Using DNA technology called a microarray, researchers are able to see which genes are being actively transcribed in a cell. In the microarray seen on the computer screen, each spot corresponds to a different gene within the cell being studied.
DNA TECHNOLOGY

Today, scientists manipulate DNA for many practical purposes by using techniques collectively called DNA technology. For example, DNA technology can be used as evidence in a criminal case by providing positive identification of DNA left at the scene of a crime. Scientists also use DNA technology to improve food crops, to determine if a person has the genetic information for certain diseases before symptoms appear, and to do research on treatments and cures for genetic diseases. This chapter discusses the tools of DNA technology, how scientists apply these tools to study entire genomes, and how they use them to improve human lives.

DNA IDENTIFICATION

Except for identical twins, no two people in the world are genetically alike. A majority of DNA is actually the same in all humans, but about 0.10 percent of the human genome (the complete genetic material in an individual) varies from person to person. Because of the 0.10 percent variation, scientists are able to identify people based on their DNA. DNA identification compares DNA samples in regions of a chromosome that differ. DNA identification is useful for many purposes, including determining a person’s paternity, identifying human remains, tracing human origins, and providing evidence in criminal cases.

Noncoding DNA

Amazingly, about 98 percent of our genetic makeup does not code for any protein. The regions of DNA that do not code for proteins are called noncoding DNA. Noncoding DNA contains many length polymorphisms, or variations in the length of the DNA molecule between known genes. Some length polymorphisms in noncoding regions come from short, repeating sequences of DNA. For example, a repeating nucleotide sequence might be CACACA, and so on. These sequences can repeat a few or many times in tandem (one “behind” another) and so are called variable number tandem repeats (VNTR). The number of tandem repeats at specific places (loci) in DNA varies among individuals. For each of the many VNTR loci in a person’s DNA, he or she will have a certain number of repeats. Geneticists have calculated how frequently VNTR used in DNA identification occur in the general population. With these numbers, they can determine how rare a particular DNA profile is.
CHAPTER 13

STEPS IN DNA IDENTIFICATION

The main steps involved in DNA identification are (1) isolate the DNA in a sample and, if needed, make copies, (2) cut the DNA into shorter fragments that contain known VNTR areas, (3) sort the DNA by size, and (4) compare the size fragments in the unknown sample of DNA to those of known samples of DNA. If a match occurs between the unknown sample and a known sample, then a person’s identity can be confirmed.

Copying DNA: Polymerase Chain Reaction

Often, DNA that is recovered from a crime scene or from human tissues is present in very small amounts. In these cases, scientists need to make copies in order to have enough DNA to use for DNA identification. The polymerase chain reaction (PCR) is a technique that quickly produces many copies of a DNA fragment.

As shown in step 1 of Figure 13-1, PCR requires a template, a DNA fragment containing the sequence the scientist wants to copy. PCR also requires a supply of the four DNA nucleotides, heat-tolerant DNA polymerase, and primers. Primers are artificially made pieces of single-stranded DNA that are 20 to 30 nucleotides long that must be present for DNA polymerase to initiate replication. Primers are complementary to the ends of the DNA fragment that is to be copied.

When all the ingredients are combined, copying can begin. Primers bind to DNA, and DNA polymerase makes a copy of both DNA strands. Heating breaks the bonds holding the template DNA to the newly made strands. After cooling, as shown in step 2, primers can once more bind to the DNA. Then, in step 3, DNA polymerase can copy again. In step 4, the cycle is repeated. With each new cycle, the DNA between the two primers doubles.

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Cutting DNA: Restriction Enzymes

To cut long DNA molecules into shorter pieces, biologists use bacterial proteins called restriction enzymes. Restriction enzymes recognize specific short DNA sequences, and cut the DNA in or near the sequence, as Figure 13-2 shows. Some restriction enzymes leave DNA overhangs that function like “sticky ends” so that other pieces of DNA with complementary sequences can bind to them.

Sorting DNA by Size: Gel Electrophoresis

DNA fragments can be studied using a technique called gel electrophoresis. Gel electrophoresis separates nucleic acids or proteins according to their size and charge. As shown in step 1 of Figure 13-3, the DNA samples are cut with a restriction enzyme. The DNA is then placed in wells made on a thick gel, as shown in step 2. An electric current runs through the gel for a given period of time. Negatively charged DNA fragments migrate toward the positively charged end of the gel. Smaller DNA fragments migrate faster and farther than longer fragments, and this separates the fragments by size. In step 3, the DNA is transferred to a nylon membrane and radioactive probes are added. The probes bind to complementary DNA. In step 4, an X-ray film is exposed to the radiolabeled membrane. The resulting pattern of bands is called a DNA fingerprint.

FIGURE 13-2

The restriction enzyme in this figure recognizes the sequence GAATTTC on each DNA and cuts each chain between the G and A nucleotides. DNA fragments with sticky ends result.

FIGURE 13-3

In a DNA fingerprint, DNA samples are cut, transferred to a nylon membrane, and exposed to radioactive probes. Exposing X-ray film to the membrane makes the fingerprint visible.
Comparing DNA: DNA Fingerprints

To permanently preserve the DNA fingerprint, a technician can place a positively charged nylon membrane over the gel and transfer the negatively charged DNA to the membrane. To visualize specific DNA pieces, biologists can prepare a nucleic acid complementary to the DNA of interest and label it radioactively. A sheet of X-ray film placed over the gel will be exposed only where the desired DNA is on the gel, providing a DNA fingerprint. An example of a DNA fingerprint is shown in Figure 13-4.

Accuracy of DNA Fingerprints

What makes a DNA fingerprint so powerful as an identification tool is the combined analysis of many VNTR loci. Analyzing only one VNTR locus is like having only one digit in a person’s telephone number. DNA fingerprinting typically compares from five to thirteen VNTR loci. Thirteen loci, as often used by some crime labs in their criminal profiles, produce the odds that two people will share a DNA profile at about one in 100 billion. There are about 6.5 billion people on Earth, so this should be enough to remove any doubt.

RECOMBINANT DNA

The techniques of DNA technology are sometimes used to modify the genome of a living cell or organism. The process of altering the genetic material of cells or organisms to allow them to make new substances is called genetic engineering. Recombinant DNA results when DNA from two different organisms is joined.

An organism with recombinant DNA is shown in Figure 13-5. To study blood vessel growth, researchers combined a jellyfish gene encoding green fluorescent protein (GFP) that glows under ultraviolet light with a zebrafish gene that is involved in blood-vessel development. They inserted the GFP/blood-vessel gene into zebrafish embryos. The fish’s blood-vessel cells transcribed the recombinant DNA and made the green fluorescent proteins. As the zebrafish grew, their blood vessels glowed green, and researchers could more easily study their growth.

Cloning Vectors

A clone is an exact copy of a DNA segment, a whole cell, or a complete organism. Biologists also use the term clone as a verb, meaning to make a genetic duplicate. Researchers can clone DNA fragments by inserting them into vectors, DNAs that can replicate within a cell, usually a bacterium or yeast, and that can carry foreign DNA. When the vector carrying the foreign DNA enters bacteria and the bacteria reproduce, they grow a colony of cloned cells that includes the foreign DNA. Cloning vectors include viruses that infect bacteria and plasmids.
As shown at the top left side of Figure 13-6, **plasmids** are small rings of DNA found naturally in some bacterial cells in addition to the main bacterial chromosome. Step 1 of Figure 13-6, shown twice, illustrates the first step in making recombinant DNA using a plasmid. The plasmid and the DNA of interest (in this case, human DNA containing the gene for insulin) are isolated. In step 2, again shown for both the plasmid and the human DNA, a restriction enzyme is used to cut the DNA into many fragments. Sticky ends hold the donor and plasmid DNA together until an enzyme called **DNA ligase** joins them permanently, shown in step 3.

In step 4, the recombinant DNA plasmids, each with a different portion of the donor DNA, are transferred into bacteria. When the bacterial cells copy their own DNA, they also copy the plasmids and the donor genes the plasmids carry, as shown in step 5. After the cells have grown into colonies, the bacterial colony containing the plasmid with the desired DNA is then identified by using a probe.

**Probes**
A **probe** is a strand of RNA or single-stranded DNA that is labeled with a radioactive element or fluorescent dye and that can base-pair to specific DNA, such as the donor gene in recombinant DNA. For the donor gene in Figure 13-6, the probe is mRNA for the human insulin gene. To see which of the thousands of colonies contains the desired recombinant gene, biologists transfer the DNA from the bacteria onto filter paper. When viewed under ultraviolet light or exposed to photographic film, the clone of cells bearing the donor DNA and its attached probe glows and reveals its location. Biologists can now grow more of the specific recombinant bacterial clone.
APPLICATIONS FOR DNA TECHNOLOGY

DNA technology has been used for many purposes. For example, DNA identification has been used in forensics to identify criminals and to free the wrongly convicted. DNA has also been used to identify human remains. For example, DNA identification techniques were used to identify Czar Nicholas II of Russia and his family, who were executed by the Bolsheviks in 1918.

In addition to forensic applications, DNA technology is of great importance in other scientific efforts. Anthropologists use DNA identification techniques to trace human origins and migrations. Environmental conservationists use the same techniques to trace migrations and movements of threatened or endangered organisms in the effort to preserve their species.

Recombinant DNA techniques give microorganisms new capabilities that have useful applications. The first recombinant DNA product to be used commercially was human insulin (for the treatment of diabetes) in 1982. A recombinant DNA molecule was made by inserting a human gene for insulin into a bacterial plasmid. These bacteria are now grown in vats, from which large amounts of human insulin are extracted and used to treat diabetes.

Since 1982, more than 30 products made using DNA technology have been approved and are being used around the world. These proteins are preferred to conventional drugs because they are highly specific and have fewer side effects. Medically important proteins include factors to treat immune-system deficiencies and anemia. Clotting factors for people with hemophilia, human growth hormone for people with growth defects, interferons for viral infections and cancer, and proteins, such as growth factors to treat burns and ulcers, are just a few other genetically engineered medicines in use today.

1. Summarize the significance of noncoding DNA to DNA identification.
2. Describe the steps in a polymerase chain reaction.
3. What role do restriction enzymes play in DNA technology?
4. What are “sticky ends,” and in what way do they function in making recombinant DNA?
5. Explain the role cloning vectors play in making recombinant DNA.
6. List three ways that DNA technology could be used to improve the lives of humans.

CRITICAL THINKING

7. Relating Concepts Explain why the process of genetic fingerprinting uses only specific, short segments of DNA rather than the entire genome.
8. Evaluating Conclusions A student performing electrophoresis on a DNA sample believes that her smallest DNA fragment is the band nearest the negative pole of the gel. Do you agree with her conclusion? Explain your answer.
9. Applying Information Explain the statement that the genetic code is universal.
The Human Genome Project

One exciting application of DNA technology has been the sequencing of the entire human genome. This section discusses how researchers used modern genetic tools to sequence the human genome and what their findings mean for 21st century biology and society.

Mapping the Human Genome

In 1990, geneticists around the world tackled one of the most ambitious projects in scientific history—the Human Genome Project. The Human Genome Project is a research effort undertaken to sequence all of our DNA and locate within it all of the functionally important sequences, such as genes. That is, the project seeks to determine the sequence of all 3.3 billion nucleotides of the human genome and to map the location of every gene on each chromosome. Information from the project will provide insight into our evolutionary past, genome organization, gene expression, and cell growth.

The Human Genome Project linked more than 20 scientific laboratories in six countries. By 2001, the draft sequence of the human genome appeared in two landmark papers in the science journals Science and Nature. The high-quality sequence was completed in 2003—two years ahead of schedule. Figure 13-7 shows an example of how the sequence of nucleotides in a piece of DNA is displayed on a computer screen.

Important Insights

Scientists with the Human Genome Project were surprised by some of the discoveries they made, including the following:

1. Only about 2 percent of the human genome codes for proteins.
2. Chromosomes have unequal distribution of exons—sequences of nucleotides that are transcribed and translated.
3. The human genome is smaller than previously estimated. The human genome has only about 20,000 to 25,000 protein-coding genes—far fewer than the 100,000 originally estimated. Scientists now know that RNAs are not used only for translating DNA into proteins. Many RNAs are involved in regulating gene expression.
4. The exons of human genes are spliced in many ways, allowing the same gene to encode different versions of a protein. An organism’s complete set of proteins is called its **proteome**. The human proteome is quite complex.

5. About half of the human genome arises from the shuffling of transposons, pieces of DNA that move from one chromosome location to another. Transposons appear to have no specific role in development or physiology.

6. There are about 8 million **single nucleotide polymorphisms** (SNP), unique spots where individuals differ by a single nucleotide. SNPs are important for mapping the genome in more detail, and in the identification of human disease genes.

**Model Species**

In order to better understand how human genes control development and health and to explain how genes affect behaviors, biologists want to map similar genes in **model species**. Since the Human Genome Project, many other genome sequence projects have been completed, and more are underway. Some of the model species for genome sequencing, as seen in Table 13-1, include a bacterium, a roundworm, a fruit fly, a zebrafish, and a mouse. Researchers can induce mutations in each of these species to look for gene actions. Because they represent such a broad phylogenetic base, it is possible to make valid generalizations that relate to larger groups.

**Table 13-1** Genome Sizes of Some Species

<table>
<thead>
<tr>
<th>Domain/Kingdom</th>
<th>Organism</th>
<th>Common name</th>
<th>Genome size (million bases)</th>
<th>Number of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Archaia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archaebacteria</td>
<td><em>Pyrococcus furiosus</em></td>
<td><em>Pyrococcus</em></td>
<td>1.9</td>
<td>2,065</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eubacteria</td>
<td><em>Chlamydia trachomatis</em></td>
<td><em>Chlamydia</em></td>
<td>1.0</td>
<td>894</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td><em>E. coli</em></td>
<td>4.6</td>
<td>4,289</td>
</tr>
<tr>
<td><strong>Eukarya</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protista</td>
<td><em>Dictyostelium discoideum</em></td>
<td><em>Amoeba</em></td>
<td>34</td>
<td>~9,000</td>
</tr>
<tr>
<td>Fungi</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td><em>Yeast</em></td>
<td>12</td>
<td>6,000</td>
</tr>
<tr>
<td>Plantae</td>
<td><em>Arabidopsis thaliana</em></td>
<td><em>Mustard</em></td>
<td>125</td>
<td>23,174</td>
</tr>
<tr>
<td></td>
<td><em>Lilium longiflorum</em></td>
<td><em>Easter lily</em></td>
<td>100,000</td>
<td>~25,000</td>
</tr>
<tr>
<td>Animalia</td>
<td><em>Drosophila melanogaster</em></td>
<td><em>Fruit fly</em></td>
<td>120</td>
<td>13,600</td>
</tr>
<tr>
<td></td>
<td><em>Caenorhabditis elegans</em></td>
<td><em>Roundworm</em></td>
<td>97</td>
<td>19,049</td>
</tr>
<tr>
<td></td>
<td><em>Xenopus tropicalis</em></td>
<td><em>Frog</em></td>
<td>1,700</td>
<td>~30,000</td>
</tr>
<tr>
<td></td>
<td><em>Homo sapiens</em></td>
<td><em>Human</em></td>
<td>3,300</td>
<td>~20,000</td>
</tr>
<tr>
<td></td>
<td><em>Mus musculus</em></td>
<td><em>Mouse</em></td>
<td>3,630</td>
<td>~30,000</td>
</tr>
<tr>
<td></td>
<td><em>Danio rerio</em></td>
<td><em>Zebrafish</em></td>
<td>1,700</td>
<td>~3,000</td>
</tr>
</tbody>
</table>
**Applications**

Information from the Human Genome Project has been and will continue to be applied to different medical, industrial, commercial, and scientific purposes. For example, scientists have already discovered specific genes responsible for several genetic disorders, including cystic fibrosis, Duchenne muscular dystrophy, and colon cancer. Researchers may improve diagnoses, treatments, and therapies for the more than 4,000 human genetic disorders.

John Carpten of the National Institutes of Health, pictured in Figure 13-8, is a researcher taking such an approach to prostate cancer. Carpten knew that prostate cancer rates in men are second only to skin cancer rates and that some forms of the disease run in families. By studying male relatives with and without prostate cancer, he and his team were able to map a gene for susceptibility to prostate cancer. The gene, called **RNASEL**, occurs near a gene sequenced during the Human Genome Project. **RNASEL** encodes an RNA-digesting enzyme. Carpten isolated the **RNASEL** gene from patients, and found mutations. His discoveries may one day lead to new prostate cancer treatments.

**Figure 13-8**

Dr. John Carpten is a researcher at the National Human Genome Research Institute. He uses the results of the Human Genome Project to study prostate cancer.

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**THE FUTURE OF GENOMICS**

It is clear that although the more than 3 billion letters of the human genetic code have been sequenced, this is only the beginning of the quest to understand the human genome. New tools and fields of scientific research have arisen that enable the acquisition, analysis, storage, modeling, and distribution of the information contained within DNA and protein sequence data. Geneticists will now be able to apply their new tools to benefit human health.

**Bioinformatics**

Keeping track of the billions of nucleotide base pairs in a complex genome requires substantial computer power. Much of the progress in studying genomes comes from advances in the new scientific field of bioinformatics. **Bioinformatics** combines biological science, computer science, and information technology to enable the discovery of new biological insights and unifying principles. Bioinformatics utilizes databases to store and integrate the data coming out of genomic research. One such database called the BLAST search allows rapid comparisons between a gene or protein sequence from one organism and similar sequences from all other organisms in a database at the National Center for Biotechnology Information. The BLAST tool allows a worker to find corresponding genes in different organisms. By knowing the function of a gene from a related model species such as a mouse, the researcher can then infer the function of a previously unstudied human gene.
Proteomics
As important as genomes are, it is the proteins they encode that actually carry out the work of cells. To understand how genes work, biologists must understand proteins. **Proteomics** is the study of all of an organism’s proteins, including their identities, structures, interactions, and abundances. A key tool in proteomics is **two-dimensional gel electrophoresis**, a method that separates the proteins in a sample into individual spots, as shown in Figure 13-9. A researcher can cut out a protein spot from the gel, then use special methods to determine the amino acid sequence in part of the protein. Using bioinformatics, he or she can then search the DNA of a sequenced genome and match an individual gene to that unique protein. Proteomics and bioinformatics will allow medical researchers to identify new targets for therapeutic drugs and develop new markers for diagnosing disease.

Microarrays
An important tool of the genomic revolution is a technique called **DNA microarray**, a two-dimensional arrangement of DNA molecules representing thousands of cloned genes. A DNA microarray can show which genes are active in a cell. To prepare the DNA microarray, robotic machines arrange tiny amounts of sequences from thousands of genes on a single microscope slide. To investigate, for example, how tumor cells differ from normal cells, mRNA from the tumor is labeled with fluorescent dyes and poured onto the microarray slides. The more mRNA that binds to its complement at a particular spot on the slide, the brighter the color, indicating that this specific gene is highly active. Physicians use DNA microarray analysis to classify patients’ cancers. Such classification can lead to better-informed decisions about which type of treatment is best. A DNA microarray is shown on the first page of this chapter.

**SECTION 2 REVIEW**

1. Describe two major goals of the Human Genome Project.
2. Summarize four insights from the initial analysis of the complete sequence of the human genome.
3. Why are model species useful to study genes?
4. List and describe two genetic disorders that might be treated using DNA technology information gained in the Human Genome Project.
5. Distinguish between proteomics and bioinformatics.

**CRITICAL THINKING**

6. **Relating Concepts** Describe how the rapid growth of the computer technology industry in the 1990s relates to the Human Genome Project.
7. **Analyzing Models** Some people might assume that the more nucleotides in a genome, the more genes it contains. Make a graph of the data in Table 13-1. Explain why you agree or disagree.
8. **Evaluating Choices** If you were the scientist who developed the model organism list, which organisms would you include? Justify your choices.
WHO OWNS GENES?

The very large U.S. government investment in the Human Genome Project has spurred research in genetics. Within months of the genetic map’s release, researchers around the country applied for patents on thousands of different genes and parts of genes. By the end of 2004, more than three million gene-related patent applications had been filed. Most gene-related patents have been issued to the government, the University of California, and three top biotechnology companies. Some observers criticize the idea of patenting human genes. Others support it.

The Patent Process
The United States Commerce Department issues patents on inventions. By proving that he or she is the first to invent something, an inventor can get exclusive rights to develop and sell products based on the invention for 20 years.

The patent office must determine that an invention is practically useful, new, clever, and creative in design, and useful to a skilled technician for the practical purpose intended.

Gene Patents
The patent office has ruled that whole genes as they occur in nature on chromosomes are not patentable. If a person isolates a gene, however, or modifies the gene, it becomes a candidate for a patent. Most patent applications are for gene fragments that contain one or more changed base pairs and may represent gene sequences that lead to disease or new drugs.

Arguments Against Gene Patents
One critic of human gene patents, Dr. Jonathan King, points out that the human genome mapping project came from 50 years of public investment and therefore shouldn’t be patentable by private companies. Others object to patenting any part of nature. Still others argue that patenting gene fragment base-pair sequences rewards the easy part. The cost of identifying a gene is only a small fraction of the total cost of developing useful applications from genetic information. It is argued that gene patents penalize anyone (such as pharmaceutical companies) who would invest in the research for drugs or therapies derived from the gene sequence. Most critics agree that gene patenting could slow the study of the human genome.

Arguments in Favor of Gene Patents
Some companies say patents are necessary because of high research and development costs. Companies fear they will waste millions of dollars on duplicated efforts because only patented information is made public. Unpatented information is kept secret. They point out that owning a patent draws investors, which helps fund research. The public nature of a patent reduces secrecy and allows researchers to share techniques.

1. What characteristics of an invention must be present for a gene to be patented?
2. What are the arguments for and against gene patents?
3. Critical Thinking Are you in favor of gene patenting? Why or why not?
In addition to DNA fingerprints and genomics, genetic engineering techniques are being used in medical, industrial, commercial, and agriculture settings. This section discusses some of these applications and the ethical issues the techniques raise.

**MEDICAL APPLICATIONS**

Genetic engineering has allowed biologists to study how genes function. For example, researchers in Montreal used genetic engineering to study brain development in mice. They wanted to determine what activates the gene \( Hoxd4 \) as the hindbrain develops in an embryo. This is important because abnormal hindbrain development may contribute to autism, a disorder that disrupts a child’s ability to socialize and communicate.

The researchers combined the \( Hoxd4 \) gene and a region adjacent to the gene with a “reporter gene.” The reporter gene encodes an enzyme that can make a blue-colored product. They inserted the recombinant DNA into mouse cells, grew embryos, and found that the region adjacent to the \( Hoxd4 \) gene could turn on the reporter gene and its blue product, as shown in Figure 13-10a. When they mutated the adjacent region, they discovered (by the lack of blue color) that it was expressed in the spinal cord, but not in the embryo’s hindbrain, as shown in Figure 13-10b. They concluded that the DNA sequence adjacent to \( Hoxd4 \) helps control hindbrain development. Experiments such as these are unraveling the mysteries of gene function during development and may eventually provide therapies for disease.
Gene Therapy

Genetic engineering has also allowed biologists to try to treat genetic disorders in different ways. One method is a technique called gene therapy. In gene therapy a genetic disorder is treated by introducing a gene into a patient’s cells. Gene therapy works best for disorders that result from the loss of a single protein. For example, the lung disease cystic fibrosis results from the lack of a functional gene called the CFTR gene. When functional, the gene encodes a protein that helps transport ions into and out of cells in the breathing passages. Without that gene, poor ion exchange causes the symptoms of cystic fibrosis, including the buildup of sticky mucus that blocks the airways.

Figure 13-11 summarizes the steps involved in gene therapy. In step 1, researchers isolate the functional gene (such as the CFTR gene). In step 2, they insert the healthy gene into a viral vector. In step 3, they introduce the recombinant virus to the patient by infecting the patient’s airway by means of a nasal spray. The healthy copy of the CFTR gene temporarily produces the missing protein and improves ion exchange. The traditional treatment for cystic fibrosis involves thumping sessions—clapping on the back and chest for half-hour periods several times a day to dislodge mucus.

Cystic fibrosis research has accelerated since the discovery of the CFTR gene in 1989. In the laboratory, researchers were able to add a healthy copy of CFTR into the DNA of cystic fibrosis cells. The result was an immediate return to a normal ion transport mechanism. However, trials in the laboratory are different from trials on living humans. Apparently, the cells that express the highest levels of CFTR are deeper in the lungs than the surface cells that current forms of gene therapy can reach. Because the cells that line the airway slough off periodically, the treatment must be repeated. In addition, patients may suffer immune reactions to the treatment. Researchers hope to overcome these obstacles and to one day provide a permanent cure.

People with certain kinds of hemophilia, acquired immunodeficiency syndrome (AIDS), or some cancers are future candidates for gene therapy. Until recombinant DNAs can be inserted into the correct cells, however, and immune reactions can be prevented, gene therapy may continue to be a short-term solution.
Cloning

In the 1990s, biologists began cloning whole organisms, such as sheep and mice. The name for this procedure is **cloning by nuclear transfer**, the introduction of a nucleus from a body cell into an egg cell to generate an organism identical to the nucleus donor. The first animal successfully cloned from an adult tissue was a sheep named Dolly in 1996.

As shown in Figure 13-12, scientists in Scotland isolated a mature, functioning mammary cell nucleus from an adult sheep. They also isolated an egg cell from a second sheep and removed the nucleus. They then fused the mammary cell with the “empty” egg cell. The egg was stimulated to divide and grew into an embryo. The researchers implanted this embryo into the uterus of a surrogate mother who gave birth to a lamb, which they called Dolly. Dolly’s nuclear DNA was identical to the original donor of the mammary gland cell.

Despite the successful cloning, Dolly suffered premature aging and disease and died at age 6, only half of a normal sheep’s lifespan. Researchers found that Dolly had short telomeres, or repeated DNA sequences at the ends of chromosomes that shorten with each round of cell division. Short telomeres may be associated with premature aging. Other cloned species, however, have not experienced similar telomere shortening.

The goal of most animal cloning is to alter the genome in some useful way. For example, researchers have altered and cloned goats so that they secrete human blood clotting factors into their milk. Cloned pigs have been altered in the hope that pig livers, hearts, and other organs might not trigger organ rejection if transplanted into human recipients. Some researchers are cloning animals as models for the study of human disease, such as cystic fibrosis.
After a 5-month pregnancy, a lamb was born that was genetically identical to the sheep from which the mammary cell was extracted.

The embryo developed \textit{in vitro} and was later implanted into a surrogate mother.

**Vaccines**

A vaccine is a substance containing all or part of a harmless version of a pathogen that physicians introduce into the body to produce immunity to disease. The immune system recognizes the pathogen’s surface proteins and responds by making defensive proteins called \textit{antibodies}. A \textbf{DNA vaccine} is a vaccine made from the DNA of a pathogen but does not have disease-causing capability. The DNA vaccine is injected into a patient where it directs the synthesis of a protein. The immune system mounts a defense against the protein. If the vaccinated person contacts the disease agent in the future, his or her new immunity should provide protection. Researchers are currently working on developing DNA vaccines to prevent AIDS, malaria, and certain cancers.

**AGRICULTURAL APPLICATIONS**

Plant researchers are using genetic engineering to develop new strains of plants called \textit{genetically modified (GM) crops}. In a world of exponentially increasing human population, the need for more food with better nutritional value presents a challenge to plant biologists.

**Increasing Yields and Improving Nutrition**

To feed the planet’s hungry population, biologists have made crop plants that are more tolerant to environmental conditions. They have also added genes to strains of wheat, cotton, and soybeans that make the plants resistant to weed-controlling chemicals called \textit{herbicides}. To increase the amount of food a crop will yield, researchers have transferred genes for proteins that are harmful to insects and other pests into crop plants. The plants are protected from serious damage and yield more food. Similar techniques have been used to make plants resistant to certain diseases.

**Word Roots and Origins**

\textit{herbicide}

from the Latin \textit{herba}, meaning “plant,” and \textit{cida}, meaning “to kill”
Genetic engineers have also been able to improve the nutritional value of many crop plants. For example, in Asia many people use rice as a major food source, yet rice has low levels of iron and beta carotene, which the body uses to make vitamin A. As a result, millions suffer from iron and vitamin A deficiencies. Genetic engineers have added genes to rice to overcome these deficiencies.

**ETHICAL ISSUES**

**Bioethics** is the study of ethical issues related to DNA technology. Many scientists and nonscientists are involved in identifying and addressing any ethical, legal, and social issues that may arise as genetic engineering techniques continue to be developed. They want to make sure that none of the tools turn out to be dangerous or have unwanted results and that any technology and data that arise are carefully used. Almost all scientists agree that continued restraint and oversight are needed.

For example, some people are concerned that GM food crops might harm the environment in unusual ways. What would happen if introduced genes for herbicide resistance jumped to the wild, weedy relative of a GM crop? To this end, in the mid-1970s, government agencies set standards for safety procedures and required permits and labels for certain GM products. Most biologists agree that rigorous testing should be conducted and safeguards required before farmers release GM organisms into the environment.

Most scientists currently consider gene therapy to be unethical if it involves reproductive cells that would affect future generations. Most people consider cloning of human embryos for reproduction unethical.

The welfare of each patient is most important. Confidentiality of each individual’s genetic make up is vital to prohibit discrimination in the workplace. Decisions about ethical issues must be made not just by scientists but by the involvement of an informed public.

**SECTION 3 REVIEW**

1. List two types of medical products that can be produced using DNA technology.
2. How have medical researchers used gene therapy to help people with cystic fibrosis?
3. What are the main steps in cloning a sheep?
4. Describe a potential safety and environmental concern with regard to genetically modified (GM) crops.
5. Relate bioethics to the continued development of genetic engineering techniques.

**CRITICAL THINKING**

6. **Forming Reasoned Opinions** Should genetically engineered food products require special labels? Why or why not?
7. **Applying Information** If you were to genetically engineer a crop, what would it be, and how would you improve it?
8. **Evaluating Information** In what way might employers discriminate against a person if his or her genome were known to them?
**SECTION 1**

**DNA Technology**

- DNA technology provides the tools to manipulate DNA molecules for practical purposes.
- The repeating sequences in noncoding DNA vary between individuals and thus can be used to identify an individual.
- To identify a DNA sample, scientists isolate the DNA and copy it using the polymerase chain reaction (PCR). The DNA is then cut into fragments using restriction enzymes. The fragments are separated by size using gel electrophoresis.

**Vocabulary**

<table>
<thead>
<tr>
<th>length polymorphism (p. 255)</th>
<th>polymerase chain reaction (PCR) (p. 256)</th>
<th>gel electrophoresis (p. 257)</th>
</tr>
</thead>
<tbody>
<tr>
<td>variable number tandem repeats (VNTR) (p. 255)</td>
<td>primer (p. 256)</td>
<td>DNA fingerprint (p. 257)</td>
</tr>
<tr>
<td>restriction enzyme (p. 257)</td>
<td>genetic engineering (p. 258)</td>
<td>recombinant DNA (p. 258)</td>
</tr>
<tr>
<td>DNA vaccine (p. 258)</td>
<td>bioethics (p. 270)</td>
<td></td>
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</tbody>
</table>

**SECTION 2**

**The Human Genome Project**

- The goals of the Human Genome Project were to determine the nucleotide sequence of the entire human genome and map the location of every gene on each chromosome. This information will advance the diagnosis, treatment, and prevention of human genetic disorders.
- The Human Genome Project included sequencing the genes of many model species to provide insights into gene function.
- Information from the Human Genome Project has been applied to medical, commercial, and scientific purposes.
- Bioinformatics uses computers to catalog and analyze genomes. Microarrays, two-dimensional arrangements of cloned genes, allow researchers to compare specific genes such as those that cause cancer. Proteomics studies the identities, structures, interactions, and abundances of an organism’s proteins.

**Vocabulary**

<table>
<thead>
<tr>
<th>Human Genome Project (p. 261)</th>
<th>single nucleotide polymorphism (SNP) (p. 262)</th>
<th>bioinformatics (p. 263)</th>
<th>two-dimensional gel electrophoresis (p. 264)</th>
</tr>
</thead>
<tbody>
<tr>
<td>proteome (p. 262)</td>
<td>proteomics (p. 264)</td>
<td></td>
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</tbody>
</table>

**SECTION 3**

**Genetic Engineering**

- Genetic engineering is being used to provide therapies for certain genetic diseases.
- Gene therapy refers to treating genetic disorders by correcting a defect in a gene or by providing a normal form of a gene. Researchers hope that gene therapy can be used to cure genetic disorders in the future.
- In cloning by nuclear transfer, a nucleus from a body cell of one individual is introduced into an egg cell (without its nucleus) from another individual. An organism identical to the nucleus donor results.
- Genetic engineering is used to produce disease-resistant, pest-resistant, and herbicide-resistant crops in an effort to improve the yields and nutrition of the human food supply.
- Some people fear that the release of genetically modified organisms would pose a separate environmental risk. Many safety, environmental, and ethical issues involved in genetic engineering have not been resolved.

**Vocabulary**

<table>
<thead>
<tr>
<th>gene therapy (p. 267)</th>
<th>cloning by nuclear transfer (p. 268)</th>
<th>telomere (p. 268)</th>
<th>DNA vaccine (p. 269)</th>
<th>bioethics (p. 270)</th>
</tr>
</thead>
<tbody>
<tr>
<td>© Holt, Rinehart and Winston. All rights reserved.</td>
<td></td>
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</tbody>
</table>
USING VOCABULARY

1. Explain the relationship between length polymorphisms and variable number tandem repeats (VNTR).

2. For each pair of terms, explain how the meanings of the terms differ.
   a. gel electrophoresis and DNA fingerprint
   b. restriction enzyme and recombinant DNA
   c. vector and plasmid

3. Use the following terms in the same sentence: cloning by nuclear transfer and telomeres.

4. Word Roots and Origins The word herbicide derives from the Latin herba, meaning “plant” and cida, meaning “to kill.” Using this information, explain how the term herbicide relates to an increase in food crop yields.

UNDERSTANDING KEY CONCEPTS

5. Describe how variable number tandem repeats (VNTR) are used to identify DNA.

6. Name the four major steps used in DNA identification.

7. Identify the purpose for using the polymerase chain reaction (PCR).

8. Explain how restriction enzymes are used.

9. Describe how a DNA fingerprint is prepared.

10. Relate cloning vectors to recombinant DNA.

11. Name two applications of DNA identification.

12. State the two major goals of the Human Genome Project.

13. Discuss three insights gained from the Human Genome Project.

14. Describe the importance of sequencing the genomes of model species.

15. State how information from the Human Genome Project will be applied to future projects.

16. Discuss the role of bioinformatics in the Human Genome Project.

17. Explain how gene therapy has been used to treat cystic fibrosis.

18. Describe how biologists used cloning by nuclear transfer to clone animals.

19. List two ways that genetic engineering may increase crop yields.

20. Describe the steps scientists are taking to ensure that genetic engineering techniques are carefully used in society.

CONCEPT MAPPING Use the following terms to create a concept map about genetic engineering: DNA of interest, vectors, recombinant DNA, plasmids, restriction enzymes, and sticky ends.

CRITICAL THINKING

22. Applying Information In the past, breeders developed new plant and animal varieties by selecting organisms with desirable traits and breeding them. Name an advantage and a disadvantage of genetic engineering techniques over this method.

23. Unit 6—Gene Expression The United States has regulations regarding the release of genetically engineered organisms into the environment. Write a report about reasons why people have concerns regarding the release of such organisms.

24. Interpreting Graphics The photograph below shows eight lanes (columns) in a gel. Several of these lanes contain DNA fingerprints of samples taken from a crime scene, a victim, and four suspects. State which suspect’s DNA fingerprint matches the blood found at the crime scene. Is it likely that the blood found at the crime scene belongs to the suspect? Explain.

KEY
1 Control 5 Suspect 1
2 Blood 6 Suspect 2
3 Victim 7 Suspect 3
4 Standard 8 Suspect 4
Standardized Test Preparation

DIRECTIONS: Choose the letter of the answer choice that best answers the question.

1. Which is a molecule containing DNA from two different organisms?
   A. vector DNA
   B. a DNA clone
   C. plasmid DNA
   D. recombinant DNA

2. Which of the following is used to cut DNA molecules in specific locations?
   F. cloning vectors
   G. cloning enzymes
   H. restriction enzymes
   J. polymerase chain reaction

3. What is the term used for inserting a healthy copy of a gene into a person who has a defective gene?
   A. cloning vector
   B. gene therapy
   C. recombinant DNA
   D. polymerase chain reaction (PCR)

4. Which is the process used in animal cloning?
   F. DNA cloning
   G. recombinant DNA
   H. polymerase chain reaction
   J. cloning by nuclear transfer

INTERPRETING GRAPHICS: The graphic below shows a bacterial cell. Use the graphic to answer the questions that follow.

5. Which best describes molecule A?
   A. It is an insulin gene.
   B. It is recombinant DNA.
   C. It is a bacterial plasmid.
   D. It is a disease-causing virus.

6. How is a bacterial plasmid described after donor DNA is inserted into the bacterium’s DNA?
   F. vector DNA
   G. cloned DNA
   H. plasmid DNA
   J. recombinant DNA

7. Proteomics : proteins :: genomics :
   A. lipids
   B. genes
   C. proteins
   D. carbohydrates

INTERPRETING GRAPHICS: The diagram below is of two pieces of DNA that were cut with the same restriction enzyme. Use the diagram to answer the question that follows.

8. Which nucleotide sequence must the sticky end labeled 2 have if it is to bond with the sticky end labeled 1?
   F. UGGCCU
   G. TCCGGA
   H. ACCGGT
   J. CT TAAG

SHORT RESPONSE

A probe is a strand of RNA or single-stranded DNA that is labeled with a radioactive element or fluorescent dye.

How do biologists use a probe to find cloned DNA?

EXTENDED RESPONSE

One concern about genetic engineering involves confidentiality and insurance.

Part A How could the human genome be misused, relative to confidentiality issues?

Part B What might people’s concern be about health insurance, and why?
Analyzing DNA Using Gel Electrophoresis

OBJECTIVES
- Use restriction enzymes to cut DNA.
- Separate DNA fragments of different sizes.

PROCESS SKILLS
- experimenting
- analyzing
- calculating

MATERIALS
- protective clothing
- ice, crushed
- ice bucket
- microtube rack
- microtube A—Uncut DNA
- microtube B—HindIII
- microtube C—BamHI
- microtube D—EcoRI
- microtube E—Unknown
- permanent marker
- micropipetter (0.5–10 µL)
- micropipetter tips
- 10× restriction buffer for each restriction enzyme
- Lambda virus DNA (34 µL)
- water bath (37°C)
- gel-casting tray
- gel comb, 6-well
- hot-water bath (65°C)
- agarose (0.8%)
- hot mitt
- zipper-lock plastic bag
- graduated cylinder (10 mL)
- 1× TBE buffer
- freezer
- loading dye (5 µL)
- gel chamber and power supply
- WARD'S DNA stain
- staining trays
- water, distilled
- ruler, metric
- 1/1000 TBE buffer
- 1/1000 TBE
- gel chamber and power supply
- WARD’S DNA stain
- staining trays
- water, distilled
- ruler, metric

Background
1. DNA has a negative charge and flows toward the positive end of a gel during electrophoresis. Small DNA fragments move faster than larger fragments.
2. The distance that each DNA fragment moves is used to calculate the $R_f$, or relative mobility, of a fragment. The $R_f$ is used to calculate the number of base pairs in the fragment.

PART A Cutting DNA
1. Wear safety goggles, gloves, and a lab apron at all times.

2. Fill an ice bucket with ice. Obtain one each of the following microtubes: A—Uncut DNA, B—HindIII, C—BamHI, D—EcoRI, and E—Unknown. Microtubes B–D contain 1 µL of the indicated restriction enzyme. Place all microtubes in the ice. Restriction enzymes MUST be kept on ice until step 6.

3. With a permanent marker, write the initials for everyone in your group on the top of microtubes A–E.

4. **CAUTION** If you get a chemical on your skin or clothing, wash it off at the sink while calling to your teacher. Set a micropipetter to 1 µL, and put a tip on the end of the micropipetter, as shown in (a) below. Using a new tip for each microtube, add 1 µL of the corresponding 10× restriction buffer to each of microtubes B–D. Place the buffer on the side of the tube. Do not touch the micropipetter tips to the solutions in the microtubes.

5. Reset the micropipetter to 8 µL. Using the micropipetter and a new tip for each microtube, add 8 µL of the Lambda virus DNA to the side of each of microtubes B–D. Gently flick the bottom of each microtube with your finger to mix the solutions. Do not shake the microtubes! Reset the micropipetter to 10 µL, and add 10 µL of Lambda DNA to microtube A.

6. Place all of the microtubes into a 37°C water bath. After 50–60 minutes, remove the microtubes from the water bath, and immediately put them into a freezer. If the class period ends before 50 minutes have passed, your teacher will give you further directions. While the restriction enzymes are working, go to Part B.
**PART B  Preparing an Agarose Gel**

7. Set up a gel-casting tray, as shown in (b). Place a gel comb in the grooves of the gel-casting tray. Make sure that the comb does not touch the bottom of the tray. If it does, get another comb from your teacher.

8. Write the names of the members of your group on a paper towel. Carry your tray to the table with the melted agarose, and place your tray on the paper towel.

9. Using a hot mitt, pour melted 0.8% agarose into your gel-casting tray until the agarose reaches a depth of 3 mm. Make sure that the agarose spreads evenly throughout the tray. Do not move your gel tray before the agarose solidifies.

10. Let the gel cool (about 20–30 minutes) until the agarose solidifies.

11. While the gel is cooling, write your name, the date, and your class period on a zipper-lock plastic bag. Pour 5 mL of 1×TBE buffer into the bag.

12. When the gel has solidified, carefully remove the gel comb by pulling it straight up. If the comb does not come up easily, pour a little 1×TBE buffer on the comb area. After removing the gel comb, open the plastic bag and carefully slide the gel tray into the bag. Do not remove the gel from the gel-casting tray. Store the gel according to your teacher’s instructions.

**PART C  Running a Gel**

13. Retrieve your microtubes (A–E) and your gel. If the materials in the microtubes are frozen, hold each tube in your hand until the solutions thaw.

14. Set a micropipette to 1 µL, and place a tip on the end. Add 1µL of loading dye to each microtube. Use a new tip for each microtube. Gently tap each microtube on your lab table to thoroughly mix the solutions. Do not shake the microtubes.

15. Remove your gel (still in the gel-casting tray) from the plastic bag, and place it in a gel chamber. Orient the gel so that the wells are closest to the black wire, or anode.

16. Set a micropipette to 10 µL, and place a new tip on the end. Open microtube A, and remove 10 µL of solution. Carefully place the solution into the well in lane 1, the left-most lane. To do this, place both elbows on the lab table, lean over the gel, and slowly lower the micropipette tip into the opening of the well before depressing the plunger. Do not jab the micropipette tip through the bottom of the well.

17. Using a new micropipette tip for each tube, repeat step 16 for each of the remaining microtubes. Use lane 2 for microtube B, lane 3 for microtube C, lane 4 for microtube D, and lane 5 for microtube E.

18. Very slowly fill the gel chamber with 1×TBE buffer until the level of the buffer is approximately 1–2 mm above the surface of the gel.

19. **CAUTION** Follow all of the manufacturer’s precautions regarding the use of this equipment. Close the gel chamber, and connect it to a power supply according to your teacher’s instructions.

20. Allow an electric current to flow through the gel. You will see a blue line moving away from the wells. When the blue line is approximately 5 mm from the end of the gel, disconnect the power supply and remove the gel. Store the gel overnight in the plastic bag.

**PART D  Analyzing a Gel**

21. To stain a gel, carefully place the gel (wells up) into a staining tray. Pour WARD’S DNA stain into the staining tray until the gel is completely covered. Cover the staining tray, and label it with your initials. Allow the stain to sit for at least 2 hours. Next, carefully pour the stain into the sink drain, and flush it down the drain with water. Do not let the stained gel slip out of the staining tray.

22. To destain a gel, cover the gel with distilled water by pouring water to one side of the gel. Let the gel sit overnight (or at least 8–12 hours). The bands of DNA will appear as purple lines against a light background.

23. Calculate the \( R_f \) for each fragment using the following equation:

\[
R_f = \frac{\text{distance in mm that DNA fragment migrated}}{\text{distance in mm from well to the dye}}
\]

24. Dispose of your materials according to your teacher’s directions, and wash your hands before leaving the lab.

**Analysis and Conclusions**

1. Which two samples appear to have the same pattern of DNA bands?

2. Which restriction enzyme cut the DNA in the unknown sample? Justify your answer.

3. What are some measures that you took to prevent contamination of your DNA samples during this lab?