WHAT IS GENETIC ENGINEERING?

CONSIDER: Preview the title, subtitles, and illustrations found on pages A-5 through A-12 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. Then you will carry out some of the very same procedures that scientists use to produce human insulin in bacteria. But instead of producing insulin, you will engineer *E. coli* to produce a red fluorescent protein. This protein is made by a sea anemone gene that has undergone a mutation that makes the protein brighter in color. You will give *E. coli* a new protein and a trait it did not have before: *the ability to glow*.

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

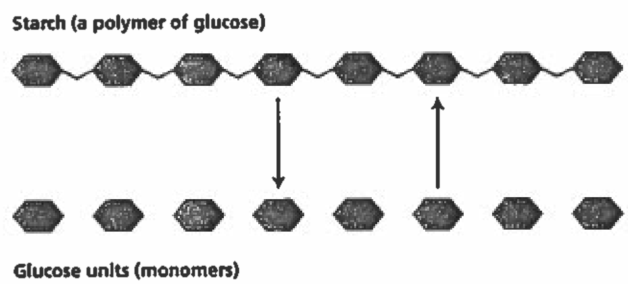


Figure P.1a: Starch is made up of subunits of glucose bonded together.

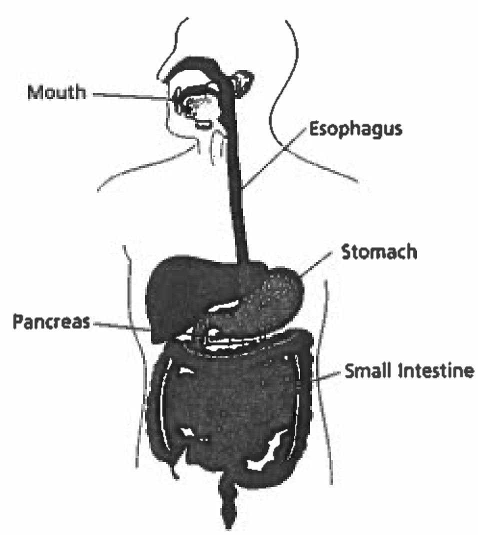


Figure P.1b: Nutrients such as starch are broken down into smaller molecules during digestion in the mouth, esophagus, and stomach.

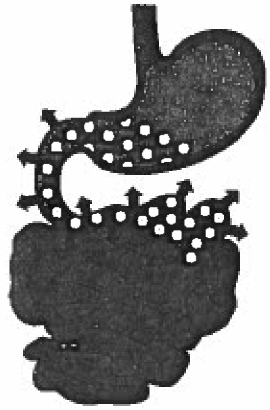
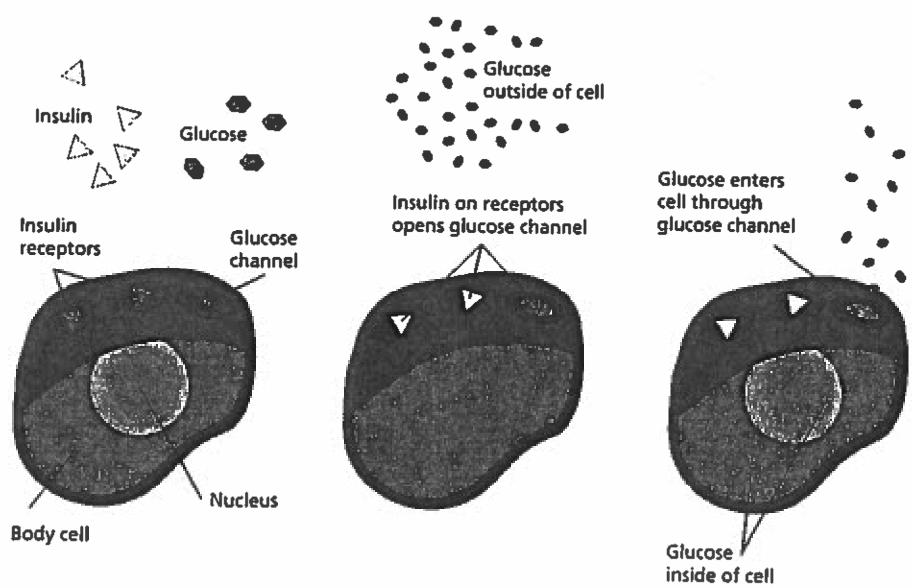


Figure P.1c: Glucose molecules pass through the small intestinal wall into the bloodstream, which delivers the glucose to cells in the body.

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 site on the cell 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

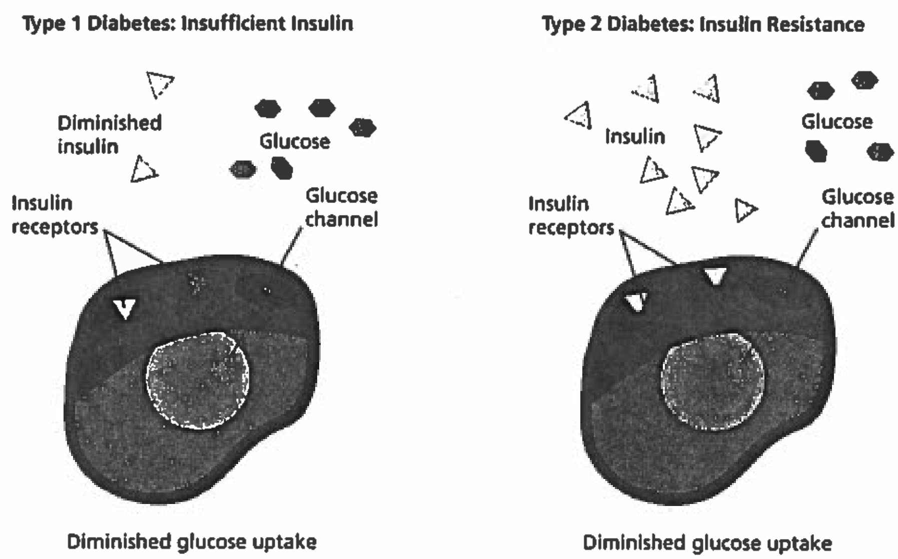


Figure P.3a: In type 1 diabetes, there is a lack of insulin in the body.

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

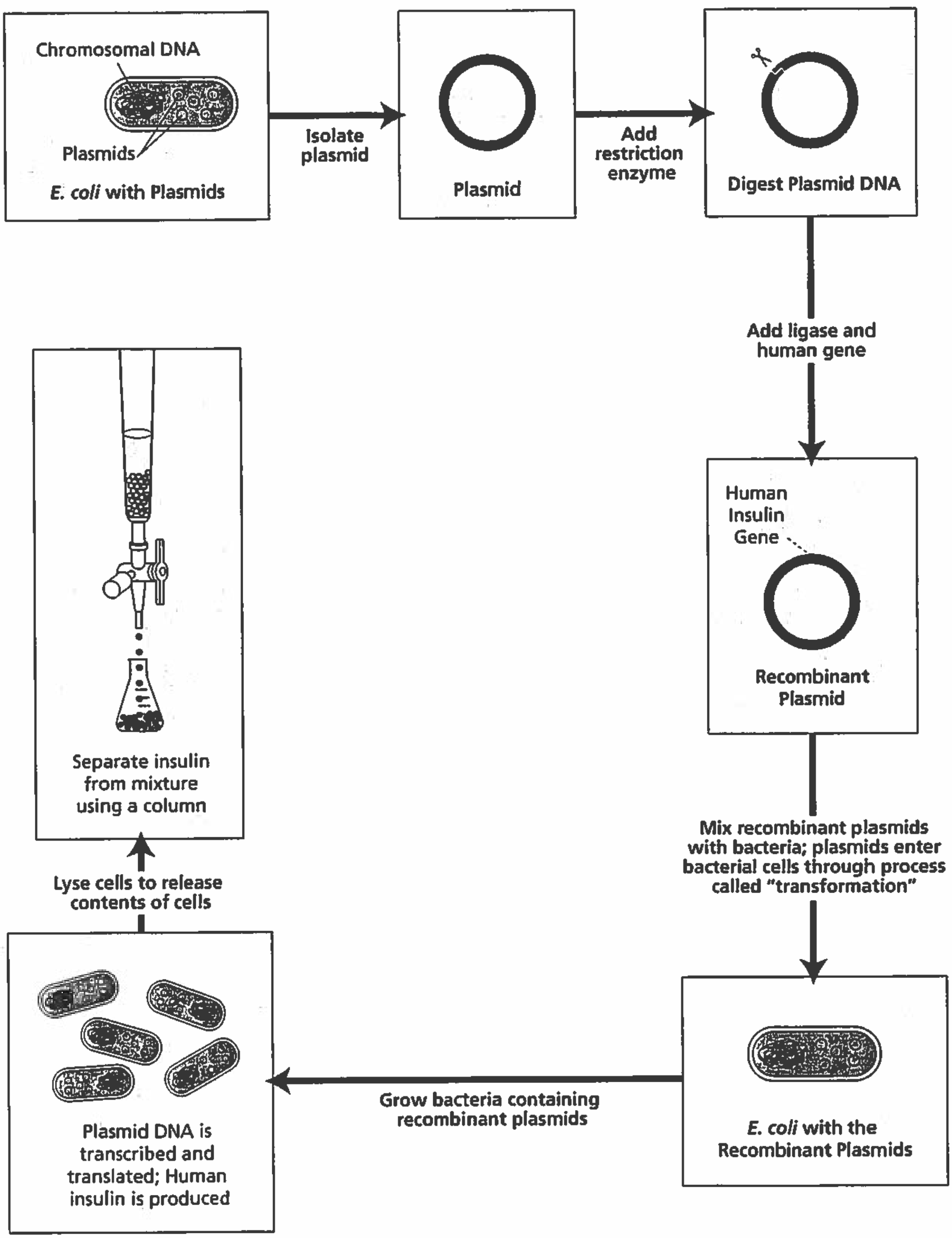
With the rise in diabetes in the population, the need for insulin for treatment is also on the rise. Originally isolated from the pancreases of pigs and cows, most of the insulin used today is genetically engineered human insulin, manufactured by bacteria. DNA sequences encoding human insulin in plasmids 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 carry out the steps of the genetic engineering process that is used to make insulin and other genetically engineered products. You will learn and practice the techniques and procedures that are part of this process. If you carry out all the steps in the program, you will create your own genetically modified bacteria.

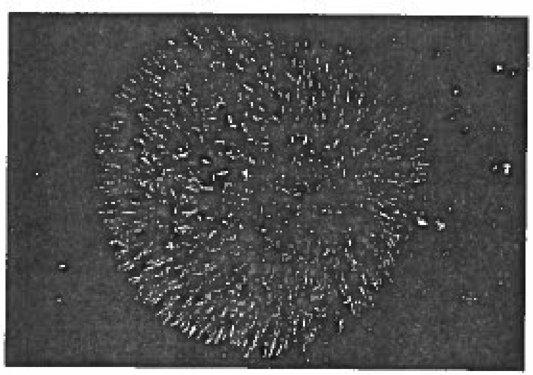
Note: The number of steps will vary depending on how much time your class has available.

Instead of cloning insulin or another human gene, you will work with a gene from a sea anemone, a soft-bodied animal related to coral and jellyfish. (The gene is called and the protein made by this gene is called red fluorescent protein *rfp*). How will you know if you are successful? The bacteria you create will have a new and highly visible trait: It will now produce red fluorescent protein!

DID YOU KNOW?

Red Fluorescent Protein in Sea Anemones

Red fluorescent protein is derived from a protein found in sea anemones. While sea anemones are sedentary, remaining attached to rocks, they are also predatory animals, using their stinging tentacles to catch their prey. The protein glows because it can absorb one color of light and then emit light of a different color—a process known as fluorescence. But why is it important for sea anemones to fluoresce? Our best guess is that fluorescent proteins somehow help sea anemones survive, but the role these proteins play is not yet well understood. Fluorescent molecules may serve as a sunblock, turning harmful UV light into light that is less damaging to the anemone's tissues. Another possibility is that while humans can't detect the fluorescence in bright sunlight, some animals may be able to, causing prey to be attracted to the glow.



PROGRAM INTRODUCTION GLOSSARY

Biomolecules: 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*): Common bacterium used in numerous molecular biology protocols. The strain of *E. coli* used in these lab protocols is relatively harmless.

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.

Plasmids: A circular molecules of DNA.

Protein: A large biomolecule (macromolecule). 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. A common response to receptors binding substances is to allow, allowing biomolecules to enter the cell.

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

Restriction enzymes: Proteins that can cut DNA at very specific sequences, which are called *recognition sites*.