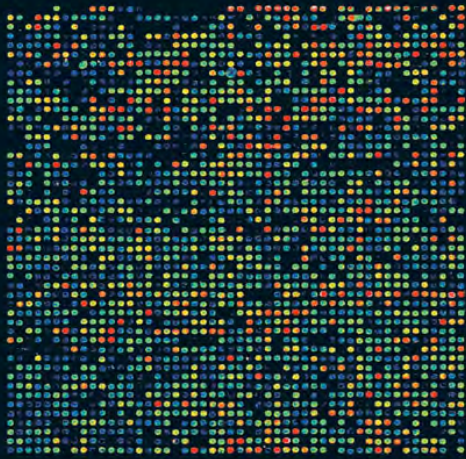


20

Biotechnology



▲ **Figure 20.1** How can this array of spots be used to compare normal and cancerous tissues?

KEY CONCEPTS

- 20.1 DNA cloning yields multiple copies of a gene or other DNA segment
- 20.2 DNA technology allows us to study the sequence, expression, and function of a gene
- 20.3 Cloning organisms may lead to production of stem cells for research and other applications
- 20.4 The practical applications of DNA technology affect our lives in many ways

OVERVIEW

The DNA Toolbox

In 2001, a major scientific milestone was announced: Researchers had completed a “first draft” sequence of all 3 billion base pairs of the human genome—only the fourth eukaryotic genome to be sequenced. This news electrified the scientific community. Few among them would have dared to dream that

a mere nine years later, genome sequencing would be under way for more than 7,000 species. By 2010, researchers had completed sequencing more than 1,000 bacterial, 80 archaeal, and 100 eukaryotic genomes, with many more in progress.

Ultimately, these achievements are attributable to advances in DNA technology—methods of working with and manipulating DNA—that had their roots in the 1970s. A key accomplishment was the invention of techniques for making **recombinant DNA**, DNA molecules formed when segments of DNA from two different sources—often different species—are combined *in vitro* (in a test tube). This advance gave rise to the development of powerful techniques for analyzing genes and gene expression. How scientists prepare recombinant DNA and use DNA technology to answer fundamental biological questions are one focus of this chapter. In the next chapter (Chapter 21), we’ll see how these techniques have allowed the sequencing of whole genomes, and we’ll consider what we’ve learned from these sequences about the evolution of species and of the genome itself.

Another focus of this chapter is how our lives are affected by **biotechnology**, the manipulation of organisms or their components to make useful products. Biotechnology includes such early practices as selective breeding of farm animals and using microorganisms to make wine and cheese. Today, biotechnology also encompasses **genetic engineering**, the direct manipulation of genes for practical purposes. Genetic engineering has launched a revolution in biotechnology, greatly expanding the scope of its potential applications. Tools from the DNA toolbox are now applied in ways that affect everything from agriculture to criminal law to medical research. For instance, on the DNA microarray in **Figure 20.1**, the colored spots represent the relative level of expression of 2,400 human genes in normal and cancerous tissue. Using microarray analysis, researchers can quickly compare gene expression in different samples, such as those tested here. The knowledge gained from such gene expression studies is making a significant contribution to the study of cancer and other diseases.

In this chapter, we’ll first describe the main techniques for manipulating DNA and analyzing gene expression and function. Next, we’ll explore advances in cloning organisms and producing stem cells, techniques that have both expanded our basic understanding of biology and enhanced our ability to apply this understanding to global problems. Finally, we’ll survey the practical applications of biotechnology and consider some of the social and ethical issues that arise as biotechnology becomes more pervasive in our lives.

CONCEPT 20.1

DNA cloning yields multiple copies of a gene or other DNA segment

The molecular biologist studying a particular gene faces a challenge. Naturally occurring DNA molecules are very long,

and a single molecule usually carries many genes. Moreover, in many eukaryotic genomes, genes occupy only a small proportion of the chromosomal DNA, the rest being noncoding nucleotide sequences. A single human gene, for example, might constitute only 1/100,000 of a chromosomal DNA molecule. As a further complication, the distinctions between a gene and the surrounding DNA are subtle, consisting only of differences in nucleotide sequence. To work directly with specific genes, scientists have developed methods for preparing well-defined segments of DNA in multiple identical copies, a process called *DNA cloning*.

DNA Cloning and Its Applications: A Preview

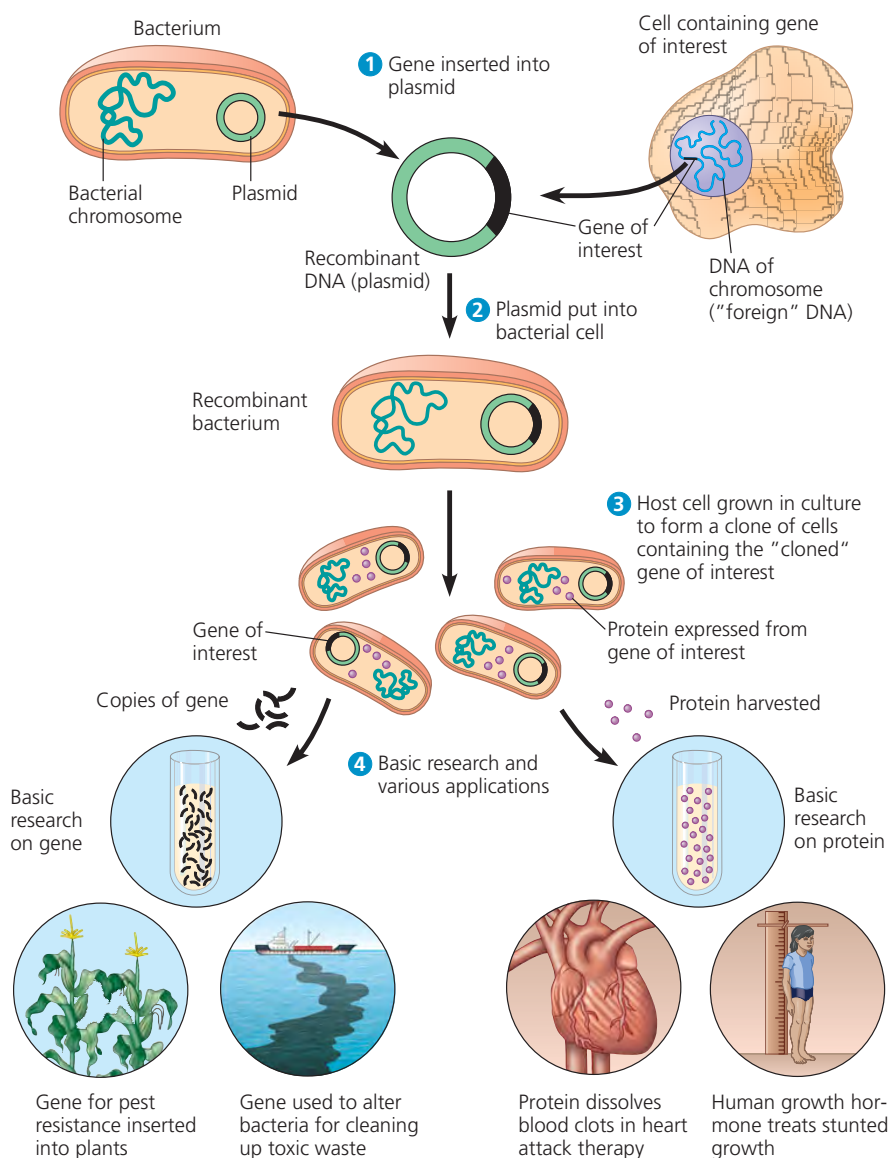
Most methods for cloning pieces of DNA in the laboratory share certain general features. One common approach uses bacteria, most often *Escherichia coli*. Recall from Figure 16.12 that the *E. coli* chromosome is a large circular molecule of DNA. In addition, *E. coli* and many other bacteria have **plasmids**, small circular DNA molecules that replicate separately from the bacterial chromosome. A plasmid has only a small number of genes; these genes may be useful when the bacterium is in a particular environment but may not be required for survival or reproduction under most conditions.

To clone pieces of DNA in the laboratory, researchers first obtain a plasmid (originally isolated from a bacterial cell and genetically engineered for efficient cloning) and insert DNA from another source ("foreign" DNA) into it (Figure 20.2). The resulting plasmid is now a recombinant DNA molecule. The plasmid is then returned to a bacterial cell, producing a *recombinant bacterium*. This single cell reproduces through repeated cell divisions to form a clone of cells, a population of genetically identical cells. Because the dividing bacteria replicate the recombinant plasmid and pass it on to their descendants, the foreign DNA and any genes it carries are cloned at the same time. The production of multiple copies of a single gene is called **gene cloning**.

Gene cloning is useful for two basic purposes: to make many copies of, or *amplify*, a particular gene and to produce a protein product. Researchers can

isolate copies of a cloned gene from bacteria for use in basic research or to endow an organism with a new metabolic capability, such as pest resistance. For example, a resistance gene present in one crop species might be cloned and transferred into plants of another species. Alternatively, a protein with medical uses, such as human growth hormone, can be harvested in large quantities from cultures of bacteria carrying the cloned gene for the protein.

A single gene is usually a very small part of the total DNA in a cell. For example, a typical gene makes up only about one-millionth of the DNA in a human cell. The ability to



▲ Figure 20.2 A preview of gene cloning and some uses of cloned genes. In this simplified diagram of gene cloning, we start with a plasmid (originally isolated from a bacterial cell) and a gene of interest from another organism. Only one plasmid and one copy of the gene of interest are shown at the top of the figure, but the starting materials would include many of each.

amplify such rare DNA fragments is therefore crucial for any application involving a single gene.

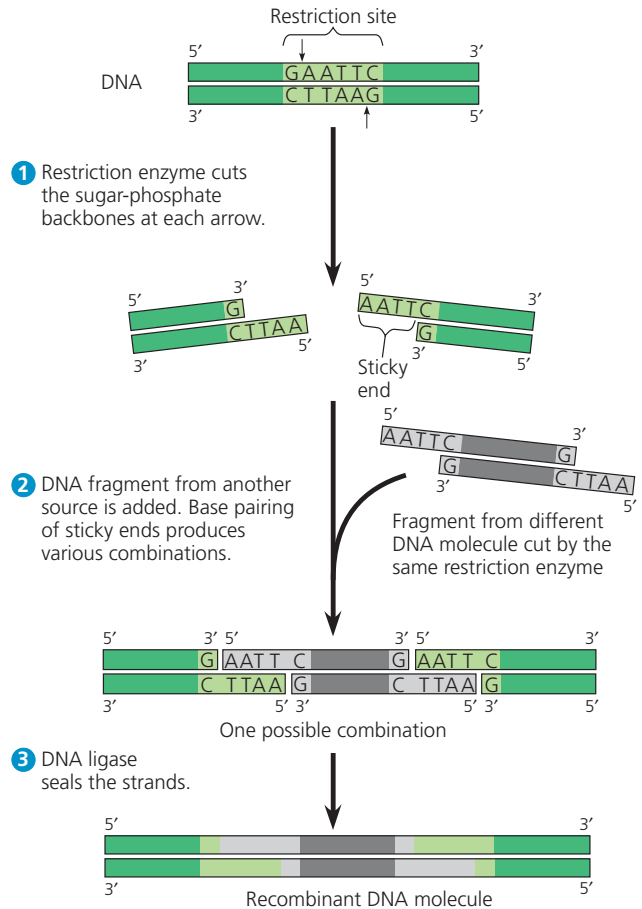
Using Restriction Enzymes to Make Recombinant DNA

Gene cloning and genetic engineering rely on the use of enzymes that cut DNA molecules at a limited number of specific locations. These enzymes, called restriction endonucleases, or **restriction enzymes**, were discovered in the late 1960s by biologists doing basic research on bacteria. Restriction enzymes protect the bacterial cell by cutting up foreign DNA from other organisms or phages (see Chapter 19).

Hundreds of different restriction enzymes have been identified and isolated. Each restriction enzyme is very specific, recognizing a particular short DNA sequence, or **restriction site**, and cutting both DNA strands at precise points within this restriction site. The DNA of a bacterial cell is protected from the cell's own restriction enzymes by the addition of methyl groups ($-\text{CH}_3$) to adenines or cytosines within the sequences recognized by the enzymes.

The top of **Figure 20.3** illustrates a restriction site recognized by a particular restriction enzyme from *E. coli*. As shown in this example, most restriction sites are symmetrical. That is, the sequence of nucleotides is the same on both strands when read in the 5' → 3' direction. The most commonly used restriction enzymes recognize sequences containing four to eight nucleotides. Because any sequence this short usually occurs (by chance) many times in a long DNA molecule, a restriction enzyme will make many cuts in a DNA molecule, yielding a set of **restriction fragments**. All copies of a particular DNA molecule always yield the same set of restriction fragments when exposed to the same restriction enzyme. In other words, a restriction enzyme cuts a DNA molecule in a reproducible way. (Later you will learn how the different fragments can be separated and distinguished from each other.)

The most useful restriction enzymes cleave the sugar-phosphate backbones in the two DNA strands in a staggered manner, as indicated in **Figure 20.3**. The resulting double-stranded restriction fragments have at least one single-stranded end, called a **sticky end**. These short extensions can form hydrogen-bonded base pairs with complementary sticky ends on any other DNA molecules cut with the same enzyme. The associations formed in this way are only temporary but can be made permanent by the enzyme **DNA ligase**. As you saw in **Figure 16.16**, this enzyme catalyzes the formation of covalent bonds that close up the sugar-phosphate backbones of DNA strands; for example, it joins Okazaki fragments during replication. You can see at the bottom of **Figure 20.3** that the ligase-catalyzed joining of DNA from two different sources produces a stable recombinant DNA molecule.



▲ Figure 20.3 Using a restriction enzyme and DNA ligase to make recombinant DNA. The restriction enzyme in this example (called *EcoRI*) recognizes a specific six-base-pair sequence, the restriction site, and makes staggered cuts in the sugar-phosphate backbones within this sequence, producing fragments with sticky ends. Any fragments with complementary sticky ends can base-pair, including the two original fragments. If the fragments come from different DNA molecules, the ligated product is recombinant DNA.

DRAW IT The restriction enzyme *HindIII* recognizes the sequence 5'-AAGCTT-3', cutting between the two 'A's. Draw the double-stranded sequence before and after the enzyme cuts.

Cloning a Eukaryotic Gene in a Bacterial Plasmid

Now that you've learned about restriction enzymes and DNA ligase, we can see how genes are cloned in plasmids. The original plasmid is called a **cloning vector**, defined as a DNA molecule that can carry foreign DNA into a host cell and replicate there. Bacterial plasmids are widely used as cloning vectors for several reasons: They can be readily obtained from commercial suppliers, manipulated to form recombinant plasmids by insertion of foreign DNA *in vitro*, and then introduced into bacterial cells. Moreover, recombinant bacterial plasmids (and the foreign DNA they carry) multiply rapidly owing to the high reproductive rate of their host cells.

Producing Clones of Cells Carrying Recombinant Plasmids

Let's say we are researchers interested in studying the β -globin gene in a particular species of hummingbird. We start by cloning all the hummingbird genes; later we'll isolate the β -globin gene from all the others, a task very much like finding a needle in a haystack. **Figure 20.4** details one method for cloning hummingbird genes using a bacterial plasmid as the cloning vector.

1 First, we isolate hummingbird genomic DNA from hummingbird cells. We also obtain our chosen vector, a particular bacterial plasmid from *E. coli* cells. The plasmid has been engineered to carry two genes that will later prove useful: *amp^R*, which makes *E. coli* cells resistant to the antibiotic ampicillin, and *lacZ*, which encodes the enzyme β -galactosidase, which hydrolyzes lactose (see p. 354). This enzyme can also hydrolyze a similar synthetic molecule called X-gal to form a blue product. The plasmid contains only one copy of the restriction site recognized by the restriction enzyme used in the next step, and that site is within the *lacZ* gene.

2 Both the plasmid and the hummingbird DNA are cut with the same restriction enzyme, and then 3 the fragments are mixed together, allowing base pairing between their complementary sticky ends. We then add DNA ligase, which covalently bonds the sugar-phosphate backbones of the fragments whose sticky ends have base-paired. Many of the resulting recombinant plasmids contain single hummingbird DNA fragments (three are shown in Figure 20.4), and at least one of them is expected to carry all or part of the β -globin gene. This step will also generate other products, such as plasmids containing multiple hummingbird DNA fragments, a combination of two plasmids, or a rejoined, nonrecombinant version of the original plasmid.

4 The DNA mixture is then added to bacteria that have a mutation in the *lacZ* gene on their own chromosome, making them unable to hydrolyze

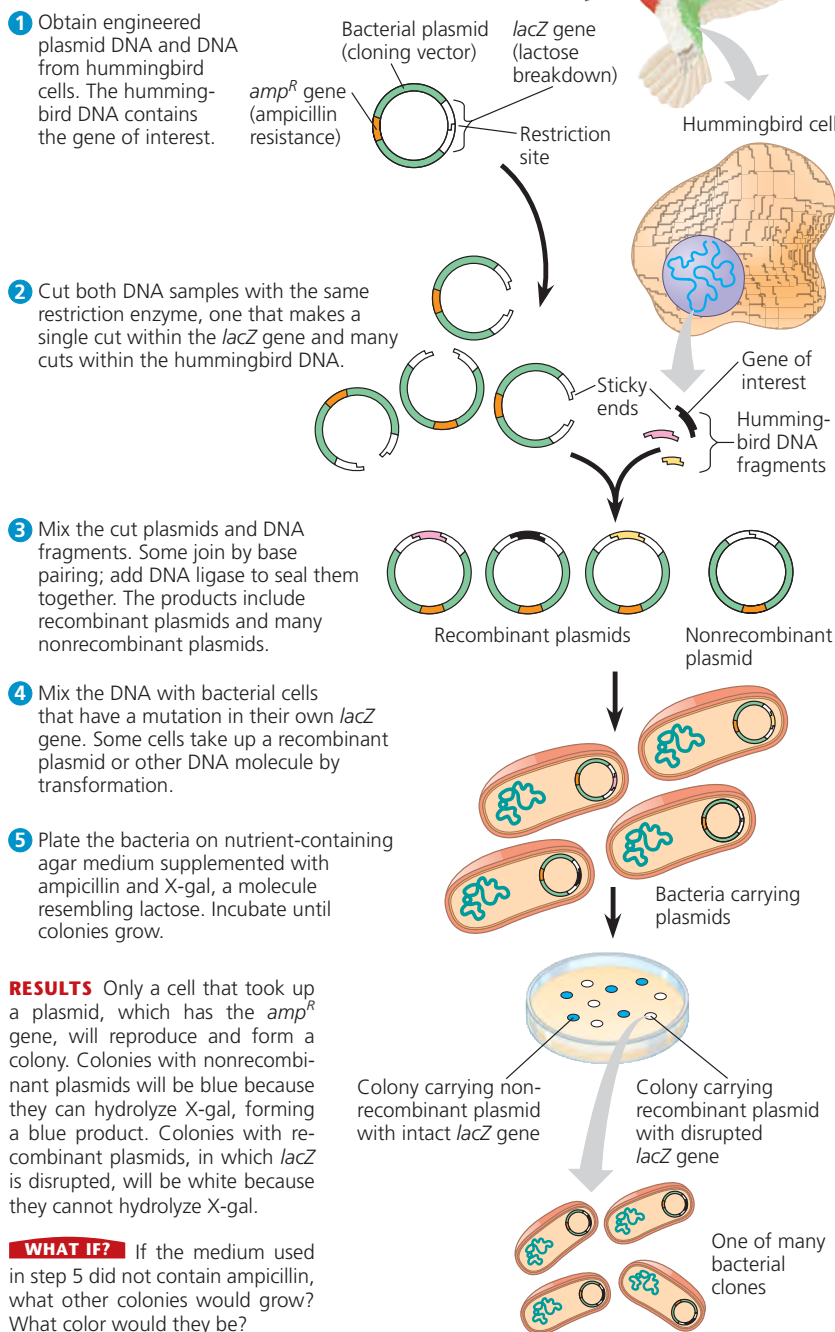
▼ **Figure 20.4**

RESEARCH METHOD

Cloning Genes in Bacterial Plasmids

APPLICATION Gene cloning is a process that produces many copies of a gene of interest. These copies can be used in sequencing the gene, in producing its encoded protein, or in basic research or other applications.

TECHNIQUE In this example, hummingbird genes are inserted into plasmids from *E. coli*. Only three plasmids and three hummingbird DNA fragments are shown, but millions of copies of the plasmid and a mixture of millions of different hummingbird DNA fragments would be present in the samples.



lactose or X-gal. Under suitable experimental conditions, the cells take up foreign DNA by transformation (see p. 306). Some cells acquire a recombinant plasmid carrying a gene, while others may take up a nonrecombinant plasmid, a fragment of noncoding hummingbird DNA, or nothing at all. The *amp^R* and *lacZ* genes on the plasmid can help us sort out these possibilities.

5 First, plating out all the bacteria on solid nutrient medium containing ampicillin allows us to distinguish the cells that have taken up plasmids, whether recombinant or not, from the other cells. Under these conditions, only cells with a plasmid will reproduce because only they have the *amp^R* gene conferring resistance to the ampicillin in the medium. Each reproducing bacterium forms a clone of cells. Once the clone contains between 10^5 and 10^8 cells, it is visible as a mass, or *colony*, on the agar. As cells reproduce, any foreign genes carried by recombinant plasmids are also copied (cloned).

Second, the presence of X-gal in the medium allows us to distinguish colonies with recombinant plasmids from those with nonrecombinant plasmids. Colonies containing nonrecombinant plasmids have the *lacZ* gene intact and will produce functional β -galactosidase. These colonies will be blue because the enzyme hydrolyzes the X-gal in the medium, forming a blue product. In contrast, no functional β -galactosidase is produced in colonies containing recombinant plasmids with foreign DNA inserted into the *lacZ* gene; these colonies will therefore be white.

The procedure to this point will have cloned many different hummingbird DNA fragments, not just the β -globin gene that interests us. In fact, taken together, the white colonies should represent all the DNA sequences from the hummingbird genome, including noncoding regions as well as genes. And because restriction enzymes do not recognize gene boundaries, some genes will be cut and divided up among two or more clones. Shortly, we will discuss the procedure we use to find the colony (cell clone) or colonies carrying the β -globin gene sequences among the many clones carrying other pieces of hummingbird DNA. To understand that procedure, we must first consider how the clones are stored.

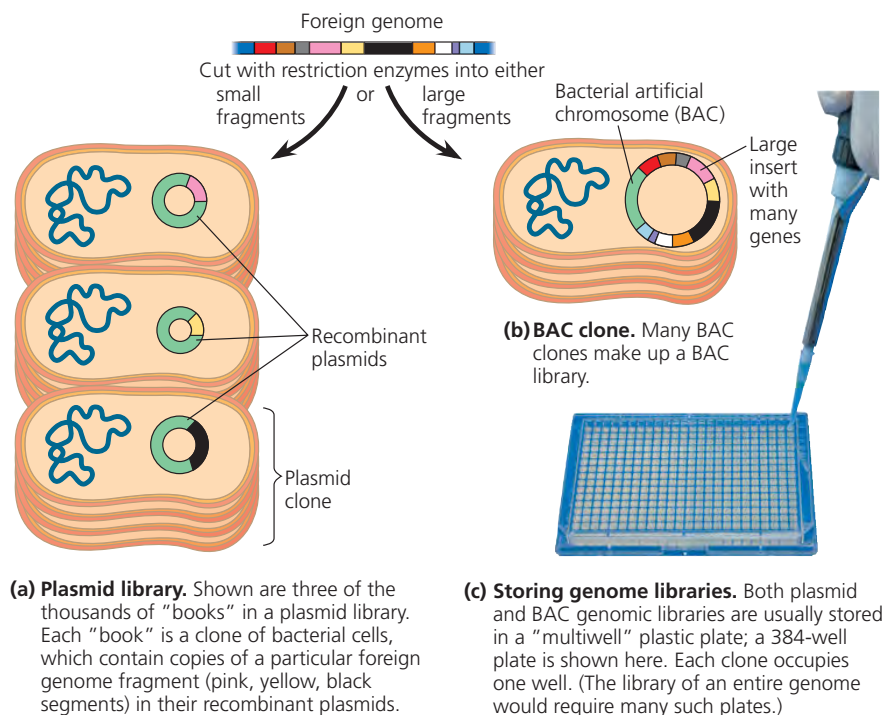
Storing Cloned Genes in DNA Libraries

The cloning procedure in Figure 20.4, which starts with a mixture of fragments from the entire genome of an organism, is called a “shotgun” approach;

no single gene is targeted for cloning. Numerous different recombinant plasmids are produced in step 3, and a clone of cells carrying each type of plasmid ends up as a white colony in step 5. The complete set of plasmid-containing cell clones, each carrying copies of a particular segment from the initial genome, is referred to as a **genomic library** (Figure 20.5a). Each “plasmid clone” in the library is like a book containing specific information. Today, scientists often obtain such libraries (or even particular cloned genes) from another researcher, a commercial source, or a sequencing center.

Historically, certain bacteriophages have also been used as cloning vectors for making genomic libraries. Fragments of foreign DNA can be spliced into a trimmed-down version of a phage genome, as into a plasmid, by using a restriction enzyme and DNA ligase. The normal infection process allows production of many new phage particles, each carrying the foreign DNA insert. Today, phages are generally used for making genomic libraries only in special cases.

Another type of vector widely used in library construction is a **bacterial artificial chromosome (BAC)**. In spite of the name, these are simply large plasmids, trimmed down so they contain just the genes necessary to ensure replication. An advantage of using BACs as vectors is that while a standard plasmid can carry a DNA insert no larger than 10,000 base pairs (10 kb), a BAC can carry an insert of 100–300 kb

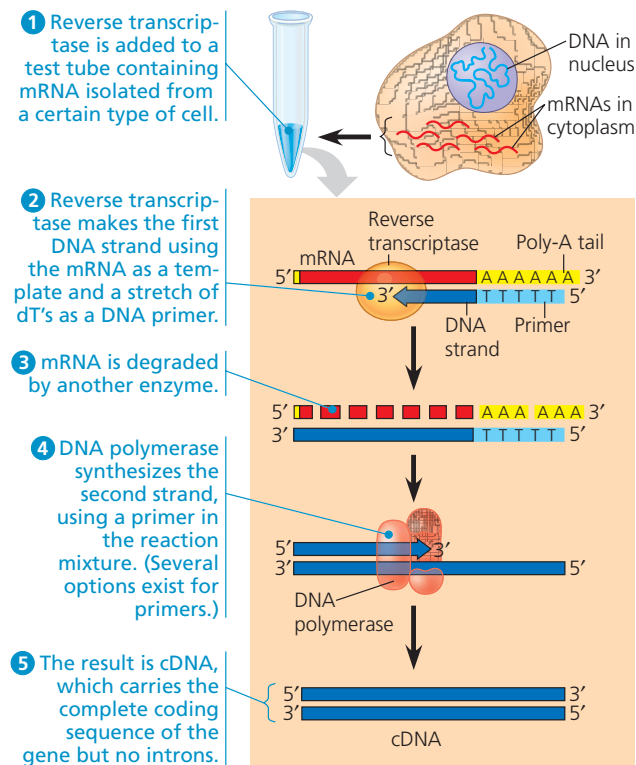


▲ Figure 20.5 Genomic libraries. A genomic library is a collection of many clones. Each clone carries copies of a particular DNA segment from a foreign genome, integrated into an appropriate DNA vector, such as a plasmid or a bacterial artificial chromosome (BAC). In a complete genomic library, the foreign DNA segments cover the entire genome of an organism. Note that the BACs are not drawn to scale; the genes carried are actually about 1,000 times larger than the vector itself.

(Figure 20.5b). The very large insert size minimizes the number of clones needed to make up the genomic library, but it also makes them more challenging to work with in the lab, so the insert may later be cut up into smaller pieces that are “subcloned” into plasmid vectors.

Clones are usually stored in multiwelled plastic plates, with one clone per well (Figure 20.5c). This orderly storage of clones, identified by their location in the plate, makes screening for the gene of interest very efficient, as you will see.

In a genomic library, the cloned β -globin gene would include not just exons containing the coding sequence, but also the promoter, untranslated regions, and any introns. Some biologists might be interested in the hummingbird β -globin protein itself—they might wonder, for instance, if this oxygen-carrying protein is different from its counterpart in other, less metabolically active species. Such researchers can make another kind of DNA library by starting with fully processed mRNA extracted from cells where the gene is expressed (Figure 20.6). The enzyme reverse transcriptase (obtained from retroviruses) is used *in vitro* to make a single-stranded DNA *reverse transcript* of each mRNA molecule. Recall that the 3' end of the mRNA has a stretch of



▲ **Figure 20.6 Making complementary DNA (cDNA) from eukaryotic genes.** Complementary DNA is DNA made *in vitro* using mRNA as a template for the first strand. Because the mRNA contains only exons, the resulting double-stranded cDNA carries the complete coding sequence of the gene but no introns. Although only one mRNA is shown here, the final collection of cDNAs would reflect all the mRNAs that were present in the cell.

adenine (A) ribonucleotides called a poly-A tail. This feature allows use of a short strand of thymine deoxyribonucleotides (dT's) as a primer for the reverse transcriptase. Following enzymatic degradation of the mRNA, a second DNA strand, complementary to the first, is synthesized by DNA polymerase. The resulting double-stranded DNA is called **complementary DNA (cDNA)**. To create a library, the researchers must now modify the cDNA by adding restriction enzyme recognition sequences at each end. Then the cDNA is inserted into vector DNA in a manner similar to the insertion of genomic DNA fragments. The extracted mRNA is a mixture of all the mRNA molecules in the original cells, transcribed from many different genes. Therefore, the cDNAs that are cloned make up a **cDNA library** containing a collection of genes. However, a cDNA library represents only part of the genome—only the subset of genes that were transcribed in the cells from which the mRNA was isolated.

Genomic and cDNA libraries each have advantages, depending on what is being studied. If you want to clone a gene but don't know what cell type expresses it or cannot obtain enough cells of the appropriate type, a genomic library is almost certain to contain the gene. Also, if you are interested in the regulatory sequences or introns associated with a gene, a genomic library is necessary, since these sequences are absent from mRNAs used in making a cDNA library. On the other hand, to study a specific protein (like β -globin), a cDNA library made from cells expressing the gene (like red blood cells) is ideal. A cDNA library can also be used to study sets of genes expressed in particular cell types, such as brain or liver cells. Finally, by making cDNA from cells of the same type at different times in the life of an organism, researchers can trace changes in patterns of gene expression during development.

Screening a Library for Clones Carrying a Gene of Interest

Now, returning to the results in Figure 20.4, we're ready to screen all the colonies with recombinant plasmids (the white colonies) for a clone of cells containing the hummingbird β -globin gene. We can detect this gene's DNA by its ability to base-pair with a complementary sequence on another nucleic acid molecule, using **nucleic acid hybridization**. The complementary molecule, a short, single-stranded nucleic acid that can be either RNA or DNA, is called a **nucleic acid probe**. If we know at least part of the nucleotide sequence of the gene of interest (perhaps from knowing the amino acid sequence of the protein it encodes or, as in our case, the gene's nucleotide sequence in a closely related species), we can synthesize a probe complementary to it. For example, if part of the sequence on one strand of the desired gene were



then we would synthesize this probe:



Each probe molecule, which will hydrogen-bond specifically to a complementary sequence in the desired gene, is labeled

with a radioactive isotope, a fluorescent tag, or another molecule so we can track it.

Recall that the clones in our hummingbird genomic library have been stored in a multiwell plate (see Figure 20.5c). If we transfer a few cells from each well to a defined location on a membrane made of nylon or nitrocellulose, we can screen a large number of clones simultaneously for the presence of DNA complementary to our DNA probe (Figure 20.7).

After we've identified the location of a clone carrying the β -globin gene, we can grow some cells from that colony in liquid culture in a large tank and then easily isolate many copies of the gene for our studies. We can also use the cloned gene as a probe to identify similar or identical genes in DNA from other sources, such as other species of birds.

Expressing Cloned Eukaryotic Genes

Once a particular gene has been cloned in host cells, its protein product can be produced in large amounts for research

purposes or valuable practical applications, which we'll explore in Concept 20.4. Cloned genes can be expressed as protein in either bacterial or eukaryotic cells; each option has advantages and disadvantages.

Bacterial Expression Systems

Getting a cloned eukaryotic gene to function in bacterial host cells can be difficult because certain aspects of gene expression are different in eukaryotes and bacteria. To overcome differences in promoters and other DNA control sequences, scientists usually employ an **expression vector**, a cloning vector that contains a highly active bacterial promoter just upstream of a restriction site where the eukaryotic gene can be inserted in the correct reading frame. The bacterial host cell will recognize the promoter and proceed to express the foreign gene now linked to that promoter. Such expression vectors allow the synthesis of many eukaryotic proteins in bacterial cells.

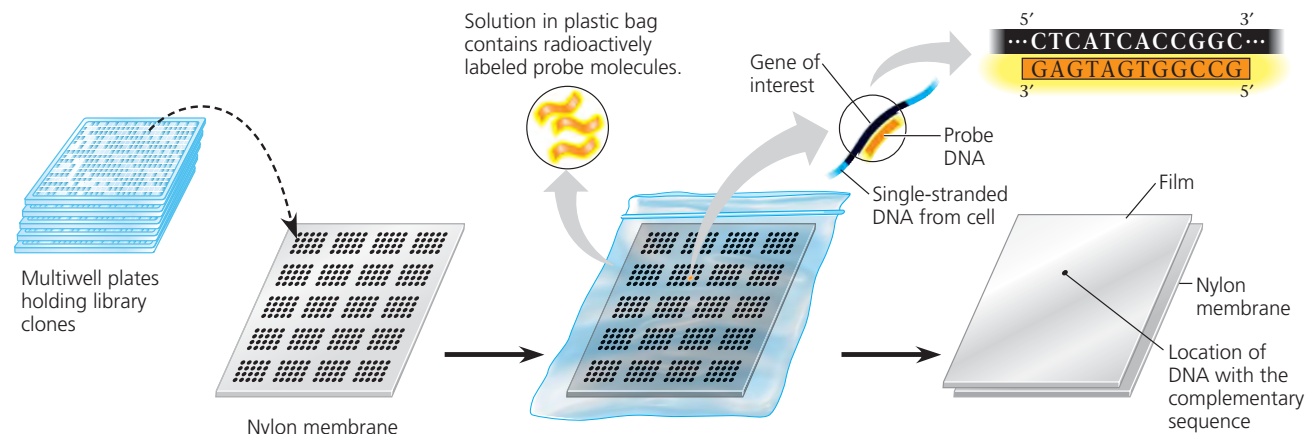
▼ Figure 20.7

RESEARCH METHOD

Detecting a Specific DNA Sequence by Hybridization with a Nucleic Acid Probe

APPLICATION Hybridization with a complementary nucleic acid probe detects a specific DNA sequence within a mixture of DNA molecules. In this example, a collection of bacterial clones from a hummingbird genomic library is screened to identify clones that carry a recombinant plasmid bearing the gene of interest. The library is stored in many multiwell plates, with one clone per well (see Figure 20.5c).

TECHNIQUE Cells from each clone are applied to a special nylon membrane. Each membrane has room for thousands of clones (many more than are shown here), so only a few membranes are needed to hold samples of all the clones in the library. This set of membranes is an *arrayed library* that can be screened for a specific gene using a labeled probe. Here the label is a radioactive nucleotide, but other labels are also commonly linked covalently to the probe nucleotides. These include fluorescent tags or enzymes that can produce either a colored or luminescent product.



1 Plate by plate, cells from each well, representing one clone, are transferred to a defined spot on a special nylon membrane. The nylon membrane is treated to break open the cells and denature their DNA; the resulting single-stranded DNA molecules stick to the membrane.

2 The membrane is then incubated in a solution of radioactive probe molecules complementary to the gene of interest. Because the DNA immobilized on the membrane is single-stranded, the single-stranded probe can base-pair with any complementary DNA on the membrane. Excess DNA is then rinsed off. (One spot with radioactive probe–DNA hybrids is shown here in orange but would not be distinguishable yet.)

3 The membrane is laid under photographic film, allowing any radioactive areas to expose the film. Black spots on the film correspond to the locations on the membrane of DNA that has hybridized to the probe. Each spot can be traced back to the original well containing the bacterial clone that holds the gene of interest.

RESULTS For a radioactive probe, the location of the black spot on a piece of photographic film identifies the clone containing the gene of interest. (Probes labeled in other ways use other detection systems.) By using probes with different nucleotide sequences in different experiments, researchers can screen the collection of bacterial clones for different genes.

Another problem with expressing cloned eukaryotic genes in bacteria is the presence of noncoding regions (introns) in most eukaryotic genes. Introns can make a eukaryotic gene very long and unwieldy, and they prevent correct expression of the gene by bacterial cells, which do not have RNA-splicing machinery. This problem can be surmounted by using a cDNA form of the gene, which includes only the exons.

Eukaryotic Cloning and Expression Systems

Molecular biologists can avoid eukaryotic-bacterial incompatibility by using eukaryotic cells such as yeasts, rather than bacteria, as hosts for cloning and/or expressing eukaryotic genes of interest. Yeasts, single-celled fungi, offer two advantages: They are as easy to grow as bacteria, and they have plasmids, a rarity among eukaryotes. Scientists have even constructed recombinant plasmids that combine yeast and bacterial DNA and can replicate in either type of cell.

Another reason to use eukaryotic host cells for expressing a cloned eukaryotic gene is that many eukaryotic proteins will not function unless they are modified after translation, for example, by the addition of carbohydrate (glycosylation) or lipid groups. Bacterial cells cannot carry out these modifications, and if the gene product requiring such processing is from a mammal, even yeast cells may not be able to modify the protein correctly. Several cultured cell types have proved successful as host cells for this purpose, including some mammalian cell lines and an insect cell line that can be infected by a virus (called baculovirus) carrying recombinant DNA.

Besides using vectors, scientists have developed a variety of other methods for introducing recombinant DNA into eukaryotic cells. In **electroporation**, a brief electrical pulse applied to a solution containing cells creates temporary holes in their plasma membranes, through which DNA can enter. (This technique is now commonly used for bacteria as well.) Alternatively, scientists can inject DNA directly into single eukaryotic cells using microscopically thin needles. To get DNA into plant cells, the soil bacterium *Agrobacterium* can be used, as well as other methods you will learn about later. If the introduced DNA is incorporated into a cell's genome by genetic recombination, then it may be expressed by the cell.

Cross-Species Gene Expression and Evolutionary Ancestry

EVOLUTION The ability to express eukaryotic proteins in bacteria (even if the proteins aren't glycosylated properly) is quite remarkable when we consider how different eukaryotic and bacterial cells are. Examples abound of genes that are taken from one species and function perfectly well when transferred into another very different species. These observations underscore the shared evolutionary ancestry of species living today.

One example involves a gene called *Pax-6*, which has been found in animals as diverse as vertebrates and fruit flies. The vertebrate *Pax-6* gene product (the PAX-6 protein) triggers a

complex program of gene expression resulting in formation of the vertebrate eye, which has a single lens. The fly *Pax-6* gene leads to formation of the compound fly eye, which is quite different from the vertebrate eye. When scientists cloned the mouse *Pax-6* gene and introduced it into a fly embryo, they were surprised to see that it led to formation of a compound fly eye (see Figure 50.16). Conversely, when the fly *Pax-6* gene was transferred into a vertebrate embryo—a frog, in this case—a frog eye formed. Although the genetic programs triggered in vertebrates and flies generate very different eyes, both versions of the *Pax-6* gene can substitute for each other, evidence of their evolution from a gene in a common ancestor.

Simpler examples are seen in Figure 17.6, where a firefly gene is expressed in a tobacco plant, and a jellyfish gene product is seen in a pig. The basic mechanisms of gene expression have ancient evolutionary roots, which is the basis of many recombinant DNA techniques described in this chapter.

Amplifying DNA *in Vitro*: The Polymerase Chain Reaction (PCR)

DNA cloning in cells remains the best method for preparing large quantities of a particular gene or other DNA sequence. However, when the source of DNA is scanty or impure, the **polymerase chain reaction**, or **PCR**, is quicker and more selective. In this technique, any specific target segment within one or many DNA molecules can be quickly amplified in a test tube. With automation, PCR can make billions of copies of a target segment of DNA in a few hours, significantly faster than the days it would take to obtain the same number of copies by screening a DNA library for a clone with the desired gene and letting it replicate within host cells. In fact, PCR is being used increasingly to make enough of a specific DNA fragment to insert it directly into a vector, entirely skipping the steps of making and screening a library. To continue our literary analogy, PCR is like photocopying a few pages rather than checking out a book from the library.

In the PCR procedure, a three-step cycle brings about a chain reaction that produces an exponentially growing population of identical DNA molecules. During each cycle, the reaction mixture is heated to denature (separate) the DNA strands and then cooled to allow annealing (hydrogen bonding) of short, single-stranded DNA primers complementary to sequences on opposite strands at each end of the target sequence; finally, a heat-stable DNA polymerase extends the primers in the 5' → 3' direction. If a standard DNA polymerase were used, the protein would be denatured along with the DNA during the first heating step and would have to be replaced after each cycle. The key to automating PCR was the discovery of an unusual heat-stable DNA polymerase called Taq polymerase, named after the bacterial species from which it was first isolated. This bacterial species, *Thermus aquaticus*, lives in hot springs, so natural selection has

resulted in a heat-stable DNA polymerase that can withstand the heat at the start of each cycle. **Figure 20.8** illustrates the steps in PCR.

Just as impressive as the speed of PCR is its specificity. Only minute amounts of DNA need be present in the starting material, and this DNA can be in a partially degraded state, as long as a few molecules contain the complete target sequence. The key to this high specificity is the primers, which hydrogen-bond *only* to sequences at opposite ends of the target segment. (For high specificity, the primers must be at least 15 or so nucleotides long.) By the end of the third cycle, one-fourth of the molecules are identical to the target segment, with both strands the appropriate length. With each successive cycle, the number of target segment molecules of the correct length doubles, so the number of molecules equals 2^n , where n is the number of cycles. After 30 more cycles, about a billion copies of the target sequence are present!

Despite its speed and specificity, PCR amplification cannot substitute for gene cloning in cells when large amounts of a gene are desired. Occasional errors during PCR replication impose limits on the number of good copies that can be made by this method. When PCR is used to provide the specific DNA fragment for cloning, the resulting clones are sequenced to select clones with error-free inserts. PCR errors also impose limits on the length of DNA fragments that can be copied.

Devised in 1985, PCR has had a major impact on biological research and biotechnology. PCR has been used to amplify DNA from a wide variety of sources: fragments of ancient DNA from a 40,000-year-old frozen woolly mammoth; DNA from fingerprints or from tiny amounts of blood, tissue, or semen found at crime scenes; DNA from single embryonic cells for rapid prenatal diagnosis of genetic disorders; and DNA of viral genes from cells infected with viruses that are difficult to detect, such as HIV. We'll return to applications of PCR later in the chapter.

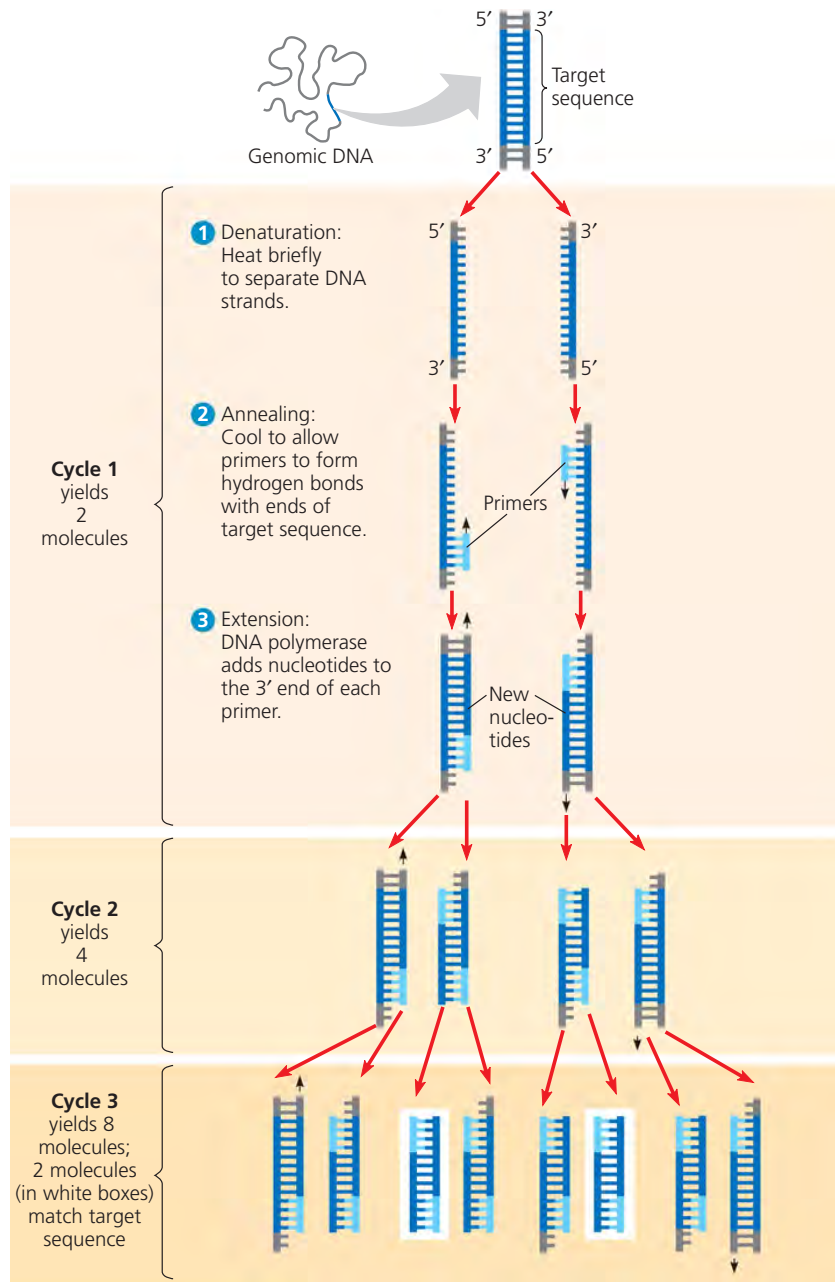
▼ **Figure 20.8**

RESEARCH METHOD

The Polymerase Chain Reaction (PCR)

APPLICATION With PCR, any specific segment—the target sequence—within a DNA sample can be copied many times (amplified), completely *in vitro*.

TECHNIQUE PCR requires double-stranded DNA containing the target sequence, a heat-resistant DNA polymerase, all four nucleotides, and two 15- to 20-nucleotide DNA strands that serve as primers. One primer is complementary to one end of the target sequence on one strand; the second primer is complementary to the other end of the sequence on the other strand.



RESULTS After 3 cycles, two molecules match the target sequence exactly. After 30 more cycles, over 1 billion (10^9) molecules match the target sequence.

CONCEPT CHECK 20.1

1. The restriction site for an enzyme called *PvuI* is the following sequence:
5'-C G A T C G-3'
3'-G C T A G C-5'
Staggered cuts are made between the T and C on each strand. What type of bonds are being cleaved?
2. **DRAW IT** One strand of a DNA molecule has the following sequence: 5'-CCTTGACGATCGTTACCG-3'. Draw the other strand. Will *PvuI* cut this molecule? If so, draw the products.
3. What are some potential difficulties in using plasmid vectors and bacterial host cells to produce large quantities of proteins from cloned eukaryotic genes?
4. **MAKE CONNECTIONS** Compare Figure 20.8 with Figure 16.20 (p. 319). How does replication of DNA ends during PCR proceed without shortening the fragments each time?
For suggested answers, see Appendix A.

CONCEPT 20.2

DNA technology allows us to study the sequence, expression, and function of a gene

Once DNA cloning has provided us with large quantities of specific DNA segments, we can tackle some interesting questions about a particular gene and its function. For example, does the sequence of the hummingbird β -globin gene suggest a protein structure that can carry oxygen more efficiently than its counterpart in less metabolically active species? Does a particular human gene differ from person to person, and are certain alleles of that gene associated with a hereditary disorder? Where in the body and when is a given gene expressed? And, ultimately, what role does a certain gene play in an organism?

Before we can begin to address such compelling questions, we must consider a few standard laboratory techniques that are used to analyze the DNA of genes.

Gel Electrophoresis and Southern Blotting

Many approaches for studying DNA molecules involve **gel electrophoresis**. This technique uses a gel made of a polymer, such as the polysaccharide *agarose*. The gel acts as a molecular sieve to separate nucleic acids or proteins on the basis of size, electrical charge, and other physical properties (Figure 20.9). Because nucleic acid molecules carry negative charges on their phosphate groups, they all travel toward the positive pole in an

▼ Figure 20.9

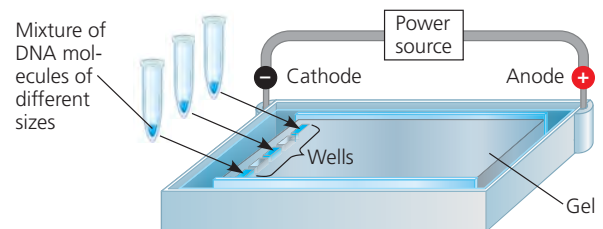
RESEARCH METHOD

Gel Electrophoresis

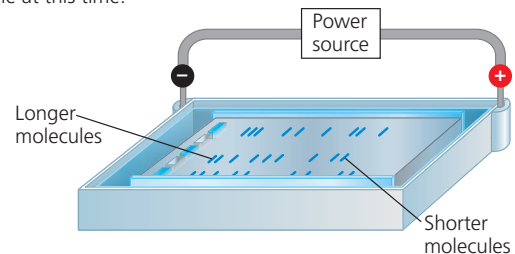
APPLICATION Gel electrophoresis is used for separating nucleic acids or proteins that differ in size, electrical charge, or other physical properties. DNA molecules are separated by gel electrophoresis in restriction fragment analysis of both cloned genes (see Figure 20.10) and genomic DNA (see Figure 20.11).

TECHNIQUE Gel electrophoresis separates macromolecules on the basis of their rate of movement through an agarose gel in an electric field: The distance a DNA molecule travels is inversely proportional to its length. A mixture of DNA molecules, usually fragments produced by restriction enzyme digestion (cutting) or PCR amplification, is separated into bands. Each band contains thousands of molecules of the same length.

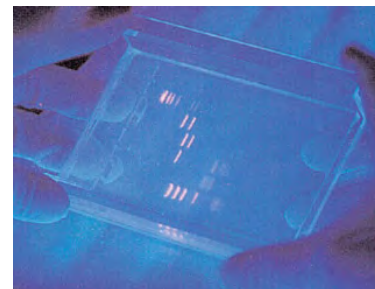
- 1 Each sample, a mixture of DNA molecules, is placed in a separate well near one end of a thin slab of agarose gel. The gel is set into a small plastic support and immersed in an aqueous, buffered solution in a tray with electrodes at each end.



- 2 When the current is turned on, the negatively charged DNA molecules move toward the positive electrode, with shorter molecules moving faster than longer ones. Bands are shown here in blue, but in an actual gel, the bands would not be visible at this time.



RESULTS After the current is turned off, a DNA-binding dye (ethidium bromide) is added. This dye fluoresces pink in ultraviolet light, revealing the separated bands to which it binds. In the gel below, the pink bands correspond to DNA fragments of different lengths separated by electrophoresis. If all the samples were initially cut with the same restriction enzyme, then the different band patterns indicate that they came from different sources.



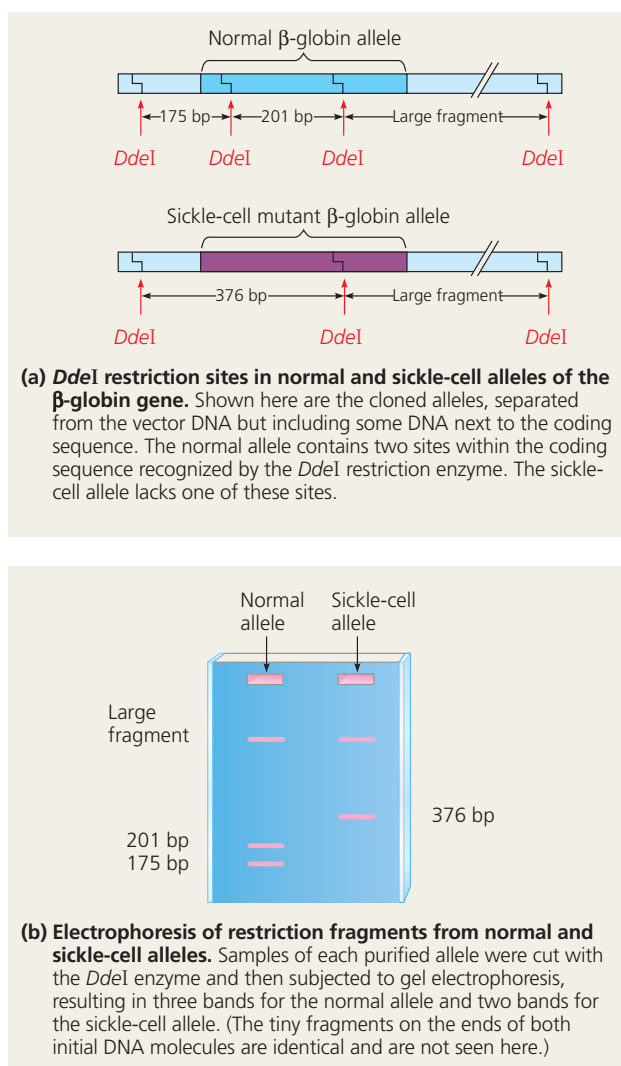
electric field. As they move, the thicket of agarose fibers impedes longer molecules more than it does shorter ones, separating them by length. Thus, gel electrophoresis separates a mixture of linear DNA molecules into bands, each band consisting of many thousands of DNA molecules of the same length.

One historically useful application of this technique has been *restriction fragment analysis*, which rapidly provides information about DNA sequences. With advances in sequencing technology, the approach taken in labs today is often simply to sequence the DNA sample in question. However, restriction fragment analysis is still done in some cases, and understanding how it is done will give you a better grasp of recombinant DNA technology. In this type of analysis, the DNA fragments produced by restriction enzyme digestion (cutting) of a DNA molecule are separated by gel electrophoresis. When the mixture of restriction fragments undergoes electrophoresis, it yields a band pattern characteristic of the starting molecule and the restriction enzyme used. In fact, the relatively small DNA molecules of viruses and plasmids can be identified simply by their restriction fragment patterns. Because DNA can be recovered undamaged from gels, the procedure also provides a way to prepare pure samples of individual fragments—assuming the bands can be clearly resolved. (Very large DNA molecules, such as those of eukaryotic chromosomes, yield so many fragments that they appear as a smear instead of distinct bands.)

Restriction fragment analysis can be used to compare two different DNA molecules—for example, two alleles of a gene—if the nucleotide difference affects a restriction site. A change in even one base pair of that sequence will prevent a restriction enzyme from cutting there. Variations in DNA sequence among a population are called *polymorphisms* (from the Greek for “many forms”), and this particular type of sequence change is called a **restriction fragment length polymorphism (RFLP)**, pronounced “Rif-lip”). If one allele contains a RFLP, digestion with the enzyme that recognizes the site will produce a different mixture of fragments for each of the two alleles. Each mixture will give its own band pattern in gel electrophoresis.

For example, sickle-cell disease is caused by mutation of a single nucleotide located within a restriction sequence (a RFLP) in the human β -globin gene (see pp. 277–278 and Figure 17.23). Consequently, while other assays are preferred today, restriction fragment analysis was used for many years to distinguish the normal and sickle-cell alleles of the β -globin gene, as shown in **Figure 20.10**.

The starting materials in Figure 20.10 are samples of the cloned and purified β -globin alleles. But how could this test be done if we didn’t have purified alleles to start with? If we wanted to determine whether a person is a heterozygous carrier of the mutant allele for sickle-cell disease, we would directly compare the genomic DNA from that person with DNA from both a person who has sickle-cell disease (and is homozygous for the mutant allele) and a person who is



▲ Figure 20.10 Using restriction fragment analysis to distinguish the normal and sickle-cell alleles of the human β -globin gene. (a) The sickle-cell mutation destroys one of the *DdeI* restriction sites within the gene. (b) As a result, digestion with the *DdeI* enzyme generates different sets of fragments from the normal and sickle-cell alleles.

WHAT IF? Given bacterial clones with recombinant plasmids carrying each of these alleles, how would you isolate the pure DNA samples run on the gel in (b)? (Hint: Study Figures 20.4 and 20.9.)

homozygous for the normal allele. As we mentioned already, electrophoresis of genomic DNA digested with a restriction enzyme and stained with a DNA-binding dye yields too many bands to distinguish them individually. However, a classic method called **Southern blotting** (developed by British biochemist Edwin Southern), which combines gel electrophoresis and nucleic acid hybridization, allows us to detect just those bands that include parts of the β -globin gene. The principle is the same as in nucleic acid hybridization for screening bacterial clones (see Figure 20.7). In Southern

blotting, the probe is usually a radioactively or otherwise labeled single-stranded DNA molecule that is complementary to the gene of interest. **Figure 20.11** outlines the entire procedure and demonstrates how it can differentiate a heterozygote (in this case, for the sickle-cell allele) from an individual homozygous for the normal allele.

The identification of carriers of mutant alleles associated with genetic diseases is only one of the ways Southern blotting has been used. In fact, this technique was a laboratory

workhorse for many years. Recently, however, it has been supplanted by more rapid methods, often involving PCR amplification of the specific parts of genomes that may differ.

DNA Sequencing

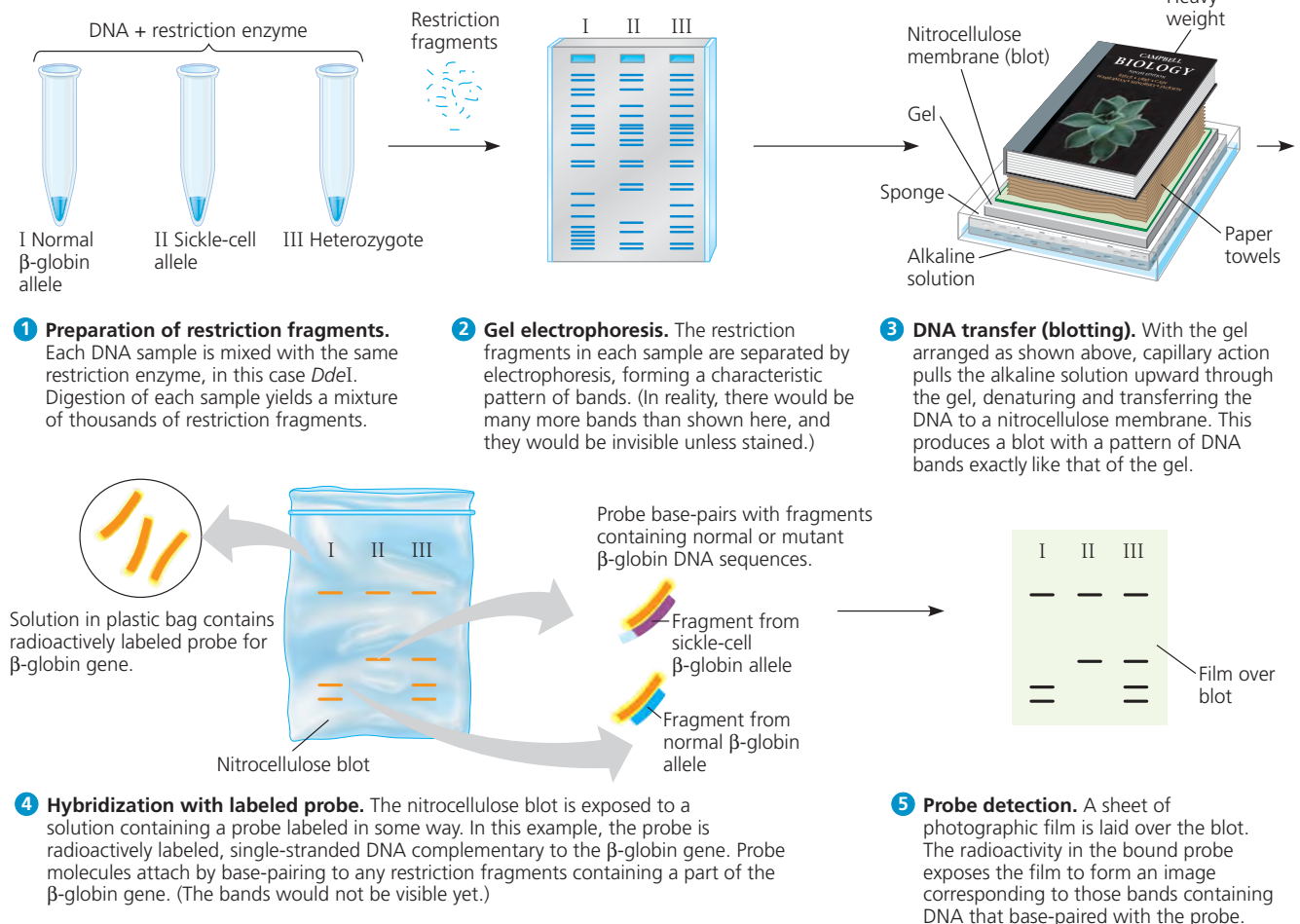
Once a gene is cloned, its complete nucleotide sequence can be determined. Today, sequencing is automated, carried out by sequencing machines (see Figure 1.12). The first automated procedure was based on a technique called the *dideoxyribonucleotide*

▼ Figure 20.11 RESEARCH METHOD

Southern Blotting of DNA Fragments

APPLICATION Researchers can detect specific nucleotide sequences within a complex DNA sample with this method. In particular, Southern blotting can be used to compare the restriction fragments produced from different samples of genomic DNA.

TECHNIQUE In this example, we compare genomic DNA samples from three individuals: a homozygote for the normal β -globin allele (I), a homozygote for the mutant sickle-cell allele (II), and a heterozygote (III). As in Figure 20.7, we show a radioactively labeled probe, but other methods of probe labeling and detection are also used.



RESULTS The band patterns for the three samples are clearly different, so this method can be used to identify heterozygous carriers of the sickle-cell allele (III), as well as those with the disease, who have two mutant alleles (II), and unaffected individuals, who have two normal

alleles (I). Band patterns for samples I and II resemble those seen for the purified normal and mutant alleles, respectively, seen in Figure 20.10b. The band pattern for the sample from the heterozygote (III) is a combination of the patterns for the two homozygotes (I and II).

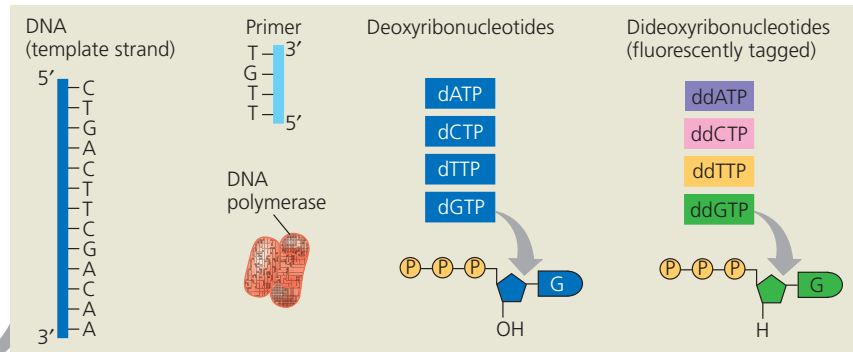
▼ **Figure 20.12** **RESEARCH METHOD**

Dideoxy Chain Termination Method for Sequencing DNA

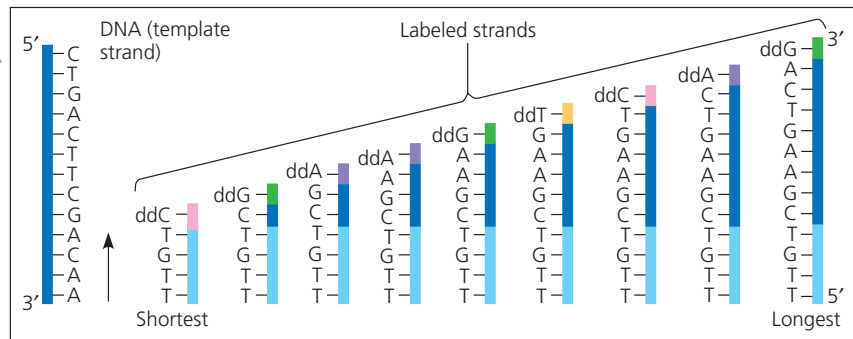
APPLICATION The sequence of nucleotides in any cloned DNA fragment of up to 800–1,000 base pairs in length can be determined rapidly with machines that carry out sequencing reactions and separate the labeled reaction products by length.

TECHNIQUE This method synthesizes a set of DNA strands complementary to the original DNA fragment. Each strand starts with the same primer and ends with a dideoxyribonucleotide (ddNTP), a modified nucleotide. Incorporation of a ddNTP terminates a growing DNA strand because it lacks a 3' —OH group, the site for attachment of the next nucleotide (see Figure 16.14). In the set of strands synthesized, each nucleotide position along the original sequence is represented by strands ending at that point with the complementary ddNTP. Because each type of ddNTP is tagged with a distinct fluorescent label, the identity of the ending nucleotides of the new strands, and ultimately the entire original sequence, can be determined.

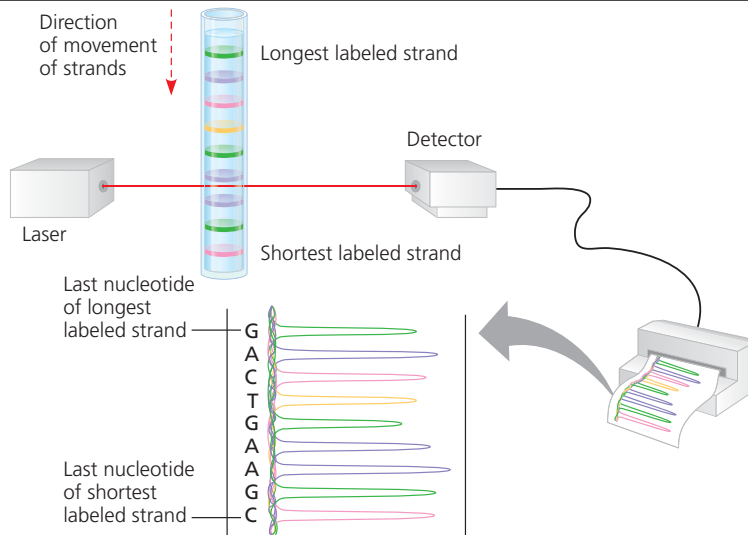
- 1 The fragment of DNA to be sequenced is denatured into single strands and incubated in a test tube with the necessary ingredients for DNA synthesis: a primer designed to base-pair with the known 3' end of the template strand, DNA polymerase, the four deoxyribonucleotides, and the four dideoxyribonucleotides, each tagged with a specific fluorescent molecule.



- 2 Synthesis of each new strand starts at the 3' end of the primer and continues until a dideoxyribonucleotide is inserted, at random, instead of the normal equivalent deoxyribonucleotide. This prevents further elongation of the strand. Eventually, a set of labeled strands of various lengths is generated, with the color of the tag representing the last nucleotide in the sequence.



- 3 The labeled strands in the mixture are separated by passage through a polyacrylamide gel, with shorter strands moving through more quickly. For DNA sequencing, the gel is formed in a capillary tube rather than a slab like that shown in Figure 20.9. The small size of the tube allows a fluorescence detector to sense the color of each fluorescent tag as the strands come through. Strands differing in length by as little as one nucleotide can be distinguished from each other.



RESULTS The color of the fluorescent tag on each strand indicates the identity of the nucleotide at its end. The results can be printed out as a spectrogram, and the sequence, which is complementary to the template strand, can then be read from bottom (shortest strand) to top (longest strand). (Notice that the sequence here begins after the primer.)

(or *dideoxy*) *chain termination method*, for reasons you can see in **Figure 20.12**. This method was developed by British biochemist Frederick Sanger, who received the Nobel Prize in 1980 for this accomplishment. (One of only four people to win two Nobel Prizes, Sanger also won one in 1975 for determining the amino acid sequence of insulin.)

In the last ten years, “next-generation sequencing” techniques have been developed that do not rely on chain termination. Instead, a single template strand is immobilized, and reagents are added that allow so-called *sequencing by synthesis* of a complementary strand, one nucleotide at a time. A chemical trick enables electronic monitors to identify which of the four nucleotides is added, allowing determination of the sequence. Further technical changes have given rise to “third-generation sequencing,” with each new technique being faster and less expensive than the previous. In Chapter 21, you’ll learn more about how this rapid acceleration of sequencing technology has enhanced our study of genes and whole genomes.

Knowing the sequence of a gene allows researchers to compare it directly with genes in other species, where the function of the gene product may be known. If two genes from different species are quite similar in sequence, it is reasonable to suppose that their gene products perform similar functions. In this way, sequence comparisons provide clues to a gene’s function, a topic we’ll return to shortly. Another set of clues is provided by experimental approaches that analyze when and where a gene is expressed.

Analyzing Gene Expression

Having cloned a given gene, researchers can make labeled nucleic acid probes that will hybridize with mRNAs transcribed from the gene. The probes can provide information about when or where in the organism the gene is transcribed. Transcription levels are commonly used as a measure of gene expression.

Studying the Expression of Single Genes

Suppose we want to find out how the expression of the β -globin gene changes during the embryonic development of the hummingbird. There are at least two ways to do this.

The first is called **Northern blotting** (a play on words based on this method’s close similarity to Southern blotting). In this method, we carry out gel electrophoresis on samples of mRNA from hummingbird embryos at different stages of development, transfer the samples to a nitrocellulose membrane, and then allow the mRNAs on the membrane to hybridize with a labeled probe recognizing β -globin mRNA. If we expose a film to the membrane, the resulting image will look similar to the Southern blot in Figure 20.11, with one band of a given size showing up in each sample. If the mRNA band is seen at a particular stage, we can hypothesize that the protein functions during events taking place at that stage. Like Southern blotting, Northern blotting has been a mainstay over the years, but it is being supplanted in many labs by other techniques.

A method that is quicker and more sensitive than Northern blotting (because it requires less mRNA) and therefore becoming more widely used is the **reverse transcriptase–polymerase chain reaction**, or **RT-PCR** (**Figure 20.13**). Analysis of hummingbird β -globin gene expression with RT-PCR begins similarly to Northern blotting, with the isolation of mRNAs from different developmental stages of hummingbird embryos. Reverse transcriptase is added next to make cDNA, which then serves as a template for PCR amplification using primers from the β -globin gene. When the products are run on a gel, copies of the amplified region will be observed as bands only in samples that originally contained the β -globin mRNA. In the case of hummingbird β -globin, for instance, we might expect to see a band appear at the stage when red blood cells begin forming, with all subsequent stages

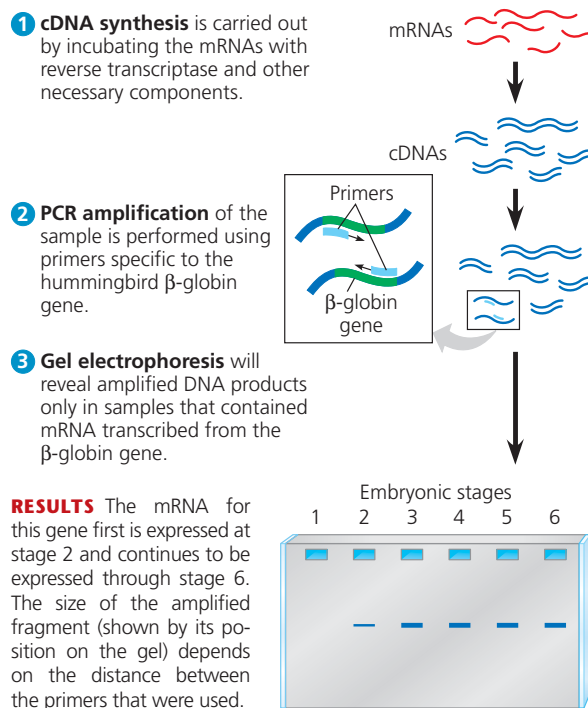
▼ **Figure 20.13**

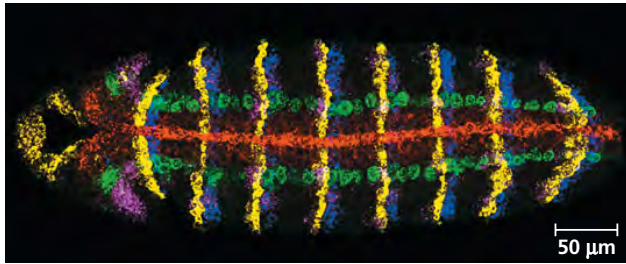
RESEARCH METHOD

RT-PCR Analysis of the Expression of Single Genes

APPLICATION RT-PCR uses the enzyme reverse transcriptase (RT) in combination with PCR and gel electrophoresis. RT-PCR can be used to compare gene expression between samples—for instance, in different embryonic stages, in different tissues, or in the same type of cell under different conditions.

TECHNIQUE In this example, samples containing mRNAs from six embryonic stages of hummingbird were processed as shown below. (The mRNA from only one stage is shown.)





▲ **Figure 20.14 Determining where genes are expressed by *in situ* hybridization analysis.** This *Drosophila* embryo was incubated in a solution containing probes for five different mRNAs, each probe labeled with a different fluorescently colored tag. The embryo was then viewed using fluorescence microscopy. Each color marks where a specific gene is expressed as mRNA.

showing the same band. RT-PCR can also be carried out with mRNAs collected from different tissues at one time to discover which tissue is producing a specific mRNA.

An alternative way to determine which tissues or cells are expressing certain genes is to track down the location of specific mRNAs using labeled probes in place, or *in situ*, in the intact organism. This technique, called ***in situ* hybridization**, is most often carried out with probes labeled by attachment of fluorescent dyes (see Chapter 6). Different probes can be labeled with different dyes, sometimes with strikingly beautiful results (**Figure 20.14**).

Studying the Expression of Interacting Groups of Genes

A major goal of biologists is to learn how genes act together to produce and maintain a functioning organism. Now that the entire genomes of a number of organisms have been sequenced, it is possible to study the expression of large groups of genes—a systems approach. Researchers use genome sequences as probes to investigate which genes are transcribed in different situations, such as in different tissues or at different stages of development. They also look for groups of genes that are expressed in a coordinated manner, with the aim of identifying networks of gene expression across an entire genome.

The basic strategy in such global (genome-wide) expression studies is to isolate the mRNAs made in particular cells, use these molecules as templates for making the corresponding cDNAs by reverse transcription, and then employ nucleic acid hybridization to compare this set of cDNAs with a collection of DNA fragments representing all or part of the genome. The results identify the subset of genes in the genome that are being expressed at a given time or under certain conditions. DNA technology makes such studies possible; with automation, they are easily performed on a large scale. Scientists can now measure the expression of thousands of genes at one time.

Genome-wide expression studies are made possible by **DNA microarray assays**. A DNA microarray consists of tiny amounts of a large number of single-stranded DNA fragments representing different genes fixed to a glass slide in a tightly spaced array, or grid (see Figure 20.1). (The microarray is also called a *DNA chip* by analogy to a computer chip.) Ideally, these fragments represent all the genes of an organism. **Figure 20.15** outlines how the DNA fragments on a microarray are tested for hybridization with cDNA molecules that have been prepared from the mRNAs in particular cells of interest and labeled with fluorescent dyes.

Using this technique, researchers have performed DNA microarray assays on more than 90% of the genes of the nematode *Caenorhabditis elegans* during every stage of its life cycle. The results show that expression of nearly 60% of *C. elegans* genes changes dramatically during development and that many genes are expressed in a sex-specific pattern. This study supports the model held by most developmental biologists that embryonic development involves a complex and elaborate program of gene expression, rather than simply the expression of a small number of important genes. This example illustrates the ability of DNA microarrays to reveal general profiles of gene expression over the lifetime of an organism.

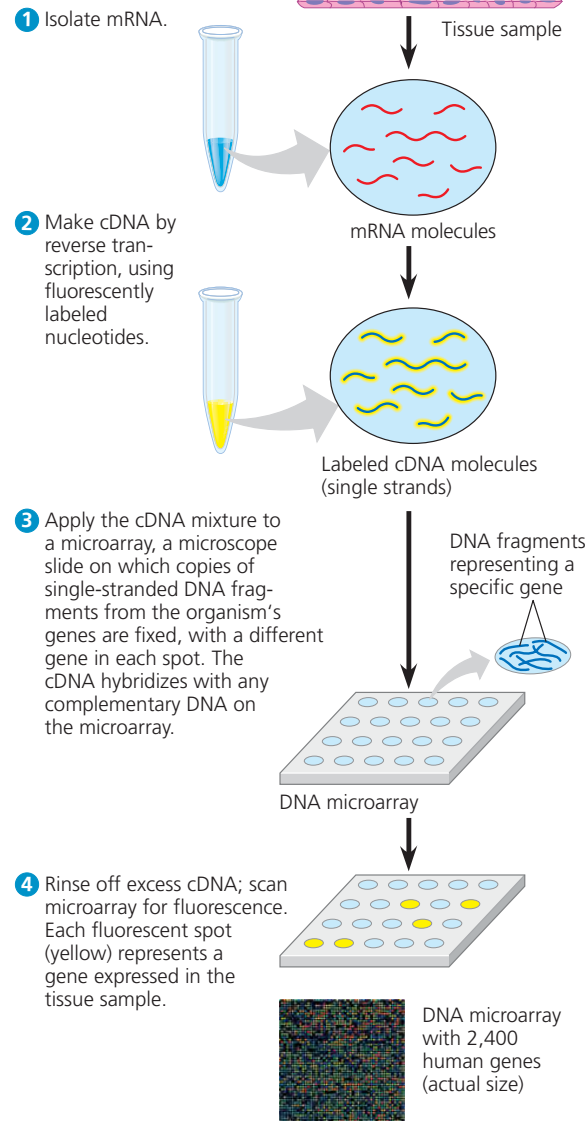
In addition to uncovering gene interactions and providing clues to gene function, DNA microarray assays may contribute to a better understanding of diseases and suggest new diagnostic techniques or therapies. For instance, comparing patterns of gene expression in breast cancer tumors and non-cancerous breast tissue has already resulted in more informed and effective treatment protocols. Ultimately, information from DNA microarray assays should provide a grander view of how ensembles of genes interact to form an organism and maintain its vital systems.

Determining Gene Function

How do scientists determine the function of a gene identified by the techniques described thus far in the chapter? Perhaps the most common approach is to disable the gene and then observe the consequences in the cell or organism. In one application of this approach, called ***in vitro* mutagenesis**, specific mutations are introduced into a cloned gene, and then the mutated gene is returned to a cell in such a way that it disables (“knocks out”) the normal cellular copies of the same gene. If the introduced mutations alter or destroy the function of the gene product, the phenotype of the mutant cell may help reveal the function of the missing normal protein. Using molecular and genetic techniques worked out in the 1980s, researchers can even generate mice with any given gene disabled, in order to study the role of that gene in development and in the adult. Mario Capecchi, Martin Evans, and Oliver Smithies received the Nobel Prize in 2007 for first accomplishing this feat.

DNA Microarray Assay of Gene Expression Levels

APPLICATION With this method, researchers can test thousands of genes simultaneously to determine which ones are expressed in a particular tissue, under different environmental conditions, in various disease states, or at different developmental stages. They can also look for coordinated gene expression.

TECHNIQUE

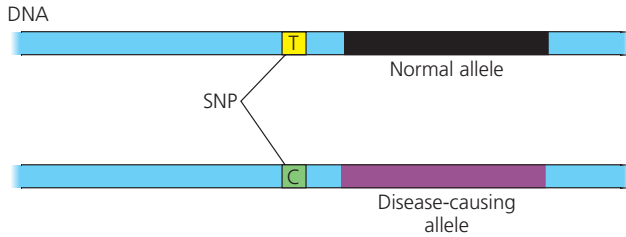
RESULTS The intensity of fluorescence at each spot is a measure of the expression in the tissue sample of the gene represented by that spot. Most often, as in the actual microarray above, two different samples are tested together by labeling the cDNAs prepared from each sample with labels of different colors, often green and red. The resulting color at a spot reveals the relative levels of expression of a particular gene in the two samples: Green indicates expression in one sample, red in the other, yellow in both, and black in neither. (See Figure 20.1 for a larger view.)

A newer method for silencing expression of selected genes exploits the phenomenon of **RNA interference (RNAi)**, described in Chapter 18. This experimental approach uses synthetic double-stranded RNA molecules matching the sequence of a particular gene to trigger breakdown of the gene's messenger RNA or to block its translation. In organisms such as the nematode and the fruit fly, RNAi has already proved valuable for analyzing the functions of genes on a large scale. In one study, RNAi was used to prevent expression of 86% of the genes in early nematode embryos, one gene at a time. Analysis of the phenotypes of the worms that developed from these embryos allowed the researchers to classify most of the genes into a small number of groups by function. This type of analysis, in which the functions of multiple genes are considered in a single study, is sure to become more common as research focuses on the importance of interactions between genes in the system as a whole. This is the basis of systems biology (see Chapter 21).

In humans, ethical considerations prohibit knocking out genes to determine their functions. An alternative approach is to analyze the genomes of large numbers of people with a certain phenotypic condition or disease, such as heart disease or diabetes, to try to find differences they all share compared with people without that condition. These large-scale analyses, called **genome-wide association studies**, do not require complete sequencing of all the genomes in the two groups. Instead, researchers test for *genetic markers*, DNA sequences that vary in the population. In a gene, such sequence variation is the basis of different alleles, as we saw earlier for sickle-cell disease. And just like coding sequences, noncoding DNA at a specific locus on a chromosome may exhibit small nucleotide differences (polymorphisms) among individuals.

Among the most useful of these genetic markers are single base-pair variations in the genomes of the human population. A single base-pair site where variation is found in at least 1% of the population is called a **single nucleotide polymorphism (SNP)**, pronounced "snip"). A few million SNPs occur in the human genome, about once in 100–300 base pairs of both coding and noncoding DNA sequences. (Roughly 98.5% of our genome doesn't code for protein, as you will learn in Chapter 21.) It isn't necessary to sequence the DNA of multiple individuals to find SNPs; today they can be detected by very sensitive microarray analysis or by PCR.

Once a region is found that has a SNP shared by affected but not unaffected people, researchers focus on that region and sequence it. In the vast majority of cases, the SNP itself does not contribute to the disease, and most SNPs are in noncoding regions. Instead, if the SNP and a disease-causing allele are close enough, scientists can take advantage of the fact that crossing over between the marker and the gene is very unlikely during gamete formation. Therefore, the marker and gene will almost always be inherited together, even though the marker is not



▲ Figure 20.16 Single nucleotide polymorphisms (SNPs) as genetic markers for disease-causing alleles. This diagram depicts homologous segments of DNA from two groups of individuals, those in one group having a particular disease or condition with a genetic basis. Unaffected people have a T at a particular SNP locus, while affected people have a C at that locus. A SNP that varies in this way is likely to be closely linked to one or more alleles of genes that contribute to the disease in question. (Here, only a single strand is shown for each DNA molecule.)

MAKE CONNECTIONS What does it mean for a SNP to be “closely linked” to a disease-causing allele, and how does this allow the SNP to be used as a genetic marker? (See Concept 15.3, p. 296.)

part of the gene (Figure 20.16). SNPs have been found that correlate with diabetes, heart disease, and several types of cancer, and the search is on for genes that might be involved.

The techniques and experimental approaches you have learned about thus far have already taught us a great deal about genes and the functions of their products. This research is now being augmented by the development of powerful techniques for cloning whole multicellular organisms. An aim of this work is to obtain special types of cells, called stem cells, that give rise to all the different kinds of tissues. On a basic level, stem cells would allow scientists to use the DNA-based methods previously discussed to study the process of cell differentiation. On a more applied level, recombinant DNA techniques could be used to alter stem cells for the treatment of disease. Methods involving the cloning of organisms and production of stem cells are the subject of the next section.

CONCEPT CHECK 20.2

1. If you isolated DNA from human cells, treated it with a restriction enzyme, and analyzed the sample by gel electrophoresis, what would you see? Explain.
2. Describe the role of complementary base pairing during Southern blotting, DNA sequencing, Northern blotting, RT-PCR, and microarray analysis.
3. Distinguish between a SNP and a RFLP.
4. **WHAT IF?** Consider the microarray in Figure 20.1, a larger image of the one in Figure 20.15. If a sample from normal tissue is labeled with a green fluorescent dye, and a sample from cancerous tissue is labeled red, what can you conclude about a spot that is green? Red? Yellow? Black? Which genes would you be interested in examining further if you were studying cancer? Explain.

For suggested answers, see Appendix A.

CONCEPT 20.3

Cloning organisms may lead to production of stem cells for research and other applications

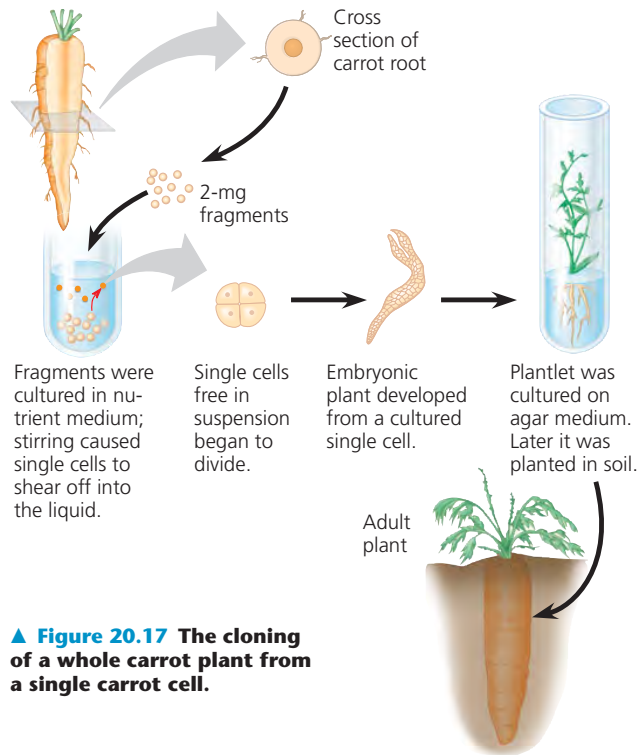
In parallel with advances in DNA technology, scientists have been developing and refining methods for cloning whole multicellular organisms from single cells. In this context, cloning produces one or more organisms genetically identical to the “parent” that donated the single cell. This is often called *organismal cloning* to differentiate it from gene cloning and, more significantly, from cell cloning—the division of an asexually reproducing cell into a collection of genetically identical cells. (The common theme for all types of cloning is that the product is genetically identical to the parent. In fact, the word *clone* comes from the Greek *klon*, meaning “twig.”) The current interest in organismal cloning arises primarily from its potential to generate stem cells, which can in turn generate many different tissues.

The cloning of plants and animals was first attempted over 50 years ago in experiments designed to answer basic biological questions. For example, researchers wondered if all the cells of an organism have the same genes (a concept called *genomic equivalence*) or if cells lose genes during the process of differentiation (see Chapter 18). One way to answer this question is to see whether a differentiated cell can generate a whole organism—in other words, whether cloning an organism is possible. Let’s discuss these early experiments before we consider more recent progress in organismal cloning and procedures for producing stem cells.

Cloning Plants: Single-Cell Cultures

The successful cloning of whole plants from single differentiated cells was accomplished during the 1950s by F. C. Steward and his students at Cornell University, who worked with carrot plants (Figure 20.17). They found that differentiated cells taken from the root (the carrot) and incubated in culture medium could grow into normal adult plants, each genetically identical to the parent plant. These results showed that differentiation does not necessarily involve irreversible changes in the DNA. In plants, at least, mature cells can “dedifferentiate” and then give rise to all the specialized cell types of the organism. Any cell with this potential is said to be **totipotent**.

Plant cloning is now used extensively in agriculture. For some plants, such as orchids, cloning is the only commercially practical means of reproducing plants. In other cases, cloning has been used to reproduce a plant with valuable characteristics, such as the ability to resist a plant pathogen. In fact, you yourself may be a plant cloner: If you have ever grown a new plant from a cutting, you have practiced cloning!



▲ Figure 20.17 The cloning of a whole carrot plant from a single carrot cell.

Cloning Animals: Nuclear Transplantation

Differentiated cells from animals generally do not divide in culture, much less develop into the multiple cell types of a new organism. Therefore, early researchers had to use a different approach to the question of whether differentiated animal cells can be totipotent. Their approach was to remove the nucleus of an unfertilized or fertilized egg and replace it with the nucleus of a differentiated cell, a procedure called *nuclear transplantation*. If the nucleus from the differentiated donor cell retains its full genetic capability, then it should be able to direct development of the recipient cell into all the tissues and organs of an organism.

Such experiments were conducted on one species of frog (*Rana pipiens*) by Robert Briggs and Thomas King in the 1950s and on another (*Xenopus laevis*) by John Gurdon in the 1970s. These researchers transplanted a nucleus from an embryonic or tadpole cell into an enucleated (nucleus-lacking) egg of the same species. In Gurdon's experiments, the transplanted nucleus was often able to support normal development of the egg into a tadpole (Figure 20.18). However, he found that the potential of a transplanted nucleus to direct normal development was inversely related to the age of the donor: the older the donor nucleus, the lower the percentage of normally developing tadpoles.

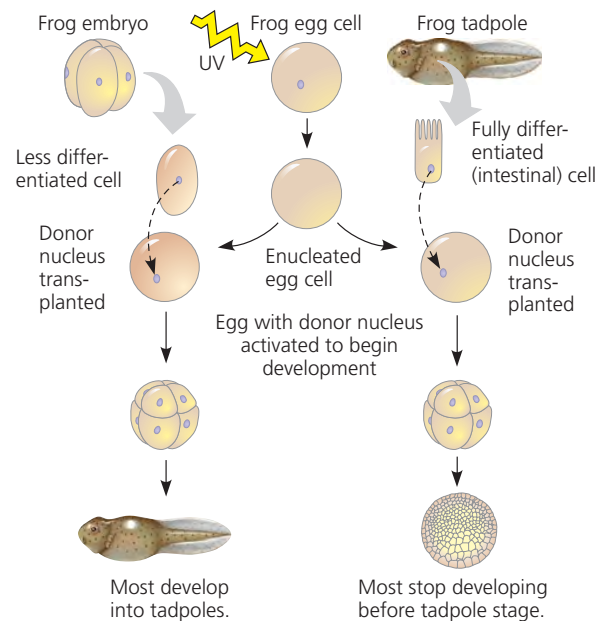
From these results, Gurdon concluded that something in the nucleus *does* change as animal cells differentiate. In frogs and most other animals, nuclear potential tends to be restricted more and more as embryonic development and cell differentiation progress.

▼ Figure 20.18

INQUIRY

Can the nucleus from a differentiated animal cell direct development of an organism?

EXPERIMENT John Gurdon and colleagues at Oxford University, in England, destroyed the nuclei of frog (*Xenopus laevis*) eggs by exposing the eggs to ultraviolet light. They then transplanted nuclei from cells of frog embryos and tadpoles into the enucleated eggs.



RESULTS When the transplanted nuclei came from an early embryo, whose cells are relatively undifferentiated, most of the recipient eggs developed into tadpoles. But when the nuclei came from the fully differentiated intestinal cells of a tadpole, fewer than 2% of the eggs developed into normal tadpoles, and most of the embryos stopped developing at a much earlier stage.

CONCLUSION The nucleus from a differentiated frog cell can direct development of a tadpole. However, its ability to do so decreases as the donor cell becomes more differentiated, presumably because of changes in the nucleus.

SOURCE J. B. Gurdon et al., The developmental capacity of nuclei transplanted from keratinized cells of adult frogs, *Journal of Embryology and Experimental Morphology* 34:93–112 (1975).

WHAT IF? If each cell in a four-cell embryo was already so specialized that it was not totipotent, what results would you predict for the experiment on the left side of the figure?

Reproductive Cloning of Mammals

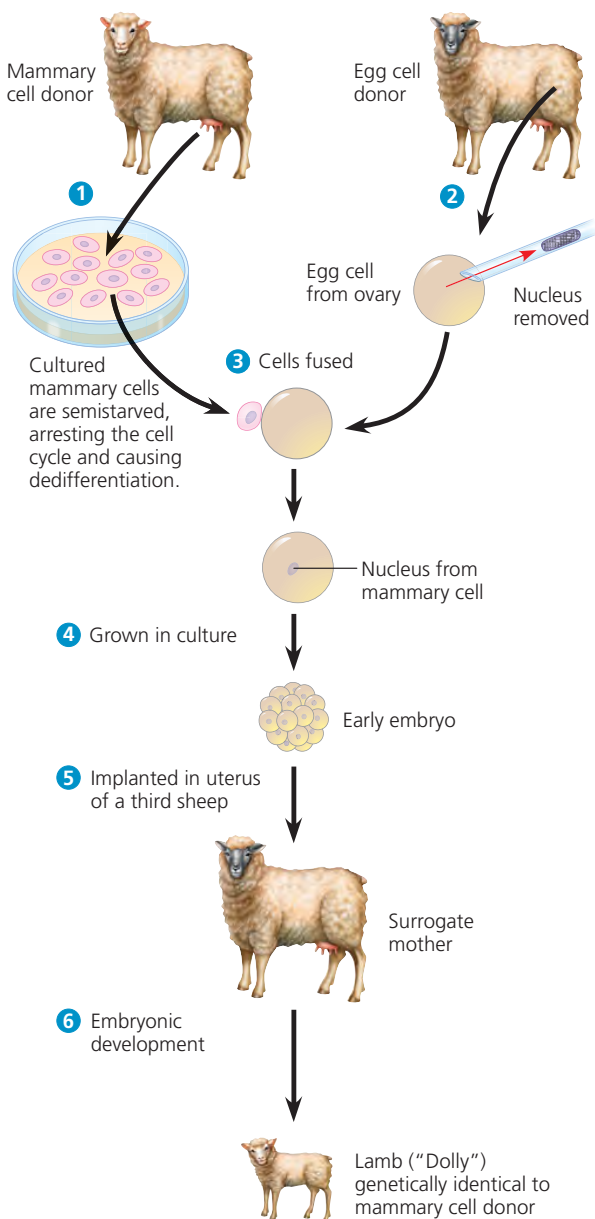
In addition to cloning frogs, researchers have long been able to clone mammals by transplanting nuclei or cells from a variety of early embryos. But it was not known whether a nucleus from a fully differentiated cell could be reprogrammed to succeed in acting as a donor nucleus. In 1997, however, researchers at the Roslin Institute in Scotland captured newspaper headlines when they announced the birth of Dolly, a

▼ **Figure 20.19** **RESEARCH METHOD**

Reproductive Cloning of a Mammal by Nuclear Transplantation

APPLICATION This method is used to produce cloned animals whose nuclear genes are identical to those of the animal supplying the nucleus.

TECHNIQUE Shown here is the procedure used to produce Dolly, the first reported case of a mammal cloned using the nucleus of a differentiated cell.



RESULTS The genetic makeup of the cloned animal is identical to that of the animal supplying the nucleus but differs from that of the egg donor and surrogate mother. (The latter two are "Scottish blackface" sheep, with dark faces.)

lamb cloned from an adult sheep by nuclear transplantation from a differentiated cell (Figure 20.19). These researchers achieved the necessary dedifferentiation of donor nuclei by culturing mammary cells in nutrient-poor medium. They then fused these cells with enucleated sheep eggs. The resulting diploid cells divided to form early embryos, which were implanted into surrogate mothers. Out of several hundred implanted embryos, one successfully completed normal development, and Dolly was born.

Later analyses showed that Dolly's chromosomal DNA was indeed identical to that of the nucleus donor. (Her mitochondrial DNA came from the egg donor, as expected.) At the age of 6, Dolly suffered complications from a lung disease usually seen only in much older sheep and was euthanized. Dolly's premature death, as well as her arthritic condition, led to speculation that her cells were in some way not quite as healthy as those of a normal sheep, possibly reflecting incomplete reprogramming of the original transplanted nucleus.

Since 1997, researchers have cloned numerous other mammals, including mice, cats, cows, horses, pigs, dogs, and monkeys. In most cases, their goal has been the production of new individuals; this is known as *reproductive cloning*. We have already learned a lot from such experiments. For example, cloned animals of the same species do *not* always look or behave identically. In a herd of cows cloned from the same line of cultured cells, certain cows are dominant in behavior and others are more submissive. Another example of nonidentity in clones is the first cloned cat, named CC for Carbon Copy (Figure 20.20). She has a calico coat, like her single female parent, but the color and pattern are different because of random X chromosome inactivation, which is a normal occurrence during embryonic development (see Figure 15.8). And identical human twins, which



▲ **Figure 20.20** CC, the first cloned cat, and her single parent. Rainbow (left) donated the nucleus in a cloning procedure that resulted in CC (right). However, the two cats are not identical: Rainbow is a classic calico cat with orange patches on her fur and has a "reserved personality," while CC has a gray and white coat and is more playful.

are naturally occurring “clones,” are always slightly different. Clearly, environmental influences and random phenomena can play a significant role during development.

The successful cloning of so many mammals has heightened speculation about the cloning of humans. Scientists in several labs around the world have tackled the first steps of human cloning. In the most common approach, nuclei from differentiated human cells are transplanted into unfertilized enucleated eggs, and the eggs are stimulated to divide. In 2001, a research group at a biotechnology company in Massachusetts observed a few early cell divisions in such an experiment. A few years later, researchers at Seoul National University, in South Korea, reported cloning embryos to an early stage called the blastocyst stage, but the scientists were later found guilty of research misconduct and data fabrication. This episode sent shock waves through the scientific community. In 2007, the first primate (macaque) embryos were cloned by researchers at the Oregon National Primate Research Center; these clones reached the blastocyst stage. This achievement has moved the field one step closer to human cloning, the prospect of which raises unprecedented ethical issues.

Problems Associated with Animal Cloning

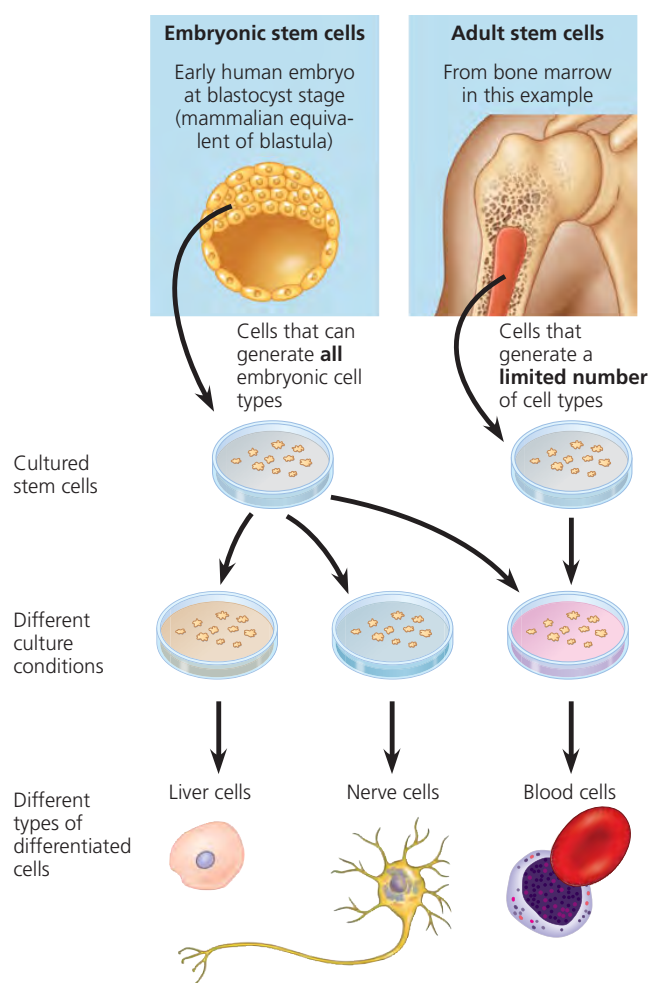
In most nuclear transplantation studies thus far, only a small percentage of cloned embryos develop normally to birth. And like Dolly, many cloned animals exhibit defects. Cloned mice, for instance, are prone to obesity, pneumonia, liver failure, and premature death. Scientists assert that even cloned animals that appear normal are likely to have subtle defects.

In recent years, we have begun to uncover some reasons for the low efficiency of cloning and the high incidence of abnormalities. In the nuclei of fully differentiated cells, a small subset of genes is turned on and expression of the rest is repressed. This regulation often is the result of epigenetic changes in chromatin, such as acetylation of histones or methylation of DNA (see Figure 18.7). During the nuclear transfer procedure, many of these changes must be reversed in the later-stage nucleus from a donor animal for genes to be expressed or repressed appropriately in early stages of development. Researchers have found that the DNA in cells from cloned embryos, like that of differentiated cells, often has more methyl groups than does the DNA in equivalent cells from normal embryos of the same species. This finding suggests that the reprogramming of donor nuclei requires chromatin restructuring, which occurs incompletely during cloning procedures. Because DNA methylation helps regulate gene expression, misplaced methyl groups in the DNA of donor nuclei may interfere with the pattern of gene expression necessary for normal embryonic development. In fact, the success of a cloning attempt may depend in large part on whether or not the chromatin in the donor nucleus can be artificially modified to resemble that of a newly fertilized egg.

Stem Cells of Animals

The major goal of cloning human embryos is not reproduction, but the production of stem cells for treating human diseases. A **stem cell** is a relatively unspecialized cell that can both reproduce itself indefinitely and, under appropriate conditions, differentiate into specialized cells of one or more types. Thus, stem cells are able both to replenish their own population and to generate cells that travel down specific differentiation pathways.

Many early animal embryos contain stem cells capable of giving rise to differentiated embryonic cells of any type. Stem cells can be isolated from early embryos at a stage called the blastula stage or its human equivalent, the blastocyst stage (Figure 20.21). In culture, these *embryonic stem (ES) cells*



▲ **Figure 20.21 Working with stem cells.** Animal stem cells, which can be isolated from early embryos or adult tissues and grown in culture, are self-perpetuating, relatively undifferentiated cells. Embryonic stem cells are easier to grow than adult stem cells and can theoretically give rise to *all* types of cells in an organism. The range of cell types that can arise from adult stem cells is not yet fully understood.

reproduce indefinitely; and depending on culture conditions, they can be made to differentiate into a wide variety of specialized cells, including even eggs and sperm.

The adult body also has stem cells, which serve to replace nonreproducing specialized cells as needed. In contrast to ES cells, *adult stem cells* are not able to give rise to all cell types in the organism, though they can generate multiple types. For example, one of the several types of stem cells in bone marrow can generate all the different kinds of blood cells (see Figure 20.21), and another can differentiate into bone, cartilage, fat, muscle, and the linings of blood vessels. To the surprise of many, the adult brain has been found to contain stem cells that continue to produce certain kinds of nerve cells there. And recently, researchers have reported finding stem cells in skin, hair, eyes, and dental pulp. Although adult animals have only tiny numbers of stem cells, scientists are learning to identify and isolate these cells from various tissues and, in some cases, to grow them in culture. With the right culture conditions (for instance, the addition of specific growth factors), cultured stem cells from adult animals have been made to differentiate into multiple types of specialized cells, although none are as versatile as ES cells.

Research with embryonic or adult stem cells is a source of valuable data about differentiation and has enormous potential for medical applications. The ultimate aim is to supply cells for the repair of damaged or diseased organs: for example, insulin-producing pancreatic cells for people with type 1 diabetes or certain kinds of brain cells for people with Parkinson's disease or Huntington's disease. Adult stem cells from bone marrow have long been used as a source of immune system cells in patients whose own immune systems are nonfunctional because of genetic disorders or radiation treatments for cancer.

The developmental potential of adult stem cells is limited to certain tissues. ES cells hold more promise than adult stem cells for most medical applications because ES cells are **pluripotent**, capable of differentiating into many different cell types. The only way to obtain ES cells thus far, however, has been to harvest them from human embryos, which raises ethical and political issues.

ES cells are currently obtained from embryos donated by patients undergoing infertility treatment or from long-term cell cultures originally established with cells isolated from donated embryos. If scientists were able to clone human embryos to the blastocyst stage, they might be able to use such clones as the source of ES cells in the future. Furthermore, with a donor nucleus from a person with a particular disease, they might be able to produce ES cells for treatment that match the patient and are thus not rejected by his or her immune system. When the main aim of cloning is to produce ES cells to treat disease, the process is called *therapeutic cloning*. Although most people believe that reproductive cloning of humans is unethical, opinions vary about the morality of therapeutic cloning.

Resolving the debate now seems less imperative because researchers have been able to turn back the clock in fully differentiated cells, reprogramming them to act like ES cells. The accomplishment of this feat, which posed formidable obstacles, was announced in 2007, first by labs using mouse skin cells and then by additional groups using cells from human skin and other organs or tissues. In all these cases, researchers transformed the differentiated cells into ES cells by using retroviruses to introduce extra cloned copies of four “stem cell” master regulatory genes. All the tests that were carried out at the time indicated that the transformed cells, known as *induced pluripotent stem (iPS) cells*, could do everything ES cells can do. More recently, however, several research groups have uncovered differences between iPS and ES cells in gene expression and other cellular functions, such as cell division. At least until these differences are fully understood, the study of ES cells will continue to make important contributions to the development of stem cell therapies. (In fact, ES cells will likely always be a focus of basic research as well.) In the meantime, work is proceeding using the iPS cells in hand.

There are two major potential uses for human iPS cells. First, cells from patients suffering from diseases can be reprogrammed to become iPS cells, which can act as model cells for studying the disease and potential treatments. Human iPS cell lines have already been developed from individuals with type 1 diabetes, Parkinson's disease, and at least a dozen other diseases. Second, in the field of regenerative medicine, a patient's own cells could be reprogrammed into iPS cells and then used to replace nonfunctional tissues (Figure 20.22). Developing techniques that direct iPS cells to become specific cell types for this purpose is an area of intense research, one that has already seen some success. The iPS cells created in this way could eventually provide tailor-made “replacement” cells for patients without using any human eggs or embryos, thus circumventing most ethical objections.

CONCEPT CHECK 20.3

1. Based on current knowledge, how would you explain the difference in the percentage of tadpoles that developed from the two kinds of donor nuclei in Figure 20.18?
2. If you were to clone a carrot using the technique shown in Figure 20.17, would all the progeny plants (“clones”) look identical? Why or why not?
3. **WHAT IF?** If you were a doctor who wanted to use iPS cells to treat a patient with severe type 1 diabetes, what new technique would have to be developed?
4. **MAKE CONNECTIONS** Compare an individual carrot cell in Figure 20.17 with the fully differentiated muscle cell in Figure 18.18 (p. 369) in terms of their potential to develop into different cell types.

For suggested answers, see Appendix A.

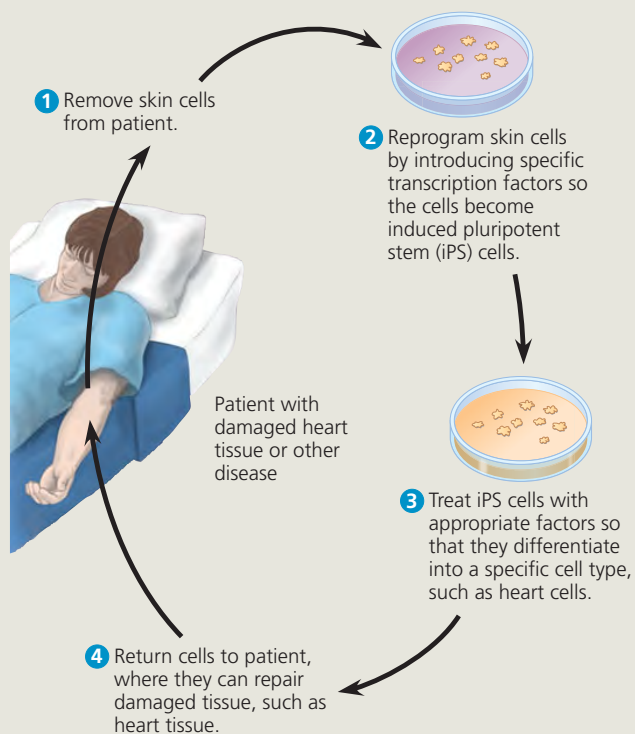
▼ Figure 20.22

IMPACT

The Impact of Induced Pluripotent Stem (iPS) Cells on Regenerative Medicine

While embryonic stem (ES) cells can generate every cell in an organism, the use of human embryos as their source is controversial. Several research groups have developed similar procedures for reprogramming fully differentiated cells to become induced pluripotent stem (iPS) cells, which act like ES cells. The technique is based on introducing transcription factors that are characteristic of stem cells into differentiated cells, such as skin cells.

WHY IT MATTERS Patients with diseases such as heart disease, diabetes, or Alzheimer's could have their own skin cells reprogrammed to become iPS cells. Once procedures have been developed for converting iPS cells into heart, pancreatic, or nervous system cells, the patients' own iPS cells might be used to treat their disease. This technique has already been used successfully to treat sickle-cell disease in a mouse that had been genetically engineered to have the disease. Shown below is how this therapy could work in humans, once researchers learn how iPS cells can be triggered to differentiate as desired (step 3).



FURTHER READING G. Vogel and C. Holden, Field leaps forward with new stem cell advances, *Science* 318:1224–1225 (2007); K. Hochedlinger, Your inner healers, *Scientific American* 302:46–53 (2010).

WHAT IF? When organs are transplanted from a donor to a diseased recipient, the recipient's immune system may reject the transplant, a condition with serious and often fatal consequences. Would using converted iPS cells be expected to carry the same risk? Why or why not?

CONCEPT 20.4

The practical applications of DNA technology affect our lives in many ways

DNA technology is in the news almost every day. Most often, the topic is a new and promising application in medicine, but this is just one of numerous fields benefiting from DNA technology and genetic engineering.

Medical Applications

One important use of DNA technology is the identification of human genes whose mutation plays a role in genetic diseases. These discoveries may lead to ways of diagnosing, treating, and even preventing such conditions. DNA technology is also contributing to our understanding of “nongenetic” diseases, from arthritis to AIDS, since a person's genes influence susceptibility to these diseases. Furthermore, diseases of all sorts involve changes in gene expression within the affected cells and often within the patient's immune system. By using DNA microarray assays or other techniques to compare gene expression in healthy and diseased tissues, as seen in Figure 20.1, researchers hope to find many of the genes that are turned on or off in particular diseases. These genes and their products are potential targets for prevention or therapy.

Diagnosis and Treatment of Diseases

A new chapter in the diagnosis of infectious diseases has been opened by DNA technology, in particular the use of PCR and labeled nucleic acid probes to track down pathogens. For example, because the sequence of the RNA genome of HIV is known, RT-PCR can be used to amplify, and thus detect, HIV RNA in blood or tissue samples (see Figure 20.13). RT-PCR is often the best way to detect an otherwise elusive infective agent.

Medical scientists can now diagnose hundreds of human genetic disorders by using PCR with primers that target the genes associated with these disorders. The amplified DNA product is then sequenced to reveal the presence or absence of the disease-causing mutation. Among the genes for human diseases that have been identified are those for sickle-cell disease, hemophilia, cystic fibrosis, Huntington's disease, and Duchenne muscular dystrophy. Individuals afflicted with such diseases can often be identified before the onset of symptoms, even before birth. PCR can also be used to identify symptomless carriers of potentially harmful recessive alleles, essentially replacing Southern blotting for this purpose.

As you learned earlier, genome-wide association studies have pinpointed SNPs (single nucleotide polymorphisms) that are linked to disease-causing alleles. Individuals can be tested by PCR and sequencing for a SNP that is correlated with

the abnormal allele. The presence of particular SNPs is correlated with increased risk for conditions such as heart disease, Alzheimer's, and some types of cancer. Companies that offer individual genetic testing for risk factors like these are looking for the presence of previously identified, linked SNPs. It may be helpful for an individual to learn about their health risks, with the understanding that such genetic tests merely reflect correlations and do not make predictions.

The techniques described in this chapter have also prompted improvements in disease treatments. By analyzing the expression of many genes in breast cancer patients, researchers carrying out one genome-wide association study were able to identify 70 genes whose expression pattern could be correlated with the likelihood that the cancer would recur. Given that low-risk patients have a 96% survival rate over a ten-year period with no treatment, gene expression analysis allows doctors and patients access to valuable information when they are considering treatment options.

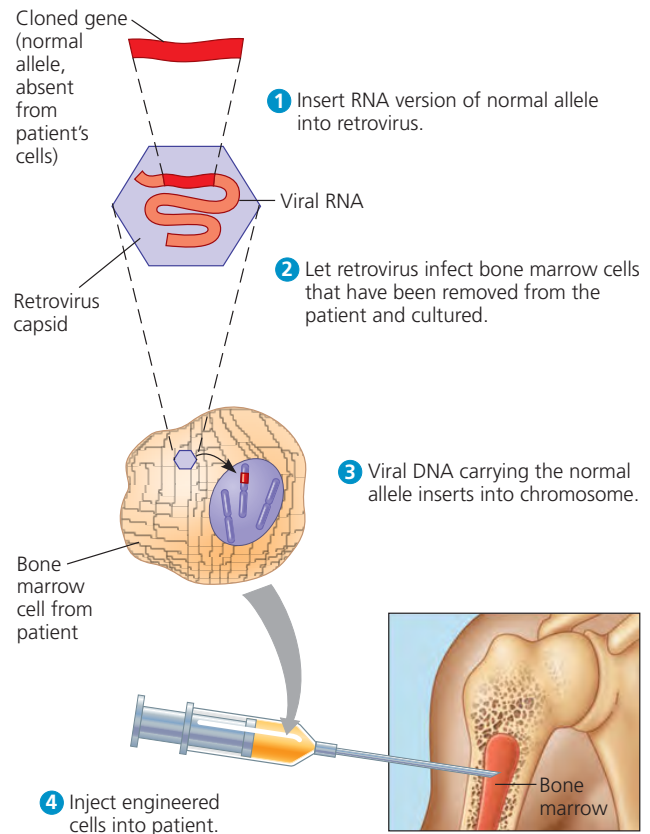
Many envision a future of "personalized medicine" where each person's genetic health profile can inform them about diseases or conditions for which they are especially at risk and help them make treatment choices. A genetic profile is currently taken to mean a set of genetic markers such as SNPs, but ultimately it could mean the complete DNA sequence of each individual—once sequencing becomes inexpensive enough.

Human Gene Therapy

Gene therapy—introducing genes into an afflicted individual for therapeutic purposes—holds great potential for treating the relatively small number of disorders traceable to a single defective gene. In theory, a normal allele of the defective gene could be inserted into the somatic cells of the tissue affected by the disorder.

For gene therapy of somatic cells to be permanent, the cells that receive the normal allele must be ones that multiply throughout the patient's life. Bone marrow cells, which include the stem cells that give rise to all the cells of the blood and immune system, are prime candidates. **Figure 20.23** outlines one possible procedure for gene therapy of an individual whose bone marrow cells do not produce a vital enzyme because of a single defective gene. One type of severe combined immunodeficiency (SCID) is caused by just this kind of defect. If the treatment is successful, the patient's bone marrow cells will begin producing the missing protein, and the patient may be cured.

The procedure shown in Figure 20.23 has been used in gene therapy trials for SCID. In a trial begun in France in 2000, ten young children with SCID were treated by the same procedure. Nine of these patients showed significant, definitive improvement after two years, the first indisputable success of gene therapy. However, three of the patients subsequently developed leukemia, a type of blood cell cancer, and one of them died. Two factors may have contributed to the development of leukemia: the insertion of the retroviral



▲ Figure 20.23 Gene therapy using a retroviral vector. A retrovirus that has been rendered harmless is used as a vector in this procedure, which exploits the ability of a retrovirus to insert a DNA transcript of its RNA genome into the chromosomal DNA of its host cell (see Figure 19.8). If the foreign gene carried by the retroviral vector is expressed, the cell and its descendants will possess the gene product. Cells that reproduce throughout life, such as bone marrow cells, are ideal candidates for gene therapy.

vector near a gene involved in the proliferation of blood cells and an unknown function of the replacement gene itself. Two other genetic diseases have recently been treated somewhat successfully with gene therapy: one causing progressive blindness (see Figure 50.21) and the other leading to degeneration of the nervous system. The successful trials involve very few patients, but are still cause for cautious optimism.

Gene therapy raises many technical issues. For example, how can the activity of the transferred gene be controlled so that cells make appropriate amounts of the gene product at the right time and in the right place? How can we be sure that the insertion of the therapeutic gene does not harm some other necessary cell function? As more is learned about DNA control elements and gene interactions, researchers may be able to answer such questions.

In addition to technical challenges, gene therapy provokes ethical questions. Some critics believe that tampering with

human genes in any way is immoral. Other observers see no fundamental difference between the transplantation of genes into somatic cells and the transplantation of organs. You might wonder whether scientists are considering engineering human germ-line cells in the hope of correcting a defect in future generations. At present, no one in the mainstream scientific community is pursuing this goal—it is considered much too risky. Such genetic engineering is routinely done in laboratory mice, though, and the technical problems relating to similar genetic engineering in humans will eventually be solved. Under what circumstances, if any, should we alter the genomes of human germ lines? Would this inevitably lead to the practice of eugenics, a deliberate effort to control the genetic makeup of human populations? While we may not have to resolve these questions right now, considering them is worthwhile because they will probably arise at some point in the future.

Pharmaceutical Products

The pharmaceutical industry derives significant benefit from advances in DNA technology and genetic research, applying them to the development of useful drugs to treat diseases. Pharmaceutical products are synthesized using methods of either organic chemistry or biotechnology, depending on the nature of the product.

Synthesis of Small Molecules for Use as Drugs Determining the sequence and structure of proteins crucial for tumor cell survival has led to the identification of small molecules that combat certain cancers by blocking the function of these proteins. One drug, imatinib (trade name Gleevec), is a small molecule that inhibits a specific receptor tyrosine kinase (see Figure 11.7). The overexpression of this receptor, resulting from a chromosomal translocation, is instrumental in causing chronic myelogenous leukemia (CML; see Figure 15.16). Patients in the early stages of CML who are treated with imatinib have exhibited nearly complete, sustained remission from the cancer. Drugs that work like this have also been used with success to treat a few types of lung and breast cancers. This approach is feasible only for cancers for which the molecular basis is fairly well understood.

Pharmaceutical products that are proteins can be synthesized on a large scale, using cells or whole organisms. Cell cultures are more widely used at present.

Protein Production in Cell Cultures You learned earlier in the chapter about DNA cloning and gene expression systems for producing large quantities of proteins that are present naturally in only minute amounts. The host cells used in such expression systems can even be engineered to secrete a protein as it is made, thereby simplifying the task of purifying it by traditional biochemical methods.

Among the first pharmaceutical products “manufactured” in this way were human insulin and human growth hormone (HGH). Some 2 million people with diabetes in the United

States depend on insulin treatment to control their disease. Human growth hormone has been a boon to children born with a form of dwarfism caused by inadequate amounts of HGH. Another important pharmaceutical product produced by genetic engineering is tissue plasminogen activator (TPA). If administered shortly after a heart attack, TPA helps dissolve blood clots and reduces the risk of subsequent heart attacks.

Protein Production by “Pharm” Animals In some cases, instead of using cell systems to produce large quantities of protein products, pharmaceutical scientists can use whole animals. They can introduce a gene from an animal of one genotype into the genome of another individual, often of a different species. This individual is then called a **transgenic** animal. To do this, they first remove eggs from a female of the recipient species and fertilize them *in vitro*. Meanwhile, they have cloned the desired gene from the donor organism. They then inject the cloned DNA directly into the nuclei of the fertilized eggs. Some of the cells integrate the foreign DNA, the *transgene*, into their genomes and are able to express the foreign gene. The engineered embryos are then surgically implanted in a surrogate mother. If an embryo develops successfully, the result is a transgenic animal that expresses its new, “foreign” gene.

Assuming that the introduced gene encodes a protein desired in large quantities, these transgenic animals can act as pharmaceutical “factories.” For example, a transgene for a human blood protein such as antithrombin can be inserted into the genome of a goat in such a way that the transgene’s product is secreted in the animal’s milk (Figure 20.24). The protein is then purified from the milk (which is easier than purification from a cell culture). Researchers have also engineered transgenic chickens that express large amounts of the transgene’s product in eggs. Biotechnology companies consider the characteristics of candidate animals in deciding which to use for engineering. For



▲ **Figure 20.24** Goats as “pharm” animals. This transgenic goat carries a gene for a human blood protein, antithrombin, which she secretes in her milk. Patients with a rare hereditary disorder in which this protein is lacking suffer from formation of blood clots in their blood vessels. Easily purified from the goat’s milk, the protein has been approved in the United States and Europe for treating these patients.

example, goats reproduce faster than cows, and it is possible to harvest more protein from goat milk than from the milk of other rapidly reproducing mammals, such as rabbits.

Human proteins produced in transgenic farm animals for use in humans may differ in some ways from the naturally produced human proteins, possibly because of subtle differences in protein modification. Therefore, such proteins must be tested very carefully to ensure that they (or contaminants from the farm animals) will not cause allergic reactions or other adverse effects in patients who receive them.

Forensic Evidence and Genetic Profiles

In violent crimes, body fluids or small pieces of tissue may be left at the scene or on the clothes or other possessions of the victim or assailant. If enough blood, semen, or tissue is available, forensic laboratories can determine the blood type or tissue type by using antibodies to detect specific cell-surface proteins. However, such tests require fairly fresh samples in relatively large amounts. Also, because many people have the same blood or tissue type, this approach can only exclude a suspect; it cannot provide strong evidence of guilt.

DNA testing, on the other hand, can identify the guilty individual with a high degree of certainty, because the DNA sequence of every person is unique (except for identical twins). Genetic markers that vary in the population can be analyzed for a given person to determine that individual's unique set of genetic markers, or **genetic profile**. (This term is preferred over "DNA fingerprint" by forensic scientists, who want to emphasize the heritable aspect of these markers rather than the fact that they produce a pattern on a gel that, like a fingerprint, is visually recognizable.) The FBI started applying DNA technology to forensics in 1988, using RFLP analysis by Southern blotting to detect similarities and differences in DNA samples. This method required much smaller samples of blood or tissue than earlier methods—only about 1,000 cells.

Today, forensic scientists use an even more sensitive method that takes advantage of variations in length of genetic markers called **short tandem repeats (STRs)**. These are tandemly repeated units of two- to five-base sequences in specific regions of the genome. The number of repeats present in these regions is highly variable from person to person (polymorphic), and for one individual, the two alleles of an STR may even differ from each other. For example, one individual may have the sequence ACAT repeated 30 times at one genome locus and 15 times at the same locus on the other homolog, whereas another individual may have 18 repeats at this locus on each homolog. (These two genotypes can be expressed by the two repeat numbers: 30,15 and 18,18.) PCR is used to amplify particular STRs, using sets of primers that are labeled with different-colored fluorescent tags; the length of the region, and thus the number of repeats, can then be determined by electrophoresis. Because Southern blotting is not required, this method is quicker than RFLP analysis. And the PCR step allows use of the method even

when the DNA is in poor condition or available only in minute quantities. A tissue sample containing as few as 20 cells can be sufficient for PCR amplification.

In a murder case, for example, this method can be used to compare DNA samples from the suspect, the victim, and a small amount of blood found at the crime scene. The forensic scientist tests only a few selected portions of the DNA—usually 13 STR markers. However, even this small set of markers can provide a forensically useful genetic profile because the probability that two people (who are not identical twins) would have exactly the same set of STR markers is vanishingly small. The Innocence Project, a nonprofit organization dedicated to overturning wrongful convictions, uses STR analysis of archived samples from crime scenes to revisit old cases. As of 2010, more than 250 innocent people had been released from prison as a result of forensic and legal work by this group (**Figure 20.25**).

Genetic profiles can also be useful for other purposes. A comparison of the DNA of a mother, her child, and the purported father can conclusively settle a question of paternity. Sometimes paternity is of historical interest: Genetic profiles

(a) In 1984, Earl Washington was convicted and sentenced to death for the 1982 rape and murder of Rebecca Williams. His sentence was commuted to life in prison in 1993 due to new doubts about the evidence. In 2000, STR analysis by forensic scientists associated with The Innocence Project showed conclusively that he was innocent. This photo shows Washington just before his release in 2001, after 17 years in prison.



Source of sample	STR marker 1	STR marker 2	STR marker 3
Semen on victim	17,19	13,16	12,12
Earl Washington	16,18	14,15	11,12
Kenneth Tinsley	17,19	13,16	12,12

(b) In STR analysis, selected STR markers in a DNA sample are amplified by PCR, and the PCR products are separated by electrophoresis. The procedure reveals how many repeats are present for each STR locus in the sample. An individual has two alleles per STR locus, each with a certain number of repeats. This table shows the number of repeats for three STR markers in three samples: from semen found on the victim, from Washington, and from another man named Kenneth Tinsley, who was in prison because of an unrelated conviction. These and other STR data (not shown) exonerated Washington and led Tinsley to plead guilty to the murder.

▲ **Figure 20.25** STR analysis used to release an innocent man from prison.

provided strong evidence that Thomas Jefferson or one of his close male relatives fathered at least one of the children of his slave Sally Hemings. Genetic profiles can also identify victims of mass casualties. The largest such effort occurred after the attack on the World Trade Center in 2001; more than 10,000 samples of victims' remains were compared with DNA samples from personal items, such as toothbrushes, provided by families. Ultimately, forensic scientists succeeded in identifying almost 3,000 victims using these methods.

Just how reliable is a genetic profile? The greater the number of markers examined in a DNA sample, the more likely it is that the profile is unique to one individual. In forensic cases using STR analysis with 13 markers, the probability of two people having identical DNA profiles is somewhere between one chance in 10 billion and one in several trillion. (For comparison, the world's population in 2009 was about 6.8 billion.) The exact probability depends on the frequency of those markers in the general population. Information on how common various markers are in different ethnic groups is critical because these marker frequencies may vary considerably among ethnic groups and between a particular ethnic group and the population as a whole. With the increasing availability of frequency data, forensic scientists can make extremely accurate statistical calculations. Thus, despite problems that can still arise from insufficient data, human error, or flawed evidence, genetic profiles are now accepted as compelling evidence by legal experts and scientists alike.

Environmental Cleanup

Increasingly, the remarkable ability of certain microorganisms to transform chemicals is being exploited for environmental cleanup. If the growth needs of such microbes make them unsuitable for direct use, scientists can now transfer the genes for their valuable metabolic capabilities into other microorganisms, which can then be used to treat environmental problems. For example, many bacteria can extract heavy metals, such as copper, lead, and nickel, from their environments and incorporate the metals into compounds such as copper sulfate or lead sulfate, which are readily recoverable. Genetically engineered microbes may become important in both mining minerals (especially as ore reserves are depleted) and cleaning up highly toxic mining wastes. Biotechnologists are also trying to engineer microbes that can degrade chlorinated hydrocarbons and other harmful compounds. These microbes could be used in wastewater treatment plants or by manufacturers before the compounds are ever released into the environment.

Agricultural Applications

Scientists are working to learn more about the genomes of agriculturally important plants and animals. For a number of years, they have been using DNA technology in an effort to

improve agricultural productivity. The selective breeding of both livestock (animal husbandry) and crops has exploited naturally occurring mutations and genetic recombination for thousands of years.

As we described earlier, DNA technology enables scientists to produce transgenic animals, which speeds up the selective breeding process. The goals of creating a transgenic animal are often the same as the goals of traditional breeding—for instance, to make a sheep with better quality wool, a pig with leaner meat, or a cow that will mature in a shorter time. Scientists might, for example, identify and clone a gene that causes the development of larger muscles (muscles make up most of the meat we eat) in one breed of cattle and transfer it to other cattle or even to sheep. However, problems such as low fertility or increased susceptibility to disease are not uncommon among farm animals carrying genes from other species. Animal health and welfare are important issues to consider when developing transgenic animals.

Agricultural scientists have already endowed a number of crop plants with genes for desirable traits, such as delayed ripening and resistance to spoilage and disease. In one striking way, plants are easier to genetically engineer than most animals. For many plant species, a single tissue cell grown in culture can give rise to an adult plant (see Figure 20.17). Thus, genetic manipulations can be performed on an ordinary somatic cell and the cell then used to generate an organism with new traits.

The most commonly used vector for introducing new genes into plant cells is a plasmid, called the **Ti plasmid**, from the soil bacterium *Agrobacterium tumefaciens*. This plasmid integrates a segment of its DNA, known as T DNA, into the chromosomal DNA of its host plant cells. For vector purposes, researchers work with versions of the plasmid that do not cause disease (unlike the wild-type version) and that have been engineered to carry genes of interest within the borders of the T DNA. **Figure 20.26** (on the next page) outlines one method for using the Ti plasmid to produce transgenic plants.

Genetic engineering is rapidly replacing traditional plant-breeding programs, especially for useful traits, such as herbicide or pest resistance, determined by one or a few genes. Crops engineered with a bacterial gene making the plants resistant to herbicides can grow while weeds are destroyed, and genetically engineered crops that can resist destructive insects reduce the need for chemical insecticides. In India, the insertion of a salinity resistance gene from a coastal mangrove plant into the genomes of several rice varieties has resulted in rice plants that can grow in water three times as salty as seawater. The research foundation that carried out this feat of genetic engineering estimates that one-third of all irrigated land has high salinity owing to overirrigation and intensive use of chemical fertilizers, representing a serious threat to the food supply. Thus, salinity-resistant crop plants would be enormously valuable worldwide.

▼ Figure 20.26

RESEARCH METHOD

Using the Ti Plasmid to Produce Transgenic Plants

APPLICATION Genes conferring useful traits, such as pest resistance, herbicide resistance, delayed ripening, and increased nutritional value, can be transferred from one plant variety or species to another using the Ti plasmid as a vector.

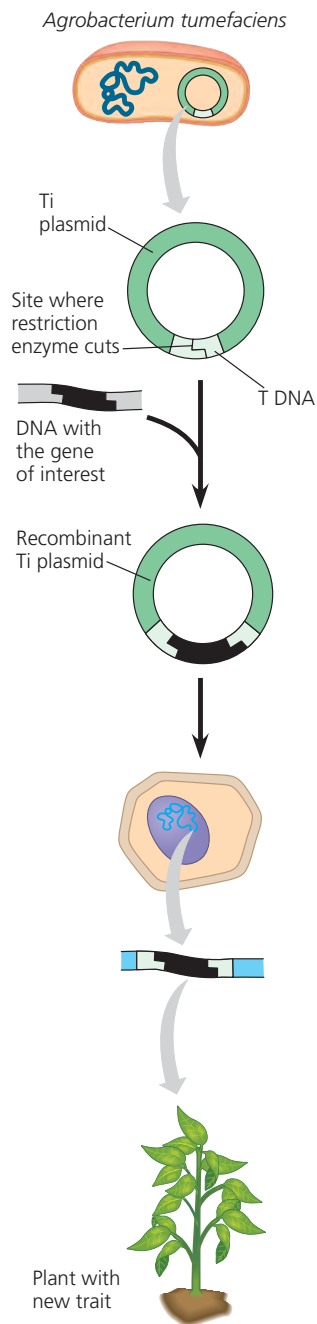
TECHNIQUE

1 The Ti plasmid is isolated from the bacterium *Agrobacterium tumefaciens*. The segment of the plasmid that integrates into the genome of host cells is called T DNA.

2 The foreign gene of interest is inserted into the middle of the T DNA using methods shown in Figure 20.4.

3 Recombinant plasmids can be introduced into cultured plant cells by electroporation. Or plasmids can be returned to *Agrobacterium*, which is then applied as a liquid suspension to the leaves of susceptible plants, infecting them. Once a plasmid is taken into a plant cell, its T DNA integrates into the cell's chromosomal DNA.

RESULTS Transformed cells carrying the transgene of interest can regenerate complete plants that exhibit the new trait conferred by the transgene.



Safety and Ethical Questions Raised by DNA Technology

Early concerns about potential dangers associated with recombinant DNA technology focused on the possibility that hazardous new pathogens might be created. What might happen, for instance, if cancer cell genes were transferred into bacteria or viruses? To guard against such rogue microbes, scientists developed a set of guidelines that were adopted as formal government regulations in the United States and some other countries. One safety measure is a set of strict laboratory procedures designed to protect researchers from infection by engineered microbes and to prevent the microbes from accidentally leaving the laboratory. In addition, strains of microorganisms to be used in recombinant DNA experiments are genetically crippled to ensure that they cannot survive outside the laboratory. Finally, certain obviously dangerous experiments have been banned.

Today, most public concern about possible hazards centers not on recombinant microbes but on **genetically modified (GM) organisms** used as food. A GM organism is one that has acquired by artificial means one or more genes from another species or even from another variety of the same species. Some salmon, for example, have been genetically modified by addition of a more active salmon growth hormone gene. However, the majority of the GM organisms that contribute to our food supply are not animals, but crop plants.

GM crops are widespread in the United States, Argentina, and Brazil; together these countries account for over 80% of the world's acreage devoted to such crops. In the United States, most corn, soybean, and canola crops are genetically modified, and GM products are not required to be labeled. However, the same foods are an ongoing subject of controversy in Europe, where the GM revolution has met with strong opposition. Many Europeans are concerned about the safety of GM foods and the possible environmental consequences of growing GM plants. Early in 2000, negotiators from 130 countries agreed on a Biosafety Protocol that requires exporters to identify GM organisms present in bulk food shipments and allows importing countries to decide whether the products pose environmental or health risks. (Although the United States declined to sign the agreement, it went into effect anyway because the majority of countries were in favor of it.) Since then, European countries have, on occasion, refused crops from the United States and other countries, leading to trade disputes. Although a small number of GM crops have been grown on European soil, these products have generally failed in local markets, and the future of GM crops in Europe is uncertain.

Advocates of a cautious approach toward GM crops fear that transgenic plants might pass their new genes to close relatives in nearby wild areas. We know that lawn and crop grasses, for example, commonly exchange genes with wild

relatives via pollen transfer. If crop plants carrying genes for resistance to herbicides, diseases, or insect pests pollinated wild ones, the offspring might become “super weeds” that are very difficult to control. Another worry concerns possible risks to human health from GM foods. Some people fear that the protein products of transgenes might lead to allergic reactions. Although there is some evidence that this could happen, advocates claim that these proteins could be tested in advance to avoid producing ones that cause allergic reactions.

Today, governments and regulatory agencies throughout the world are grappling with how to facilitate the use of biotechnology in agriculture, industry, and medicine while ensuring that new products and procedures are safe. In the United States, such applications of biotechnology are evaluated for potential risks by various regulatory agencies, including the Food and Drug Administration, the Environmental Protection Agency, the National Institutes of Health, and the Department of Agriculture. Meanwhile, these same agencies and the public must consider the ethical implications of biotechnology.

Advances in biotechnology have allowed us to obtain complete genome sequences for humans and many other species, providing a vast treasure trove of information about genes. We can ask how certain genes differ from species to species, as well as how genes and, ultimately, entire genomes have evolved. (These are the subjects of Chapter 21.) At the same time, the increasing speed and falling cost of sequencing the genomes of

individuals are raising significant ethical questions. Who should have the right to examine someone else’s genetic information? How should that information be used? Should a person’s genome be a factor in determining eligibility for a job or insurance? Ethical considerations, as well as concerns about potential environmental and health hazards, will likely slow some applications of biotechnology. There is always a danger that too much regulation will stifle basic research and its potential benefits. However, the power of DNA technology and genetic engineering—our ability to profoundly and rapidly alter species that have been evolving for millennia—demands that we proceed with humility and caution.

CONCEPT CHECK 20.4

1. What is the advantage of using stem cells for gene therapy?
2. List at least three different properties that have been acquired by crop plants via genetic engineering.
3. **WHAT IF?** As a physician, you have a patient with symptoms that suggest a hepatitis A infection, but you have not been able to detect viral proteins in the blood. Knowing that hepatitis A is an RNA virus, what lab tests could you perform to support your diagnosis? Explain what the results would mean.

For suggested answers, see Appendix A.

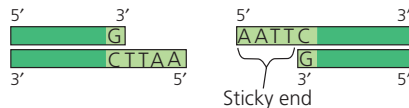
20 CHAPTER REVIEW

SUMMARY OF KEY CONCEPTS

CONCEPT 20.1

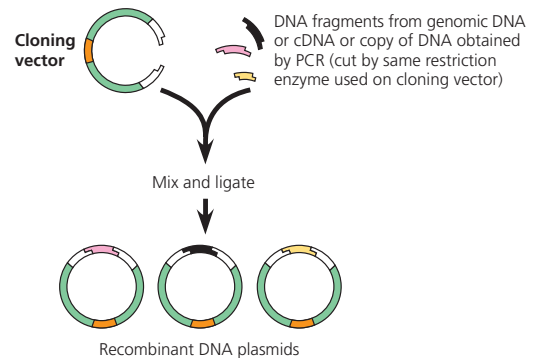
DNA cloning yields multiple copies of a gene or other DNA segment (pp. 396–405)

- **Gene cloning** and other techniques, collectively termed DNA technology, can be used to manipulate and analyze DNA and to produce useful new products and organisms.
- In **genetic engineering**, bacterial **restriction enzymes** are used to cut DNA molecules within short, specific nucleotide sequences (**restriction sites**), yielding a set of double-stranded DNA fragments with single-stranded **sticky ends**.



- The sticky ends on **restriction fragments** from one DNA source can base-pair with complementary sticky ends on fragments from other DNA molecules; sealing the base-paired fragments with **DNA ligase** produces **recombinant DNA** molecules.

- Cloning a eukaryotic gene in a bacterial plasmid:



- Cloning vectors** include **plasmids** and **bacterial artificial chromosomes (BACs)**. Recombinant plasmids are returned to host cells, each of which divides to form a clone of cells. Collections of clones are stored as **genomic** or **complementary DNA (cDNA) libraries**. Libraries can be screened for a gene of interest using **nucleic acid hybridization** with a **nucleic acid probe**.
- Several technical difficulties hinder the expression of cloned eukaryotic genes in bacterial host cells. The use of cultured

eukaryotic cells (such as yeasts, insect cells, or cultured mammalian cells) as host cells, coupled with appropriate **expression vectors**, helps avoid these problems.

- The **polymerase chain reaction (PCR)** can produce many copies of (amplify) a specific target segment of DNA *in vitro*, using primers that bracket the desired sequence and a heat-resistant DNA polymerase.

? Describe how the process of gene cloning results in a cell clone containing a recombinant plasmid.

CONCEPT 20.2

DNA technology allows us to study the sequence, expression, and function of a gene (pp. 405–412)

- DNA restriction fragments of different lengths can be separated by **gel electrophoresis**. Specific fragments can be identified by **Southern blotting**, using labeled probes that hybridize to the DNA immobilized on a “blot” of the gel. Historically, **restriction fragment length polymorphisms (RFLPs)** were used to screen for some disease-causing alleles, such as the sickle-cell allele.
- Relatively short DNA fragments can be sequenced by the dideoxy chain termination method, which can be performed in automated sequencing machines. The rapid development of faster and cheaper methods is ongoing.
- Expression of a gene can be investigated using hybridization with labeled probes to look for specific mRNAs, either on a gel (**Northern blotting**) or in a whole organism (***in situ* hybridization**). Also, RNA can be transcribed into cDNA by reverse transcriptase and the cDNA amplified by PCR with specific primers (**RT-PCR**). **DNA microarrays** allow researchers to compare the expression of many genes at once in different tissues, at different times, or under different conditions.
- For a gene of unknown function, experimental inactivation of the gene and observation of the resulting phenotypic effects can provide clues to its function. In humans, **genome-wide association studies** use **single nucleotide polymorphisms (SNPs)** as genetic markers for alleles that are associated with particular conditions.

? Complementary base pairing is the basis of most procedures used to analyze gene expression. Explain.

CONCEPT 20.3

Cloning organisms may lead to production of stem cells for research and other applications (pp. 412–417)

- Studies showing genomic equivalence (that an organism’s cells have the same genome) provided the first examples of organismal cloning.
- Single differentiated cells from mature plants are often **totipotent**: capable of generating all the tissues of a complete new plant.
- Transplantation of the nucleus from a differentiated animal cell into an enucleated egg can sometimes give rise to a new animal.
- Certain embryonic stem (ES) or adult **stem cells** from animal embryos or adult tissues can reproduce and differentiate *in vitro* as well as *in vivo*, offering the potential for medical use. ES cells are **pluripotent** but difficult to acquire. Induced pluripotent stem (iPS) cells resemble ES cells in their capacity to differentiate; they can be generated by reprogramming differentiated cells. iPS cells hold promise for medical research and regenerative medicine.

? Describe how a researcher could carry out organismal cloning, production of ES cells, and generation of iPS cells, focusing on how the cells are reprogrammed and using mice as an example. (The procedures are basically the same in humans and mice.)

CONCEPT 20.4

The practical applications of DNA technology affect our lives in many ways (pp. 417–423)

- DNA technology, including the analysis of genetic markers such as SNPs, is increasingly being used in the diagnosis of genetic and other diseases and offers potential for better treatment of genetic disorders (or even permanent cures through **gene therapy**), as well as more informed cancer therapies. Large-scale production of protein hormones and other proteins with therapeutic uses is possible with DNA technology. Some therapeutic proteins are being produced in **transgenic** “pharm” animals.
- Analysis of genetic markers such as **short tandem repeats (STRs)** in DNA isolated from tissue or body fluids found at crime scenes leads to a **genetic profile** that can provide definitive evidence that a suspect is innocent or strong evidence of guilt. Such analysis is also useful in parenthood disputes and in identifying the remains of crime victims.
- Genetically engineered microorganisms can be used to extract minerals from the environment or degrade various types of toxic waste materials.
- The aims of developing transgenic plants and animals are to improve agricultural productivity and food quality.
- The potential benefits of genetic engineering must be carefully weighed against the potential for harm to humans or the environment.

? What factors affect whether a given genetic disease would be a good candidate for successful gene therapy?

TEST YOUR UNDERSTANDING

LEVEL 1: KNOWLEDGE/COMPREHENSION

1. Which of the following tools of recombinant DNA technology is *incorrectly* paired with its use?
 - a. restriction enzyme—analysis of RFLPs
 - b. DNA ligase—cutting DNA, creating sticky ends of restriction fragments
 - c. DNA polymerase—polymerase chain reaction to amplify sections of DNA
 - d. reverse transcriptase—production of cDNA from mRNA
 - e. electrophoresis—separation of DNA fragments
2. Plants are more readily manipulated by genetic engineering than are animals because
 - a. plant genes do not contain introns.
 - b. more vectors are available for transferring recombinant DNA into plant cells.
 - c. a somatic plant cell can often give rise to a complete plant.
 - d. genes can be inserted into plant cells by microinjection.
 - e. plant cells have larger nuclei.
3. A paleontologist has recovered a bit of tissue from the 400-year-old preserved skin of an extinct dodo (a bird). To compare a specific region of the DNA from the sample with DNA from living birds, which of the following would be most useful for increasing the amount of dodo DNA available for testing?
 - a. RFLP analysis
 - b. polymerase chain reaction (PCR)
 - c. electroporation
 - d. gel electrophoresis
 - e. Southern blotting

- DNA technology has many medical applications. Which of the following is *not* done routinely at present?
 - production of hormones for treating diabetes and dwarfism
 - production of microbes that can metabolize toxins
 - introduction of genetically engineered genes into human gametes
 - prenatal identification of genetic disease alleles
 - genetic testing for carriers of harmful alleles
- In recombinant DNA methods, the term *vector* can refer to
 - the enzyme that cuts DNA into restriction fragments.
 - the sticky end of a DNA fragment.
 - a SNP marker.
 - a plasmid used to transfer DNA into a living cell.
 - a DNA probe used to identify a particular gene.

LEVEL 2: APPLICATION/ANALYSIS

- Which of the following would *not* be true of cDNA produced using human brain tissue as the starting material?
 - It could be amplified by the polymerase chain reaction.
 - It could be used to create a complete genomic library.
 - It was produced from mRNA using reverse transcriptase.
 - It could be used as a probe to detect genes expressed in the brain.
 - It lacks the introns of the human genes.
- Expression of a cloned eukaryotic gene in a bacterial cell involves many challenges. The use of mRNA and reverse transcriptase is part of a strategy to solve the problem of
 - post-transcriptional processing.
 - electroporation.
 - post-translational processing.
 - nucleic acid hybridization.
 - restriction fragment ligation.
- Which of the following sequences in double-stranded DNA is most likely to be recognized as a cutting site for a restriction enzyme?

a. AAGG	b. AGTC	c. GGCC	d. ACCA	e. AAAA
TTCC	TCAG	CCGG	TGGT	TTTT
- DRAW IT** You are making a genomic library for the aardvark, using a bacterial plasmid as a vector. The green diagram below shows the plasmid, which contains the restriction site for the enzyme used in Figure 20.3. Above the plasmid is a segment of linear aardvark DNA. Diagram your cloning procedure, showing what would happen to these two molecules during each step. Use one color for the aardvark DNA and its bases and another color for those of the plasmid. Label each step and all 5' and 3' ends.



LEVEL 3: SYNTHESIS/EVALUATION

- WHAT IF?** Imagine you want to study one of the human crystallins, proteins present in the lens of the eye. To obtain a sufficient amount of the protein of interest, you decide to clone the gene that codes for it. Would you construct a genomic library or a cDNA library? What material would you use as a source of DNA or RNA?
- EVOLUTION CONNECTION**
Ethical considerations aside, if DNA-based technologies became widely used, how might they change the way evolution proceeds, as compared with the natural evolutionary mechanisms of the past 4 billion years?
- SCIENTIFIC INQUIRY**
You hope to study a gene that codes for a neurotransmitter protein produced in human brain cells. You know the amino acid sequence of the protein. Explain how you might (a) identify what genes are expressed in a specific type of brain cell, (b) identify (isolate) the neurotransmitter gene, (c) produce multiple copies of the gene for study, and (d) produce large quantities of the neurotransmitter for evaluation as a potential medication.
- SCIENCE, TECHNOLOGY, AND SOCIETY**
Is there danger of discrimination based on testing for “harmful” genes? What policies can you suggest that would prevent such abuses?
- SCIENCE, TECHNOLOGY, AND SOCIETY**
Government funding of embryonic stem cell research has been a contentious political issue. Why has this debate been so heated? Summarize the arguments for and against embryonic stem cell research, and explain your own position on the issue.
- WRITE ABOUT A THEME**
The Genetic Basis of Life In a short essay (100–150 words), discuss how the genetic basis of life plays a central role in biotechnology.

For selected answers, see Appendix A.

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1. MasteringBiology® Assignments

Video Tutor Session DNA Profiling

Tutorial Restriction Enzymes, Recombinant DNA, and Gene Cloning

Activities Cloning a Gene in Bacteria • Producing Human Growth Hormone • Restriction Enzymes • The Polymerase Chain Reaction • Gel Electrophoresis of DNA • Analyzing DNA Fragments Using Gel Electrophoresis • Discovery Channel Video: Cloning • DNA Fingerprinting • Making Decisions About DNA Technology: Golden Rice • Discovery Channel Videos: DNA Forensics; Transgenics

Questions Student Misconceptions • Reading Quiz • Multiple Choice • End-of-Chapter

2. eText

Read your book online, search, take notes, highlight text, and more.

3. The Study Area

Practice Tests • Cumulative Test • **BioFlix** 3-D Animations • MP3 Tutor Sessions • Videos • Activities • Investigations • Lab Media • Audio Glossary • Word Study Tools • Art