Chapter 15

Gene Technologies and Human Applications

Preview

Why It Matters

Gene technologies aid the study of basic biology. They have many other applications, such as producing food and treating disease.

Why would scientists make a pig that glows green? So they can study how genes work.

InquiryLab

15 min

Code Comparison

All humans have very similar DNA, with slight individual variations. The differences that are easiest to observe are among DNA stretches that have many short, repeating base sequences, as shown below. Different people have different numbers of repeats.

GATATATAGACTACTACTACTA

AGATATAGACTACTACTGACTT

GATATAGACTACTACTACTAGC

Procedure

1. Copy and then examine the three DNA sequences shown here.
2. Mark the portions of the code that include repeating bases.

Analysis

1. **Identify** what the four letters in the code sequences represent.
2. **State** how many kinds of repeating sequences you find.
3. **Identify** the basic repeating unit(s) among all segments.
4. **Explain** how each person can have a unique genetic code, even though some people may share an identical pattern of repeating base sequences.

The green-glowing gene was inserted into cloned pig cells by scientists using modern gene technologies. This gene is often used as a "marker" in genetic experiments because it is easy to see if the gene is present in an organism.

READING TOOLBOX

These reading tools can help you learn the material in this chapter. For more information on how to use these and other tools, see **Appendix: Reading and Study Skills.**

Using Words

Word Parts You can tell a lot about a word by taking it apart and examining its parts, such as the prefix and root.

Your Turn Use the information in the table to define the following terms:

1. *electrophoresis*
2. *microarray*

Word Parts

Word Part	Meaning
electro-	using electricity
phore	to carry
micro-	very small
array	orderly arrangement

Using Language

Analogies Analogies compare words with similar relationships. You can write analogies with words or with colons. For example, the analogy "up is related to down in the same way that top is related to bottom" can be written "up : down :: top : bottom." To answer an analogy problem, you must figure out how the words are related. In this example, up is above down, and top is above bottom.

Your Turn Use information found in prior chapters to complete the following analogy:

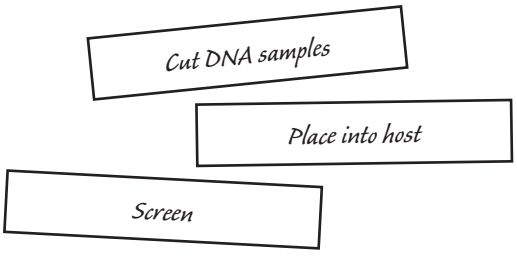
transcription : RNA :: translation : ___.

Using Graphic Organizers

Pattern Puzzles You can use pattern puzzles to help you remember sequential information. Exchanging puzzles with a classmate can help you study.

Your Turn Make a pattern puzzle for the steps of a recombinant gene cloning process, as shown in this chapter.

1. Write the steps of the process on a sheet of notebook paper, one step per line. Do not number the steps.
2. Cut the paper so that there is one step per strip of paper.
3. Shuffle the paper strips so that they are out of sequence.
4. Try to place the strips in their proper sequence.
5. Check your sequence by consulting your textbook, class notes, or a classmate.



Section 1

The Human Genome

Key Ideas	Key Terms	Why It Matters
❯ Why is the Human Genome Project so important? ❯ How do genomics and gene technologies affect our lives? ❯ What questions about the human genome remain to be studied?	genomics microarray DNA fingerprint	Many diseases may someday be cured by genetic technologies.

In 2000, headlines announced that scientists had deciphered the "book of life" by listing almost the entire sequence of bases in human DNA. This major feat was only the beginning of a new era.

Secrets of the Human Genome

The term *genome* refers to all of the genetic material in an organism, population, or species. **Genomics** is the study of entire genomes, especially by using technology to compare genes within and between species. A major part of genomics is to *sequence* genomes, or to identify every DNA base pair that makes up each genome. Only recently has it been possible to sequence the human genome.

The *Human Genome Project* (HGP) was an international cooperative effort to sequence the human genome. More than 20 laboratories in six countries worked together to sequence the 3.2 billion DNA base pairs that make up the human genome. ❯ **The sequencing of the human genome has advanced the study of human biology yet created new questions.**

genomics (juh NOH miks) the study of entire genomes, especially by using technology to compare genes within and between species

Surprising Findings The major draft of the human genome sequence was completed and reported in 2003. Scientists were surprised and excited by findings such as these:

- **Humans have few genes.** Scientists expected to find 120,000 genes but found only about 25,000.
- **Most human DNA is noncoding.** Less than 2% of human DNA seems to code for proteins. The rest is either introns or is not yet fully explained.
- **Many human genes are identical to those of other species.** Much of what we learn about mice and flies can be used to understand ourselves.
- **All humans are genetically close.** If the DNA of any two people is compared, 99.9% is identical.

❯ **Reading Check** ***How big is the human genome?*** ***(See the Appendix for answers to Reading Checks.)***

Figure 1 Despite differences in appearance, the DNA of any two humans is 99.9% similar.

Applications of Human Genetics

Studying the human genome opens new doors to understanding our bodies. In addition, we have new ways to apply this knowledge. *Gene technologies* allow us to find genes, copy them, turn them on or off, and even move them between organisms. **› Genomics and gene technologies have many applications in human healthcare and society.**

A major part of gene technologies is *genetic engineering,* which usually refers to the transfer of genes from one organism to another. For example, the human gene for insulin has been inserted into bacteria. Insulin is lacking in people with some forms of diabetes. So, the engineered bacteria are used to produce insulin to treat diabetes.

Diagnosing and Preventing Disease The first challenge to fighting disease is simply to diagnose, or identify, the problem. Modern gene technologies can help. For example, a **microarray,** shown in **Figure 2,** shows which genes are being actively transcribed in a sample from a cell. Some patterns of gene activity can be recognized as signs of genetic disorders or cancer.

Although most genetic disorders cannot be cured, they may be avoided in the future. For example, a person with a family history of genetic disorders may wish to undergo genetic counseling before becoming a parent. *Genetic counseling* informs people about the risk of genetic problems that could affect them or their offspring.

Many viral diseases are best prevented by vaccination. However, vaccines can be dangerous because they are made from disease-causing agents. Vaccines made through genetic engineering may limit such dangers by being more carefully designed. Various vaccines are now produced through genetic engineering. Some of these vaccines prevent diseases that were not preventable before.

› Reading Check ***When might a person seek genetic counseling?***

microarray (MIE kroh uh RAY) a device that contains a micro-scale, orderly arrangement of biomolecules; used to rapidly test for the presence of a range of similar substances, such as specific DNA sequences

DNA fingerprint a pattern of DNA characteristics that is unique, or nearly so, to an individual organism

Figure 2 A microarray contains an assortment of gene sequences, each set in a dot. The colors indicate whether a sample of genetic material has bound to the sequence at that dot. Thus, a pattern of gene expression can be seen. **› What conditions could be detected this way?**

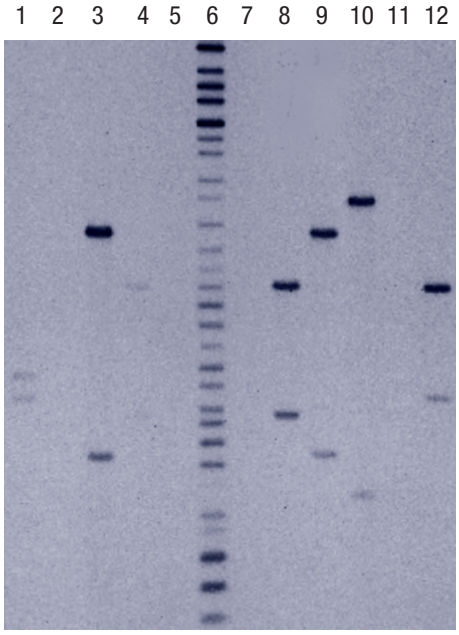
Data

QuickLab

15 min

Forensic DNA Fingerprints

DNA "fingerprinting" is useful in forensics because it can be performed on a sample of DNA from body tissues such as hair or blood. Samples can be compared to find genetically identical or closely related people. Identical segments of DNA will form identical patterns of bands in the columns of a DNA fingerprint, as shown here.



1 Control
3 Sample from crime scene
4 Victim
6 Standard size marker
8 Suspect A
9 Suspect B
10 Suspect C
12 Suspect D

Analysis

1. **Identify** the number of individuals whose DNA samples are being analyzed in this DNA fingerprint.
2. CRITICAL THINKING **Interpreting Graphics** Identify the suspect sample that matches the sample from the crime scene.
3. CRITICAL THINKING **Analyzing Methods** Column 6 shows every possible position of a DNA fingerprint band. Propose a purpose for these columns in this method.

Treating Disease Many genetic disorders occur when a specific protein, such as insulin, is missing or malformed because a gene has been mutated. So, the disorder can often be treated by supplying the needed protein. Many drug companies are now genetically engineering organisms to produce specific proteins for human use.

Another possible treatment for genetic disorders is to insert a functional "replacement" gene into a person's cells by using a genetically engineered virus. This technique is called *gene therapy*. However, gene therapy has had limited success because the human body has many protections against the invasion and genetic change that viruses cause.

The use of genomics to produce drugs is called *pharmacogenomics*. Currently, most drugs are made to combat diseases in a broad way. The drugs are generally effective for many people but not tailored to individuals. Soon, drugs could be custom-made for individuals based on a personal genetic profile. Such a profile could be produced by technologies that rapidly sequence a person's DNA.

Identifying Individuals Each person (other than identical twins) has some parts of the DNA sequence that are unique. So, samples of DNA can be compared to determine if the samples came from the same person or from people related by ancestry. These samples of DNA are cut, sorted, and "tagged" to produce a pattern of banding called a **DNA fingerprint.** DNA fingerprints are now used regularly to confirm the identity of criminals, family members, or dead bodies.

› **Reading Check** *Why is insulin used to treat genetic diabetes?*

READING TOOLBOX

Word Parts The prefix *pharma-* means "medicine" or "drug." Use this information to analyze the meaning of the term *pharmacogenomics*.

Figure 3 The human genome contains as much information as 180 phone books from different major cities.

SCILINKS
www.scilinks.org
Topic: Human Genome Project
Code: HX80770

Ongoing Work

Making a list of all of the bases in the human genome was only a first step. Understanding this "book of life" will take much more work. First, a huge amount of information is involved, as **Figure 3** shows. Second, although we know how to read the "letters" of this "book," we do not understand most of its meaning. We have compiled a long list of genes, but we do not know what many of the genes actually do. **› Many important questions about the human genome remain to be investigated or decided.** These questions include the following:

- **How do our genes interact?** To understand how genes interact, scientists are looking closely at the processes of gene expression. For example, they study how the protein that results from one gene may regulate the expression of other genes.
- **How unique are we?** Scientists are increasingly comparing our genome to those of other organisms to find out how small differences in genomes result in different species. Genome projects for many other species have been completed or are under way.
- **Can genetics help us live longer?** Gene technologies and genomics are thus leading to increased knowledge of how we could live longer, healthier lives. We are just beginning to find genetic clues about complex conditions such as asthma, obesity, schizophrenia, cancer, and aging. These conditions are affected by complex interactions between many genes as well as our environment. For many disorders, we are not likely to find a single cause, much less a simple cure.
- **How should we deal with ethical issues?** With so much information about human DNA being recorded, many questions arise that cannot be answered by scientific lab work. For example, Who should get the information? Who owns it? Should it be used to make decisions about individuals? Scientists and governments expect these issues to arise. In the United States, a portion of the federal funds for the HGP are dedicated to a special program of the HGP called *Ethical Legal and Social Implications* (ELSI).

ACADEMIC VOCABULARY
implication something involved or resulting from

› Reading Check ***Why is asthma difficult to cure?***

Section 1 Review

› KEY IDEAS

1. **Describe** the major findings of the Human Genome Project.
2. **Identify** some applications of genomics and genetic engineering that benefit humans.
3. **List** remaining questions about the human genome.

CRITICAL THINKING

4. **Proposing Explanations** Propose some possible explanations for the large volume of noncoding DNA in the human genome.
5. **Applying Logic** Scientists say that knowing the sequence of nucleotides in the human genome is only the first step in understanding the genome. What are some possible next steps?

WRITING FOR SCIENCE

6. **Genetics on Trial** When were gene technologies first used as evidence in criminal cases? Research the early history of this field, and summarize your findings in a news-style oral report.

Why It Matters

Cleanup Microbes

Using microbes for environmental cleanup is called *bioremediation*. For example, oil-devouring microbes are used to help clean up oil spills. Increasingly, genetically modified organisms (GMOs) are being engineered for use in bioremediation.

Oil Spills

Spills of fuel oil can be devastating to environments because the oil is toxic, floats on water, and soaks into soils. Fortunately, scientists have found that some marine bacteria are capable of using oil as food. Some of the first genetically modified (GM) microbes were derived from such bacteria. In fact, the first organism to be patented was an oil-eating, genetically engineered bacterium.

Radioactive Waste

Nuclear waste is another bioremediation challenge with which GM microbes may help. Water near nuclear waste dumps may become polluted with radioactive substances. Again, bacteria naturally exist that can break down most of these substances, but those bacteria cannot survive high levels of radiation. So, scientists have turned to another kind of bacteria that can withstand 3,000 times the normal radiation levels. They hope to engineer a solution by transferring genes between these species.

An impossible job? Cleaning oil and dangerous chemical spills out of sand or soil can be nearly impossible for humans, even with tools. However, this cleanup is simple work for a microbe.

An Enormous Mess Oil spills at sea are dangerous to wildlife, dangerous to the people involved in fighting them, and difficult to contain.

Quick Project Find out the date that the first patent for a GMO was awarded in the United States. Also find out the name of the scientist to whom it was awarded.

Section 2

Gene Technologies in Our Lives

Key Ideas	Key Terms	Why It Matters
❯ For what purposes are genes and proteins manipulated? ❯ How are cloning and stem cell research related? ❯ What ethical issues arise with the uses of gene technologies?	genetic engineering recombinant DNA clone stem cell	Gene technologies have many applications in modern life, but ethical issues exist for each of these applications.

Recall that a gene has a DNA sequence that is translated into the sequence of amino acids in a protein. In a sense, proteins are the "actors" in biology, and genes are the "directors." To understand how genes work, scientists have studied both the instructions in the genes and the actions of the proteins. Meanwhile, some have tried to modify the instructions to change the actions that result.

Manipulating Genes

Gene technologies include a wide range of procedures that analyze, decode, or manipulate genes from organisms. ❯ **Gene technologies are now widely applied to study organisms in new ways, to alter organisms for human use, and to improve human lives.** Gene technologies have rapidly changed over the past two decades, yet the basic applications are not so new. Human beings have been influencing the lives and genes of organisms for thousands of years. The first farmers and herders did so when they selected plants and animals to breed. But today, we have more specific knowledge, molecular tools, and the ability to move genes between organisms.

Genetic Engineering The application of science for specific purposes is often referred to as *engineering*. **Genetic engineering** is the deliberate alteration of the genetic material of an organism. The process often involves inserting copies of a gene from one organism into another. DNA that has been recombined by genetic engineering is called **recombinant DNA.** Organisms with recombinant genes may be called *recombinant, transgenic,* or *genetically modified.* In everyday use, they are often referred to as *genetically modified organisms* (GMOs). An example of a GMO is shown in **Figure 4.**

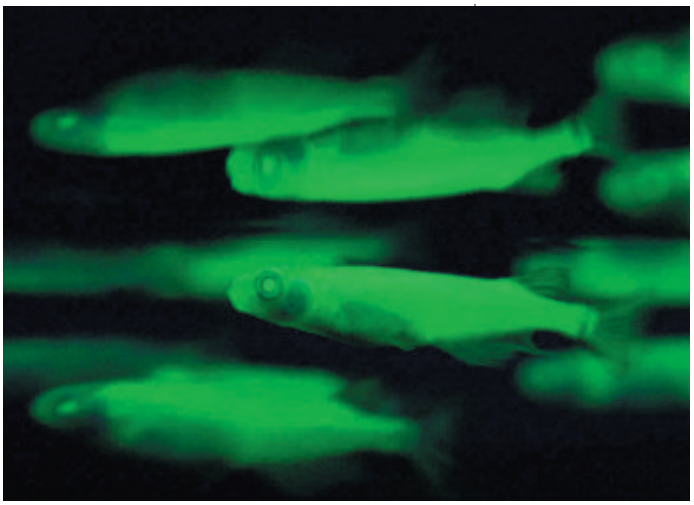


Figure 4 These fish "glow" because scientists have copied a gene from a naturally "glowing" jellyfish and inserted it into the fishes' genomes.

Many applications of gene technologies have become part of our everyday lives, from food to healthcare. In some ways, we are starting to depend on gene technologies, just as we depend on electricity and telephones. As with other technologies, gene technologies raise new social and ethical issues.

❯ **Reading Check** ***What is a GMO?***

Everyday Applications Genetic engineering was first applied to bacteria, viruses, and plants and is now applied to many life-forms. Today, GMOs are widely used in agriculture, medicine, industry, and basic research. Following are examples of the many uses of GMOs.

- **Food Crops** Most corn and soybean products sold in grocery stores in the United States are made from GMOs. In many cases, the crops have a gene added from the bacterium *Bacillus thuringiensis* (*Bt*). The gene produces an insecticide and thus benefits the crop grower. Many food crops are engineered to be easier to grow or to be more nutritious.
- **Livestock** New breeds of livestock are being engineered to grow faster or to have more muscle or less fat. Some are made to produce milk with specific proteins. Some GMOs are sold as unusual pets.
- **Medical Treatment** As you have learned, many genetic disorders, such as hemophilia and diabetes, result from a missing or abnormal protein. If the normal human gene for needed protein has been identified, the gene can be spliced into bacterial cells. Then, the recombinant bacteria will rapidly produce the human protein in large quantities. People with hemophilia and diabetes are being treated with proteins produced in this way.
- **Basic Research Tools** A variety of GMOs have been made just for laboratory research. Some plants and animals have been engineered with genes from other organisms that "glow." Often, this engineering is done so that researchers can study another, less obvious gene. In this case, the two foreign genes are spliced into the GMO at the same time. The "glow" gene then serves as a "marker" of the presence of the second gene being studied.

Manipulating Cell Interactions Gene technologies involve more than just inserting genes. Cells and bodies are affected by when and where each gene is expressed. So, gene technologies are also used to control the expression of genes or to redirect the products.

The study of how proteins interact within cells is called *proteomics* (PROH tee OHM iks). As you have learned, these interactions are very complex. Gene technologies can be used to manipulate the production of specific proteins at specific times and in specific cells, tissues, organs, or individuals. This manipulation can be done for medical treatment or simply for research.

One way to study the actions of genes in cells is to work with living tissues. To do so, scientists can remove living cells from an organism and grow them in a laboratory as tissue culture, as **Figure 5** shows. Then, the cells can be studied closely and experimentally controlled.

› **Reading Check** ***What is the* Bt *gene used for?***

genetic engineering a technology in which the genome of a living cell is modified for medical or industrial use

recombinant DNA (ree KAHM buh nuhnt) DNA molecules that are artificially created by combining DNA from different sources

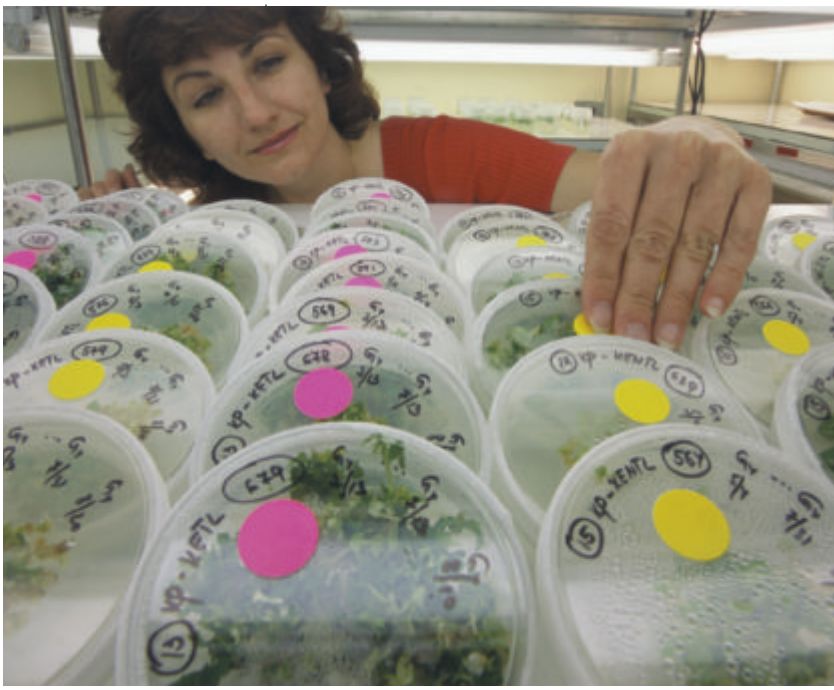


Figure 5 Tissue culture is often used to study living cells. › **What can we learn about genes from tissue culture?**

clone an organism, cell, or piece of genetic material that is genetically identical to one that was preexisting; to make a genetic duplicate

stem cell a cell that can divide repeatedly and can differentiate into specialized cell types

SCILINKS
www.scilinks.org
Topic: Cloning
Code: HX80303

Manipulating Bodies and Development

Biologists still have much to learn about the development of multicellular organisms. To do so, they must study cells in the process of multiplying and differentiating into the many types of cells found in a body. **› Cloning and stem cell techniques are used in research on animal development and have potential for treating certain diseases.**

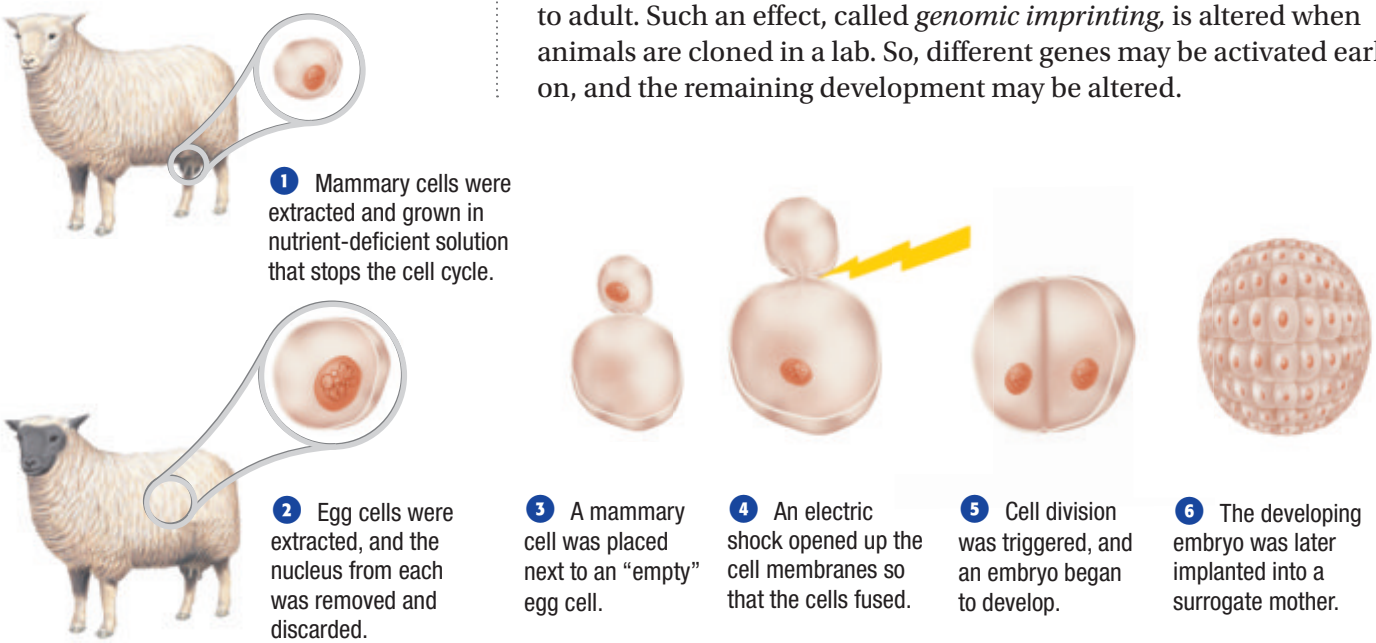
Cloning A **clone** is an organism or piece of genetic material that is genetically identical to one that was preexisting. Making a clone in a lab is called *cloning,* but the process does occur in nature. Organisms clone themselves whenever they reproduce asexually. Single-celled organisms clone themselves by simple division. Multicellular organisms may clone themselves by budding off parts, as some plants and fungi do, or by self-fertilization, as many plants and some animals do.

Very few large animals can clone themselves. Also, animals have complex processes of fertilization and embryo development. So, scientists are still experimenting with cloning animals. The first such experiments made clones from eggs or embryos. Then, a clone was made from an adult mammal, as **Figure 6** shows. The clone was made using a process called *somatic-cell nuclear transfer* (SCNT). In this process, the nucleus of an egg cell is replaced with the nucleus of an adult cell. Then, the egg begins to develop into an embryo.

Problems with Cloning Although scientists have successfully cloned many kinds of animals, only a few of the cloned offspring have survived for long. In some cases, the fetuses have grown beyond normal size. Many have failed to develop normally with age. Because of such problems and because of ethical issues, efforts to clone humans are illegal in most countries.

Genomic Imprinting Some problems with cloning may be related to the ways that eggs and sperm normally develop. Chemicals in the reproductive system turn "on" or "off" certain genes in the developing gametes. These genes later affect development from embryo to adult. Such an effect, called *genomic imprinting,* is altered when animals are cloned in a lab. So, different genes may be activated early on, and the remaining development may be altered.

Figure 6 Dolly, a cloned sheep, was born in 1997. Dolly was the first successful clone produced from the nucleus of an adult somatic cell.



1 Mammary cells were extracted and grown in nutrient-deficient solution that stops the cell cycle.

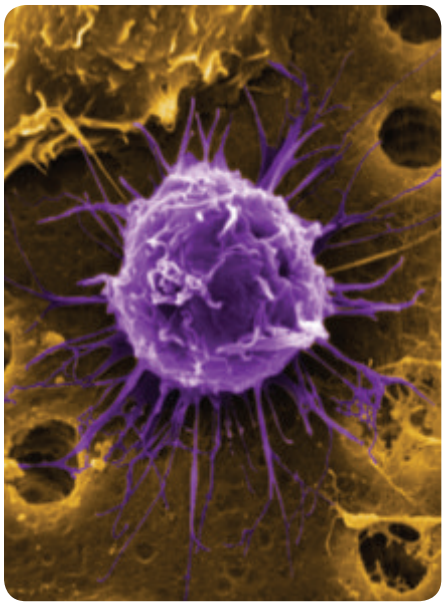
2 Egg cells were extracted, and the nucleus from each was removed and discarded.

3 A mammary cell was placed next to an "empty" egg cell.

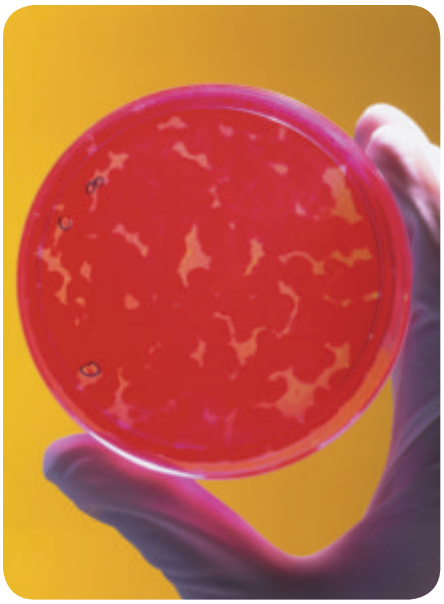
4 An electric shock opened up the cell membranes so that the cells fused.

5 Cell division was triggered, and an embryo began to develop.

6 The developing embryo was later implanted into a surrogate mother.



1 An adult stem cell can be removed from a specific tissue, such as bone marrow.



2 The cell can be grown in tissue culture to produce more cells of a specific tissue type.

3 The cells can be re-implanted into a patient whose tissues are lacking or damaged.

Figure 7 Adult stem cells can be removed and used to grow more cells of specific tissue types. This kind of therapy can replace tissue that is damaged or deficient due to disease or other medical treatment. **› How do adult stem cells differ from embryonic stem cells?**

Using Stem Cells A **stem cell** is a cell that can continuously divide and differentiate into various tissues. Some stem cells have more potential to differentiate than others. *Totipotent* cells can give rise to any cell or tissue type, *pluripotent* cells can give rise to all types except germ cells, and *multipotent* cells can give rise to just a few other cell types. The state of the cell depends on the stage of development of the body and the tissue of which the cell is part.

Adults' bodies have some multipotent cells, such as bone marrow cells, that give rise to various blood cells. These cells can be removed, frozen or cultured, and used for medical treatments, as **Figure 7** shows. The cells of new embryos have more potential uses. These cells are totipotent at first and pluripotent during development.

Issues with Stem Cell Research The first major source of human embryos for stem cell research was fertility clinics. Such clinics help people have children, often by uniting people's gametes and culturing embryos in a lab. Many extra embryos are stored in a frozen state in clinics. In some cases, the parents have given scientists permission to use the embryos for research. But such uses of human embryos pose ethical problems. In the United States, there have been strong debates about the use of federal funds for this kind of research.

Stem Cells from SCNT A newer source of embryonic stem cells is through cloning using SCNT. Some people believe that using this kind of stem cell for medical research and treatment should be ethically acceptable. One reason is that an embryo made through SCNT does not have true parents. Another reason is that the cells of the embryo are separated early in its development, so there is no chance of the embryo developing further.

› Reading Check ***What are the two main types of stem cells?***

READING TOOLBOX

Analogies Use the information in this section to help you write an analogy that relates adult stem cells to embryonic stem cells. Try to use the terms *pluripotent* and *multipotent* in your analogy.

Figure 8 This corn has been genetically modified to carry the *Bt* gene, which causes the corn plant to produce an insect-killing chemical. As with any use of pesticides, this practice presents risks. An additional danger is that the gene may be transferred to other plants.

Ethical and Social Issues

Ethical issues involve differing values and perspectives. For example, the use of GMOs is prohibited or tightly controlled by laws in some countries. In others, GMOs are widely used, and GM foods are sold with few restrictions. **› Ethical issues can be raised for every use of gene technologies.**

Safety One danger of GMOs is that they can "escape" and have unforeseen effects. For example, the *Bt* toxin gene from GM corn crops, such as those in **Figure 8,** has been transferred to other plants. In addition, the toxic corn pollen seems to be harming populations of the monarch butterfly. Ecologists worry that we do not know enough to safely manipulate genes on a large scale.

Human Rights Being able to predict disease before it happens is a major achievement of modern medicine. Today, the DNA of individuals can be tested to find the risk of genetic disorders. But what should we do with this information? Many decisions could be influenced by such genetic information, such as whom to marry or what to eat. Who should have this information? Who should make these decisions? How can future probabilities be weighed against current human needs and rights? There are no easy answers to these ethical questions, but the questions need to be considered carefully.

Property Laws Gene technologies have also created new issues for old laws, especially those related to intellectual property and patents. Intellectual property (IP) is the ownership of the ideas or plans that a person creates. A patent is a specific set of rights that allows an inventor to control and profit from the uses of his or her idea. In the 1980s, the first patent for a GMO was awarded to a scientist who had engineered an oil-eating bacterium. Before this event, living organisms were considered a part of nature and, as such, were not patentable. Now, specific DNA sequences can be patented.

› Reading Check ***What issues does the use of genetic testing raise?***

ACADEMIC VOCABULARY

ethical conforming to moral standards

Section 2 Review

› KEY IDEAS

1. **Identify** applications of manipulating genes and proteins.
2. **Relate** stem cell research to the potential use of cloning.
3. **Describe** a specific ethical issue related to a gene technology.

CRITICAL THINKING

4. **Inferring Relationships** How can manipulating gene expression help advance the study of proteomics?
5. **Evaluating Risks** Given the difficulties that researchers have had with raising cloned animals, do you think it is safe to grow tissues or organs from cloned embryonic stem cells for the purpose of transplanting? Explain.

ALTERNATIVE ASSESSMENT

6. **Debate** Suppose that genetic analysis could predict a person's ability in sports, math, or music. Should genetic screening be used to determine the course selections and team assignments of every student in school? Prepare and conduct a formal debate on the subject.

Section 3

Gene Technologies in Detail

Key Ideas	Key Terms	Why It Matters
❯ What are the basic tools of genetic manipulation? ❯ How are these tools used in the major processes of modern gene technologies? ❯ How do scientists study entire genomes?	restriction enzyme DNA polymorphisms electrophoresis polymerase chain reaction (PCR) DNA sequencing bioinformatics genome mapping genetic library	Humans now have the ability to identify and manipulate genes in many organisms.

How do you find a needle in a haystack? This phrase is often used to speak of a nearly impossible task. But if the haystack is a genome and the needle is a gene, the task is now possible!

Basic Tools for Genetic Manipulation

Molecular biologists spent many years developing tools and methods to manipulate genetic material. The methods continue to be used and adapted for a wide range of applications, but the basic tools are similar. ❯ **The basic tools of DNA manipulation rely on the chemical nature of genetic material and are adapted from natural processes discovered in cells.** These tools include restriction enzymes, polymorphisms, gel electrophoresis, denaturation, and hybridization. For example, the first GMOs were made by using plasmids and enzymes that are naturally present in some bacterial cells.

Restriction Enzymes Among the first tools used to manipulate DNA were enzymes that are made by bacteria as a defense. The enzymes serve to slice up any invading DNA sequences or genes from other organisms. These **restriction enzymes** recognize a specific sequence of DNA, called a *restriction site*. The enzymes will cut DNA strands at all such sites, as **Figure 9** shows.

restriction enzyme an enzyme that cuts double-stranded DNA into fragments by recognizing specific nucleotide sequences and cutting the DNA at those sequences

These enzymes are useful in two ways. First, different enzymes recognize different sequences, so the enzymes can be used to cut up a DNA sample in specific ways. Second, the cuts of most restriction enzymes create sticky ends. A *sticky end* has a few bases on one strand that are unpaired but complementary to unpaired bases on other sticky ends. So, sticky ends will easily bind to one another.

❯ **Reading Check** ***Which basic genetic tools were used to make the first GMOs?***

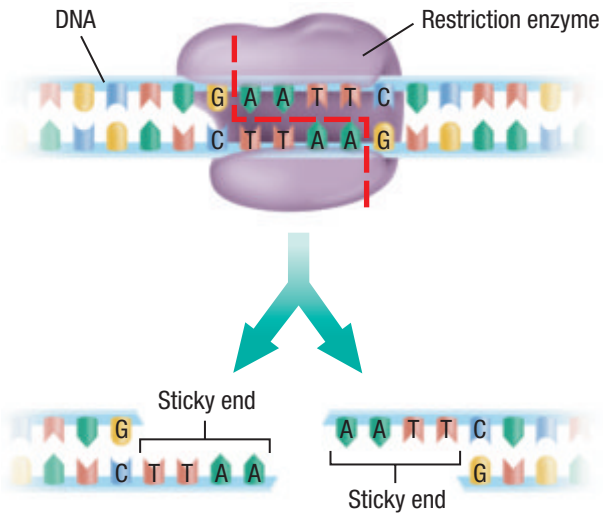


Figure 9 Restriction enzymes recognize and cut DNA at specific sequences. Usually, complementary ("sticky") ends are created. ❯ **In what ways are restriction enzymes useful?**

Hands-On

QuickLab

15 min

Gel Electrophoresis Model

You can use beads to model how DNA fragments are separated in a gel during electrophoresis.

Procedure

1. Fill a **large jar** with the largest of **three sets of beads** (each set should be a different size and different color). The filled jar represents a gel.
2. Mix the smaller sets of beads in a **500 mL beaker,** and then pour them slowly on top of the "gel." The smaller beads represent DNA fragments.
3. Observe the flow of the beads through the "gel." Lightly agitate the jar if the beads do not flow easily.

Analysis

1. **Identify** which beads flowed through faster.
2. **Relate** this model to how electrophoresis works.
3. CRITICAL THINKING **Using Models** Why did the beads identified in item 1 pass through the "gel" more quickly?

ACADEMIC VOCABULARY

slight very small or barely detectable

Polymorphisms Differences between the DNA sequences of individuals are called **DNA polymorphisms.** These differences may be slight but can be compared and analyzed for several purposes, as you will learn. Differences of just one nucleotide are called *single nucleotide polymorphisms* (SNPs). SNPs result from point mutations and are usually unique to individuals or populations. At a broader level, each species has a unique pattern of restriction sites. When different DNA samples are cut with the same restriction enzyme, the segments that result will have different lengths. These differences are called *restriction fragment length polymorphisms* (RFLPs).

Gel Electrophoresis DNA carries an electric charge, so an electric current can be used to push or pull DNA fragments. This process is called **electrophoresis.** Often, the DNA fragments are forced though a *gel,* a semisolid that allows molecules to move slowly through it. When a current is applied, shorter fragments will move faster through the gel than longer fragments will. The result is a lane of fragments sorted by size, as shown in **Figure 10.** If the fragments separate clearly, each lane is called a *ladder*. If the fragments have overlapping sizes and do not separate clearly, each lane is called a *smear*.

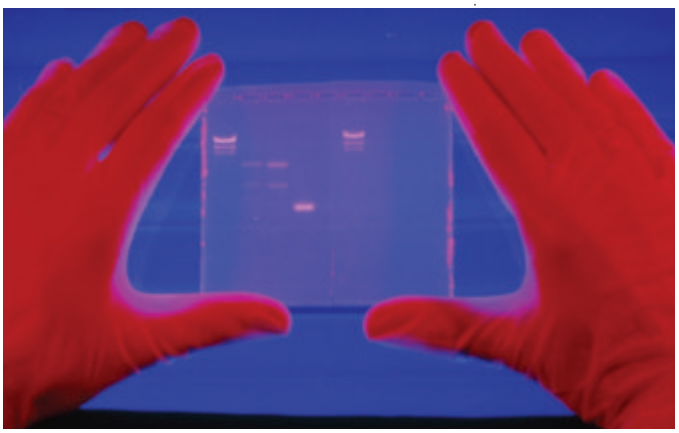


Figure 10 Gel electrophoresis separates samples of molecules, such as DNA or proteins, into bands that are ordered by size. **What is the role of the gel?**

There are many types of electrophoresis. Different kinds of gels are used to sort different sizes of DNA fragments, and other methods are used to sort RNA or proteins. Newer methods use tiny tubes of gel to sort tiny samples that can then be "read" by a machine and analyzed by a computer.

Reading Check ***What property of a gel does gel electrophoresis depend upon?***

Denaturation Recall that DNA in cells is usually double stranded, twisted, and often associated with proteins. Some conditions, such as heat or strong chemicals, can cause DNA to denature, or untwist and split into single strands. Scientists can easily denature and renature DNA and use the single strands for further manipulations.

Hybridization When single-stranded segments of DNA or RNA are mixed together under the right conditions, complementary segments will bind together, or hybridize. Genetic tools that take advantage of this natural process include the following:

- **Primers** *Primers* are short, single strands of DNA that will hybridize with a specific sequence. For this use, the sequence is one that will be recognized by an enzyme, such as DNA polymerase. Thus, primers can be used to initiate replication of single strands of DNA.
- **Probes** When DNA samples are sorted in a gel, probes are used to "tag" and find specific sequences. Probes are much like primers but carry radioactive or fluorescent materials that can be detected.
- **cDNA** Complementary DNA (cDNA) is DNA that has been made to match mRNA from cells. Recall that this mRNA is the result of transcription and has exons removed. So, making cDNA is a shortcut to getting just the expressed DNA of complete genes.

DNA polymorphisms
(PAHL ee MAWR FIZ uhmz) variations in DNA sequences; used as a basis for comparing genomes

electrophoresis (ee LEK troh fuh REE sis) the process by which electrically charged particles suspended in a liquid move through the liquid because of the influence of an electric field

polymerase chain reaction
(puh LIM uhr ays) a technique that is used to make many copies of selected segments of DNA (abbreviation, PCR)

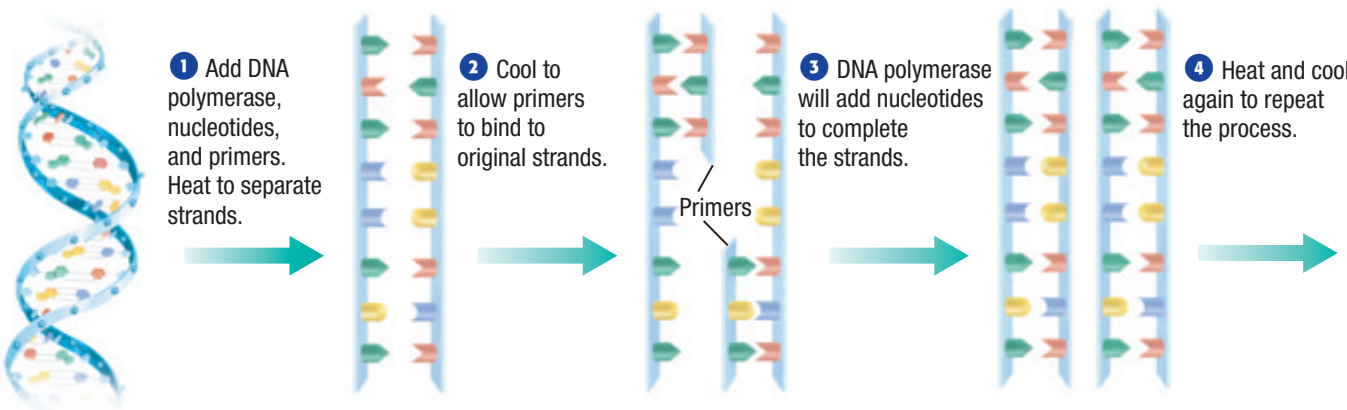
Major Gene Technology Processes

The major methods for working with genes use some combination of the basic tools and mechanisms of cellular machinery. These methods include PCR, blotting, DNA sequencing, and gene recombination.

Polymerase Chain Reaction (PCR) The **polymerase chain reaction (PCR)** process is widely used to clone DNA sequences for further study or manipulation. PCR imitates the normal process of DNA replication in cells. So, using PCR is as simple as combining the right components in a test tube and then controlling the temperature, as **Figure 11** shows. The process is called a *chain reaction* because it is repeated over and over.

Figure 11 PCR rapidly produces many copies of a DNA sample. The process can make 1 billion copies of a DNA sample within a few hours!

Polymerase Chain Reaction (PCR)



SCILINKS
www.scilinks.org
Topic: Gene Technologies
Code: HX80539

Blotting Processes and Applications Several gene technologies use a combination of restriction enzymes, gel electrophoresis, and hybridization with probes. The goal is to find or compare sequences of DNA or RNA. Many include a blotting step in which sorted segments are preserved by transferring from the gel to another surface or grid (such as a sheet of special paper). Then, probes are used to reveal the location of specific sequences.

Southern Blot The Southern blot process, shown in **Figure 12,** is used specifically for DNA, and especially for DNA fingerprints. The process may vary by using either different restriction enzymes on one DNA sample or different DNA samples with the same enzyme.

Fingerprints and Bar Codes DNA polymorphisms can be used to identify individuals or species. When restriction fragments are sorted through a Southern blot process, each person's DNA will have a unique pattern of banding called a *DNA fingerprint*. Similarly, a *DNA bar code* can be made to help identify species.

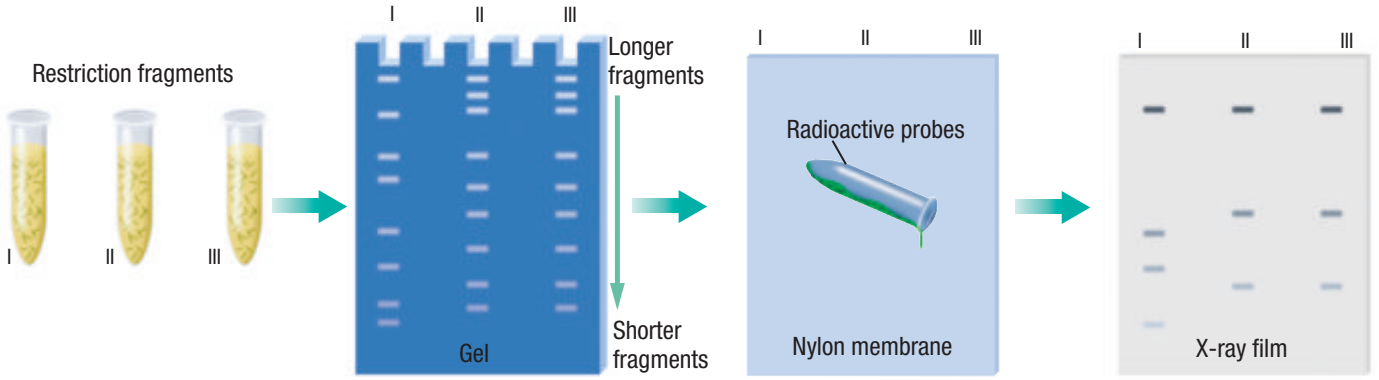
Northern Blot The Northern blot process differs from Southern blot in that the sample fragments are mRNA instead of DNA. Recall that mRNA in cells comes from genes being transcribed. So, Northern blot can be used to tell which genes in a cell are "turned on" (being expressed) or to tell the size of the expressed parts of a gene (after exons are removed).

Microarrays A *microarray* is a device that enables thousands of tiny Northern blots to be done at once. Microarrays can be used to show patterns of gene expression. For example, a cancer cell will have certain genes turned on or off. The pattern of gene activity seen in a microarray can help identify specific kinds of cancer.

› **Reading Check** *What does "blotting" refer to?*

Figure 12 In this example, a DNA sample is analyzed by using the Southern blot process. › **Which basic genetic tools are used as part of this process?**

Southern Blot Process



1 The Southern blot process begins with a sample of DNA. The DNA is cut into fragments using restriction enzymes. A unique enzyme may be used to create several batches of sample DNA.

2 The DNA fragments are sorted by gel electrophoresis. One lane in the gel is used for each batch. Then, a chemical splits all fragments into single-stranded form.

3 The DNA strands are transferred (blotted) onto a piece of nylon paper. A solution containing probes is applied to the nylon paper. The probes bind to specific sites on the single strands.

4 The exposure of photographic or X-ray film to the nylon paper reveals the location of those sample strands that hybridized with the probe. Each person's DNA will make a unique pattern.

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Chain Termination Sequencing

1 Copy The unknown DNA sequence is copied, denatured, and incubated with specific genetic molecules.

2 Terminate New copies of the original sequence begin to form, but some are terminated randomly by tagged nucelotides.

3 Sort The resulting strands are denatured and sorted in a gel. The color-coded bands in the gel will match the orginal sequence.

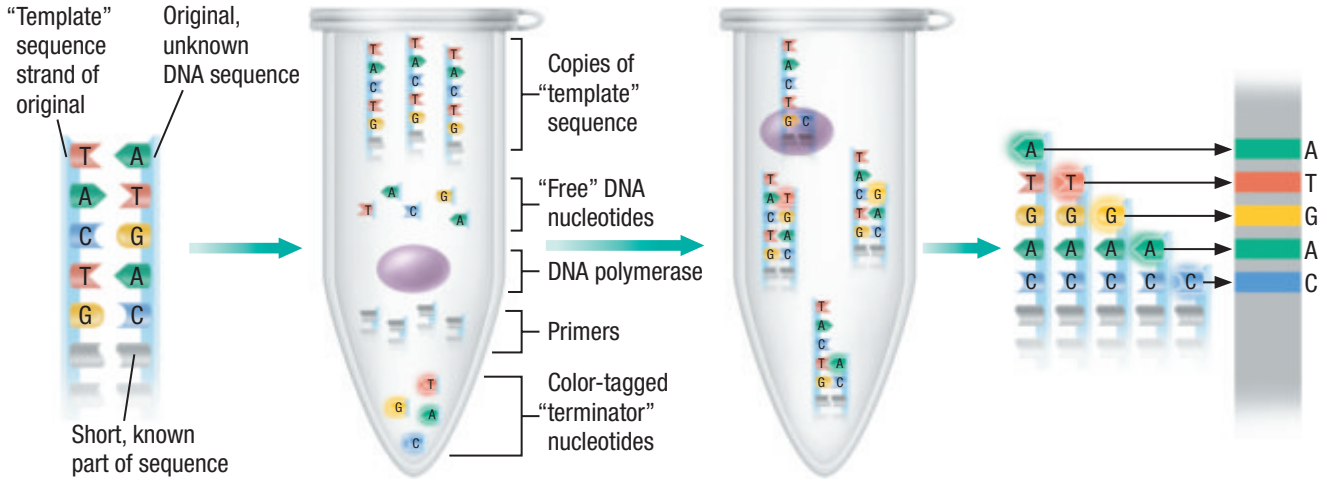

Figure 13 Chain termination sequencing modifies DNA replication processes in order to deduce a DNA sequence. **› Why is this method so important?**

DNA Sequencing Among the great achievements of modern biology are DNA sequencing methods. **DNA sequencing** is the process of determining the exact order of every nucleotide in a gene. The major modern method is *chain termination sequencing*, as shown in **Figure 13.** This method has been improved over time.

Step 1 Start Copying a Template The gene (DNA segment) of interest is copied (using PCR) and split into single strands. The copies are placed in solution with primers, DNA polymerase, and an assortment of bases. The primers will bond to the "template" strand, and then DNA polymerase will begin to add bases to the "copy" strand, as in normal DNA replication.

Step 2 Randomly Terminate the Copies Some of the bases act as "terminator" bases. When one of these bases is placed in one of the growing copy strands, copying will stop on that strand. Thus, an assortment of randomly "cut-off" sequence copies is produced.

Step 3 Sort the Copies by Size At this point, the sequence of bases can be deduced by sorting the segments by size. When sequencing was first developed, scientists would use four batches of radioactively tagged "terminators" (one for each base type). Then, they would perform electrophoresis in four lanes, side by side, which would reveal the relative order of each end-base. Today, scientists use color-coded fluorescent tags (one color for each base type) and run a single batch through a tiny tube of gel. A machine with a laser can detect the wavelengths of the tags and thus "read" the sequence.

› Reading Check ***When are primers used in DNA sequencing?***

DNA sequencing (SEE kwuhns ing)
the process of determining the order of every nucleotide in a gene or genetic fragment

Figure 14 The earliest gene cloning and recombination methods used the steps shown here. The first GMOs were produced in this way.

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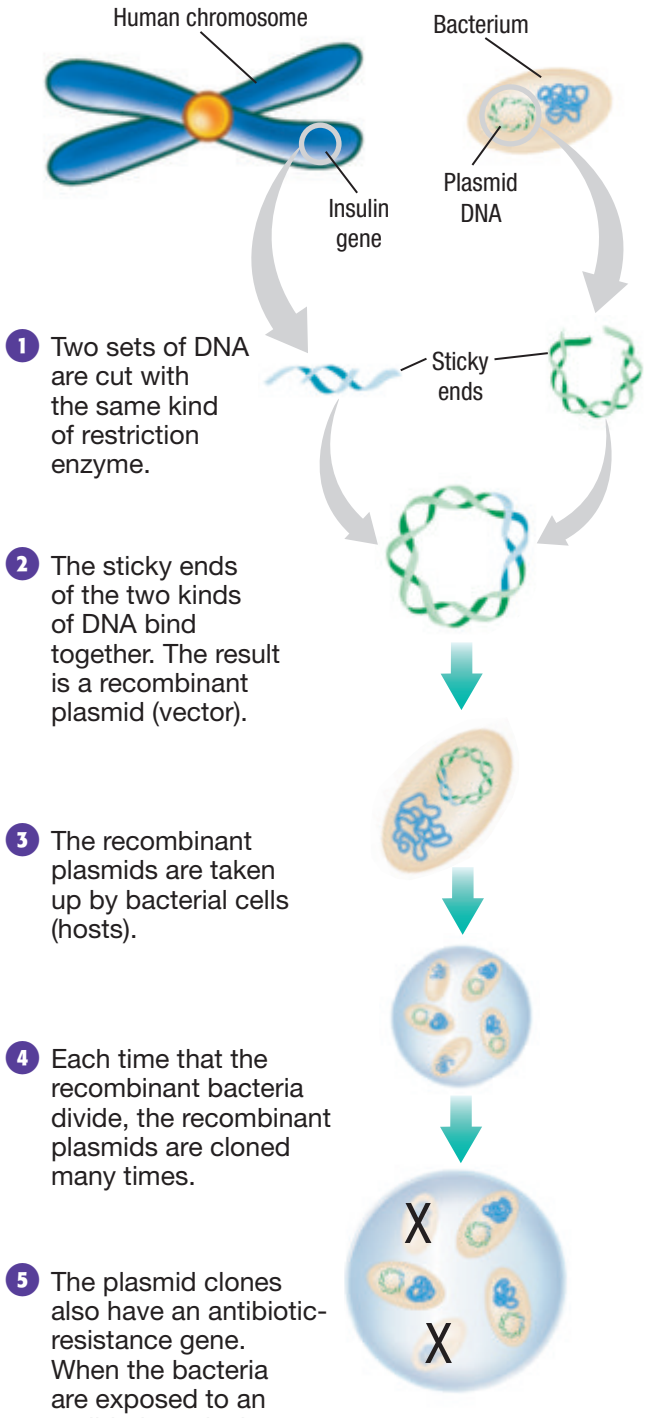
Recombinant Cloning



Gene Recombination and Cloning The first attempts at gene recombination and cloning were done by inserting a gene into an organism that replicates easily, as shown in **Figure 14.** Other methods may use similar steps.

Step 1 Cut DNA Samples Two sets of DNA are cut by the same kind of restriction enzyme so that all fragments have matching sticky ends. One set of DNA is from an organism containing a specific gene (in this case, the human insulin gene). The other DNA is part of a vector, such as a virus or a bacterial plasmid, that can carry or move DNA between cells. The vector will be replicated when placed in a host, such as a bacterial cell.

Step 2 Splice Pieces Together The DNA fragments from the first organism are combined with the fragments from the vector. Then, an enzyme called *DNA ligase* is added to help bond the sticky ends of all the fragments together.

Step 3 Place into Host At this point, some plasmids are recombinant with human DNA. When the plasmids are placed in a culture of bacteria, some cells take up the plasmids. The cells are allowed to replicate normally.

Step 4 Replicate Gene Each time that a bacterial cell divides, its plasmids are copied many times. In a few generations, the cells make millions of clones of the recombinant plasmids.

Step 5 Screen for Gene At this point, only some of the bacterial cells contain the recombinant plasmids. These cells must be identified in some way. One clever solution is to use vectors that contain another gene that is easy to detect. In this example, the original plasmids contained a gene that makes bacteria resistant to an antibiotic chemical. When the bacteria from step 4 are exposed to that chemical, only the cells that have taken up the vectors will survive.

These steps are just the beginning of genetic-engineering applications. Before PCR, this process of recombination was the main way to clone genes for further research. Another use is simply to produce a protein, such as insulin, from a cloned gene. As you have learned, recombinant organisms are created for many applications, from agriculture to medicine.

› **Reading Check** *What is a vector?*

Exploring Genomes

Until recently, the human genome was largely "unexplored." But now, specific genes are being identified and their locations "mapped." These first steps lead to understanding how each gene works. Like geographic maps, maps of genetic data can have different levels of detail or scale. For example, one can view a map of an entire nation or "zoom in" to view a particular state, city, neighborhood, or street. In a similar way, **› one can explore and map a genome at many levels, including species, individual, chromosome, gene, or nucleotide.**

Managing Genomic Data Your school library has a system for organizing and keeping track of books, as **Figure 15** shows. Similarly, scientists need systems for managing the vast amounts of data in a genome. Today, they use information technologies. The application of information technologies in biology is **bioinformatics.** **› Genomic bioinformatics starts with the mapping and assembly of the many parts of each genome.** The major stages of this work include the following:

- **Mapping and Assembly** Many genes have been "mapped" to reveal their location relative to other genes. In addition, large collections of sequences are being pieced together like a puzzle.
- **Organized Storage** Genomic information is stored in a logical system or database. This way, the information can be sorted and searched, and new information can be added easily.
- **Annotation** Each gene or sequence is named and categorized according to its location, structure, or function in each genome.
- **Analysis** The ultimate goal of genomics is to understand the exact function of each gene or sequence. This analysis includes studying the complex interactions among genes and proteins.

› Reading Check ***What are the first steps of studying genomes?***

bioinformatics the application of information technologies in biology, especially in genetics

READING TOOLBOX

Learning Steps If you have not yet completed your pattern puzzle for **Figure 14,** do so now. Then, close your book, scramble the pieces, and see if you can put them in order.

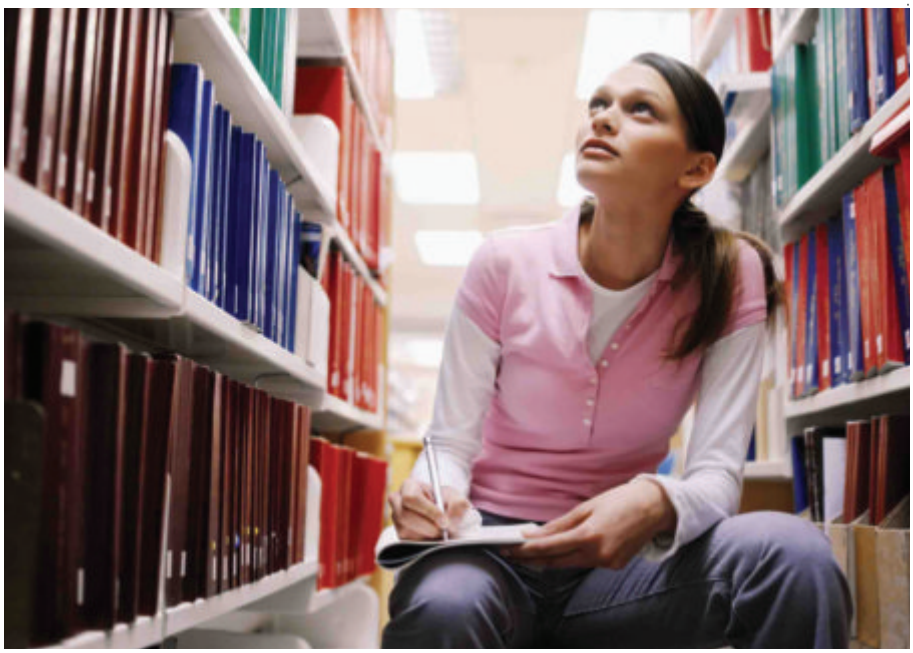


Figure 15 Like a library full of books, genomic data must be organized in order to be useful. **› What other actions are needed to manage genomic data?**

genome mapping the process of determining the relative position of genes in a genome

genetic library a collection of genetic sequence clones that represent all of the genes in a given genome

Mapping Methods

Genome mapping is the process of determining the relative position of all of the genes on chromosomes in an organism's genome. To make a city map from scratch, you might start with landmarks that are easy to find and recognize. Similarly, genome mapping methods use *genetic markers,* or traits that can be easily detected, to trace the movement and location of genes. Examples are shown in **Figure 16.** Any detectable physical, behavioral, or chemical trait can be used as a marker. As the next step in making a map, you might try to determine the location of each thing relative to other things. Similarly, genome mapping uses several methods.

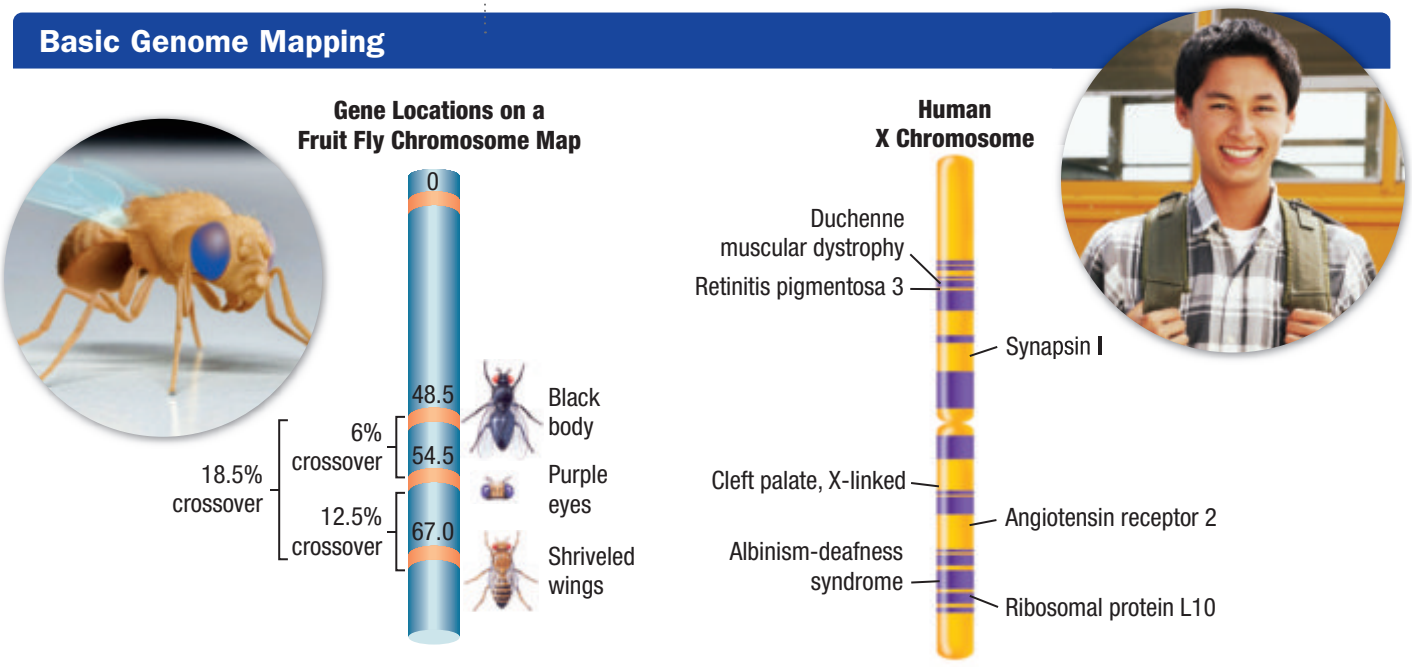
Linkage Mapping Linkage mapping methods identify the relative order of genes along a chromosome. Recall that the closer together that two genes are, the less frequently they will be separated during chromosome crossover. So, closely linked genes are more often associated, or found together, in the same individual. By comparing how often genes are associated, scientists can deduce their location relative to one another, as **Figure 16** shows.

Physical Mapping Physical mapping methods determine the exact number of base pairs between specific genes. These methods manipulate DNA to deduce exactly how close together genes are.

Human Chromosome Mapping Early attempts to map human genes used historical family records. By studying the patterns of inheritance of specific traits, scientists could infer which genes tend to be inherited together. This method was especially useful for initial mapping of the X chromosome. Such maps have since been filled in with data from physical mapping, as **Figure 16** shows.

Figure 16 Each of these maps shows the relative positions of genes on chromosomes. The physical map is more specific than the linkage map. **Why was the X chromosome mapped more easily than other chromosomes?**

Basic Genome Mapping



Linkage Map This map shows the frequencies of crossover of specific genes in the *Drosophila* genome.

Physical Chromosome Map This map shows the locations of some genes on the human X chromosome.

Genome Sequence Assembly As they zoom in on the map of genes, scientists want to record all of the nucleotide sequences in a genome. The process of deducing and recording the exact order of every base and gene in a genome is called *sequence assembly*. The process involves collecting, sorting, and comparing large samples of genetic material.

Genetic Libraries To study an entire genome, scientists break up the genome into small fragments and clone all of the fragments. A collection of clones that represent all of the genes in a given genome is called a **genetic library.** Two kinds of genetic libraries are made. A *genomic library* is made by cloning all of the DNA in a cell. A genomic library includes all functional genes as well as all noncoding DNA. An *expressed sequence tag* (EST) *library* starts with the mRNA that results from transcription. The mRNA is used to make cDNA segments, which are then cloned to make the library.

Using the Libraries Once the clones are assembled, they can be sequenced, sorted, and organized. Early methods involved sorting through libraries one gene at a time by repeated probing and deduction. More recently, a method called *shotgun sequencing* was developed. In this method, an entire genome is cut up randomly into segments of varying size. All resulting segments are cloned and sequenced. Then, by looking for overlapping parts, researchers put together the entire sequence like a puzzle. The resulting genome sequence is stored as data and can be searched for specific genes or sequences of any size.

Automated Sequencing Robotic devices are now used to sequence a genome in a fraction of the time that it took to complete such a project only decades ago. Automated sequencing devices can quickly "read" many tiny sequence gels at one time. In such a device, a laser beam scans each gel tube, and detectors identify each of the four kinds of tags. Finally, a computer compiles the data into a string of letters, as **Figure 17** shows.

› **Reading Check** *What are the two kinds of genetic libraries?*

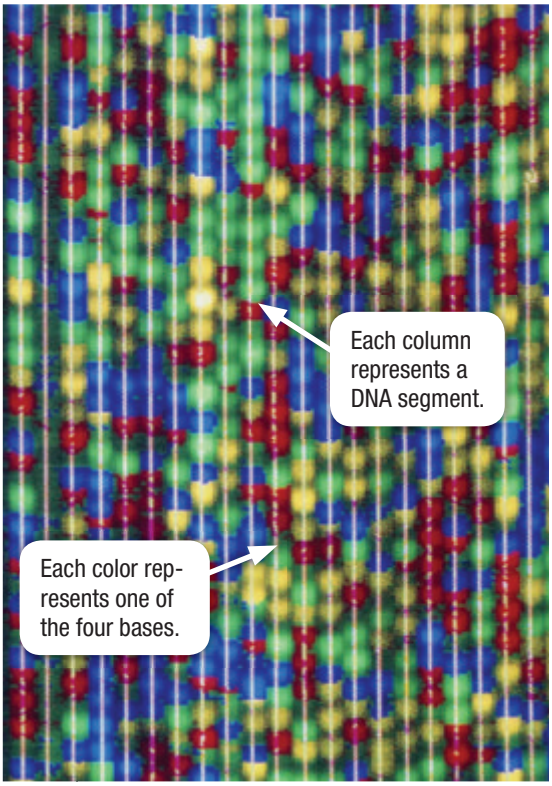


Figure 17 This computer screen shows the output of an automated sequencing device. The device "reads" DNA sequences by detecting color-coded, "tagged" bases in tiny gel-electrophoresis tubes. › **What advantages do computers provide?**

Section 3

Review

› KEY IDEAS

1. **Identify** the basic tools of genetic manipulation.
2. **Outline** any one of the major processes of modern gene technologies.
3. **Identify** the major stages of the work of genomics, in terms of bioinformatics.

CRITICAL THINKING

4. **Relating Concepts** Differentiate between SNPs and RFLPs.
5. **Predicting Outcomes** If samples of nerve cells and bone cells from the same person were run through the same type of microarray, would the results differ? Explain.
6. **Analyzing Information** Why is *expressed sequence tag library* a fitting name for a collection of clones made from mRNA?

METHODS OF SCIENCE

7. **Choosing Appropriate Tools** Suppose that you are a genetic scientist who has been asked to help stop the illegal killing of some tropical bird species. These birds are being killed so that their feathers can be sold for fashionable hat decorations. Propose some ways that you could use gene technologies to help protect these birds.

Chapter 15 Lab

Objectives

- Model the forensic analysis of evidence from a crime scene.
- Use restriction enzymes, PCR, and gel electrophoresis to manipulate DNA samples.
- Compare DNA fingerprints to match identical DNA samples.

Materials

- lab apron, safety goggles, and disposable gloves
- marker, permanent, waterproof
- microcentrifuge tubes (5)
- micropipettes, sterile, disposable (25)
- DNA samples (5)
- restriction enzyme buffer
- restriction enzyme
- incubator or hot water bath
- ice, crushed
- cup, plastic-foam
- gel, agarose, precast for electrophoresis chamber
- electrophoresis chamber with power supply and wires
- running buffer
- loading dye
- bag, plastic, resealable
- DNA staining solution
- tray for staining gel
- water, distilled
- paper, white, or light table

Safety

DNA Fingerprint Analysis

Each person's DNA is unique. This fact can be used to match crime suspects to DNA samples taken from crime scenes. *DNA fingerprints* can be made by using restriction enzymes and gel electrophoresis to reveal unique patterns in each individual's DNA.

Procedure

Cut DNA with Restriction Enzyme

1. Read all procedures, and prepare to collect your data. Label each microcentrifuge tube with a code for each DNA sample provided. For example, label one tube "C" for "crime scene sample" and the remaining tubes "S1" to "S4," one for each suspect.
2. Wear a lab apron, safety goggles, and gloves during all parts of this lab.
3. **CAUTION: Never taste chemicals or allow them to contact your skin.** Using a clean pipette each time, transfer 10 µL of each DNA sample to the microcentrifuge tube that has the matching label.
4. Using a clean pipette each time, transfer 2 µL of restriction enzyme buffer to each of the tubes.
5. Using a clean pipette each time, transfer 2 µL of restriction enzyme to each of the tubes. Close all of the tubes. Gently flick the bottom of each tube to mix the DNA and reagents.
6. **CAUTION: Use caution when working with heating devices.** Transfer the tubes to the incubator or water bath set at 37 °C. Let the samples incubate for one hour.
7. Stand the tubes in crushed ice in the plastic-foam cup.
8. If you need to pause this lab at this point, store the cup at 4 °C.

Separate Fragments by Gel Electrophoresis

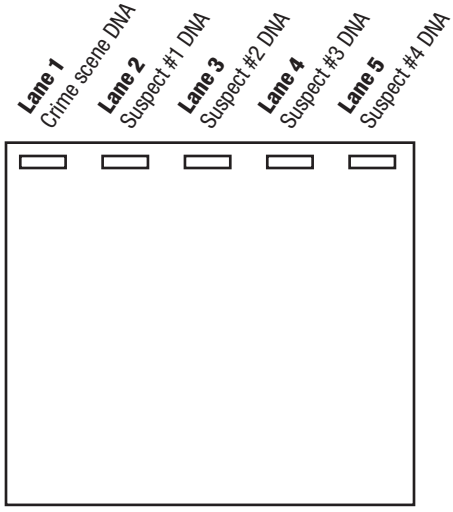
9. Place the precast gel on the level surface of the electrophoresis chamber. The wells in the gel should be closest to the black, or negative, electrode. Keep the gel level and flat at all times.
10. Fill the chamber with enough buffer to barely cover the gel. Do not pour the buffer directly onto the gel. Sketch a diagram of your gel in your lab notebook, as the sample diagram shows.
11. Using a clean pipette each time, transfer 2 µL of loading dye to each of the tubes. Gently flick the tubes to mix the contents.
12. Using a clean pipette, load the crime scene DNA into the well for Lane 1 of your gel. Be careful not to overflow or puncture the well.
13. Repeat step 12 for the remaining DNA samples and gel lanes. End with the DNA from Suspect 4. Use a clean pipette for each transfer.

14. **CAUTION: Use caution when working with electrical equipment; use only as directed by your teacher.** Make sure that everything outside the chamber is dry before proceeding. Attach the power connectors to the chamber and power supply as directed by your teacher. Set the power supply to the voltage determined by your teacher, and turn on the power supply.
15. Allow the gel to run undisturbed for the time directed by your teacher. Observe the gel periodically, and stop the process when the dye front is about 3 cm away from the end of the gel. At that point, turn off the power supply. Then, disconnect the power connectors from the power supply and chamber.
16. **CAUTION: Dispose of all waste materials as directed by your teacher.** Carefully remove the casting tray from the chamber. Pour off the running buffer according to your teacher's instructions.
17. If you need to pause this lab at this point, carefully slide the gel into a resealable bag. Add 2 mL running buffer, seal the bag, and store the bag in a refrigerator. Remember to keep the gel flat.

View Separated DNA Fragments

18. Gently slide the gel onto the staining tray. Pour enough stain into the tray to barely cover the gel. Do not pour the stain directly onto the gel. Let the gel sit for at least 30 min.
19. Carefully pour off the stain as directed by your teacher.
20. Gently pour distilled water into the tray to cover the gel. Do not pour the water directly onto the gel. After 5 min, carefully pour off the water as directed by your teacher.
21. Repeat step 20 until bands are clearly visible on the gel.
22. Gently transfer the gel to a white sheet of paper or to a light table. Sketch and describe your observations in your lab notebook.
23. Clean up your lab materials according to your teacher's instructions. Wash your hands before leaving the lab.

Loading the gel



Sample gel diagram

Analyze and Conclude

1. SCIENTIFIC METHODS **Organizing Data** Organize your data into a table. How many different fragment sizes resulted from the treatment of each DNA sample?
2. **Analyzing Data** Identify any bands of fragments that are the same size among any of the samples. Mark these bands on your sketch.
3. **Forming Conclusions** Use this evidence to determine which suspect most likely committed the crime. Explain your answer.
4. SCIENTIFIC METHODS **Evaluating Methods** Do these results provide enough evidence to convict the suspect? Explain your answer.

Extension

5. **Applying Concepts** Some bands appeared in the same position in several lanes. Propose an explanation for this result.
6. **Predicting Results** How might the results have been affected if a different restriction enzyme had been used? Explain your answer.

Chapter 15 Summary

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SUPER SUMMARY
Keyword: HX8GTCS

Key Ideas	Key Terms

1 The Human Genome

- The sequencing of the human genome has advanced the study of human biology yet created new questions
- Genomics and gene technologies have many applications in human healthcare and society.
- Many important questions about the human genome remain to be investigated or decided.

genomics (345)
microarray (346)
DNA fingerprint (347)

2 Gene Technologies in Our Lives

- Today, gene technologies are widely applied to study organisms in new ways, to alter organisms for human use, and to improve human lives.
- Cloning and stem cell techniques are used in research on animal development and have potential for treating certain diseases.
- Ethical issues can be raised for every use of gene technologies.

genetic engineering (350)
recombinant DNA (350)
clone (352)
stem cell (353)

3 Gene Technologies in Detail

- The basic tools of DNA manipulation rely on the chemical nature of genetic material and are adapted from natural processes discovered in cells. These tools include restriction enzymes, polymorphisms, gel electrophoresis, denaturation, and hybridization.
- The major methods for working with genes use some combination of the basic tools of cellular machinery. These methods include PCR, blotting, DNA sequencing, and gene recombination.
- One can explore and map a genome at many levels, including species, individual, chromosome, gene, or nucleotide. Genomic bioinformatics starts with the mapping and assembly of the many parts of each genome.

restriction enzyme (355)
DNA polymorphisms (356)
electrophoresis (356)
polymerase chain reaction (PCR) (357)
DNA sequencing (359)
bioinformatics (361)
genome mapping (362)
genetic library (363)