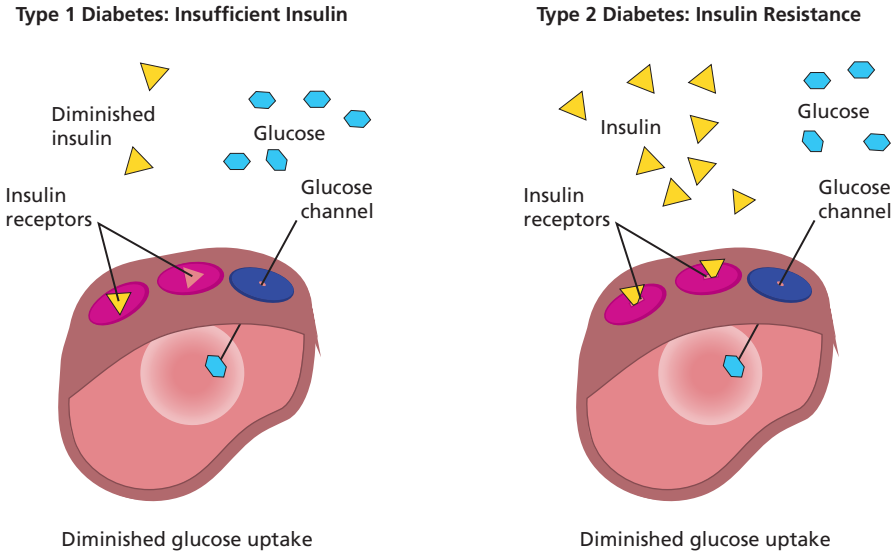


Figure P.3a: In type 1 diabetes, there is a lack of insulin in the body.

Figure P.3b: In type 2 diabetes, cells cannot bind the insulin.

THE PROBLEMS OF TOO LITTLE INTRACELLULAR GLUCOSE

When cells cannot get glucose, they cannot get the energy and biological molecules they need. The body responds by breaking down fats and proteins to obtain its needed energy. Loss of proteins and fats can cause serious damage to tissues and organs, leading to the symptoms of diabetes that patients like Jennifer experience, such as blindness and nerve damage (which can result in amputation).

TREATING DIABETES

Individuals with type 1 diabetes can regulate their sugar levels by monitoring their blood and injecting insulin as needed. Those with type 2 diabetes can sometimes regulate their blood sugar levels by changing their diet and increasing the amount that they exercise. However, in many cases, medications that reduce insulin resistance in cells and increase the levels of insulin in the blood are required to maintain normal blood sugar levels.

Currently, there is no cure for either type of diabetes.

E. COLI WITH A HUMAN GENE

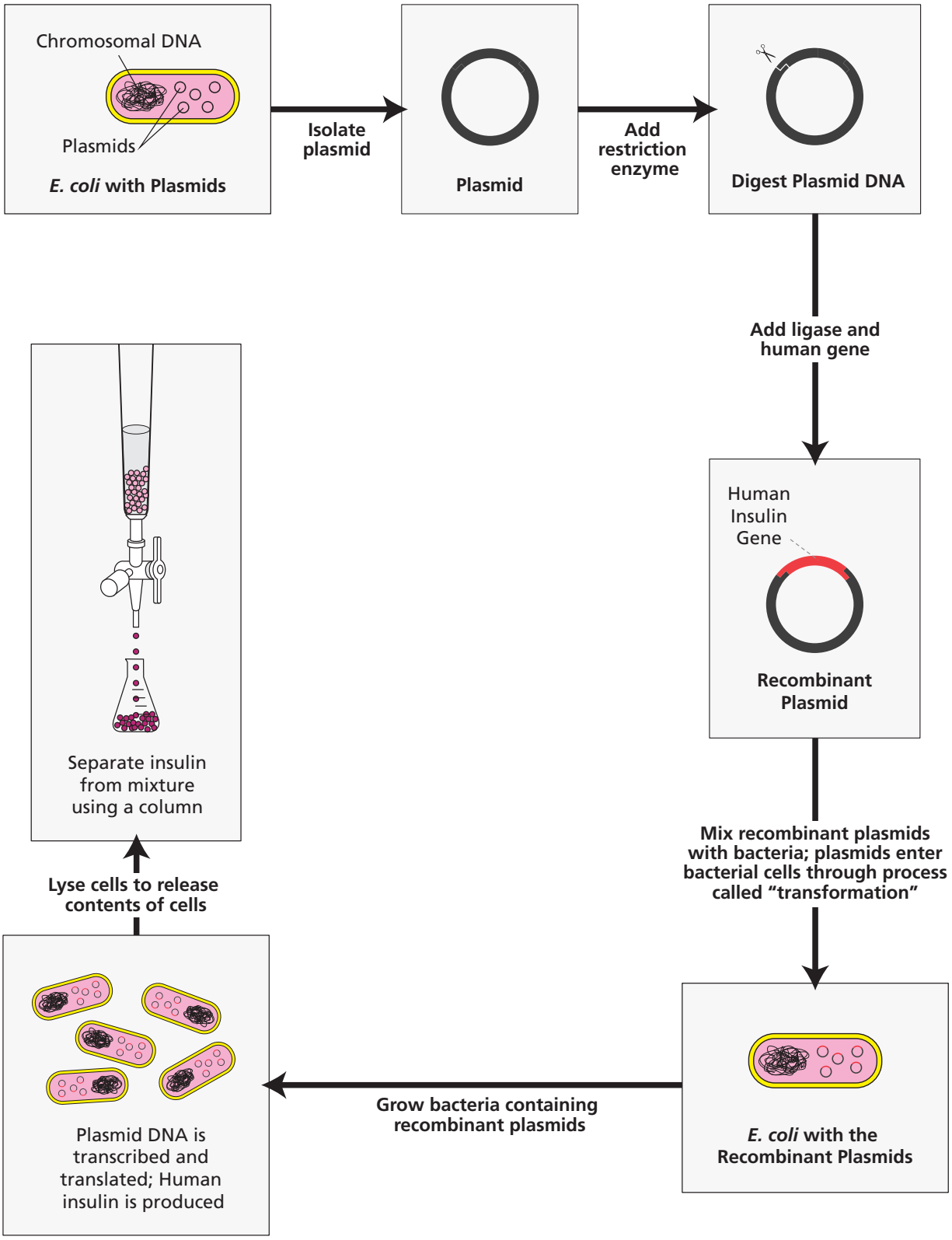
With the rise in diabetes in the population, the need for insulin for treatment is also on the rise. Originally isolated from the pancreases of pigs and cows, most of the insulin used today is genetically engineered human insulin, manufactured by bacteria. DNA sequences encoding human insulin in plasmids are taken up by bacteria, which make the hormone along with all of its bacterial proteins. Insulin is then isolated from the bacteria. In 1982, human insulin was the first commercially successful product made by recombinant DNA technology. (*Recombinant DNA* refers to DNA that contains sequences or genes from two or more sources.)

CONSIDER: **Why might diabetes be on the rise, especially in teenagers?**

MAKING NEW PROTEINS IN BACTERIA

Figure P.4 shows how a human protein—in this case, insulin—can be made in bacteria. The insulin is then purified so that it can be used by people with diabetes.

Figure P.4: Making insulin in bacteria



YOUR CHALLENGE

Your challenge in the Amgen Biotech Experience is to successfully learn and practice two techniques that are used in biotechnology research laboratories. You will acquire laboratory skills and be able to explain how these techniques are used in the genetic engineering process.

PROGRAM INTRODUCTION GLOSSARY

Biomolecule: A molecule produced by living cells. Examples include proteins, carbohydrates, lipids, and nucleic acids.

Cells: The basic units of any living organism that carry on the biochemical processes of life.

Diabetes: A disease that occurs when the body doesn't produce or properly use insulin.

DNA (deoxyribonucleic acid): A double-stranded biomolecule that encodes genetic information.

***Escherichia coli* (*E. coli*):** *E. coli* is a common bacterium found in the gut of warm blooded animals. Most strains are harmless, including the strain used in these lab protocols.

Fluorescence: The production of light by a molecule (e.g., red fluorescent protein will release red light when exposed to ultraviolet light).

Gene cloning: Using genetic engineering techniques to create exact copies, or clones, of a gene or DNA sequence of interest.

Genetic engineering: A branch of biotechnology that uses specific procedures and techniques to change an organism's DNA.

Glucose: A sugar that is a major source of energy and biomolecules to sustain life processes. Glucose is absorbed through the intestine and travels in the blood to cells, where it is transported through the cell membrane to be used as energy, to synthesize cells and tissues, and to carry out other essential processes.

Hemophilia: A disease that occurs when the ability of blood to clot is reduced due to lack of one or more blood clotting factors.

Insulin: A hormone produced in the pancreas that controls the amount of glucose in the blood. Insulin is a protein.

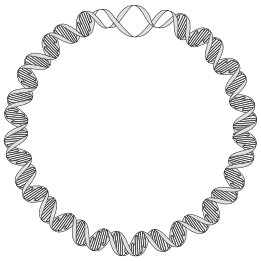
Plasmid: A circular molecule of DNA.

Protein: A large biomolecule. Proteins carry out essential functions in cells, from forming cellular structures to enabling chemical reactions to take place.

Receptor: A protein that receives signals from outside the cell. When a signal substance binds to a receptor, it directs the cell to do something, for example to allow biomolecules to enter the cell.

Recombinant DNA: DNA that contains sequences or genes from two or more sources.

Restriction enzyme: A protein that can cut DNA at a specific sequence.



CHAPTER 1

SOME TOOLS OF THE TRADE

INTRODUCTION

The year 1978 marked a major breakthrough in medicine. For the first time ever, scientists were able to induce bacterial *cells* to make human insulin by inserting human *DNA* into the cells. This new technology, termed *genetic engineering*, can be used to make products that treat the symptoms of certain genetic diseases.

To carry out genetic engineering, you need good laboratory skills. In this chapter, you'll focus on gaining practice in the use of *micropipettes* (instruments used to transfer small volumes of liquid) and *gel electrophoresis* (a technique for separating and identifying *biomolecules*)—two critical skills for biotechnology. You will complete two labs, using instruments and supplies that are identical to the ones used in biotechnology research laboratories.

CHAPTER 1 GOALS

By the end of this chapter, you will be able to do the following:

- Use micropipettes and the technique of gel electrophoresis correctly
- Explain the importance of micropipettes and gel electrophoresis in genetic engineering
- Describe how gel electrophoresis separates DNA
- Explain how genetic engineering can be used to treat some genetic diseases

WHAT DO YOU ALREADY KNOW?

Discuss the following questions with your partner and write your ideas in your notebook. Be prepared to discuss your responses with the class. Don't worry if you don't know all the answers. Discussing these questions will help you think about what you already know about genetic disease and DNA.

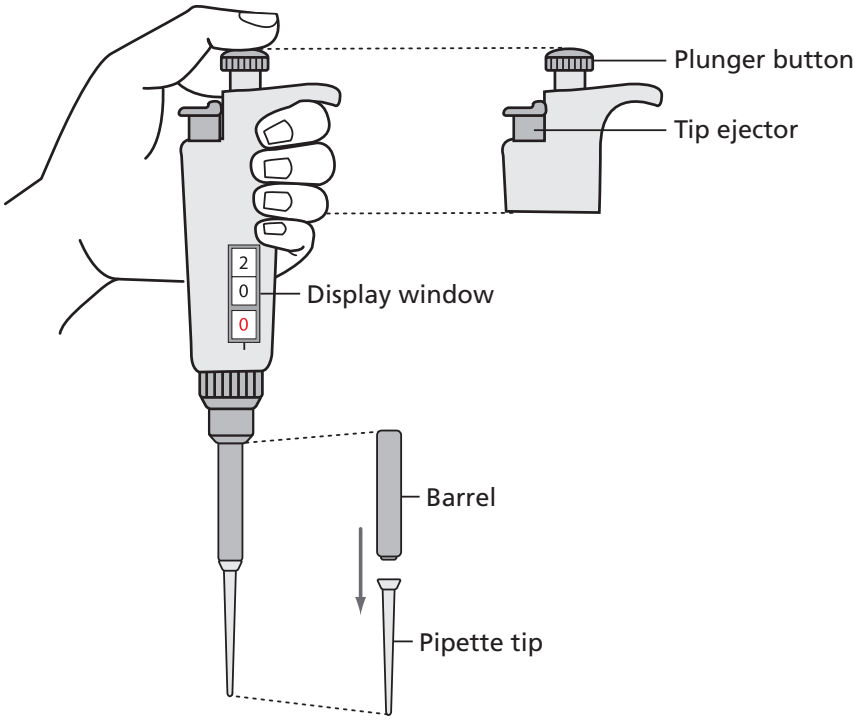
1. What does the term *genetic disease* mean? What examples of genetic diseases do you know about?

2. Adding human DNA to bacteria makes it possible to make human insulin. What do you already know about DNA? Be as detailed as possible and discuss the location of DNA in the cell, DNA structure, the replication of DNA, and the components of DNA.

LABORATORY 1.1: HOW TO USE A MICROPIPETTE

The purpose of this laboratory is to introduce you to an important tool used in genetic engineering: the micropipette, shown in **Figure 1.1**. A micropipette is used to transfer very small and exact volumes of liquids in either milliliters (mL, thousandths of a liter) or microliters (µL, millionths of a liter), which are the measurements of volume most often used in genetic engineering. This laboratory will give you the chance to learn how to use the micropipette and to see the relative size of different amounts of solution measured by this very precise tool and how precise the amounts that you can measure with it are.

Figure 1.1: A P-20 micropipette



BEFORE THE LAB

Respond to the following questions with your group and be prepared to share your answers with the class.

1. Why do you think it is necessary to use very small and exact volumes of reagents in biotechnology?
2. Read through the Methods section on pages 21 through 23 and briefly outline the steps, using words and a flowchart.

MATERIALS

Reagents

- A plastic microfuge tube rack with a microfuge tube of red dye solution (RD)

Equipment and Supplies

- P-20 micropipette (measures 2.0–20.0 µL)
- Tip box of disposable pipette tips
- Laminated micropipette practice sheet
- Waste container for used tips and microfuge tubes (will be shared among groups)

SAFETY:

- **All appropriate safety precautions and attire required for a science laboratory should be used, including safety goggles. Please refer to your teacher's instructions.**
- **Wash your hands well with soap after completing the lab.**

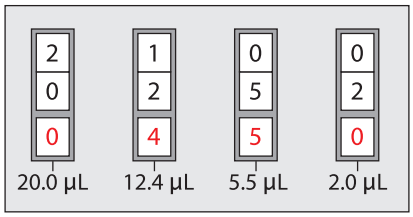
METHODS

1. Check your rack to make sure that you have the reagent listed.
2. Review the parts of the micropipette shown in (see **Figure 1.1** on page 20).
3. Find the display window on the handle of the micropipette.
4. Turn the plunger button on the top of the micropipette clockwise—to the right—to decrease the volume, or counterclockwise—to the left—to increase the volume.

5. **Figure 1.2** shows four micropipette volumes. Practice setting the micropipette to these volumes.

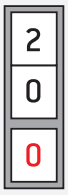
LAB TECHNIQUE: Never set the P-20 micropipette lower than 2.0 µL or higher than 20.0 µL or you could damage the equipment.

Figure 1.2: Four micropipette volumes



The display window of a micropipette shows how much fluid it will load and dispense. Four examples of displays and the corresponding amounts are shown.

6. Review the laminated micropipette practice sheet. Each group member will pipette five drops of different volumes onto the sheet. Pipetting consists of two parts: loading the liquid into the micropipette, and dispensing the liquid from the micropipette.



7. Load the micropipette with 20.0 µL of RD by doing the following:

   a. Set the micropipette to 20.0 µL.
   b. Open the tip box. Lower the micropipette onto a tip and press down firmly (do not touch the tip with your fingers). Close the box when done.
   c. Bring the micropipette and the RD tube to eye level.
   d. Use your thumb to press the plunger to the first stop position, which is your first point of resistance.

LAB TECHNIQUE: When loading the micropipette, only press the plunger to the first stop or you will draw too much solution into the pipette tip.

   e. Put your pipette tip into the RD and slowly release the plunger to draw up the solution.

LAB TECHNIQUE: Do not lay down a micropipette with fluid in the tip or hold it with the tip pointed upward. If the disposable tip is not firmly seated onto the barrel, fluid could leak back into the pipette.

8. Dispense RD onto the laminated sheet by doing the following:

   a. Place the pipette tip over the 20.0 µL circle.

b. Use your thumb to press the plunger to the first stop position and then press down to the second stop.

LAB TECHNIQUE: When dispensing liquid from the micropipette, press the plunger to the first stop to dispense most of the liquid and then press the plunger to the second stop in order to dispense the last little bit.

c. With the plunger still depressed, pull the pipette out of the tube—this prevents you from accidentally pulling the liquid back into the tip.

9. Without setting down the micropipette, twist the plunger button to set it to 15.0 µL and repeat steps 7b–8c, dispensing over the 15.0 µL circle.

10. Without setting down the micropipette, twist the plunger button to set it to 10.0 µL and repeat steps 7b–8c, dispensing it over the 10.0 µL circle when dispensing the liquid.

11. Without setting down the micropipette, twist the plunger button to set it to 5.0 µL and repeat steps 7b–8c, dispensing it over the 5.0 µL circle.

12. Without setting down the micropipette, twist the plunger button to set it to 2.0 µL and repeat steps 7b–8c, dispensing it over the 2.0 µL circle.

13. Use the tip ejector to place your pipette tip into the waste container.

STOP AND THINK:

- **When loading or dispensing a solution, why is it important to actually see the solution enter or leave the pipette tip?**
- **You were instructed to avoid contact with the pipette tips—for example, you were asked to put the pipette tip on without using your hands, to avoid setting down the micropipette, to use the ejector button to remove the tip, and to keep the tip box closed. If you were working with plasmids and bacterial cells, why would these precautions be important?**

14. Using the micropipette practice sheet, each person in your group should have a chance to load and dispense the five drops of different volumes, with each person using a new pipette tip.

15. When everyone in your group has had a chance to dispense RD onto the micropipette practice sheet, draw the approximate sizes of each drop in your notebook (or take a photograph and tape it into your notebook) and label them with the amounts.

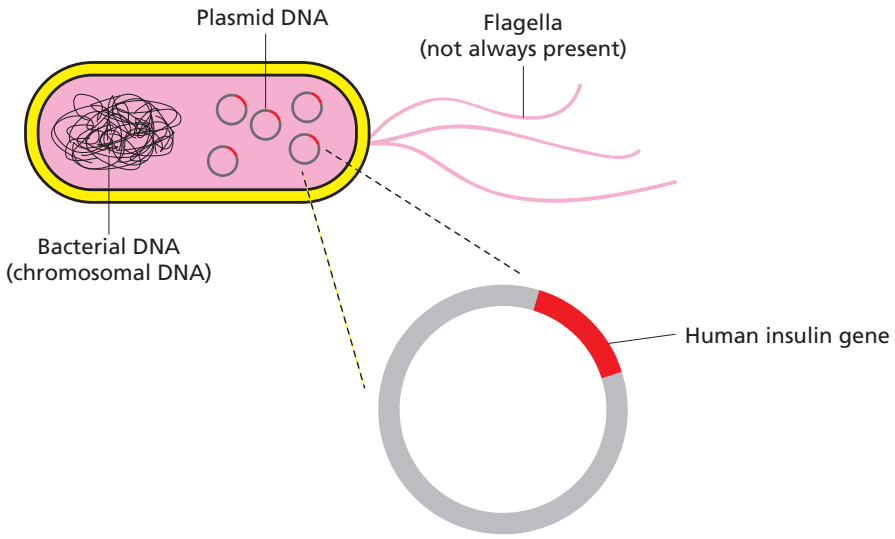
THE GENETIC ENGINEERING PROCESS

Do you know somebody who takes *insulin*, or a *blood clotting factor*, or *human growth hormone*? These substances are all *proteins* manufactured in certain human cells. If those cells fail to make these particular proteins, the diseases *diabetes*, *hemophilia*, and *growth deficiency* can result. A patient with one of these diseases must be treated with the missing protein.

CONSIDER: Prior to genetic engineering, how could people get missing proteins for a genetic disease?

Before the development of genetic engineering, it was difficult to obtain human proteins to treat people who needed them. Now, bacteria can make these proteins because scientists have figured out a way to change bacterial DNA by adding human DNA. (see **Figure 1.3**).

Figure 1.3: Bacterial cell with human DNA



What is the relationship between DNA and proteins? Both are biomolecules, large molecules made by living cells. When scientists investigated traits in organisms, they found that proteins were responsible for traits. For example, consider a plant that has the trait of red flowers. The flowers' red pigment is produced by the action of an enzyme (one kind of protein). The DNA in that plant contains instructions for making proteins, including that enzyme. The part of a DNA molecule that has the instructions for making a particular protein is called a *gene*.

In the genetic engineering process, a human gene is added to a *plasmid*, a small circular piece of DNA found in many bacteria. The plasmid is taken up by bacterial cells, and the cells make the human protein that is encoded by the human gene along with their own proteins. During this process, biotechnologists use a combination of tools, some human-made and some biological. Among the human-made tools are two that you'll work with in this chapter: micropipettes and gel electrophoresis.

DID YOU KNOW?

The DNA Code

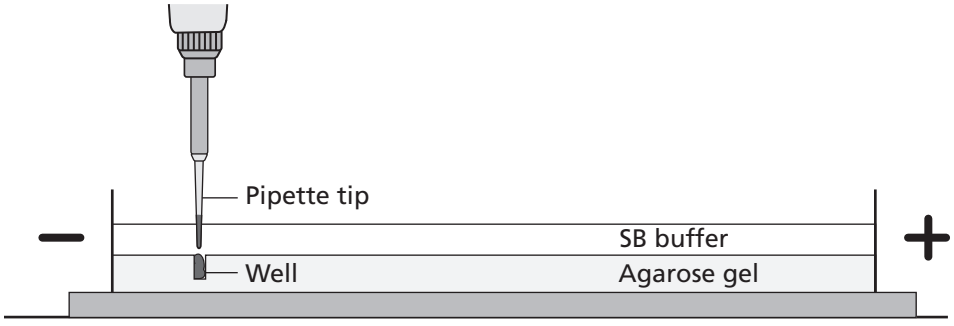
DNA information is encoded by the arrangement of *nucleotides*, small molecules that join together to form the DNA molecule. A DNA molecule has millions of nucleotides. There are four different kinds of nucleotides, and they are arranged in a specific *sequence* (order). A specific sequence of nucleotides in the DNA (i.e., a gene) is a code for how to make a specific protein. Think of a sequence of nucleotides as similar to a sequence of written musical notes—the code for how to play music. Just as different sequences of notes encode different songs, different sequences of nucleotides encode different proteins.

LABORATORY 1.2: GEL ELECTROPHORESIS

The purpose of this laboratory is to give you experience with gel electrophoresis, which is used to separate and identify a mixture of biomolecules including DNA; the components of each mixture can then be identified by their location in the gel. Gel electrophoresis works based on the fact that biomolecules have a negative charge, which means that they will move in response to an electric charge. The biomolecules move through a gel, and their speed varies primarily according to their weight, although molecular shape and degree of charge also influence their movement. In the genetic engineering process, gel electrophoresis is used to separate and identify plasmids and short linear pieces of DNA.

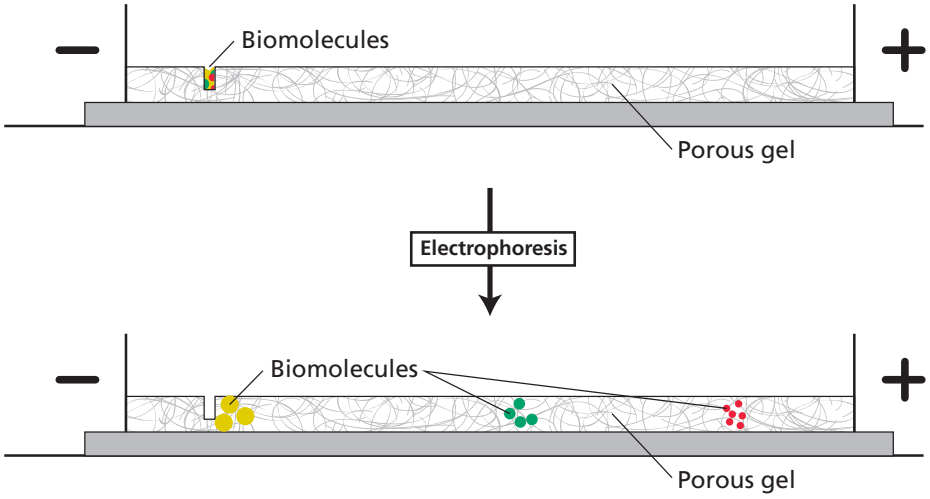
The electrophoresis setup consists of a box containing an agarose gel and two electrodes that create an electric field across the gel when the box is attached to a power supply. The negative electrode is black, and the positive electrode is red. Samples of biomolecules are pipetted into wells near the negative (black) electrode. The samples move through the gel toward the positive (red) electrode, as shown in **Figure 1.4**.

Figure 1.4: The gel electrophoresis unit



The gel that the biomolecules move through is composed of *agarose*, a polysaccharide (complex sugar) found in seaweed. Its structure is a porous matrix (like a sponge) with lots of holes through which the solution and biomolecules flow. See **Figure 1.5**.

Figure 1.5: How biomolecules, including DNA, move through the agarose gel matrix in gel electrophoresis



BEFORE THE LAB

Respond to the following questions with your group, and be prepared to share your answers with the class.

1. In what circumstances might it be important to use gel electrophoresis to separate and identify plasmids and short linear pieces of DNA?

2. Read through the Methods section on pages 28 through 31 and briefly outline the steps for *Part A* and for *Part B*, using words and a flowchart.

MATERIALS

Reagents

- A plastic microfuge tube rack with the following:
  - Microfuge tube of red dye solution (RD)
  - Microfuge tube of dye solution 1 (S1)
  - Microfuge tube of dye solution 2 (S2)
  - Microfuge tube of dye solution 3 (S3)
- 50-mL flask containing 1x sodium borate buffer (1x SB) (shared with another group)

Equipment and Supplies

- P-20 micropipette (measures 2.0–20.0 µL)
- Tip box of disposable pipette tips

- 2 pipetting practice plates loaded with 0.8% agarose gel
- Electrophoresis box loaded with 0.8% agarose gel (will be shared among groups)
- Microcentrifuge (will be shared among all groups)
- Waste container for used tips and microfuge tubes (will be shared among groups)

SAFETY:

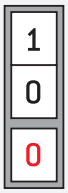
- **All appropriate safety precautions and attire required for a science laboratory should be used, including safety goggles. Please refer to your teacher's instructions.**
- **Wash your hands well with soap after completing the lab.**

METHODS

PART A: PIPETTING INTO WELLS

You will practice pipetting RD into preformed wells in an agarose gel.

1. Check your rack to make sure that you have the RD tube.
2. Fill the two pipetting practice plates with 1x SB to a level that just covers the entire surface of the gel. If you see any "dimples" over the wells, add more buffer.



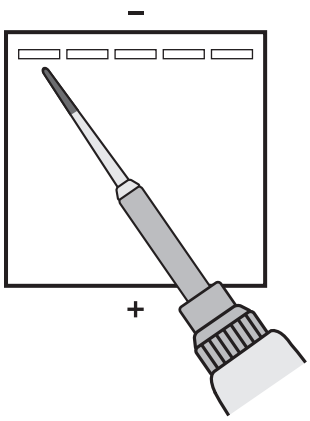
3. Set the P-20 micropipette to 10.0 µL and put on a pipette tip.
4. Load the RD into the pipette with 10.0 µL of RD.

LAB TECHNIQUE: **Do not lay down a micropipette with fluid in the tip or hold it with the tip pointed upward.**

5. Dispense RD into a well in one of the practice plates by doing the following:
   a. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.
   b. Lower the pipette tip until it is under the buffer but just above the well.

LAB TECHNIQUE: **Be careful not to place your pipette tip into the well or you might puncture the gel, which will make the well unusable.**

   c. Gently press the plunger to slowly dispense the sample. To avoid getting air into the buffer, do not go past the first stop. The sample will sink into the well.



6. Repeat steps 4 and 5 until all the practice plate wells have been filled. Everyone in your group should get an opportunity to practice pipetting into the wells.

7. Eject the pipette tip.

PART B: SEPARATING DYES WITH GEL ELECTROPHORESIS

Now you will use gel electrophoresis to separate different dyes. First you will add dyes into wells in the gel electrophoresis unit. You will then turn the unit on in order to move the negatively charged dyes through the gel. (You will share the electrophoresis boxes with one other group; your teacher will tell you which wells your group should use.)

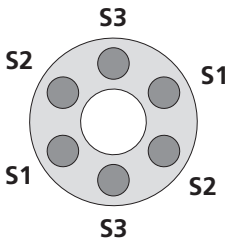
1. Check your rack to make sure that you have the three dye solutions (S1, S2, and S3).

2. Review **Figure 1.4** on page 26. Check to make sure that the wells in the gel are located near the negative (black) electrode.

3. Fill the box with 1x SB to a level that just covers the entire surface of the gel. If you see any "dimples" over the wells, add more buffer.

4. Centrifuge the S1, S2, and S3 tubes.

   LAB TECHNIQUE: **Distribute the tubes evenly in the microcentrifuge so that their weight is balanced.**



5. Make a drawing in your notebook that shows the location of the wells in the electrophoresis box. Record which solution you will place in each well.

6. Set the P-20 micropipette to 10.0 µL and put on a pipette tip.

7. Load 10.0 µL of S1 into the pipette.

8. Dispense the S1 into the well you've designated for that solution by doing the following:

   a. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.

   b. Lower the pipette tip until it is under the buffer but just above the well.

      LAB TECHNIQUE: Do not puncture the gel or it will become unusable.

   c. Gently press the plunger to slowly dispense the sample. To avoid getting air into the buffer, do not go past the first stop. The sample will sink into the well.

      LAB TECHNIQUE:

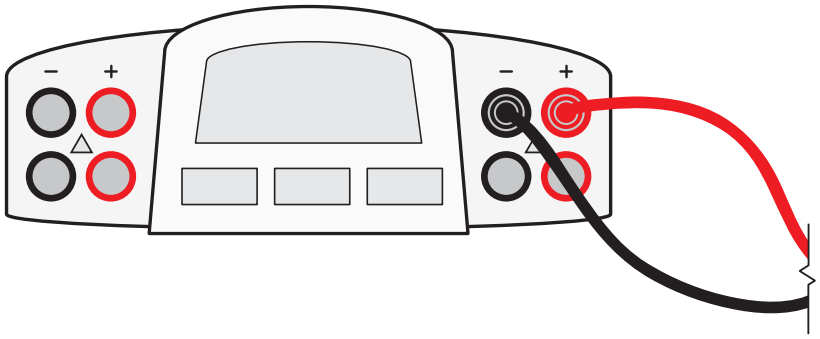
      - **While the plunger is still depressed, pull the tip out of the buffer so that you don't aspirate the solution or buffer.**
      - **Use a fresh pipette tip for each sample.**

9. Repeat steps 7 and 8 for S2 and S3, using a new pipette tip with each solution.

10. When all the samples have been loaded, close the cover tightly over the electrophoresis box. (Carefully close the cover in a horizontal motion, so that samples don't spill.)

11. Connect the electrical leads to the power supply. Connect both leads to the same channel, with cathode (–) to cathode (black to black) and anode (+) to anode (red to red). See **Figure 1.6**.

Figure 1.6: Leads from electrophoresis box connected to correct channel in power supply



12. Turn on the power supply and set the voltage to 130–135 V. (You will see bubbles form in the buffer at the red (+) end of the electrophoresis unit.)

13. After two or three minutes, check to see if the dyes are moving toward the positive (red) electrode. You should begin to see the purple dye (bromophenol blue) beginning to separate from the blue dye (xylene cyanole).

STOP AND THINK:

- **Study your gel electrophoresis results. Which solution sample contained a single dye: S1, S2, or S3? How do you know?**
- **What electrical charge do the dyes have? Explain your reasoning.**
- **The dyes that you are separating are orange G (yellow), bromophenol blue (purple), and xylene cyanole (blue). If the molecular shape and electric charge of all three dyes are similar, what is the order of the dyes from heaviest to lightest molecules, based on your initial results? Why do you think this is the correct order?**

14. In approximately 10 minutes, or when you can distinguish all three dyes, turn off the power switch and unplug the electrodes from the power supply. Do this by grasping the electrode at the plastic plug, NOT the cord.

15. Carefully remove the cover from the gel box and observe the dyes in the gel.

16. In your notebook, draw the relative location of the bands and their colors in each of the lanes containing your samples.

17. Leave the gels in the gel box.

CHAPTER 1 QUESTIONS

1. What is the importance of micropipettes and gel electrophoresis in genetic engineering?

2. How are recombinant plasmids used to treat genetic diseases?

DID YOU KNOW?

Gel Electrophoresis in DNA Fingerprinting

DNA fingerprinting uses gel electrophoresis to distinguish between samples of genetic material. In DNA fingerprinting, human DNA molecules are treated with enzymes that chop them at certain characteristic points, thereby reducing the DNA to a collection of smaller and more manageable pieces. The DNA fragments are loaded into a gel and placed in an electrical field, which electrophoretically sorts the DNA fragments into various bands. These bands can be colored with a radioactive dye to make them visible to imaging techniques. Methods of DNA identification have been applied to many branches of science and technology, including medicine (prenatal tests, genetic screening), conservation biology (guiding captive breeding programs for endangered species), and forensic science. In the latter discipline, analysis of the pattern of DNA fragments that results from the action of restriction enzymes enables us to discriminate between suspects accused of a crime, or potential fathers in a paternity suit.

CHAPTER 1 GLOSSARY

Agarose: A polymer made up of sugar molecules that is used as the matrix in gel electrophoresis procedures.

Biomolecule: A molecule produced by living cells. Most biomolecules are long polymers—made of repeating subunits called monomers—and include proteins, carbohydrates, lipids, and nucleic acids.

Blood clotting factor: A variety of proteins in blood plasma that participate in the clotting process.

Cell: The basic unit of any living organism that carries on the biochemical processes of life.

Diabetes: A disease that occurs when the body doesn't produce or properly use insulin.

DNA (deoxyribonucleic acid): A double-stranded molecule made up of nucleotide subunits that encodes genetic information.

Gel electrophoresis: The movement of charged molecules toward an electrode of the opposite charge; used to separate nucleic acids and proteins. When used to separate DNA fragments, electrophoresis will separate the fragments by size, with smaller fragments moving faster than larger fragments.

Gene: The fundamental physical and functional unit of heredity; an ordered sequence of nucleotides located in a specific place on the DNA that encode for a specific functional product.

Genetic engineering: The process of altering the genetic material of cells or organisms to enable them to make new substances or perform new functions.

Genetic disease: A disease caused by a change in DNA. Genetic diseases are often inherited from parents.

Growth deficiency: A disease that occurs when the body doesn't produce enough human growth hormone.

Hemophilia: A disease that occurs when the ability of blood to clot is reduced due to lack of one or more blood clotting factors.

Human growth hormone: A hormone secreted by the pituitary gland that stimulates growth. Human growth hormone is a protein.

Insulin: A hormone produced in the pancreas that controls the amount of glucose in the blood. Insulin is a protein.

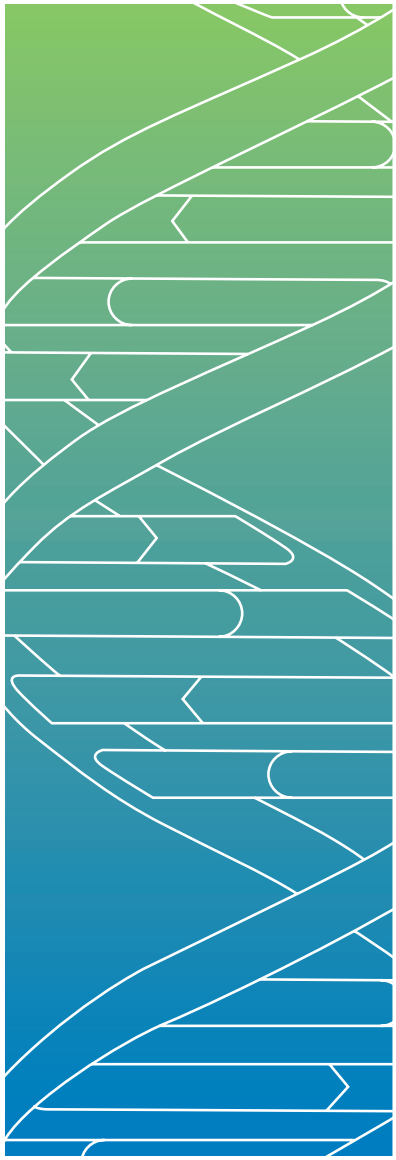
Micropipette: A laboratory instrument used to measure, dispense, and transfer very small amounts of liquid.

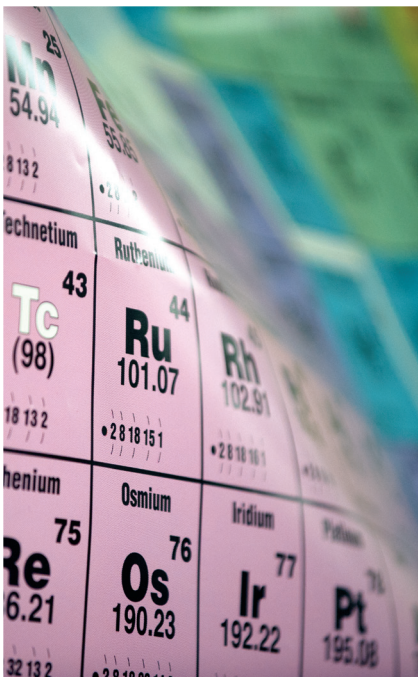
Nucleotide: A set of molecules that link together to form DNA or RNA. DNA and RNA each contain four types of nucleotides.

Plasmid: A circular molecule of DNA.

Protein: A large biomolecule. Proteins carry out essential functions in cells, from forming cellular structures to enabling chemical reactions to take place. Examples of proteins are enzymes, red fluorescent protein, cell receptors, and some hormones.

Sequence: A set of related events, movements, or items (such as nucleotides) that follow each other in a particular order.




Technetium
43
Tc
(98)
Ruthenium
44
Ru
101.07
Rh
102.91
Osmium
76
Os
190.23
Iridium
77
Ir
192.22
Pt