9.1 Manipulating DNA

KEY CONCEPT Biotechnology relies on cutting DNA at specific places.

MAIN IDEAS

- Scientists use several techniques to manipulate DNA.
- Restriction enzymes cut DNA.
- Restriction maps show the lengths of DNA fragments.

VOCABULARY

restriction enzyme, p. 265
gel electrophoresis, p. 266
restriction map, p. 267

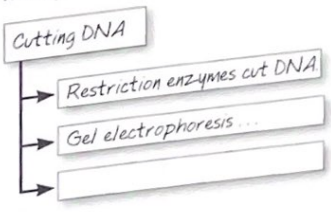
Review
DNA, enzyme, allele, nucleotide

REVIEW AT CLASSZONE.COM

Connect Many applications of genetics that are widely used today were unimaginable just 30 years ago. Our use of genetics to identify people is just one example. Biotechnology and genetics are used to produce transgenic organisms and clones. They are used to study diseases and evolution. They are used to produce medical treatments for people with life-threatening illnesses. Through many years of research and a combination of many different methods, advances in biotechnology seem to happen on a daily basis.

TAKING NOTES

Use a supporting main ideas chart to organize your notes on ways in which DNA is manipulated.



MAIN IDEA

Scientists use several techniques to manipulate DNA.

By the middle of the 1950s, scientists had concluded that DNA was the genetic material. Watson and Crick had determined the structure of DNA. Yet the field of genetics as we know it today was just beginning. For example, even the genetic code that you learned about in Chapter 8 was not fully understood until the early 1960s. Since that time, scientists have developed a combination of methods to study DNA and genes.

DNA is a very large molecule, but it is still just a molecule. It is far too small to see, and you cannot pick it up or rearrange it with your hands. Therefore, scientists must be able to work with DNA without being able to see or handle it directly. Chemicals, computers, and bacteria are just a few of the tools that have allowed advances in genetics research.

Artificial nucleotides are used to sequence genes. Artificial copies of genes are used to study gene expression. Chemical mutagens are used to change DNA sequences. Computers analyze and organize the vast amounts of data from genetics research. Enzymes, often from bacteria, are used to cut and copy DNA. Bacteria also provide one of the ways in which genes are transferred between different organisms. Throughout this chapter, you will learn about some of the techniques used in biotechnology, as well as some of its applications. You likely have heard of genetic engineering, DNA fingerprinting, and cloning, but how are they done? In many cases, one of the first steps in biotechnology and genetics research is to precisely cut DNA.

Infer **Why might so many different methods be needed to study DNA and genes?**

MAIN IDEA

estriction enzymes cut DNA.

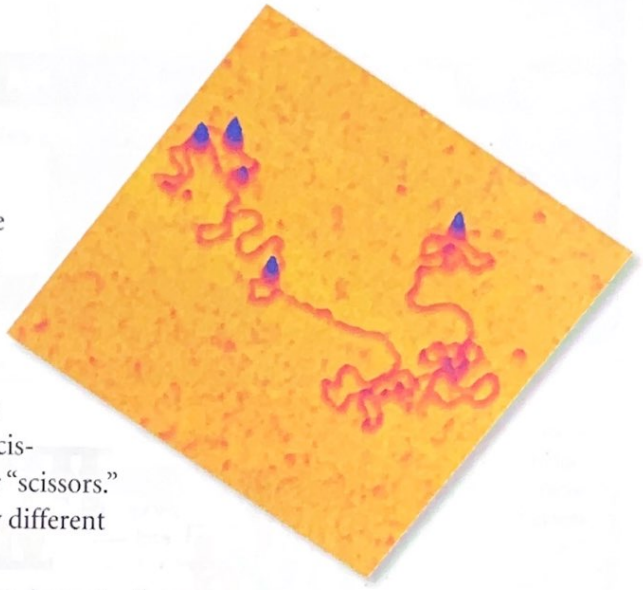
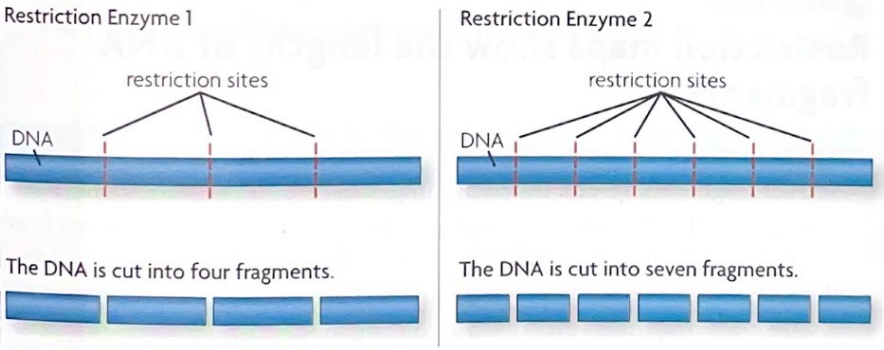


FIGURE 9.1 A restriction enzyme (blue peaks) from an *E. coli* bacterium helps protect against viruses by cutting DNA (red). This cutting "restricts" the effect of a virus on a bacterium. (colored 3D atomic force micrograph; magnification 63,000×)

Why would scientists want to cut DNA? To answer that question, you have to remember that a gene is a sequence of DNA nucleotides, and that a chromosome is one long DNA molecule. A whole chromosome is too large for scientists to study a particular gene easily, so they had to find a way to get much smaller pieces of DNA. Of course, slicing a chromosome into pieces is not as simple as picking up the molecule and cutting it with a pair of scissors. Instead, scientists use enzymes that act as molecular "scissors." These enzymes, which slice apart DNA, come from many different types of bacteria.

Bacterial cells, like your cells, can be infected by viruses. As protection against these invaders, bacteria produce enzymes that cut up the DNA of the viruses. As **FIGURE 9.1** shows, a DNA molecule can be cut apart in several places at once by several molecules of a restriction enzyme, or endonuclease. **Restriction enzymes** are enzymes that cut DNA molecules at specific nucleotide sequences. In fact, any time the enzyme finds that exact DNA sequence, it cuts the DNA molecule. The sequence of nucleotides that is identified and cut by a restriction enzyme is called a restriction site. These enzymes are called restriction enzymes because they restrict, or decrease, the effect of the virus on the bacterial cell.

Each of the hundreds of known restriction enzymes has a different restriction site. Different restriction enzymes will cut the same DNA molecule in different ways. For example, one restriction enzyme may find three of its restriction sites in a segment of DNA. Another restriction enzyme might find six of its restriction sites in the same segment. Different numbers of fragments with different lengths result. As you can see below, two different restriction enzymes can cut the same strand of DNA in very different ways.

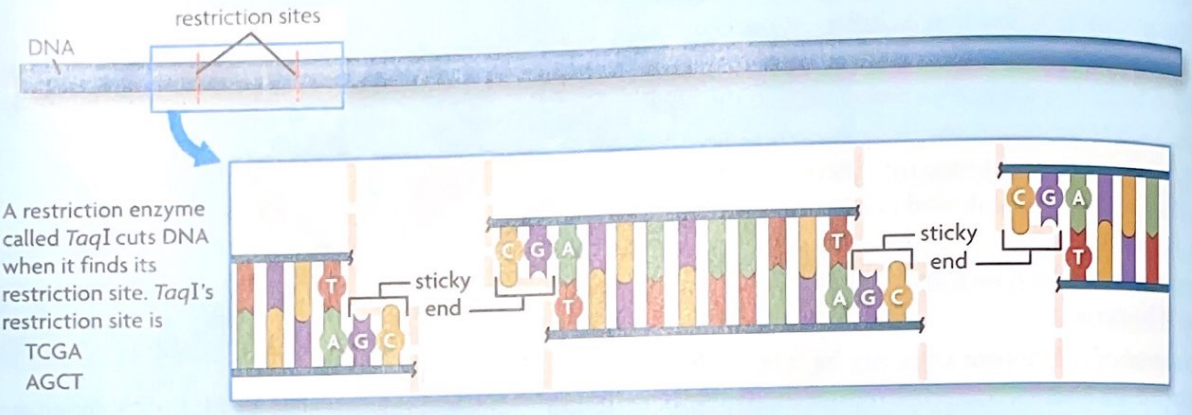


Restriction enzymes recognize nucleotide sequences that are between four and eight base pairs long, and then cut the DNA within that area. Some enzymes make cuts straight across the two strands of a DNA molecule. These cuts leave behind fragments of DNA that end in what are called "blunt ends."

FIGURE 9.2 Restriction Enzymes Cut DNA

Some restriction enzymes leave behind nucleotide tails, or "sticky ends," when they cut DNA.

Animated BIOLOGY
Use virtual restriction enzymes at ClassZone.com.



Infer How would the above illustration change if *TaqI* left behind blunt ends rather than sticky ends when it cuts DNA?

Connecting CONCEPTS

DNA Base Pairs Recall from **Chapter 8** that DNA nucleotides match up by complementary base pairing. A always pairs with T, and C always pairs with G.

Other restriction enzymes, as shown in **FIGURE 9.2**, make staggered cuts th leave tails of free DNA bases on each side of the cut. These nucleotide tails o the cut DNA strands are called "sticky ends." Sticky ends are like tiny pieces Velcro that are ready to hook on to their opposite sides. If two pieces of DN with sticky ends and complementary base pairs come close to each other, th two segments of DNA will join by hydrogen bonding. Because of this charac teristic of DNA, restriction enzymes that leave sticky ends when they cut DN are often used in biotechnology, as you will learn in Section 9.4.

Summarize How do different restriction enzymes produce different DNA fragments from the same DNA molecule?

MAIN IDEA

Restriction maps show the lengths of DNA fragments.

After a long DNA molecule has been cut by restriction enzymes into many smaller fragments, several different things can be done with the DNA. For example, the DNA sequence of a gene can be studied, or a gene cut out from the DNA can be placed into the DNA of another organism. But before any- thing else can be done, the DNA fragments have to be separated from one another. The fragments are sorted according to their sizes by a technique called gel electrophoresis (ih-LEHK-troh-fuh-REE-sihs).

In **gel electrophoresis,** an electrical current is used to separate a mixtur DNA fragments from each other. A sample of DNA is loaded into a gel, wh is like a thin slab of hard gelatin. A positive electrode is at one end of the g At the other end is a negative electrode. Because DNA has a negative charg

the fragments move toward the positive electrode, or the positively charged pole. The gel also has tiny pores running through it. The pores allow small molecules to move quickly. Larger molecules cannot easily move through the gel and they travel more slowly. Therefore, the length of a DNA fragment can be estimated from the distance it travels through a gel in a certain period of time. As shown in **FIGURE 9.3**, DNA fragments of different sizes appear as different bands, or lines, on a gel. The pattern of bands on the gel can be thought of as a map of the original strand of DNA. **Restriction maps** show the lengths of DNA fragments between restriction sites in a strand of DNA.

The bands on a gel indicate only the lengths of DNA fragments. Alone, they do not give any information about the DNA sequences of the fragments. Even though restriction maps do not directly show the makeup of a fragment of DNA, the maps are very useful in genetic engineering, which you will read about in Section 9.4. They can also be used to study gene mutations. How? First, a mutation may add or delete bases between restriction sites, which would change the lengths of DNA fragments on a gel. Second, a mutation may change a restriction site, and the DNA would not be cut in the same places.

Suppose, for example, that when a normal allele of a gene is cut by a restriction enzyme, five DNA fragments appear as five different bands on a gel. Then, when a mutant allele of the same gene is cut with the same enzyme, only three bands appear. Comparisons of restriction maps can help diagnose genetic diseases, as you will see in Section 9.6. A restriction map from a person's DNA can be compared with a restriction map from DNA that is known to be normal. If the restriction maps differ, it is an indication that the person has inherited a disease-causing allele of the gene.

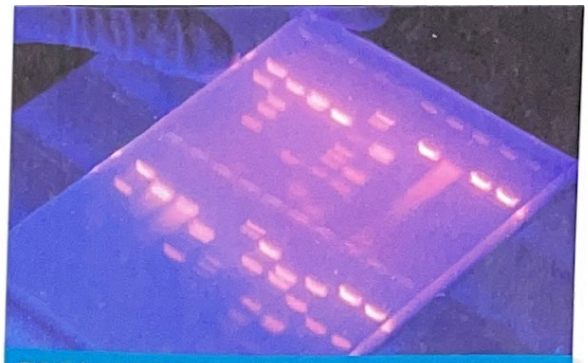
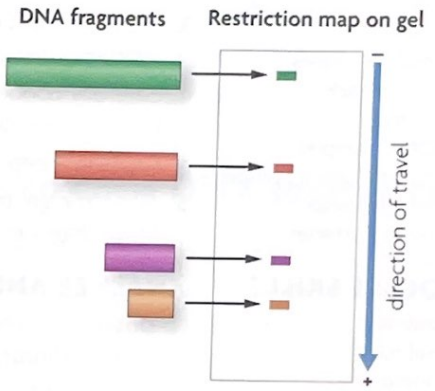


FIGURE 9.3 GEL ELECTROPHORESIS

A segment of DNA is cut with a restriction enzyme into fragments of different lengths.

Different sizes of DNA fragments show up as bands on a gel. Smaller fragments move farther down the gel.



Synthesize **How are restriction enzymes used in making restriction maps?**

9.1 ASSESSMENT

ONLINE QUIZ ClassZone.com

REVIEWING MAIN IDEAS

1. List four different ways in which scientists can manipulate DNA.
2. What determines how DNA will be cut by a **restriction enzyme**?
3. How does **gel electrophoresis** separate DNA fragments from each other?

CRITICAL THINKING

4. **Apply** Suppose you cut DNA. You know that you should find four DNA fragments on a gel, but only three appear, and one fragment is very large. Explain what happened.
5. **Synthesize** What is the relationship between restriction sites and a **restriction map**?

Connecting CONCEPTS

6. **Mutations** Would a mutation in a gene always be detectable by using restriction maps? Why or why not?

9.2 Copying DNA

KEY CONCEPT The polymerase chain reaction rapidly copies segments of DNA.

MAIN IDEAS

- PCR uses polymerases to copy DNA segments.
- PCR is a three-step process.

VOCABULARY

polymerase chain reaction (PCR), p. 269

primer, p. 271

Review

DNA polymerase, replication

REVIEW AT CLASSZONE.COM

Connect Forensic scientists use DNA from cells in a single hair at a crime scene to identify a criminal. Doctors test a patient's blood to quickly detect the presence of bacteria that cause Lyme disease. Scientists compare DNA from different species to determine how closely the species are related. However, the original amount of DNA from any of these sources is far too small to accurately study. Samples of DNA must be increased, or amplified, so that they can be analyzed.

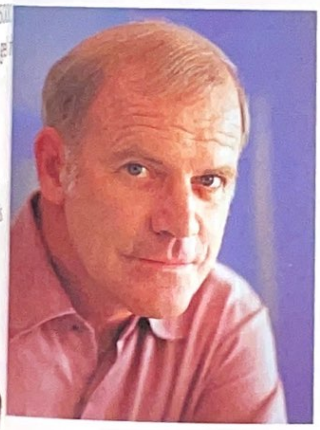
MAIN IDEA

PCR uses polymerases to copy DNA segments.

How do scientists get an amount of DNA that is large enough to be studied and manipulated? They copy the same segment of DNA over and over again. **Polymerase chain reaction (PCR)** is a technique that produces millions—or even billions—of copies of a specific DNA sequence in just a few hours. As the name indicates, the DNA polymerase enzymes that you learned about in Chapter 8 play key roles in this process.

Kary Mullis, who invented PCR, is shown in **FIGURE 9.4**. While working for a California biotechnology company in 1983, Mullis had an insight about how to copy DNA segments. He adapted the process of DNA replication that occurs in every living cell into a method for copying DNA in a test tube. Under the right set of conditions, DNA polymerases copy DNA in a test tube just as they do inside cells. However, in cells several other enzymes are needed before the polymerases can do their job. For example, before a cell can begin to copy its DNA, enzymes called helicases unwind and separate DNA molecules. Instead of using these enzymes, Mullis used heat to separate the DNA strands.

Unfortunately, heat also broke down the *E. coli* polymerases that Mullis first used. Then came Mullis's second stroke of genius: Why not use polymerases from a bacterium that lives in temperatures above 80°C (176°F)? By using this enzyme, Mullis was able to raise the temperature of the DNA to separate the strands without destroying the DNA polymerases. Here again, just as with restriction enzymes that you read about in Section 9.1, a major advance came from applying an adaptation found in nature to biotechnology. Mullis introduced PCR to the world in 1985, and in 1993 he won the Nobel Prize in chemistry for his revolutionary technique.



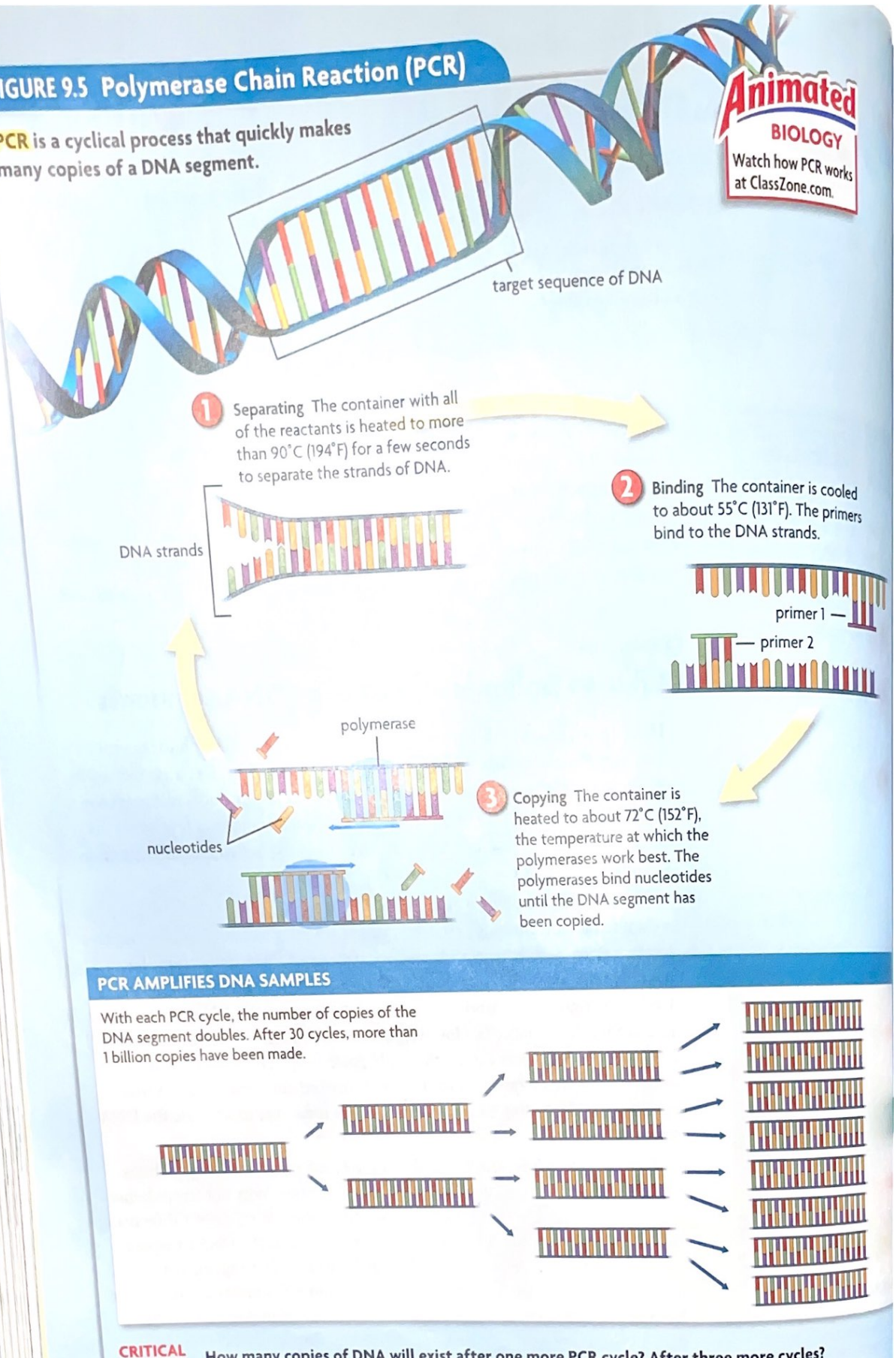
IGURE 9.4 Kary Mullis came up ith the idea for PCR while on a urfing trip in 1983. He won the lobel Prize in chemistry in 1993.

Compare and Contrast **How are replication and PCR similar? different? Explain.**

FIGURE 9.5 Polymerase Chain Reaction (PCR)

PCR is a cyclical process that quickly makes many copies of a DNA segment.

Animated BIOLOGY
Watch how PCR works at ClassZone.com.



PCR AMPLIFIES DNA SAMPLES

With each PCR cycle, the number of copies of the DNA segment doubles. After 30 cycles, more than 1 billion copies have been made.

CRITICAL VIEWING How many copies of DNA will exist after one more PCR cycle? After three more cycles?

MAIN IDEA

CR is a three-step process.

CR is a surprisingly simple process. It uses just four materials: the DNA to be copied, DNA polymerases, large amounts of each of the four DNA nucleotides (A, T, C, and G), and two primers. A **primer** is a short segment of DNA that acts as the starting point for a new strand. If DNA polymerases build new DNA strands, why are primers needed for PCR? DNA polymerases can add nucleotides to strands that have already been started, but they cannot start the strands. In PCR, two primers are used to start the copying of DNA close to the desired segment. The two primers are like bookends for the DNA strand. They limit the length of the copied DNA to one small segment of the strand.

PCR has three main steps, as shown in **FIGURE 9.5.** All of the steps of the cycle take place in the same container but at different temperatures. The main function of the first two PCR cycles is to produce the small segment of DNA that is desired. By making a copy of the desired segment, many copies of that tiny piece of DNA can be made, rather than copying an entire chromosome.

1. **Separating** The container with all of the reactants is heated to separate the double-stranded DNA into single strands.
2. **Binding** The container is cooled and the primers bind to their complementary DNA sequences. One primer binds to each DNA strand. The primers bind on opposite ends of the DNA segment being copied.
3. **Copying** The container is heated again and the polymerases begin to build new strands of DNA. Added nucleotides bind to the original DNA strands by complementary base pairing. The polymerases continue attaching nucleotides until the entire DNA segment has been copied.

Each PCR cycle doubles the number of DNA copies. The original piece of DNA becomes two copies. Those two copies become four copies. And the cycle is repeated over and over to quickly copy enough DNA for study. After only 30 cycles of PCR, for example, the original DNA sequence is copied more than a billion times. This doubling is why the process is called a chain reaction.

er Why is it necessary to keep changing the temperature in the PCR process?

VOCABULARY

The term *primer* comes from a Latin word that means "first." In PCR, a primer is the starting point for the DNA copying process.

Connecting CONCEPTS

Replication Look back at the process of DNA replication in **Chapter 8** to compare PCR with replication.

2 ASSESSMENT

ONLINE QUIZ ClassZone.com

VIEWING MAIN IDEAS

riefly describe the function of **olymerase chain reaction (PCR).**

ummarize the cycle involved in the CR process.

CRITICAL THINKING

3. **Synthesize** Describe how heating double-stranded DNA separates the strands. Why does heating also inactivate DNA polymerases from many organisms?
4. **Analyze** Explain two reasons why **primers** are important in PCR.

Connecting CONCEPTS

5. **Human Genetics** Many human genetic diseases are caused by recessive alleles of genes. How might PCR be important in the diagnosis of these illnesses?

9.3 DNA Fingerprinting

KEY CONCEPT DNA fingerprints identify people at the molecular level.

MAIN IDEAS

- A DNA fingerprint is a type of restriction map.
- DNA fingerprinting is used for identification.

VOCABULARY

DNA fingerprint, p. 272

Review

restriction enzyme, gel electrophoresis, restriction map

REVIEW AT CLASSZONE.COM

Connect You hear about it in the news all the time. DNA evidence is used to convict a criminal, release an innocent person from prison, or solve a mystery. A couple of decades ago, the lines and swirls of someone's fingertip were a detective's best hope for identifying someone. Now, investigators gather biological samples and analyze DNA for another kind of evidence: a DNA fingerprint.

MAIN IDEA

A DNA fingerprint is a type of restriction map.

Unless you have an identical twin, your complete set of DNA, or your genome, is unique. This variation in DNA among people is the basis of DNA fingerprinting. A **DNA fingerprint** is a representation of parts of an individual's DNA that can be used to identify a person at the molecular level.

A DNA fingerprint is a specific type of restriction map, which you learned about in Section 9.1. First, a DNA sample is cut with a restriction enzyme. Then the DNA fragments are run through a gel and the pattern of bands on the gel is analyzed. As you can see in **FIGURE 9.6,** a DNA fingerprint can show relationships among family members. The children (C) have similar DNA fingerprints to one another, but they are not identical. Also, their DNA fingerprints are combinations of the DNA fingerprints of the parents (M and F).

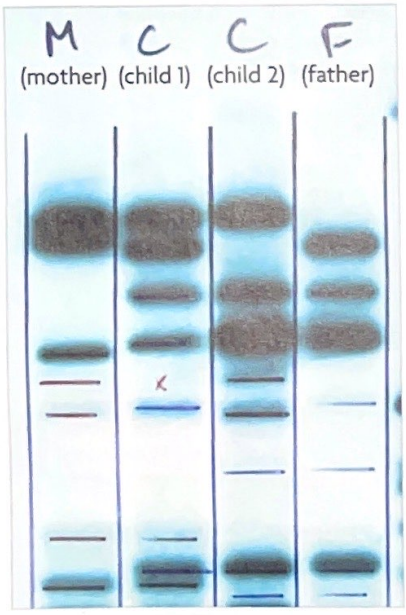


FIGURE 9.6 DNA fingerprints can be compared to identify people. Both children share some bands with each parent.

The greatest differences in DNA among people are found in regions of the genome that are not parts of genes. As a result, DNA fingerprinting focuses on noncoding regions of DNA, or DNA sequences outside genes. Noncoding DNA sequences often include stretches of nucleotides that repeat several times, one after another, as shown in **FIGURE 9.7.** Each person's DNA differs in the numbers of copies of the repeats. For example, one person may have seven repeats in one location, and another person may have three in the same place. To get to the specific regions of DNA that can be identified through DNA fingerprinting, the DNA is cut in known locations with restriction enzymes.

The differences in the number of repeats are found by separating the DNA fragments with gel electrophoresis. When there are more repeats, a DNA fragment is larger. The pattern of DNA fragments on a gel represents the uniqueness of a person's DNA. Individuals might have some of the fragments in common, but it is very unlikely that all of them would be the same.

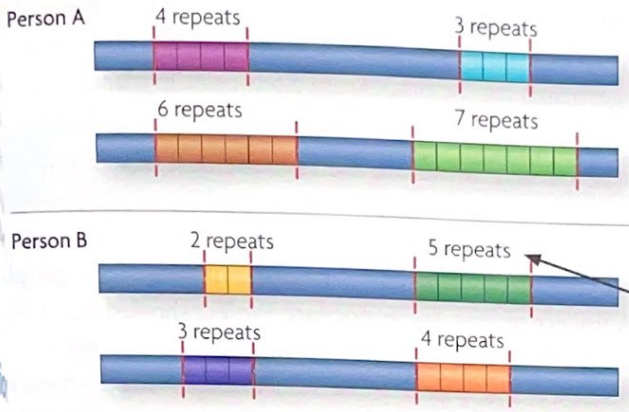
Synthesize Does a DNA fingerprint show a person's genotype? Why or why not?

GURE 9.7 DNA Fingerprinting

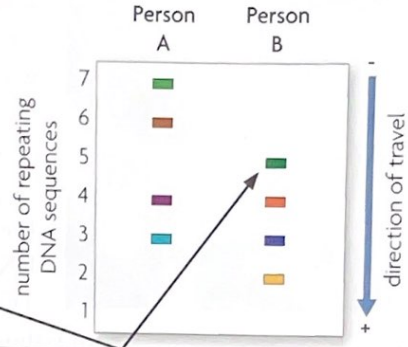
DNA fingerprint shows differences in the number of repeats of certain DNA sequences.

This DNA sequence of 33 bases can be repeated many times in a sample of a person's DNA.

Person A and person B have different numbers of repeated DNA sequences in their DNA.



A DNA fingerprint finds differences in DNA by separating the fragments on a gel.



DNA fragments with different numbers of repeated DNA sequences show up as different bands on a gel.

fer How would the DNA fingerprints change if a different restriction nzyme cut the DNA in the middle of one of the repeated DNA sequences?

MAIN IDEA

NA fingerprinting is used for identification.

DNA fingerprinting to identify people has become a reliable and widely used process since the 1990s. Why? The specific nucleotide sequences that are repeated can be found in everyone. More importantly, from one person to another, the number of repeat sequences can differ greatly, even among brothers and sisters.

DNA Fingerprints and Probability

Identification with DNA fingerprinting depends on probability. Suppose that 1 in every 500 people has three copies of the repeat at location A. This means any person has a 1-in-500 chance of having a matching DNA fingerprint for that region of a chromosome. By itself, the number of repeats in one location cannot be used for identification, because too many people would match.

But then suppose that 1 in every 90 people has six copies of the repeat sequence at location B, and 1 in every 120 people has ten copies of the repeat sequence at location C. Individual probabilities are multiplied by each other to find the total probability. Therefore, when the three separate probabilities are multiplied, suddenly the chance that two people have the same DNA fingerprint is very small.

$$\frac{1}{500} \times \frac{1}{90} \times \frac{1}{120} = \frac{1}{5{,}400{,}000} = 1 \text{ chance in } 5.4 \text{ million people}$$

Connecting CONCEPTS

Genome Recall from **Chapter 6** that a genome is the entire set of DNA in a cell. You will learn more about genome research in **Section 9.5.**

FIGURE 9.8 DNA collected at crime scenes is used as evidence in many legal cases.

Usually, DNA fingerprinting compares at least five regions of the genome. That way it is more certain that the pattern of DNA fragments in the fingerprint is unique. The more regions of DNA that are studied, the less likely it becomes that another person would have the same DNA fingerprint. For this reason, DNA fingerprinting is considered very reliable for identification purposes.

Uses of DNA Fingerprinting

DNA fingerprints are often used in legal cases. Because PCR can make a large sample of DNA even when there is a very small sample to start with, DNA fingerprints can be made from a few cells. Evidence, such as that shown in **FIGURE 9.8**, can come from just a single drop of blood.

Sometimes, DNA fingerprints are used against a suspect, but other times they are used to prove someone's innocence. The Innocence Project at Benjamin Cardozo Law School in New York City has used DNA evidence to help free more than 170 wrongfully convicted people. Through DNA fingerprinting, the Innocence Project showed that DNA from those people did not match DNA from the crime scenes. Proving a person's guilt through DNA fingerprinting is harder than proving a person's innocence. For example, a DNA sample can become contaminated with other DNA if it is not handled carefully. Investigators must also consider questions such as "What is the chance that another person has the same DNA fingerprint?" and "What probability is low enough to be acceptable?" Are chances of 1 in 100,000 low enough? One in 1 million? In fact, there is no legal standard for this probability of a random DNA fingerprint match.

The same DNA fingerprinting methods are also used for identification purposes outside of the courtroom. DNA fingerprints can prove family relationships, such as paternity and the kinship necessary for immigration requests. But DNA fingerprinting is not limited to human identification. Genetic comparisons through DNA fingerprinting are used to study biodiversity and to locate genetically engineered crops. And, as you saw at the beginning of the chapter, researchers are using DNA fingerprinting to identify tortoises native to the Galapagos Islands.

Summarize **How does identification by DNA fingerprinting depend on probability?**

NSTA SCILINKS scilinks.org

To find out more about DNA fingerprinting, go to scilinks.org. **Keycode: MLB009**

9.3 ASSESSMENT

ONLINE QUIZ ClassZone.com

REVIEWING MAIN IDEAS

1. On what, in a person's DNA, is a **DNA fingerprint** based?
2. Describe two ways in which DNA fingerprinting is used.

CRITICAL THINKING

3. **Compare and Contrast** How are DNA fingerprints and restriction maps similar? different? Explain.
4. **Synthesize** Briefly describe how restriction enzymes, gel electrophoresis, and PCR are used in DNA fingerprinting.

Connecting CONCEPTS

5. **Mutations** Why might non-coding regions of DNA outside of genes be more variable than coding regions of DNA?

9.4 Genetic Engineering

KEY CONCEPT DNA sequences of organisms can be changed.

MAIN IDEAS

- Entire organisms can be cloned.
- New genes can be added to an organism's DNA.
- Genetic engineering produces organisms with new traits.

VOCABULARY

clone, p. 275
genetic engineering, p. 276
recombinant DNA, p. 276
plasmid, p. 276
transgenic, p. 277
gene knockout, p. 279
Review
restriction enzyme

REVIEW AT CLASSZONE.COM

Connect Glowing mice are used in cancer research. Glowing plants are used to track genetically modified crops. And, in 1999, British researchers introduced glowing yeast cells that locate water pollution. The scientists put a gene for a fluorescent protein into yeast. Under normal conditions, the yeast cells do not glow. But they do glow when certain chemicals are present. The glow identifies areas that need to be cleaned. New biotechnology applications seem to be developed on a daily basis. What advances will you see during your lifetime?

MAIN IDEA

Entire organisms can be cloned.

The term *cloning* might make you think of science fiction and horror movies, but the process is quite common in nature. A **clone** is a genetically identical copy of a gene or of an organism. For example, some plants clone themselves from their roots. Bacteria produce identical genetic copies of themselves through binary fission. And human identical twins are clones of each other.

People have cloned plants for centuries. The process is fairly easy because many plants naturally clone themselves and because plants have stem cell tissues that can develop into many types of cells. Some simple animals, such as sea stars, can essentially clone themselves through a process called regeneration. Mammals, however, cannot clone themselves.

GURE 9.9 The cat named CC—for opy Cat or Carbon Copy—is the rst successful clone of a cat (right). he original cat is on the left.

To clone a mammal, scientists swap DNA between cells with a technique called nuclear transfer. First, an unfertilized egg is taken from an animal, and the egg's nucleus is removed. Then the nucleus of a cell from the animal to be cloned is implanted into the egg. The egg is stimulated and, if the procedure is successful, the egg will begin dividing. After the embryo grows for a few days, it is transplanted into a female. In 1997 a sheep named Dolly became the first clone of an adult mammal. The success of Dolly led to the cloning of adult cows, pigs, and mice. Now, a biotechnology company has even said that it can clone people's pets.

But pet owners who expect cloning to produce an exact copy of their furry friend will likely be disappointed. As you can see from the cat called CC in **FIGURE 9.9**, a clone may not look like the original, and it will probably not behave like the original, either. Why? Because, as you have learned, many factors, including environment, affect the expression of genes.

Cloning brings with it some extraordinary opportunities. For example, scientists are studying how to use organs from cloned mammals for transplant into humans. This use of cloning could save an enormous number of lives each year. Cloning could even help save endangered species. Cells from endangered species could be taken and used to produce clones that would increase the population of the species.

Cloning is also controversial for a few reasons. The success rate in cloning mammals is very low. It takes hundreds of tries to produce one clone, and sometimes the clone is not as healthy as the original. For example, Dolly seemed to develop and grow normally, but she also had health problems. She seemed to age quickly and did not live as long as a typical sheep, possibly because she was cloned from an adult sheep and had "old DNA." There are also ecological concerns about cloning. Cloned animals in a wild population would reduce biodiversity because the clones would be genetically identical.

Connecting CONCEPTS

Biodiversity In Chapter 1, you learned that biodiversity can be defined as the number of different species in an area. You will learn much more about genetic diversity within a species in Unit 4.

Apply **Given the opportunity, would you have a pet cloned? Explain your answer based on your knowledge of genetics, biotechnology, and cloning.**

MAIN IDEA

New genes can be added to an organism's DNA.

Genetic research relies on cloning, but not the cloning of organisms. Instead, it is the cloning of individual genes. A clone of a gene is a copy of that one segment of DNA. In some cases, scientists insert cloned genes from one organism into a different organism. This changing of an organism's DNA to give the organism new traits is called **genetic engineering.** Genetic engineering is possible because the genetic code is shared by all organisms.

Genetic engineering is based on the use of recombinant DNA technology. **Recombinant DNA** (ree-KAHM-buh-nuhnt) is DNA that contains genes from more than one organism. Scientists are trying to use recombinant DNA in several different ways. For example, recombinant DNA could be used to produce crop plants that make medicines and vitamins. Scientists hope that large amounts of medicines will one day be made through this process, which has been called "pharming." Scientists are also studying ways of using recombinant DNA to make vaccines to protect against HIV, the virus that causes AIDS.

Bacteria are commonly used in genetic engineering. One reason is because bacteria have tiny rings of DNA called plasmids. **Plasmids,** as shown in **FIGURE 9.10**, are closed loops of DNA that are separate from the bacterial chromosome and that replicate on their own within the cell.

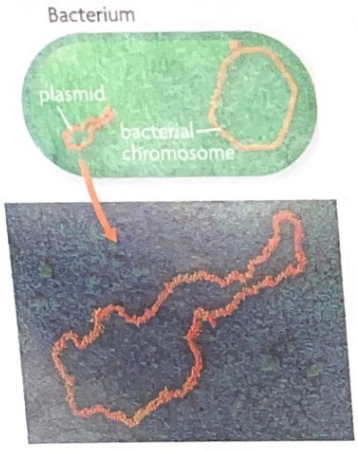
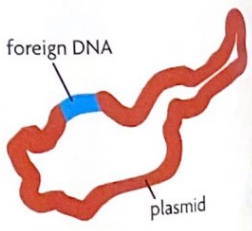


FIGURE 9.10 A plasmid is a closed loop of DNA in a bacterium that is separate from the bacterial chromosome. (colored TEM; magnification 48,000×)

VISUAL VOCAB

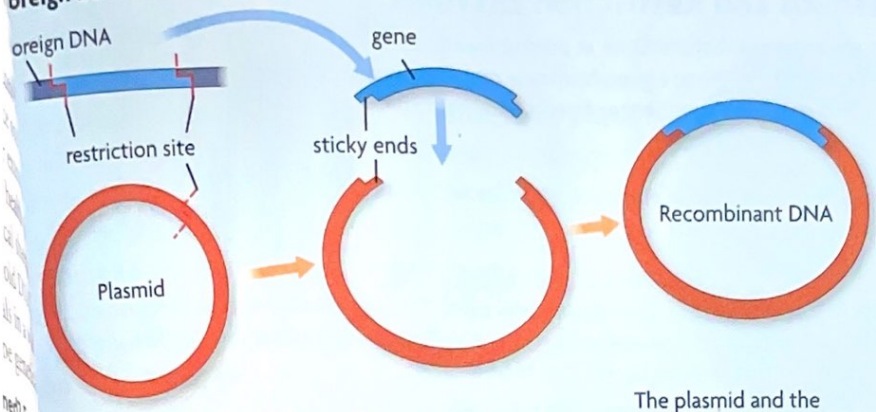
Recombinant DNA is DNA that combines genes from more than one organism.



Foreign DNA is inserted into **plasmid** to make recombinant DNA.

IGURE 9.11 Making Recombinant DNA

oreign DNA can be inserted into a plasmid to make recombinant DNA.

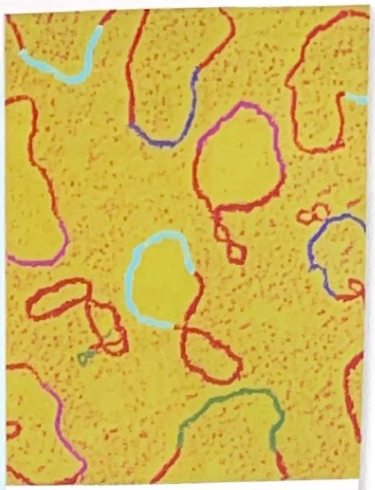


plasmid and the foreign DNA ith the gene are cut with the me restriction enzyme.

The sticky ends of the plasmid and the foreign gene match.

The plasmid and the foreign gene are bonded together to form recombinant DNA.

pply **Why are sticky ends important for making recombinant DNA?**



Plasmids are small rings of DNA used in genetic engineering. Foreign genes (blue, light blue, magenta, green) have been inserted into these plasmids (red). (colored TEM, magnification 29,000×)

Recombinant DNA is found naturally in bacteria that take in exogenous DNA (or DNA from a different organism) and add it to their own. Scientists adapted what happens in nature to make artificial recombinant DNA. First, a restriction enzyme is used to cut out the desired gene from a strand of DNA. Then plasmids are cut with the same enzyme. The plasmid opens, and when the gene is added to the plasmid, their complementary sticky ends are bonded together by a process called ligation. The resulting plasmid contains recombinant DNA, as shown in **FIGURE 9.11.**

mmarize **How does genetic engineering rely on a shared genetic code?**

MAIN IDEA

enetic engineering produces organisms with w traits.

After a gene is added to a plasmid, the genetically engineered plasmids can be ut into bacteria. In a way, bacteria are turned into tiny gene factories that make opy after copy of the plasmid. As a result, the transformed bacteria make many opies of the new gene. The bacteria will express the new gene and make that ene's product. The bacteria with the recombinant plasmid are called transenic. A **transgenic** organism has one or more genes from another organism nserted into its genome. For example, the gene for human insulin can be put nto plasmids. The plasmids are inserted into bacteria. The transgenic bacteria nake human insulin that is collected and used to treat people with diabetes.

VOCABULARY

The prefix *trans-* means "across," and the root *genic* means "referring to genes." When genes are transferred across different organisms, transgenic organisms are produced.

Genetic Engineering in Plants

Genetic engineering of plants is directly related to genetic engineering of acteria. To change a plant's DNA, a gene is inserted into a plasmid and the lasmid is inserted into bacteria. After the bacteria infect the plant, the new ene becomes a part of the plant's DNA and is expressed like any other gene.

QUICK LAB MODELING

Modeling Plasmids and Restriction Enzymes

Restriction enzymes are enzymes that cut DNA at precise locations. These enzymes allow scientists to move a gene from one organism into another. In this lab, you will use DNA sequences from a datasheet to simulate the use of restriction enzymes.

PROBLEM How do different restriction enzymes cut a plasmid?

MATERIALS

- 3 copies of Plasmid Sequence Datasheet
- scissors
- 10 cm clear tape
- 3 sets of 5 5-cm yarn pieces

PROCEDURE

1. Make models of 3 plasmids. Cut out the DNA sequences from the copies of Figure 1 on the datasheet. Use tape to attach the appropriate piece of yarn to each end where indicated. The yarn represents the entire plasmid. The finished plasmid should be a circle.
2. Use the scissors to cut a plasmid at the correct sites for *EcoR*I.
3. Use the sequences for sites of *Hind*III and *Sma*I to repeat step 3 with the other two plasmids.

ANALYZE AND CONCLUDE

1. **Apply** How many DNA fragments would you get if you cut the same plasmid with both *EcoR*I and *Sma*I ?
2. **Infer** Why might scientists use different restrictions enzymes to cut out different genes from a strand of DNA?

This technique has allowed scientists to give plants new traits, such as resistance to frost, diseases, and insects. For instance, a gene known as *Bt* makes a natural pesticide in some organisms. After *Bt* is added to crop plants, smaller amounts of chemical pesticides are needed to protect the crop from insects. Some genetically engineered crops, which are also called genetically modified (GM), are now common in the United States. These crops include potatoes and corn, and they are even more important in developing countries. By increasing crop yields, more food is produced more quickly and cheaply.

Genetic Engineering in Animals

In general, transgenic animals are much harder to produce than GM plants because animals are more resistant to genetic manipulation. To produce a transgenic animal, a researcher must first get a fertilized egg cell. Then the foreign DNA is inserted into the nucleus and the egg is implanted back into a female. However, only a small percentage of the genetically manipulated eggs mature normally. And only a portion of those that develop will be transgenic. That is, only a small number will have the foreign gene as a part of their DNA. But those animals that are transgenic will have the gene in all of their cells—including reproductive cells—and the transgenic trait will be passed on to their offspring.

Transgenic mice are often used as models of human development and disease. The first such animal was called the oncomouse. This mouse always develops cancer, because a gene that controls cell growth and differentiation

was mutated. Researchers use the oncomouse to study both cancer and anti-cancer drugs. Other types of transgenic mice are used to study diabetes, brain function and development, and sex determination.

In another type of genetic manipulation, some mice have genes that have been purposely "turned off." These mice, called **gene knockout** mice, are made by disrupting the function of a gene. Knockout mice are very useful for studying gene function and genetic diseases because a researcher can observe specific changes in gene expression and traits. For example, scientists are using a gene knockout mouse to study obesity, as you can see in **FIGURE 9.12.**

FIGURE 9.12 The knockout mouse (left) does not have a functional gene for a protein called leptin, which helps to control food intake. Researchers are using this type of mouse to study obesity.

Concerns About Genetic Engineering

Scientists have genetically engineered many useful organisms by transferring genes between species to give individuals new traits. At the same time, there are concerns about possible effects of genetically engineered organisms on both human health and the environment. And at an even more basic level, some people wonder whether genetic engineering is ethical in the first place.

Questions have been raised about GM crops, even though scientists have not yet found negative health effects of GM foods. Critics say that not enough research has been done, and that some added genes might cause allergic reactions or have other unknown side effects. Scientists also have concerns about the possible effects of GM plants on the environment and on biodiversity. For example, what would happen if genetically engineered *Bt* plants killed insects that pollinate plants, such as bees and butterflies? In some instances, transgenic plants have cross-pollinated with wild type plants in farming regions. Scientists do not yet know what long-term effect this interbreeding might have on the natural plants. In addition, all organisms in a transgenic population have the same genome. As a result, some scientists worry that a decrease in genetic diversity could leave crops vulnerable to new diseases or pests.

er Why is it important that a transgenic trait is passed on to the transgenic ganism's offspring?

4 ASSESSMENT

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VIEWING MAIN IDEAS

Why is the offspring of asexual reproduction a **clone**?

What are **plasmids,** and how are they used in **genetic engineering**?

Describe two applications of **transgenic** organisms.

CRITICAL THINKING

4. **Compare and Contrast** How is the cloning of genes different from the cloning of mammals?
5. **Summarize** How are restriction enzymes used to make both **recombinant DNA** and transgenic organisms?

Connecting CONCEPTS

6. **Ecology** Do you think cloning endangered species is a good idea? What effect might this have on an ecosystem?

9.5 Genomics and Bioinformatics

KEY CONCEPT Entire genomes are sequenced, studied, and compared.

MAIN IDEAS

- Genomics involves the study of genes, gene functions, and entire genomes.
- Technology allows the study and comparison of both genes and proteins.

VOCABULARY

genomics, p. 280
gene sequencing, p. 280
Human Genome Project, p. 281
bioinformatics, p. 282
DNA microarray, p. 282
proteomics, p. 283

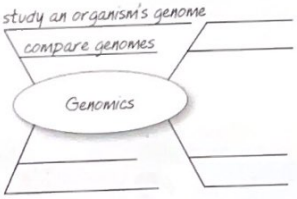
Review
genome, mRNA

REVIEW AT CLASSZONE.COM

Connect Humans and chimpanzees are identical in 98 to 99 percent of their DNA. How do scientists know this? They have sequenced all of the DNA in both species. Recent technologies are allowing scientists to look at huge amounts of genetic information at once. What might tomorrow's discoveries tell us about evolution, gene expression, and medical treatments?

TAKING NOTES

Use a mind map to organize your notes on genomics.



MAIN IDEA

Genomics involves the study of genes, gene functions, and entire genomes.

A gene, as you know, is a single stretch of DNA that codes for one or more polypeptides or RNA molecules. A genome is all of an organism's genetic information. **Genomics** is the study of genomes, which can include the sequencing of all of an organism's DNA. Scientists compare genomes both within and across species to find similarities and differences among DNA sequences. Comparing DNA from many people at one time helps researchers to find genes that cause disease and to understand how medications work. Biologists who study evolution can learn when closely related species diverged from each other. Scientists can also learn about interactions among genes and find out how an organism's genome makes the organism unique.

DNA Sequencing

All studies of genomics begin with **gene sequencing,** or determining the order of DNA nucleotides in genes or in genomes. An early sequencing method was developed in the 1970s by British scientist Frederick Sanger. The Sanger method is somewhat similar to PCR, which you read about in Section 9.2.

A radioactive primer is added to a single strand of DNA. Polymerase then builds a short segment of a new DNA strand. The lengths of the new strands are controlled so that they can be separated by gel electrophoresis. Based on the pattern of DNA fragments on the gel, the DNA sequence of the original strand can be put together like the pieces of a puzzle.

You might be surprised to learn that humans do not have the largest genome—the most DNA—among organisms. Scientists have determined the DNA sequences for the genomes of several species, including the ones listed in **FIGURE 9.13**. In some cases, the genomes are used to study basic questions about genes and genetics. In other cases, a genome is sequenced because that organism is used as a model in medical research. In all cases, the genomes of organisms that have been sequenced, including bacteria, insects, plants, and mammals, give us important clues toward finding out how genes function.

Yeast, for example, are very useful for scientists who study gene regulation. Genes that control development in the fruit fly are very similar to those genes in humans. The genomes of several plants have been sequenced so that scientists can learn ways to improve crop yields and to increase the resistance of those crops to disease and weather. The genomes of rats and mice are quite similar to the human genome. As a result, both of these species are used as models for human diseases and gene function.

FIGURE 9.13 COMPARING GENOME SIZES

Organism	Approximate Total DNA (millions of base pairs)
E. coli	4.6
Yeast	12.1
Fruit fly	165
Banana	873
Chicken	1200
Human	3000
Vanilla	7672
Crested newt	18,600
Lungfish	139,000

Source: University of Nebraska

The Human Genome Project

The genomes of yeast and fruit flies are easier to sequence than the human genome. This difficulty is not due to the number of genes that humans have. In fact, while there is still a debate about the exact number of human genes, scientists agree it is surprisingly small. It is estimated that there are somewhere between 30,000 and 40,000 genes in the human genome. But think about the amount of DNA that each of us has in our cells. The human genome has at least 3 billion base pairs. This means that there is an average of about one gene in each sequence of 100,000 bases. Now just try to imagine the huge task of finding out the exact order of all of those DNA bases. In 1990 an international effort began to do exactly that.

The two main goals of the **Human Genome Project** are (1) to map and sequence all of the DNA base pairs of the human chromosomes and (2) to identify all of the genes within the sequence. The first goal was accomplished in 2003 when scientists announced that they had sequenced the human genome. However, the Human Genome Project only analyzed the DNA from a few people. Knowing those few complete DNA sequences is only the first step in understanding the human genome.

Today, scientists continue to work on identifying genes, finding the locations of genes, and determining the functions of genes. The complete sequencing of a human genome was a giant step, but much more work still needs to be done. For example, some scientists are working on a project called the HapMap to study how DNA sequences vary among people. The goal of the HapMap is to develop a method that will quickly identify genetic differences that may play a part in human diseases.

nthesize How is genomics related to genes and DNA?

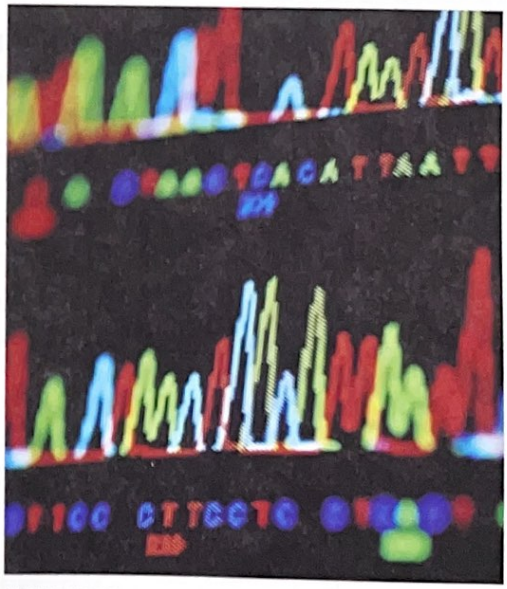


FIGURE 9.14 Computer analysis of DNA was necessary in sequencing the human genome.

DATA ANALYSIS

CONSTRUCTING HISTOGRAMS

To construct a histogram a scientist will count the number of data points in each category and then graph the number of times that category occurs. The categories are shown on the *x*-axis and the frequencies are shown on the *y*-axis. The data table to the right shows the ranges of base pair lengths for the 24 human chromosomes (chromosomes 1–22, the X chromosome, and the Y chromosome). The data are organized by these ranges.

1. **Graph Data** Construct a histogram that shows the frequency of base pair lengths for the 24 human chromosomes.
2. **Interpret** Which range of base pair length is most common for human chromosomes?
3. **Analyze** Summarize the overall trend for human chromosome length shown in the histogram.

TABLE 1. HUMAN CHROMOSOME SIZES

Millions of Base Pairs	Number of Human Chromosomes
0–50	2
51–100	6
101–150	8
151–200	6
201–250	2

Source: U.S. Department of Energy Office of Science

MAIN IDEA

Technology allows the study and comparison of both genes and proteins.

You have learned about specific genes that produce specific traits. But you also know that genes act as more than simple, separate units. They interact and affect each other's expression. Most biological processes and physical traits are the result of the interactions among many different genes.

Bioinformatics

Genes are sequenced, genomes are compared, and proteins are analyzed. What happens to the huge amounts of data that are produced? These data can be analyzed only if they are organized and searchable. **Bioinformatics** is the use of computer databases to organize and analyze biological data. Powerful computer programs are needed to compare genomes that are billions of base pairs in length, especially if the genomes differ by only a small amount.

Bioinformatics gives scientists a way to store, share, and find data. It also lets researchers predict and model the functions of genes and proteins. Because bioinformatics links different areas of research, it has become vital to the study of genes and proteins. For example, a scientist can now search databases to find the gene that is the code for a known protein.

DNA Microarrays

DNA microarrays are tools that allow scientists to study many genes, and their expression, at once. A microarray is a small chip that is dotted with all of the genes being studied. The genes are laid out in a grid pattern. Each block of the grid is so small that a one-square-inch chip can hold thousands of genes.

Connecting CONCEPTS

Computer Models Recall from **Chapter 1** how computer models are used to investigate biological systems that cannot be studied directly. Computer models are often used in genetics and genomics.

Complementary DNA (cDNA) labeled with a fluorescent dye is added to the microarray. A cDNA molecule is a single-stranded DNA molecule that is made from an mRNA molecule. The mRNA acts as a template for the cDNA. Therefore, a cDNA molecule is complementary to an mRNA molecule and is identical to a gene's DNA sequence. The cDNA binds to its complementary DNA strand in the microarray by the same base pairing that you learned about in Chapter 8.

Anywhere cDNA binds to DNA in the microarray shows up as a glowing dot because of the dye. A glowing dot in the microarray is a match between a cDNA molecule and the DNA on the chip. Therefore, a glowing dot shows which genes are expressed and how much they are expressed. Microarrays, as shown in **FIGURE 9.15**, help researchers find which genes are expressed in which tissues, and under what conditions. For example, DNA microarrays can compare gene expression in cancer cells with gene expression in healthy cells. Scientists hope that this method will lead to cancer treatments that target the faulty genes.

FIGURE 9.15 Gene expression can be studied with microarrays. The red dots show genes that are expressed after exposure to a toxic chemical.

Proteomics

You have read how genomics is the study of genomes. **Proteomics** (PROH-tee-AH-mihks) is the study and comparison of all the proteins that result from an organism's genome. Proteomics also includes the study of the functions and interactions of proteins. Identifying and studying proteins is more difficult than identifying and studying genes. A single gene, depending on how its mRNA is edited, can code for more than one polypeptide. Different proteins are found in different tissues, depending on gene expression. And, often, the functions of proteins have to be studied within a biological system.

Proteomics has potential benefits for many areas of biology. Shared evolutionary histories among organisms are studied by comparing proteins across species. Proteomics allows scientists to learn about proteins involved in human diseases. By better understanding the proteins that might play a part in cancer, arthritis, or heart disease, scientists might be able to develop new treatments that target the proteins. Proteomics even has the potential to help doctors match medical treatments to a patient's unique body chemistry.

Apply How is bioinformatics a form of data analysis?

9.5 ASSESSMENT

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REVIEWING MAIN IDEAS

1. Describe the goals of the **Human Genome Project.**
2. Why is **bioinformatics** important in genetic research?

CRITICAL THINKING

3. **Apply** Describe the difference between **gene sequencing** and DNA fingerprinting.
4. **Compare and Contrast** How is the study of specific genes different from the study of a genome?

Connecting CONCEPTS

5. **Cell Biology** How might **genomics** and **proteomics** help researchers predict how a medical treatment might affect cells in different tissues?

9.6 Genetic Screening and Gene Therapy

KEY CONCEPT Genetics provides a basis for new medical treatments.

MAIN IDEAS

- Genetic screening can detect genetic disorders.
- Gene therapy is the replacement of faulty genes.

VOCABULARY

genetic screening, p. 284
gene therapy, p. 285

Review
pedigree, genetic engine

REVIEW AT CLASSZONE.COM

Connect Anyone could be a carrier of a genetic disorder. Genetic screening is used to help people figure out whether they are at risk for passing on that diso der. If they are at risk, what do they do? Do they not have children? Do they h children and hope that a child does not get the disorder? What would you do?

MAIN IDEA

Genetic screening can detect genetic disorders.

Every one of us carries alleles that produce defective proteins. Usually, these genes do not affect us in a significant way because we have other alleles that make up for the deficiency. But about 10 percent of people will find themselves dealing with an illness related to their genes at some point in their li

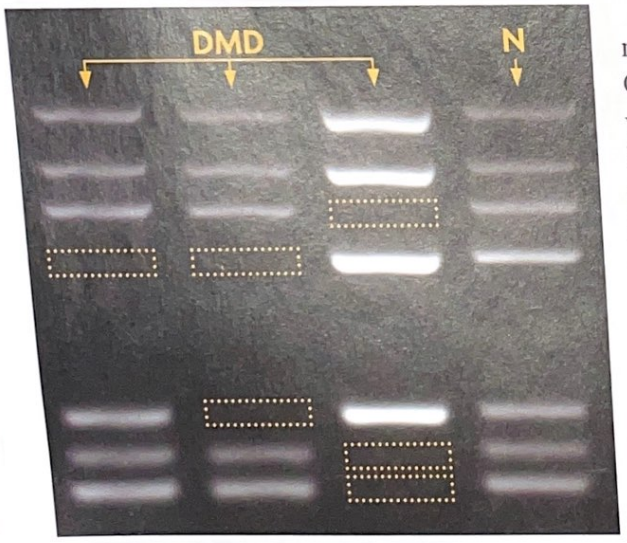

FIGURE 9.16 Genetic screening can be used to detect Duchenne's muscular dystrophy (DMD). Notice the missing bands on the gel (boxes) for three people with DMD as compared with a person without the disorder (N).

Genetic screening is the process of testing DNA to determine a person's risk of having or passing on a genetic diso Genetic screening often involves both pedigree analysis, wh you read about in Chapter 7, and DNA tests. Because our knowledge of the human genome is still limited, it is not y possible to test for every possible defect. Often, genetic scre ing is used to look for specific genes or proteins that indica particular disorder. Some tests can detect genes that are rel to an increased risk of developing a disease, such as a gene called BRCA1 that has been linked to breast cancer. There also tests for about 900 genetic disorders, including cystic fibrosis and Duchenne's muscular dystrophy (DMD). In D it is quite easy to see differences in DNA tests between peo with and without the disorder, as shown in **FIGURE 9.16.**

Genetic screening can help save lives. It can also lead to some difficult choices. Suppose a person has a family history of cancer an tested for a gene that may lead to an increased risk of cancer. Is that infor tion helpful or harmful? If a person has a chance of being a carrier of a ge disorder, should screening be required? As genetic screening becomes mo common, more questions like these will need to be answered.

Infer **Why might genetic screening raise ethical concerns about privacy?**

MAIN IDEA

ene therapy is the replacement of faulty genes.

A defective part in a car or in a computer can be easily replaced. If someone has a faulty gene that causes a disorder, is it possible to replace the gene? The goal of gene therapy is to do exactly that. **Gene therapy** is the replacement of a defective or missing gene, or the addition of a new gene, into a person's genome to treat a disease.

For any type of gene therapy to work, researchers such as Dr. Betty Pace, shown in **FIGURE 9.17**, must first get the new gene into the correct cells of a patient's body. Once in the body, the gene has to become a part of the cells' DNA. One method of gene therapy that scientists have tried is to take a sample of bone marrow stem cells and "infect" them with a virus that has been genetically engineered with the new gene. Then the stem cells are put back into the patient's bone marrow. Because they are stem cells, they divide and make more blood cells with the gene.

FIGURE 9.17 Dr. Betty Pace, director of the Sickle Cell Disease Research Center at the University of Texas at Dallas, is studying potential gene therapy treatments for sickle cell disease.

The first successful trial of gene therapy took place in 1990. The treatment was used on two children with a genetic autoimmune disorder, and the children are now adults leading normal lives. However, much of gene therapy is still experimental. For example, researchers are studying several different methods to treat cancer with gene therapy. One experimental approach involves inserting a gene that stimulates a person's immune system to attack cancer cells. Another method is to insert "suicide" genes into cancer cells. These genes activate a drug inside those cells so that only the cancer cells are killed.

Gene therapy has many technical challenges. First, the correct gene has to be added to the correct cells. And even after researchers have figured out how to transfer the desired gene, the gene's expression has to be regulated so that it does not make too much or too little protein. Scientists must also determine if the new gene will affect other genes. The many trials have produced few long-lasting positive results. But because of its great potential, research on gene therapy continues.

ynthesize **How does gene therapy rely on genetic screening?**

.6 ASSESSMENT

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EVIEWING MAIN IDEAS

How does **genetic screening** use both old and new methods of studying human genetics?

Briefly describe the goals and methods of **gene therapy.**

CRITICAL THINKING

3. **Compare and Contrast** How is gene therapy similar to, and different from, making a transgenic organism?
4. **Synthesize** How are restriction enzymes and recombinant DNA important for gene therapy?

Connecting CONCEPTS

5. **Cell Specialization** How is the type of cell into which a new gene is inserted important in gene therapy?