19

Gene Technology

Concept Outline

FIGURE 19.1
A famous plasmid. The circular molecule in this electron micrograph is pSC101, the first plasmid used successfully to clone a vertebrate gene. Its name comes from the fact that it was the one-hundred-and-first plasmid isolated by Stanley Cohen.

Over the past decades, the development of new and powerful techniques for studying and manipulating DNA has revolutionized genetics (figure 19.1). These techniques have allowed biologists to intervene directly in the genetic fate of organisms for the first time. In this chapter, we will explore these technologies and consider how they apply to specific problems of great practical importance. Few areas of biology will have as great an impact on our future lives.

19.1 The ability to manipulate DNA has led to a new genetics.

Restriction Endonucleases

In 1980, geneticists used the relatively new technique of gene splicing, which we will describe in this chapter, to introduce the human gene that encodes **interferon** into a bacterial cell's genome. Interferon is a rare blood protein that increases human resistance to viral infection, and medical scientists have been interested in its possible usefulness in cancer therapy. This possibility was difficult to investigate before 1980, however, because purification of the large amounts of interferon required for clinical testing would have been prohibitively expensive, given interferon's scarcity in the blood. An inexpensive way to produce interferon was needed, and introducing the gene responsible for its production into a bacterial cell made that possible. The cell that had acquired the human interferon gene proceeded to produce interferon at a rapid rate, and to grow and divide. Soon there were millions of interferon-producing bacteria in the culture, all of them descendants of the cell that had originally received the human interferon gene.

The Advent of Genetic Engineering

This procedure of producing a line of genetically identical cells from a single altered cell, called **cloning,** made every cell in the culture a miniature factory for producing interferon. The human insulin gene has also been cloned in bacteria, and now large amounts of insulin, a hormone essential for treating some forms of diabetes, can be manufactured at relatively little expense. Beyond these clinical applications, cloning and related molecular techniques are used to obtain basic information about how genes are put together and regulated. The interferon experiment and others like it marked the beginning of a new genetics, **genetic engineering.**

The essence of genetic engineering is the ability to cut DNA into recognizable pieces and rearrange those pieces in different ways. In the interferon experiment, a piece of DNA carrying the interferon gene was inserted into a plasmid, which then carried the gene into a bacterial cell. Most other genetic engineering approaches have used the same general strategy, bringing the gene of interest into the target cell by first incorporating it into a plasmid or an infective virus. To make these experiments work, one must be able to cut the source DNA (human DNA in the interferon experiment, for example) and the plasmid DNA in such a way that the desired fragment of source DNA can be spliced permanently into the plasmid. This cutting is performed by enzymes that recognize and cleave specific sequences of nucleotides in DNA. These enzymes are the basic tools of genetic engineering.

Discovery of Restriction Endonucleases

Scientific discoveries often have their origins in seemingly unimportant observations that receive little attention by researchers before their general significance is appreciated. In the case of genetic engineering, the original observation was that bacteria use enzymes to defend themselves against viruses.

Most organisms eventually evolve means of defending themselves from predators and parasites, and bacteria are no exception. Among the natural enemies of bacteria are bacteriophages, viruses that infect bacteria and multiply within them. At some point, they cause the bacterial cells to burst, releasing thousands more viruses. Through natural selection, some types of bacteria have acquired powerful weapons against these viruses: they contain enzymes called **restriction endonucleases** that fragment the viral DNA as soon as it enters the bacterial cell. Many restriction endonucleases recognize specific nucleotide sequences in a DNA strand, bind to the DNA at those sequences, and cleave the DNA at a particular place within the recognition sequence.

Why don't restriction endonucleases cleave the bacterial cells' own DNA as well as that of the viruses? The answer to this question is that bacteria modify their own DNA, using other enzymes known as **methylases** to add methyl (—CH_3) groups to some of the nucleotides in the bacterial DNA. When nucleotides within a restriction endonuclease's recognition sequence have been methylated, the endonuclease cannot bind to that sequence. Consequently, the bacterial DNA is protected from being degraded at that site. Viral DNA, on the other hand, has not been methylated and therefore is not protected from enzymatic cleavage.

How Restriction Endonucleases Cut DNA

The sequences recognized by restriction endonucleases are typically four to six nucleotides long, and they are often palindromes. This means the nucleotides at one end of the recognition sequence are complementary to those at the other end, so that the two strands of the DNA duplex have the same nucleotide sequence running in opposite directions for the length of the recognition sequence. Two important consequences arise from this arrangement of nucleotides.

First, because the same recognition sequence occurs on both strands of the DNA duplex, the restriction endonuclease can bind to and cleave both strands, effectively cutting the DNA in half. This ability to cut across both strands is almost certainly the reason that restriction endonucleases have evolved to recognize nucleotide sequences with twofold rotational symmetry.

Second, because the bond cleaved by a restriction endonuclease is typically not positioned in the center of the recognition sequence to which it binds, and because the DNA strands are antiparallel, the cut sites for the two strands of a duplex are offset from each other (figure 19.2). After cleavage, each DNA fragment has a single-stranded end a few nucleotides long. The single-stranded ends of the two fragments are complementary to each other.

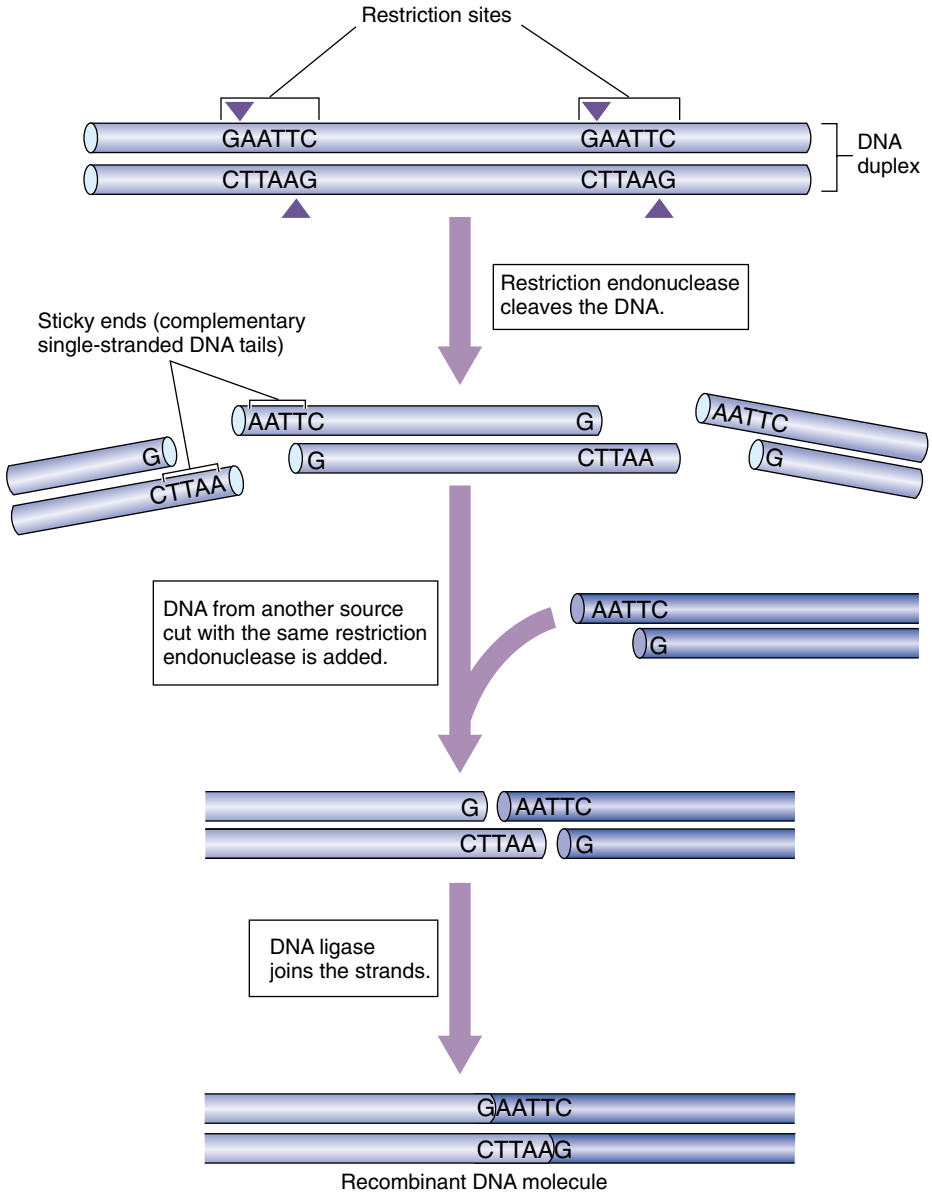


FIGURE 19.2
Many restriction endonucleases produce DNA fragments with "sticky ends." The restriction endonuclease *Eco*RI always cleaves the sequence GAATTC between G and A. Because the same sequence occurs on both strands, both are cut. However, the two sequences run in opposite directions on the two strands. As a result, single-stranded tails are produced that are complementary to each other, or "sticky."

Why Restriction Endonucleases Are So Useful

There are hundreds of bacterial restriction endonucleases, and each one has a specific recognition sequence. By chance, a particular endonuclease's recognition sequence is likely to occur somewhere in any given sample of DNA; the shorter the sequence, the more often it will arise by chance within a sample. Therefore, a given restriction endonuclease can probably cut DNA from any source into fragments. Each fragment will have complementary single-stranded ends characteristic of that endonuclease. Because of their complementarity, these single-stranded ends can pair with each other (consequently, they are sometimes called "sticky ends"). Once their ends have paired, two fragments can then be joined together with the aid of the enzyme **DNA ligase,** which re-forms the phosphodiester bonds of DNA. What makes restriction endonucleases so valuable for genetic engineering is the fact that *any* two fragments produced by the same restriction endonuclease can be joined together. Fragments of elephant and ostrich DNA cleaved by the same endonuclease can be joined to one another as readily as two bacterial DNA fragments.

Genetic engineering involves manipulating specific genes by cutting and rearranging DNA. A restriction endonuclease cleaves DNA at a specific site, generating in most cases two fragments with short single-stranded ends. Because these ends are complementary to each other, any pair of fragments produced by the same endonuclease, from any DNA source, can be joined together.

Using Restriction Endonucleases to Manipulate Genes

A **chimera** is a mythical creature with the head of a lion, body of a goat, and tail of a serpent. Although no such creatures existed in nature, biologists have made chimeras of a more modest kind through genetic engineering.

Constructing pSC101

One of the first chimeras was manufactured from a bacterial plasmid called a resistance transfer factor by American geneticists Stanley Cohen and Herbert Boyer in 1973. Cohen and Boyer used a restriction endonuclease called *Eco*RI, which is obtained from *Escherichia coli*, to cut the plasmid into fragments. One fragment, 9000 nucleotides in length, contained both the origin of replication necessary for replicating the plasmid and a gene that conferred resistance to the antibiotic tetracycline (tet^r). Because both ends of this fragment were cut by the same restriction endonuclease, they could be ligated to form a circle, a smaller plasmid Cohen dubbed pSC101 (figure 19.3).

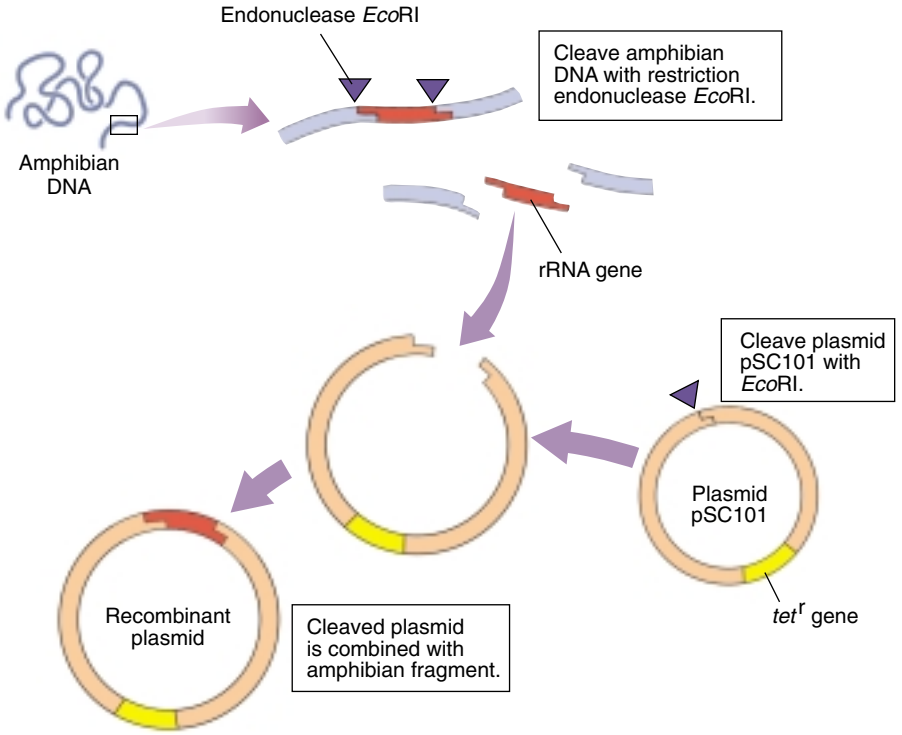


FIGURE 19.3
One of the first genetic engineering experiments. This diagram illustrates how Cohen and Boyer inserted an amphibian gene encoding rRNA into pSC101. The plasmid contains a single site cleaved by the restriction endonuclease *Eco*RI; it also contains tet^r, a gene which confers resistance to the antibiotic tetracycline. The rRNA-encoding gene was inserted into pSC101 by cleaving the amphibian DNA and the plasmid with *Eco*RI and allowing the complementary sequences to pair.

Using pSC101 to Make Recombinant DNA

Cohen and Boyer also used *Eco*RI to cleave DNA that coded for rRNA that they had isolated from an adult amphibian, the African clawed frog, *Xenopus laevis*. They then mixed the fragments of *Xenopus* DNA with pSC101 plasmids that had been "reopened" by *Eco*RI and allowed bacterial cells to take up DNA from the mixture. Some of the bacterial cells immediately became resistant to tetracycline, indicating that they had incorporated the pSC101 plasmid with its antibiotic-resistance gene. Furthermore, some of these pSC101-containing bacteria also began to produce frog ribosomal RNA! Cohen and Boyer concluded that the frog rRNA gene must have been inserted into the pSC101 plasmids in those bacteria. In other words, the two ends of the pSC101 plasmid, produced by cleavage with *Eco*RI, had joined to the two ends of a frog DNA fragment that contained the rRNA gene, also cleaved with *Eco*RI.

The pSC101 plasmid containing the frog rRNA gene is a true chimera, an entirely new genome that never existed in nature and never would have evolved by natural means. It is a form of **recombinant DNA**—that is, DNA created in the laboratory by joining together pieces of different genomes to form a novel combination.

Other Vectors

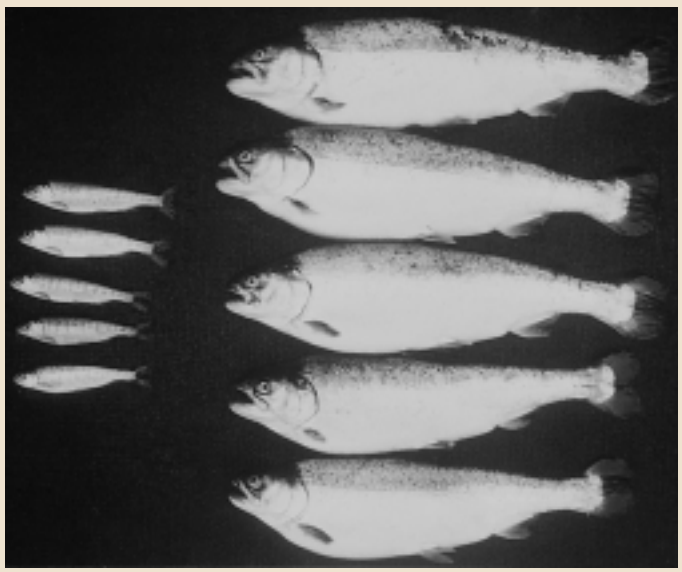
The introduction of foreign DNA fragments into host cells has become common in molecular genetics. The genome that carries the foreign DNA into the host cell is called a **vector.** Plasmids, with names like pUC18 can be induced to make hundreds of copies of themselves and thus of the foreign genes they contain. Much larger pieces of DNA can be introduced using YAKs (yeast artificial chromosomes) as a vector instead of a plasmid. Not all vectors have bacterial targets. Animal viruses such as the human cold virus adenovirus, for example, are serving as vectors to carry genes into monkey and human cells, and animal genes have even been introduced into plant cells.

One of the first recombinant genomes produced by genetic engineering was a bacterial plasmid into which an amphibian ribosomal RNA gene was inserted. Viruses can also be used as vectors to insert foreign DNA into host cells and create recombinant genomes.

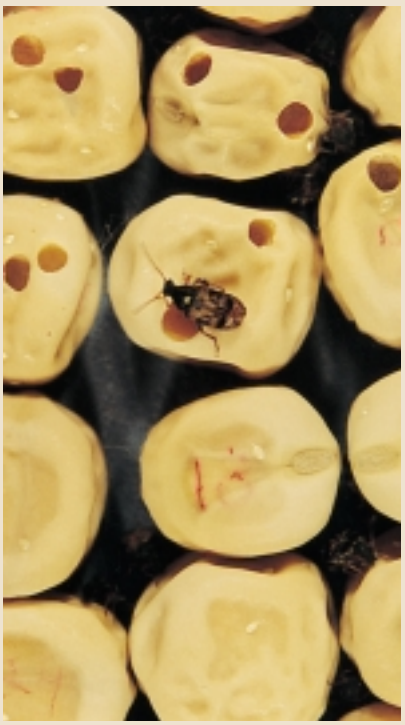
Examples of Gene Manipulation

HERMAN THE WONDER BULL
GenPharm, a California biotechnology company, engineered Herman, a bull that possesses the gene for human lactoferrin (HLF). HLF confers antibacterial and iron transport properties to humans. Many of Herman's female offspring now produce milk containing HLF, and GenPharm intends to build a herd of transgenic cows for the large-scale commercial production of HLF.

WILT-PROOF FLOWERS
Ethylene, the plant hormone that causes fruit to ripen, also causes flowers to wilt. Researchers at Purdue have found the gene that makes flower petals respond to ethylene by wilting and replaced it with a gene insensitive to ethylene. The transgenic carnations they produced lasted for 3 weeks after cutting, while normal carnations last only 3 days.



SUPER SALMON!
Canadian fisheries scientists have inserted recombinant growth hormone genes into developing salmon embryos, creating the first transgenic salmon. Not only do these transgenic fish have shortened production cycles, they are, on an average, 11 times heavier than nontransgenic salmon! The implications for the fisheries industry and for worldwide food production are obvious.



WEEVIL-PROOF PEAS
Not only has gene technology afforded agriculture viral and pest control in the field, it has also provided a pest control technique for the storage bin. A team of U.S. and Australian scientists have engineered a gene that is expressed only in the seed of the pea plant. The enzyme inhibitor encoded by this gene inhibits feeding by weevils, one of the most notorious pests affecting stored crops. The worldwide ramifications are significant as up to 40% of stored grains are lost to pests.

19.2 Genetic engineering involves easily understood procedures.

The Four Stages of a Genetic Engineering Experiment

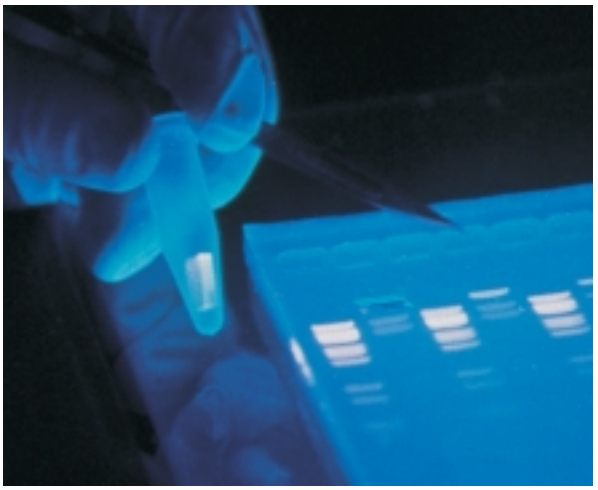
Like the experiment of Cohen and Boyer, most genetic engineering experiments consist of four stages: DNA cleavage, production of recombinant DNA, cloning, and screening.

Stage 1: DNA Cleavage

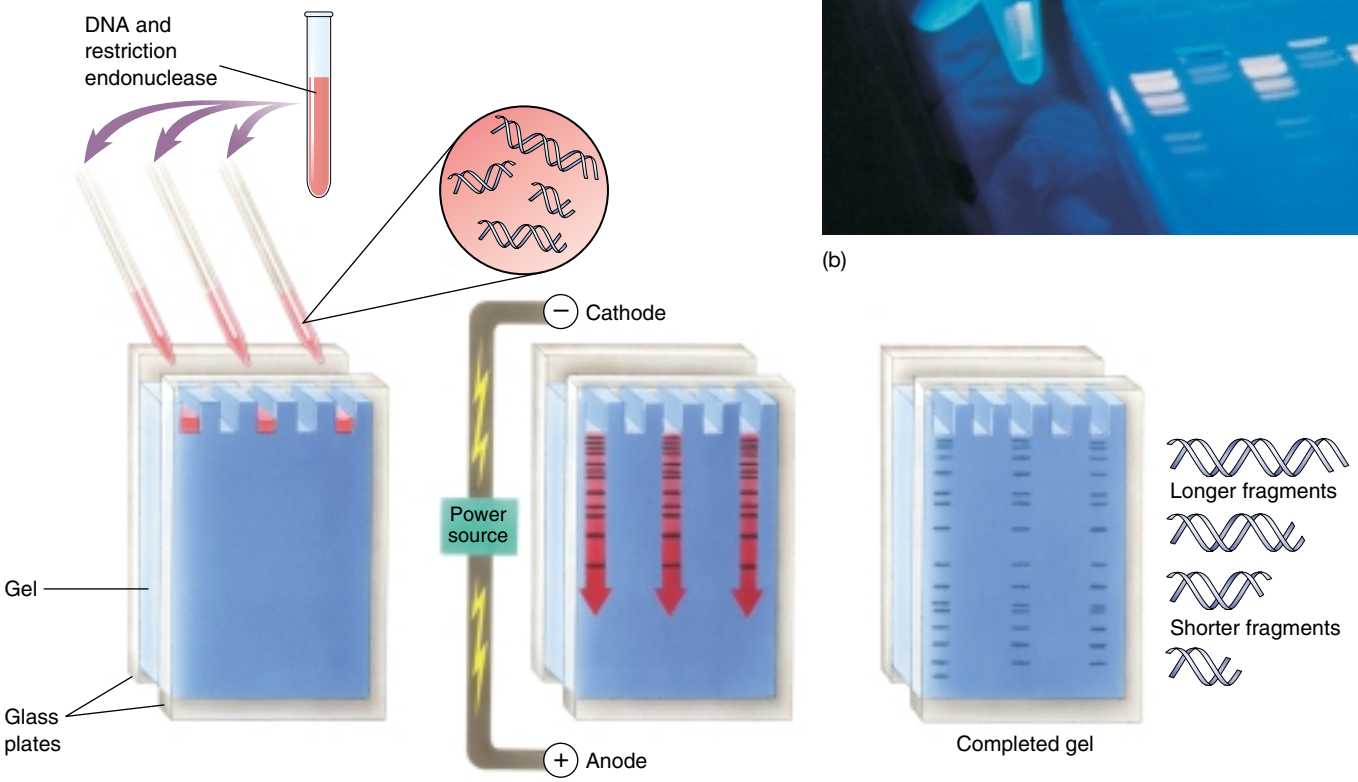
A restriction endonuclease is used to cleave the source DNA into fragments. Because the endonuclease's recognition sequence is likely to occur many times within the source DNA, cleavage will produce a large number of different fragments. A different set of fragments will be obtained by employing endonucleases that recognize different sequences. The fragments can be separated from one another according to their size by electrophoresis (figure 19.4).

Stage 2: Production of Recombinant DNA

The fragments of DNA are inserted into plasmids or viral vectors, which have been cleaved with the same restriction endonuclease as the source DNA.



(b)



(a)

FIGURE 19.4
Gel electrophoresis. (*a*) After restriction endonucleases have cleaved the DNA, the fragments are loaded on a gel, and an electric current is applied. The DNA fragments migrate through the gel, with bigger ones moving more slowly. The fragments can be visualized easily, as the migrating bands fluoresce in UV light when stained with ethidium bromide. (*b*) In the photograph, one band of DNA has been excised from the gel for further analysis and can be seen glowing in the tube the technician holds.

Stage 3: Cloning

The plasmids or viruses serve as vectors that can introduce the DNA fragments into cells—usually, but not always, bacteria (figure 19.5). As each cell reproduces, it forms a clone of cells that all contain the fragment-bearing vector. Each clone is maintained separately, and all of them together constitute a clone library of the original source DNA.

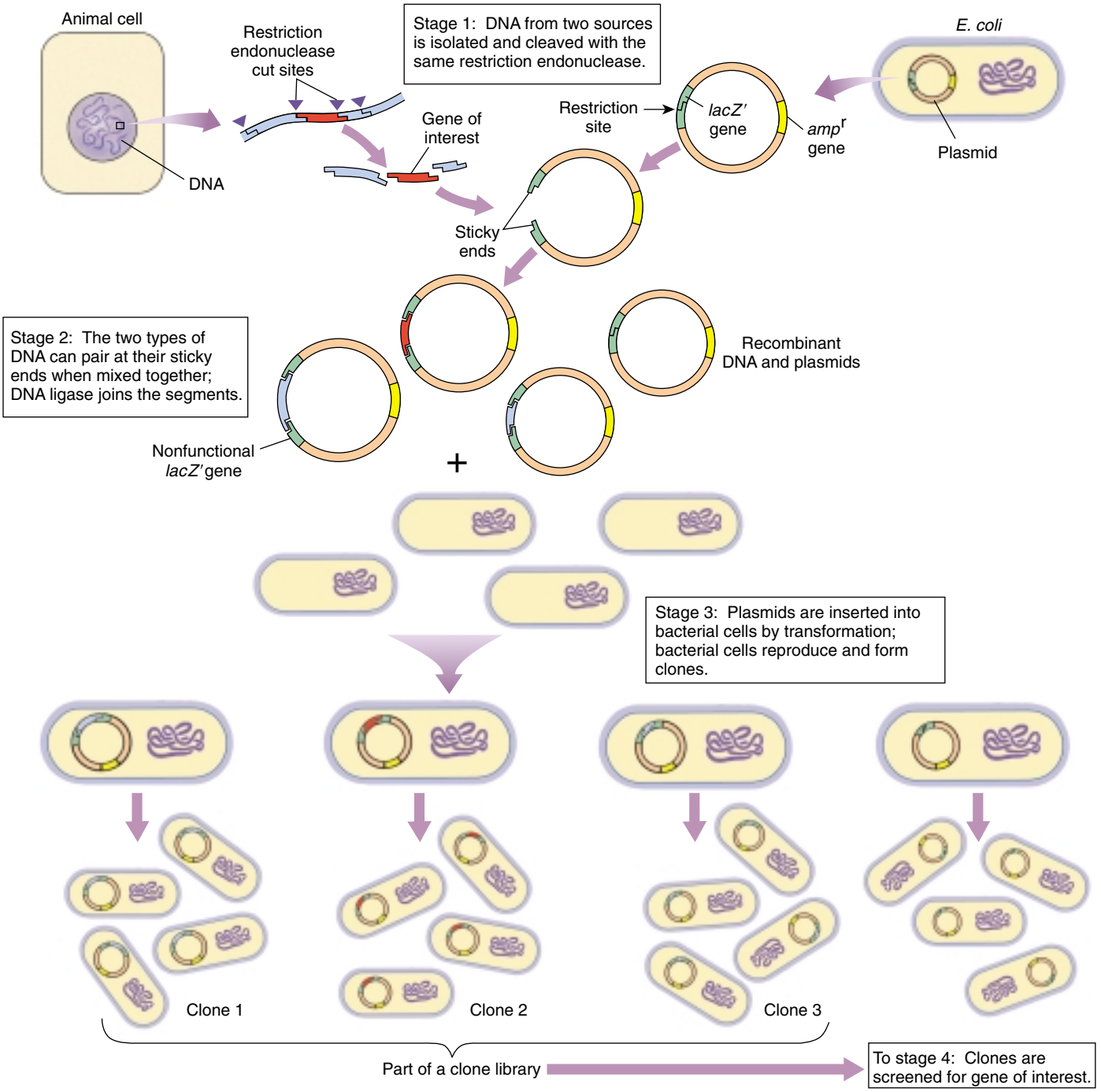


FIGURE 19.5
Stages in a genetic engineering experiment. In stage 1, DNA containing the gene of interest (in this case, from an animal cell) and DNA from a plasmid are cleaved with the same restriction endonuclease. The genes *amp*^{r} and *lacZ*' are contained within the plasmid and used for screening a clone (stage 4). In stage 2, the two cleaved sources of DNA are mixed together and pair at their sticky ends. In stage 3, the recombinant DNA is inserted into a bacterial cell, which reproduces and forms clones. In stage 4, the bacterial clones will be screened for the gene of interest.

Stage 4: Screening

The clones containing a specific DNA fragment of interest, often a fragment that includes a particular gene, are identified from the clone library. Let's examine this stage in more detail, as it is generally the most challenging in any genetic engineering experiment.

4–I: The Preliminary Screening of Clones. Investigators initially try to eliminate from the library any clones that do not contain vectors, as well as clones whose vectors do not contain fragments of the source DNA. The first category of clones can be eliminated by employing a vector with a gene that confers resistance to a specific antibiotic, such as tetracycline, penicillin, or ampicillin. In figure 19.6*a*, the gene *amp*^{r} is incorporated into the plasmid and confers resistance to the antibiotic ampicillin. When the clones are exposed to a medium containing that antibiotic, only clones that contain the vector will be resistant to the antibiotic and able to grow.

One way to eliminate clones with vectors that do not have an inserted DNA fragment is to use a vector that, in addition to containing antibiotic resistance genes, contains the *lacZ*' gene which is required to produce β-galactosidase, an enzyme that enables the cells to metabolize the sugar, X-gal. Metabolism of X-gal results in the formation of a blue reaction product, so any cells whose vectors contain a functional version of this gene will turn blue in the presence of X-gal (figure 19.6*b*). However, if one uses a restriction endonuclease whose recognition sequence lies within the *lacZ*' gene, the gene will be interrupted when recombinants are formed, and the cell will be unable to metabolize X-gal. Therefore, cells with vectors that contain a fragment of source DNA should remain colorless in the presence of X-gal.

Any cells that are able to grow in a medium containing the antibiotic but don't turn blue in the medium with X-gal must have incorporated a vector with a fragment of source DNA. Identifying cells that have a *specific* fragment of the source DNA is the next step in screening clones.

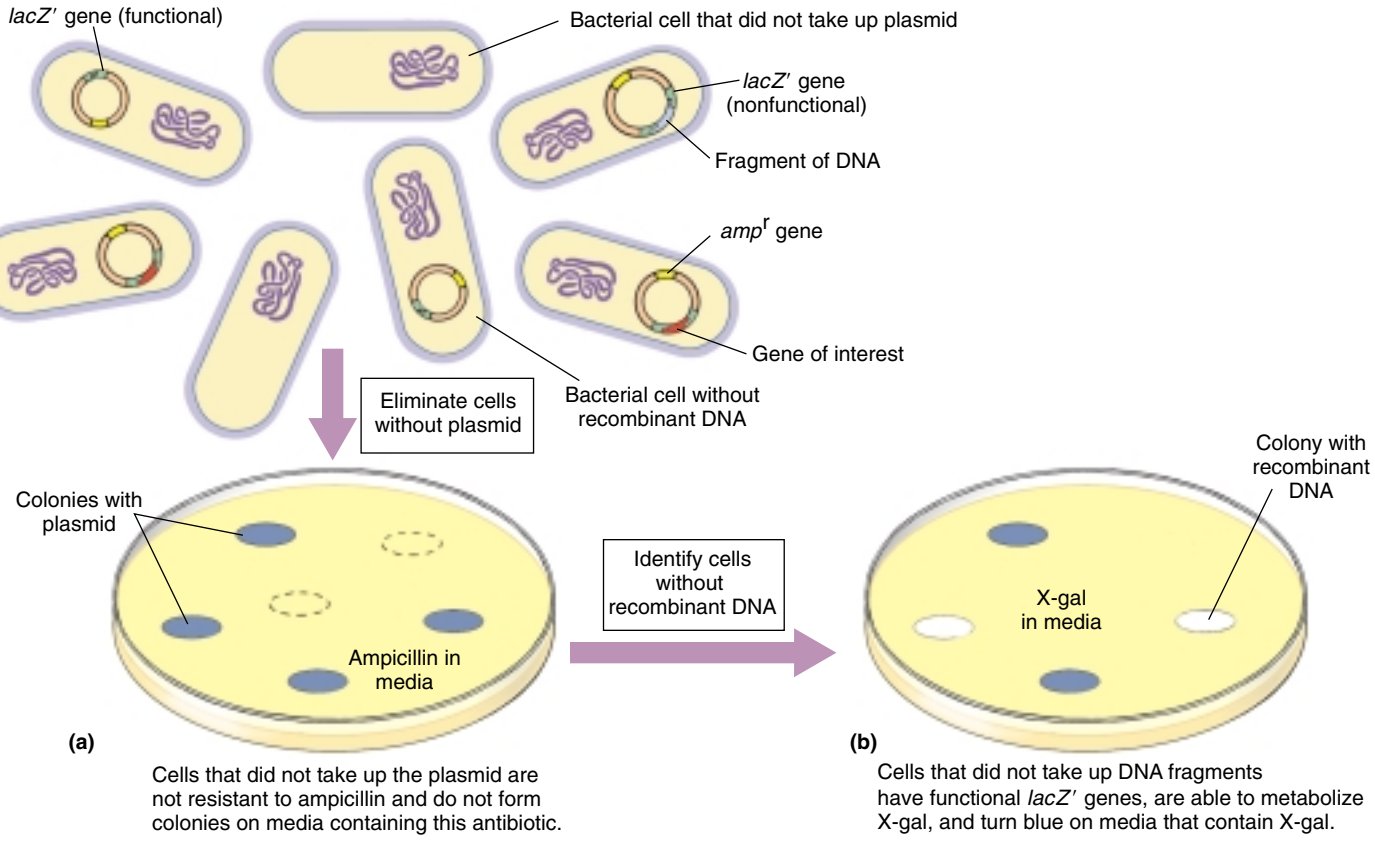


FIGURE 19.6
Stage 4-I: Using antibiotic resistance and X-gal as preliminary screens of restriction fragment clones. Bacteria are transformed with recombinant plasmids that contain a gene (*amp*^{r}) that confers resistance to the antibiotic ampicillin and a gene (*lacZ*') that is required to produce β-galactosidase, the enzyme which enables the cells to metabolize the sugar X-gal. (*a*) Only those bacteria that have incorporated a plasmid will be resistant to ampicillin and will grow on a medium that contains the antibiotic. (*b*) Ampicillin-resistant bacteria will be able to metabolize X-gal if their plasmid does *not* contain a DNA fragment inserted in the *lacZ*' gene; such bacteria will turn blue when grown on a medium containing X-gal. Bacteria with a plasmid that has a DNA fragment inserted within the *lacZ*' gene will not be able to metabolize X-gal and, therefore, will remain colorless in the presence of X-gal.

4–II: Finding the Gene of Interest. A clone library may contain anywhere from a few dozen to many thousand individual fragments of source DNA. Many of those fragments will be identical, so to assemble a complete library of the entire source genome, several hundred thousand clones could be required. A complete *Drosophila* (fruit fly) library, for example, contains more than 40,000 different clones; a complete human library consisting of fragments 20 kilobases long would require close to a million clones. To search such an immense library for a clone that contains a fragment corresponding to a particular gene requires ingenuity, but many different approaches have been successful.

The most general procedure for screening clone libraries to find a particular gene is **hybridization** (figure 19.7). In this method, the cloned genes form base-pairs with complementary sequences on another nucleic acid. The complementary nucleic acid is called a **probe** because it is used to probe for the presence of the gene of interest. At least part of the nucleotide sequence of the gene of interest must be known to be able to construct the probe.

In this method of screening, bacterial colonies containing an inserted gene are grown on agar. Some cells are transferred to a filter pressed onto the colonies, forming a replica of the plate. The filter is then treated with a solution that denatures the bacterial DNA and that contains a radioactively labeled probe. The probe hybridizes with complementary single-stranded sequences on the bacterial DNA.

When the filter is laid over photographic film, areas that contain radioactivity will expose the film (autoradiography). Only colonies which contain the gene of interest hybridize with the radioactive probe and emit radioactivity onto the film. The pattern on the film is then compared to the original master plate, and the gene-containing colonies may be identified.

Genetic engineering generally involves four stages: cleaving the source DNA; making recombinants; cloning copies of the recombinants; and screening the cloned copies for the desired gene. Screening can be achieved by making the desired clones resistant to certain antibiotics and giving them other properties that make them readily identifiable.

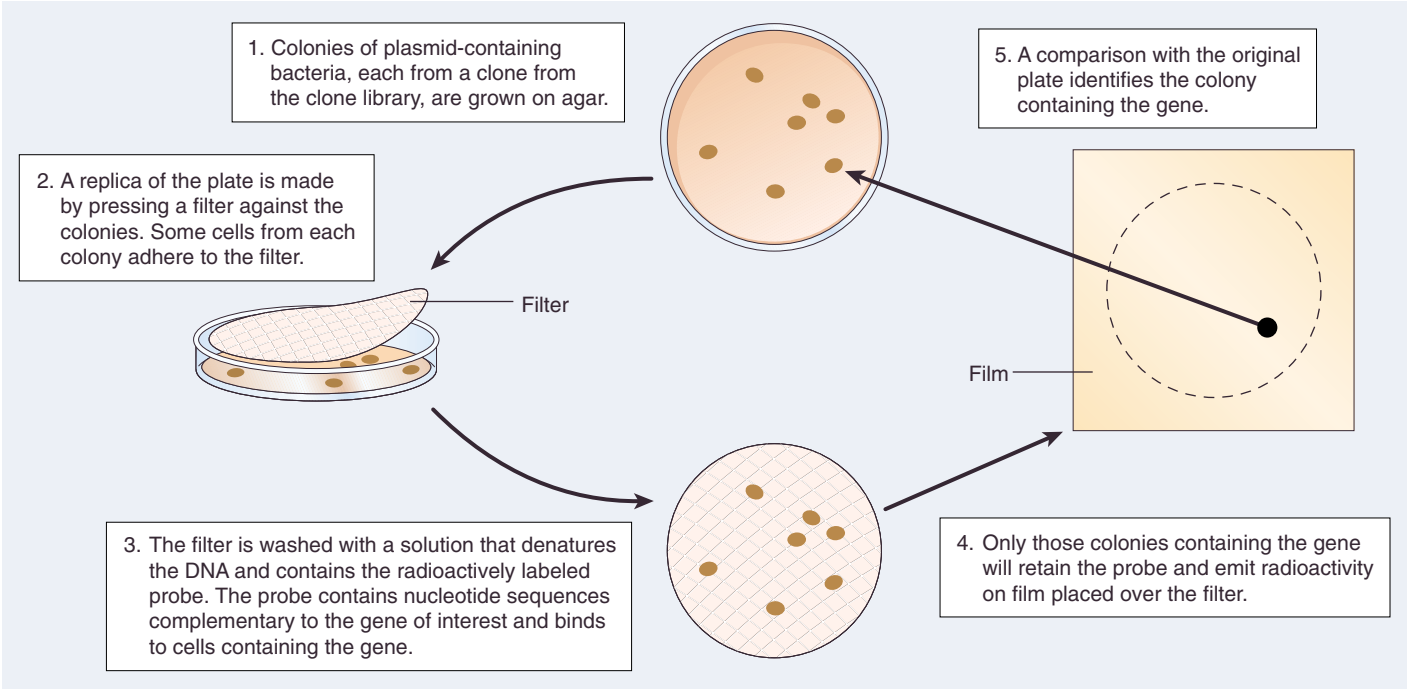


FIGURE 19.7
Stage 4-II: Using hybridization to identify the gene of interest. (*1*) Each of the colonies on these bacterial culture plates represents millions of clones descended from a single cell. To test whether a certain gene is present in any particular clone, it is necessary to identify colonies whose cells contain DNA that hybridizes with a probe containing DNA sequences complementary to the gene. (*2*) Pressing a filter against the master plate causes some cells from each colony to adhere to the filter. (*3*) The filter is then washed with a solution that denatures the DNA and contains the radioactively labeled probe. (*4*) Only those colonies that contain DNA that hybridizes with the probe, and thus contain the gene of interest, will expose film in autoradiography. (*5*) The film is then compared to the master plate to identify the gene-containing colony.

Working with Gene Clones

Once a gene has been successfully cloned, a variety of procedures are available to characterize it.

Getting Enough DNA to Work with: The Polymerase Chain Reaction

Once a particular gene is identified within the library of DNA fragments, the final requirement is to make multiple copies of it. One way to do this is to insert the identified fragment into a bacterium; after repeated cell divisions, millions of cells will contain copies of the fragment. A far more direct approach, however, is to use DNA polymerase to copy the gene sequence of interest through the **polymerase chain reaction** (**PCR;** figure 19.8). Kary Mullis developed PCR in 1983 while he was a staff chemist at the Cetus Corporation; in 1993, it won him the Nobel Prize in Chemistry. PCR can amplify specific sequences or add sequences (such as endonuclease recognition sequences) as primers to cloned DNA. There are three steps in PCR:

Step 1: Denaturation. First, an excess of primer (typically a synthetic sequence of 20 to 30 nucleotides) is mixed with the DNA fragment to be amplified. This mixture of primer and fragment is heated to about 98° C. At this temperature, the double-stranded DNA fragment dissociates into single strands.

Step 2: Annealing of Primers. Next, the solution is allowed to cool to about 60°C. As it cools, the single strands of DNA reassociate into double strands. However, because of the large excess of primer, each strand of the fragment base-pairs with a complementary primer flanking the region to be amplified, leaving the rest of the fragment single-stranded.

Step 3: Primer Extension. Now a very heat-stable type of DNA polymerase, called Taq polymerase (after the thermophilic bacterium *Thermus aquaticus*, from which Taq is extracted) is added, along with a supply of all four nucleotides. Using the primer, the polymerase copies the rest of the fragment as if it were replicating DNA. When it is done, the primer has been lengthened into a complementary copy of the entire single-stranded fragment. Because *both* DNA strands are replicated, there are now two copies of the original fragment.

Steps 1 to 3 are now repeated, and the two copies become four. It is not necessary to add any more polymerase, as the heating step does not harm this particular enzyme. Each heating and cooling cycle, which can be as short as 1 or 2 minutes, doubles the number of DNA molecules. After 20 cycles, a single fragment produces more than one million (2^{20}) copies! In a few hours, 100 billion copies of the fragment can be manufactured.

PCR, now fully automated, has revolutionized many aspects of science and medicine because it allows the investigation of minute samples of DNA. In criminal investigations, "DNA fingerprints" are prepared from the cells in a tiny speck of dried blood or at the base of a single human hair. Physicians can detect genetic defects in very early embryos by collecting a few sloughed-off cells and amplifying their DNA. PCR could also be used to examine the DNA of historical figures such as Abraham Lincoln and of now-extinct species, as long as even a minuscule amount of their DNA remains intact.

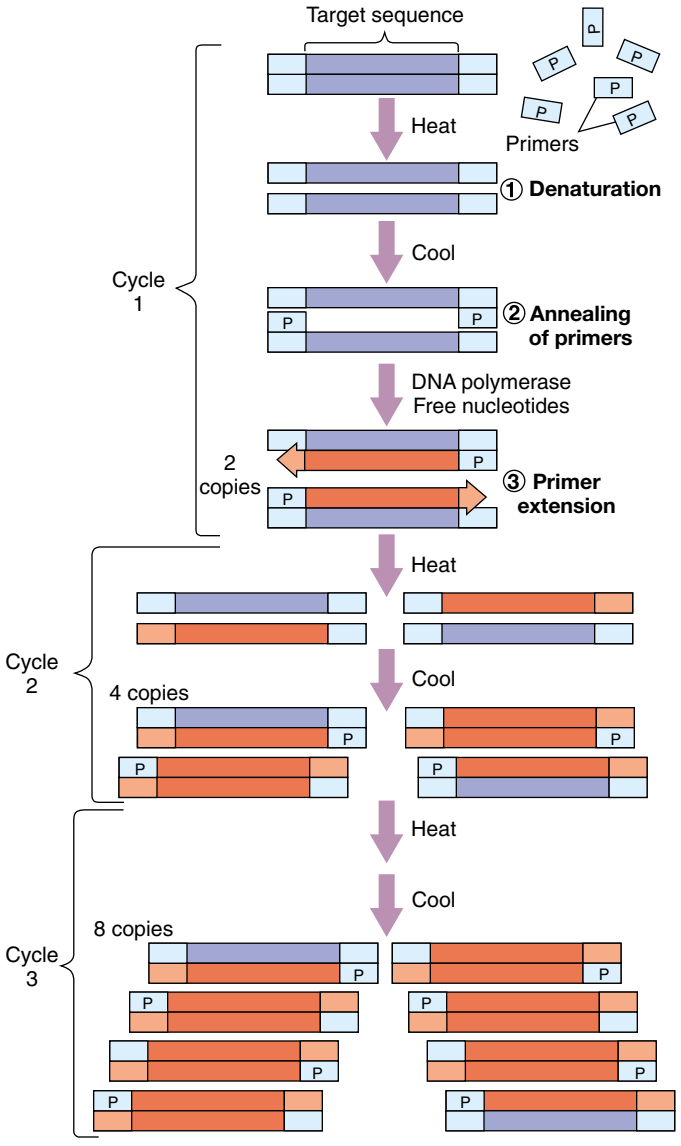


FIGURE 19.8
The polymerase chain reaction. (*1*) *Denaturation.* A solution containing primers and the DNA fragment to be amplified is heated so that the DNA dissociates into single strands. (*2*) *Annealing of primers.* The solution is cooled, and the primers bind to complementary sequences on the DNA flanking the region to be amplified. (*3*) *Primer extension.* DNA polymerase then copies the remainder of each strand, beginning at the primer. Steps 1–3 are then repeated with the replicated strands. This process is repeated many times, each time doubling the number of copies, until enough copies of the DNA fragment exist for analysis.

Identifying DNA: Southern Blotting

Once a gene has been cloned, it may be used as a probe to identify the same or a similar gene in another sample (figure 19.9). In this procedure, called a **Southern blot,** DNA from the sample is cleaved into restriction fragments with a restriction endonuclease, and the fragments are spread apart by gel electrophoresis. The double-stranded helix of each DNA fragment is then denatured into single strands by making the pH of the gel basic, and the gel is "blotted" with a sheet of nitrocellulose, transferring some of the DNA strands to the sheet. Next, a probe consisting of purified, single-stranded DNA corresponding to a specific gene (or mRNA transcribed from that gene) is poured over the sheet. Any fragment that has a nucleotide sequence complementary to the probe's sequence will hybridize (base-pair) with the probe. If the probe has been labeled with ^{32}P, it will be radioactive, and the sheet will show a band of radioactivity where the probe hybridized with the complementary fragment.

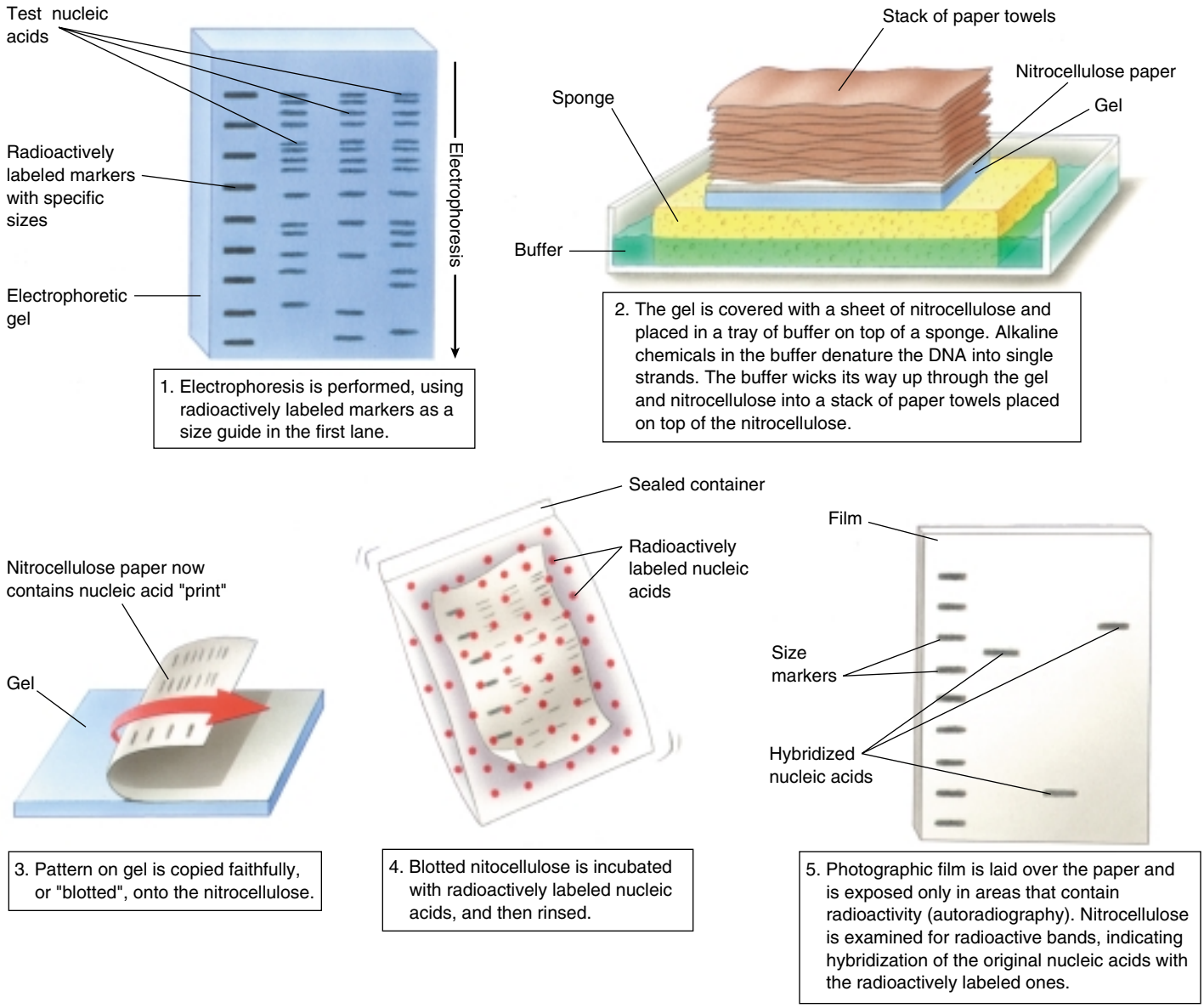


FIGURE 19.9
The Southern blot procedure. E. M. Southern developed this procedure in 1975 to enable DNA fragments of interest to be visualized in a complex sample containing many other fragments of similar size. The DNA is separated on a gel, then transferred ("blotted") onto a solid support medium such as nitrocellulose paper or a nylon membrane. It is then incubated with a radioactive single-strand copy of the gene of interest, which hybridizes to the blot at the location(s) where there is a fragment with a complementary sequence. The positions of radioactive bands on the blot identify the fragments of interest.

Distinguishing Differences in DNA: RFLP Analysis

Often a researcher wishes not to find a specific gene, but rather to identify a particular *individual* using a specific gene as a marker. One powerful way to do this is to analyze **restriction fragment length polymorphisms,** or **RFLPs** (figure 19.10). Point mutations, sequence repetitions, and transposons (see chapter 18) that occur within or between the restriction endonuclease recognition sites will alter the length of the DNA fragments (restriction fragments) the restriction endonucleases produce. DNA from different individuals rarely has exactly the same array of restriction sites and distances between sites, so the population is said to be polymorphic (having many forms) for their restriction fragment patterns. By cutting a DNA sample with a particular restriction endonuclease, separating the fragments according to length on an electrophoretic gel, and then using a radioactive probe to identify the fragments on the gel, one can obtain a pattern of bands often unique for each region of DNA analyzed. These "DNA fingerprints" are used in forensic analysis during criminal investigations. RFLPs are also useful as markers to identify particular groups of people at risk for some genetic disorders.

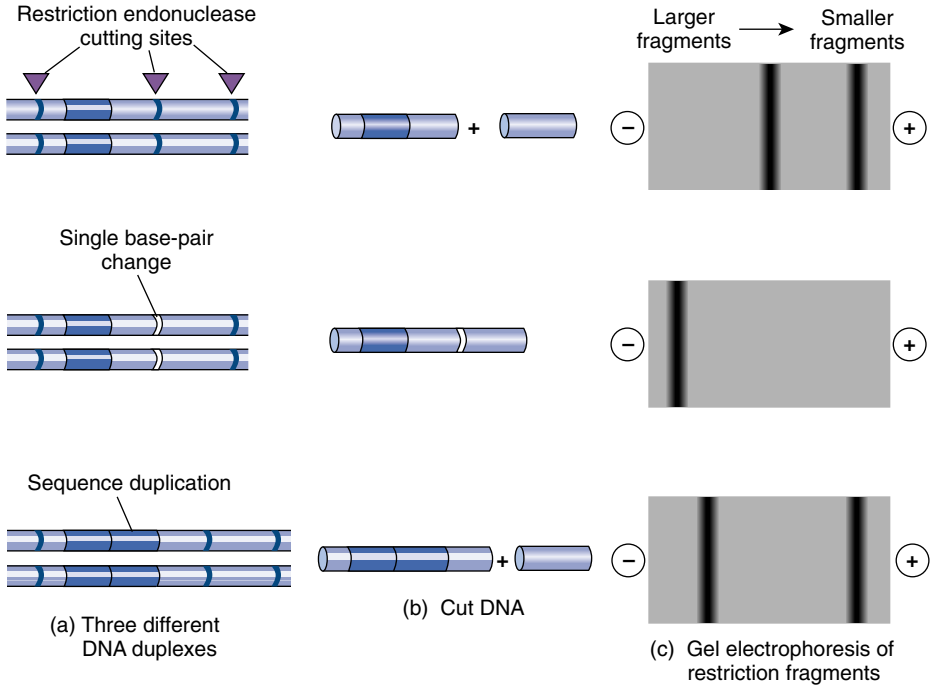


FIGURE 19.10
Restriction fragment length polymorphism (RFLP) analysis. (*a*) Three samples of DNA differ in their restriction sites due to a single base-pair substitution in one case and a sequence duplication in another case. (*b*) When the samples are cut with a restriction endonuclease, different numbers and sizes of fragments are produced. (*c*) Gel electrophoresis separates the fragments, and different banding patterns result.

Making an Intron-Free Copy of a Eukaryotic Gene

Recall from chapter 15 that eukaryotic genes are encoded in exons separated by numerous nontranslated introns. When the gene is transcribed to produce the primary transcript, the introns are cut out during RNA processing to produce the mature mRNA transcript. When transferring eukaryotic genes into bacteria, it is desirable to transfer DNA already processed this way, instead of the raw eukaryotic DNA, because bacteria lack the enzymes to carry out the processing. To do this, genetic engineers first isolate from the cytoplasm the mature mRNA corresponding to a particular gene. They then use an enzyme called reverse transcriptase to make a DNA version of the mature mRNA transcript (figure 19.11). The single strand of DNA can then serve as a template for the synthesis of a complementary strand. In this way, one can produce a double-stranded molecule of DNA that contains a gene lacking introns. This molecule is called **complementary DNA,** or **cDNA.**

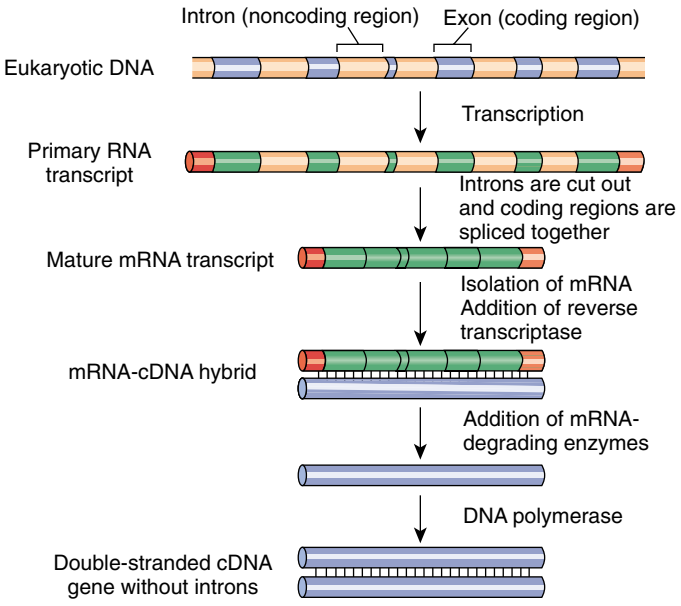


FIGURE 19.11
The formation of cDNA. A mature mRNA transcript is isolated from the cytoplasm of a cell. The enzyme reverse transcriptase is then used to make a DNA strand complementary to the processed mRNA. That newly made strand of DNA is the template for the enzyme DNA polymerase, which assembles a complementary DNA strand along it, producing cDNA, a double-stranded DNA version of the intron-free mRNA.

Sequencing DNA: The Sanger Method

Most DNA sequencing is currently done using the "chain termination" technique developed initially by Frederick Sanger, for which he earned his *second* Nobel Prize (figure 19.12). (1) A short single-stranded primer is added to the end of a single-stranded DNA fragment of unknown sequence. The primer provides a 3′ end for DNA polymerase. (2) The primed fragment is added, along with DNA polymerase and a supply of all four deoxynucleotides (d-nucleotides), to four synthesis tubes. Each contains a different *di*deoxynucleotide (dd-nucleotide); such nucleotides lack both the 2′ and the 3′ —OH groups and are thus chain-terminating. The first tube, for example, contains ddATP and stops synthesis whenever ddA is incorporated into DNA instead of dATP. Because of the relatively low concentration of ddATP compared to dATP, ddA will not necessarily be added to the first A site; this tube will contain a series of fragments of different lengths, corresponding to the different distances the polymerase traveled from the primer before a ddA was incorporated. (3) These fragments can be separated according to size by electrophoresis. (4) A radioactive label (here dATP*) allows the fragments to be visualized on X-ray film, and the newly made sequence can be read directly from the film. Try it. (5) The original fragment has the complementary sequence.

Techniques such as Southern blotting and PCR enable investigators to identify specific genes and produce them in large quantities, while RFLP analysis and the Sanger method identify individuals and unknown gene sequences.

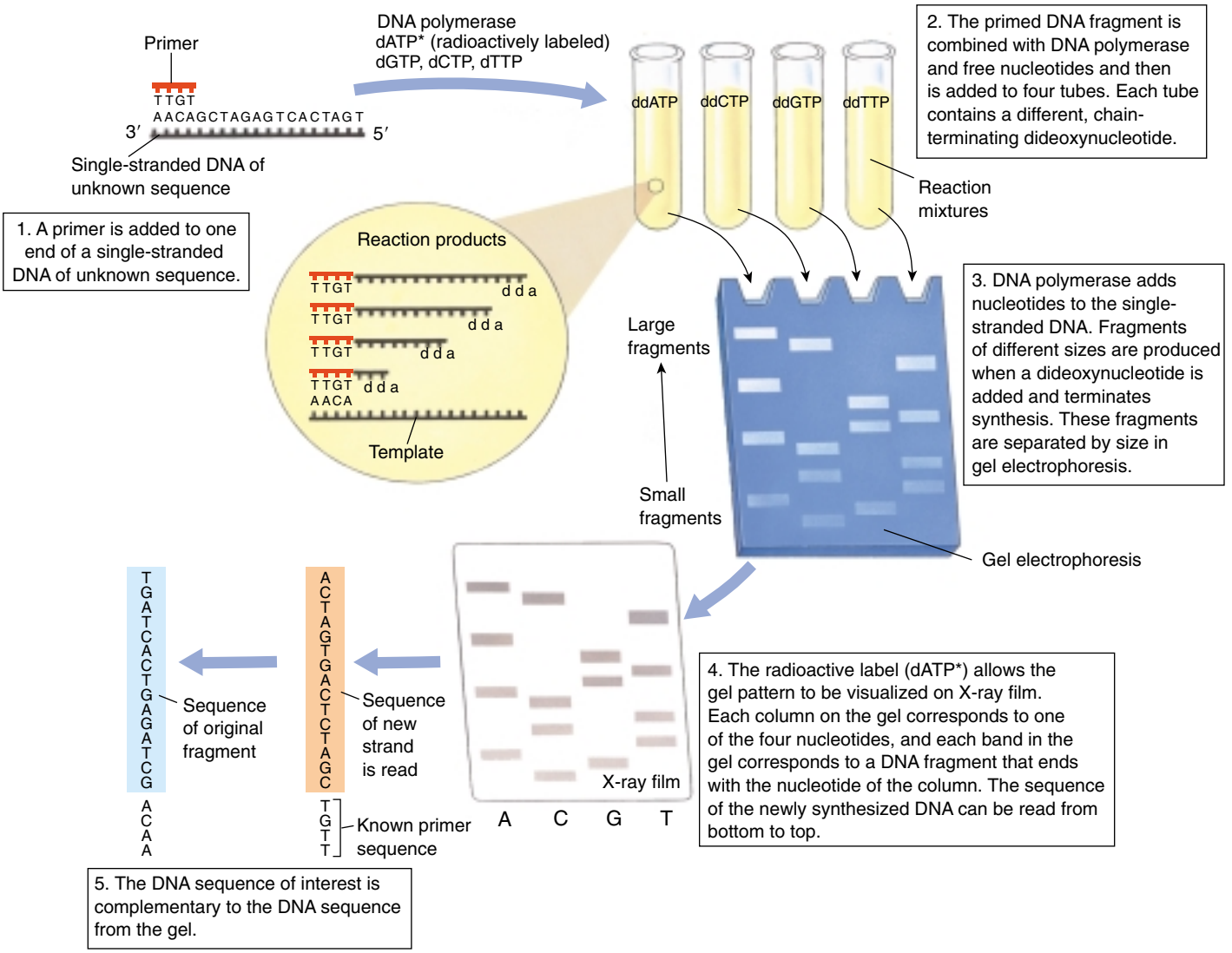


FIGURE 19.12
The Sanger dideoxynucleotide sequencing method.

19.3 Biotechnology is producing a scientific revolution.

DNA Sequence Technology

The 1980s saw an explosion of interest in **biotechnology,** the application of genetic engineering to practical human problems. Let us examine some of the major areas where these techniques have been put to use.

Genome Sequencing

Genetic engineering techniques are enabling us to learn a great deal more about the human genome. Several clonal libraries of the human genome have been assembled, using large-size restriction fragments. Any cloned gene can now be localized to a specific chromosomal site by using probes to detect in situ hybridization (that is, binding between the probe and a complementary sequence on the chromosome). Genes are now being mapped at an astonishing rate: genes that contribute to dyslexia, obesity, and cholesterol-proof blood are some of the important ones that were mapped in 1994 and 1995 alone! With an understanding of where specific genes are located in the human genome and how they work, it is not difficult to imagine a future in which virtually any genetic disease could be treated or perhaps even cured with gene therapy. As we mentioned in chapter 13, some success has already been reported in treating patients who have cystic fibrosis with a genetically corrected version of the cystic fibrosis gene.

An exciting scientific by-product of the human genome project has been the complete genome sequencing of many microorganisms with smaller genomes, on the order of a few Mb (table 19.1). In general, about half of the genes prove to have a known function; what the other half of the genes are doing is a complete mystery. The first eukaryotic genome to be sequenced in its entirety was that of brewer's yeast *Saccharomyces cerevisiae;* many of its approximately 6000 genes have a similar structure to some human genes. The complete sequences of many much larger genomes have recently been completed, including the malarial *Plasmodium* parasite (30 Mb), the nematode (100 Mb), the plant *Arabidopsis* (100 Mb) (figure 19.13), the fruit fly *Drosophila* (120 Mb), and the mouse (300 Mb).

The international scientific community has over the last several years mounted a major effort to sequence the entire human genome. Because the human genome contains some 3000 Mb (million nucleotide base-pairs), this task has presented no small challenge. Rapid progress was made possible by the use of so-called shotgun cloning techniques, in which the entire genome is first fragmented, then each of the fragments is sequenced by automated machines, and finally computers use overlaps to order the fragments. All but a small portion of the sequence was completed by the beginning of the year 2000.

Table 19.1 Genome Sequencing Projects

Organism	Genome Size (Mb)	Description
ARCHAEBACTERIA		
Methanococcus jannaschi	1.7	Extreme thermophile
EUBACTERIA		
Escherichia coli	4.6	Laboratory standard
FUNGI		
Saccharomyces cerevisiae	13	Baker's yeast
PROTIST		
Plasmodium	30	Malarial parasite
PLANT		
Arabidopsis thaliana	100	Relative of mustard plant
ANIMAL		
Caenorhabditis elegans	100	Nematode
Drosophila melanogaster	120	Fruit fly
Mus musculus	300	Mouse
Homo sapiens	3000	Human

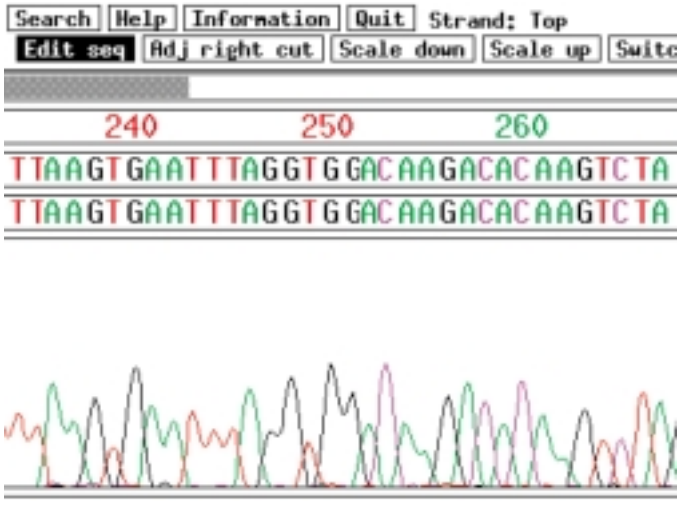


FIGURE 19.13
Part of the genome sequence of the plant *Arabidopsis*. Data from an automated DNA-sequencing run shows the nucleotide sequence for a small section of the *Arabidopsis* genome. Automated DNA sequencing has greatly increased the speed at which genomes can be sequenced.

DNA Fingerprinting

Figure 19.14 shows the DNA fingerprints a prosecuting attorney presented in a rape trial in 1987. They consisted of autoradiographs, parallel bars on X-ray film resembling the line patterns of the universal price code found on groceries. Each bar represents the position of a DNA restriction endonuclease fragment produced by techniques similar to those described in figures 19.4 and 19.10. The lane with many bars represents a standardized control. Two different probes were used to identify the restriction fragments. A vaginal swab had been taken from the victim within hours of her attack; from it semen was collected and the semen DNA analyzed for its restriction endonuclease patterns.

Compare the restriction endonuclease patterns of the semen to that of the suspect Andrews. You can see that the suspect's two patterns match that of the rapist (and are not at all like those of the victim). Clearly the semen collected from the rape victim and the blood sample from the suspect came from the same person. The suspect was Tommie Lee Andrews, and on November 6, 1987, the jury returned a verdict of guilty. Andrews became the first person in the United States to be convicted of a crime based on DNA evidence.

Since the Andrews verdict, DNA fingerprinting has been admitted as evidence in more than 2000 court cases (figure 19.15). While some probes highlight profiles shared by many people, others are quite rare. Using several probes, identity can be clearly established or ruled out.

Just as fingerprinting revolutionized forensic evidence in the early 1900s, so DNA fingerprinting is revolutionizing it today. A hair, a minute speck of blood, a drop of semen can all serve as sources of DNA to damn or clear a suspect. As the man who analyzed Andrews' DNA says: "It's like leaving your name, address, and social security number at the scene of the crime. It's that precise." Of course, laboratory analyses of DNA samples must be carried out properly—sloppy procedures could lead to a wrongful conviction. After widely publicized instances of questionable lab procedures, national standards are being developed.

The genomes of several organisms have been completely sequenced. When DNA is digested with restriction endonucleases, distinctive profiles on electrophoresis gels can be used to identify the individual that was the source of the tissue.

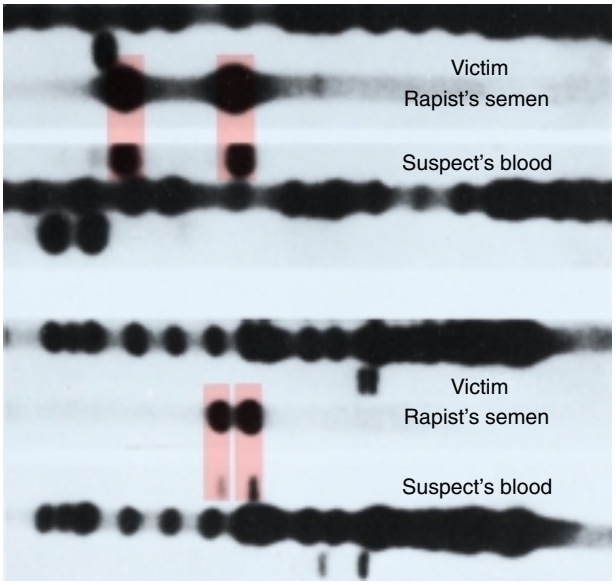


FIGURE 19.14
Two of the DNA profiles that led to the conviction of Tommie Lee Andrews for rape in 1987. The two DNA probes seen here were used to characterize DNA isolated from the victim, the semen left by the rapist, and the suspect. The dark channels are multiband controls. There is a clear match between the suspect's DNA and the DNA of the rapist's semen in these.

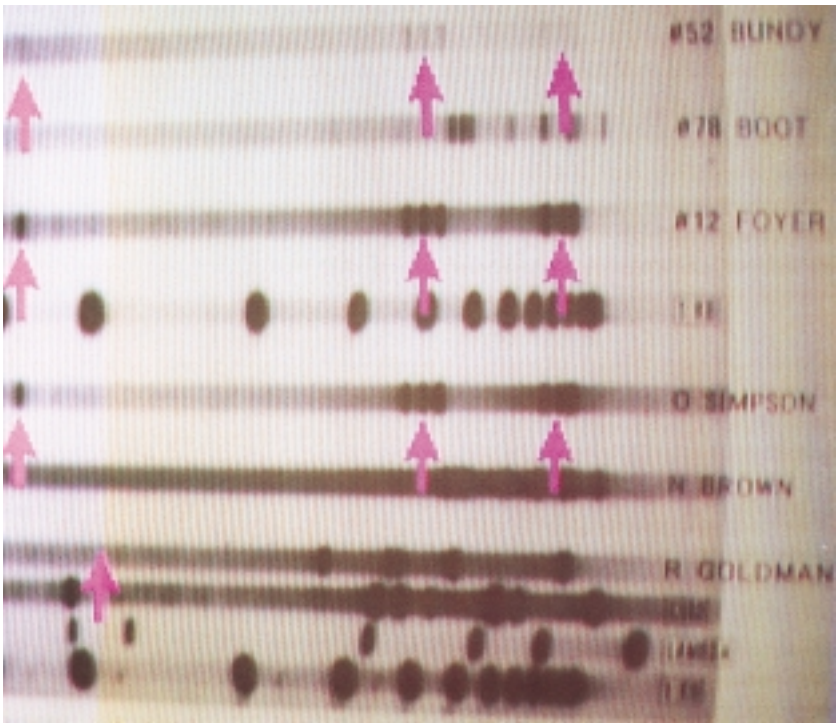


FIGURE 19.15
The DNA profiles of O. J. Simpson and blood samples from the murder scene of his former wife from his highly publicized and controversial murder trial in 1995.

Biochips

A biochip, also called a gene microarray, is a square of glass smaller than a postage stamp, covered with millions of strands of DNA like blades of grass. Biochips were invented nine years ago by gene scientist Stephen Fodor. In a flash of insight, he saw that photolithography, the process used to etch semiconductor circuits into silicon, could also be used to assemble particular DNA molecules on a chip—a biochip.

Think of the chip surface as a field of assembly sites, much as a TV screen is a field of colored dots. Just as a scanning beam moves over each individual TV dot instructing it to be red, green, or blue (the three components of color), so a scanning beam moves over each biochip spot, commanding the addition there of a base to a growing strand of DNA. A computer, by varying the wavelength of the scanning beam, determines which of four possible nucleotides is added to the growing DNA strand anchored to each spot. When the entire chip has been scanned, each DNA strand has been lengthened one nucleotide unit. The computer repeats the process, layer by layer, until each DNA strand is an entire gene or gene fragment. One biochip made in this way contains hundreds of thousands of specific gene sequences.

How could you use such a biochip to delve into a person's genes? All you would have to do is to obtain a little of the person's DNA, say from a blood sample or even a bit of hair. Flush fluid containing the DNA over the biochip surface. Every place that the DNA has a gene matching one of the biochip strands, it will stick to it in a way the computer can detect.

Now here is where it gets interesting. The mad rush to sequence the human genome is over. The gene research firm Celera has recently announced it has essentially completed the sequence, with over 90% of genes done. Already the researchers are busily comparing their consensus "reference sequence" to the DNA of individual people, and noting any differences they detect. Called single nucleotide polymorphisms, or SNPs (pronounced "snips"), these spot differences in the identity of particular nucleotides collectively record every way in which a particular individual differs from the reference sequence. Some SNPs cause diseases like cystic fibrosis or sickle cell anemia. Others may give you red hair or elevated cholesterol in your blood. As the human genome project charges toward completion, its researchers are excitedly assembling a huge database of SNPs. Research indicates that SNPs can be expected to occur at a frequency of about one per thousand nucleotides, scattered about randomly over the chromosomes. Each of us thus differs from the standard "type sequence" in several thousand nucleotide SNPs. Everything genetic about you that is diferent from a stranger you meet is caused by a few thousand SNPs; otherwise you and that stranger are identical.

How Biochips Can Be Used to Screen for Cancer

One of the biggest decisions facing an oncologist (cancer doctor) treating a tumor is to select the proper treatment. Most cancer cells look alike, although the tumors may in fact be caused by quite different forms of cancer. If the oncologist could clearly identify the cancer, very targeted therapies might be possible. Unable to tell the difference for sure, however, oncologists take no chances. Tumors are treated with therapy that attacks all cancers, usually with severe side effects.

This year Boston researchers Todd Golub and Eric Lander took a vital step towards treating cancer, using new DNA technology to sniff out the differences between different forms of a deadly cancer of the immune system. Golub and Lander worked with biochips.

The way to tell the difference between two kinds of cancer is to compare the mutations that led to the cancer in the first place. Biologists call such gene changes mutations. The mutations that cause many lung cancers are caused by a tobacco-induced alteration of a single DNA nucleotide in one gene. Such spot differences between the version of a gene one person has and another person has, or a cancer patient has, are examples of SNPs.

Golub and Lander obtained bone marrow cells from patients with two types of leukemia (cancer of white blood cells), and exposed DNA from each to biochips containing all known human genes, 6817 in all (figure 19.16). Using high-speed computer programs, Golub and Lander examined each of the 6817 positions on the chip. The two forms of leukemia each showed gene changes from normal, but, importantly, the changes were different in each case! Each had their own characteristic SNP.

Biochips thus may offer a quick and reliable way to identify any type of cancer. Just look and see what SNP is present.

The Use of Gene Chips Will Soon Be Widespread

Biochip technology is likely to dominate medicine in the coming millennium, a prospect both exciting and scary. Researchers have announced plans to compile a database of hundreds of thousands of SNPs over the next two years. Screening SNPs and comparing them to known SNP databases will soon allow doctors to screen each of us for copies of genes leading to genetic diseases. Many genetic diseases are associated with SNPs, including cystic fibrosis and muscular dystrophy.

Biochips Raise Critical Issues of Personal Privacy

The scary part is SNPs on chips. Researchers plan to have identified some 300,000 different SNPs by 2001, all of which could reside on a single biochip. When your DNA is flushed over a SNP biochip, the sequences that light up

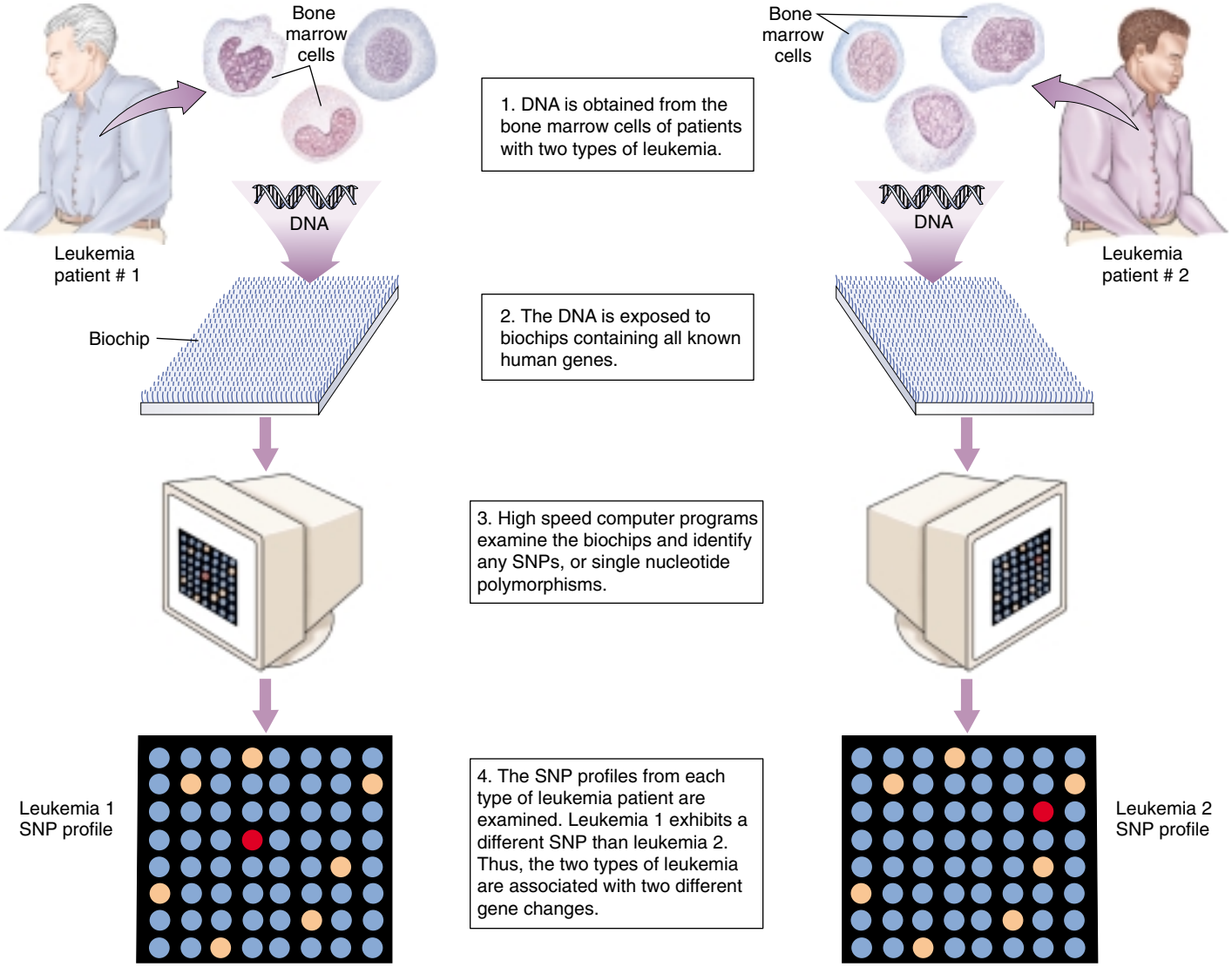


FIGURE 19.16
Biochips can help in identifying precise forms of cancer.

will instantly reveal your SNP profile. The genetic characteristics that make you you, genes that might affect your health, your behavior, your future potential—all are there to be read by anyone clever enough to interpret the profile.

To what extent are you your genes? Scientists fight about this question, and no one really knows the answer. It is clear that much of what each of us is like is strongly affected by our genetic makeup. Researchers have proven beyond any real dispute that intelligence and major personality traits like aggressiveness and inquisitiveness are about 80% heritable (that is, 80% of the variation in these traits reflects variation in genes).

Your SNP profile will reflect all of this variation, a table of contents of your chromosomes, a molecular window to who you are. When millions of such SNP profiles have been gathered over the coming years, computers will be able to identify other individuals with profiles like yours, and, by examining health records, standard personality tests, and the like, correlate parts of your profile with particular traits. Even behavioral characteristics involving many genes, which until now have been thought too complex to ever analyze, cannot resist a determined assault by a computer comparing SNP profiles.

A biochip is a discrete collection of gene fragments on a stamp-sized chip that can be used to screen for the presence of particular gene variants. Biochips allow rapid screening of gene profiles, a tool that promises to have a revolutionary impact on medicine and society.

Medical Applications

Pharmaceuticals

The first and perhaps most obvious commercial application of genetic engineering was the introduction of genes that encode clinically important proteins into bacteria. Because bacterial cells can be grown cheaply in bulk (fermented in giant vats, like the yeasts that make beer), bacteria that incorporate recombinant genes can synthesize large amounts of the proteins those genes specify. This method has been used to produce several forms of human insulin and interferon, as well as other commercially valuable proteins such as growth hormone (figure 19.17) and erythropoietin, which stimulates red blood cell production.

Among the medically important proteins now manufactured by these approaches are **atrial peptides,** small proteins that may provide a new way to treat high blood pressure and kidney failure. Another is **tissue plasminogen activator,** a human protein synthesized in minute amounts that causes blood clots to dissolve and may be effective in preventing and treating heart attacks and strokes.

A problem with this general approach has been the difficulty of separating the desired protein from the others the bacteria make. The purification of proteins from such complex mixtures is both time-consuming and expensive, but it is still easier than isolating the proteins from the tissues of animals (for example, insulin from hog pancreases), which is how such proteins used to be obtained. Recently, however, researchers have succeeded in producing RNA transcripts of cloned genes; they can then use the transcripts to produce only these proteins in a test tube containing the transcribed RNA, ribosomes, cofactors, amino acids, tRNA, and ATP.

Gene Therapy

In 1990, researchers first attempted to combat genetic defects by the transfer of human genes. When a hereditary disorder is the result of a single defective gene, an obvious way to cure the disorder is to add a working copy of the gene. This approach is being used in an attempt to combat cystic fibrosis, and it offers potential for treating muscular dystrophy and a variety of other disorders (table 19.2). One of the first successful attempts was the transfer of a gene encoding the enzyme adenosine deaminase into the bone marrow of two girls suffering from a rare blood disorder caused by the lack of this enzyme. However, while many clinical trials are underway, no others have yet proven successful. This extremely promising approach will require a lot of additional effort.

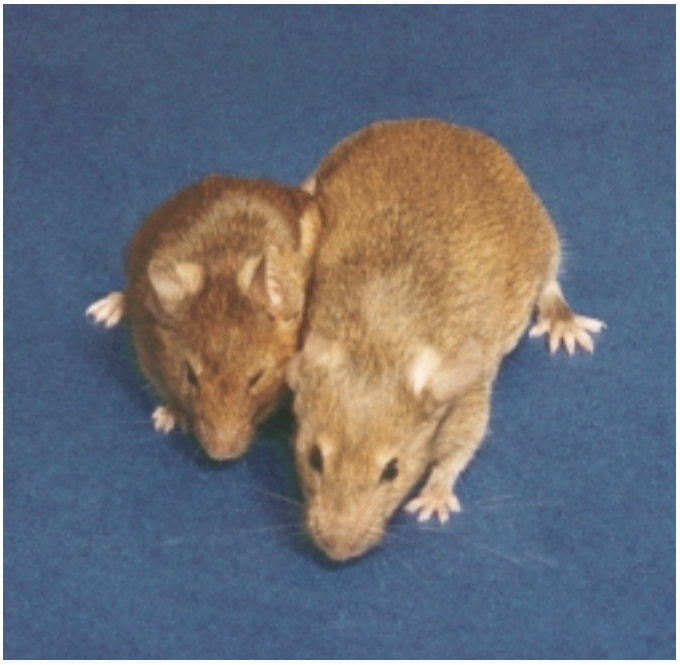


FIGURE 19.17
Genetically engineered human growth hormone. These two mice are genetically identical, but the large one has one extra gene: the gene encoding human growth hormone. The gene was added to the mouse's genome by genetic engineers and is now a stable part of the mouse's genetic endowment.

Table 19.2 Diseases Being Treated in Clinical Trials of Gene Therapy

Disease
Cancer (melanoma, renal cell, ovarian, neuroblastoma, brain, head and neck, lung, liver, breast, colon, prostate, mesothelioma, leukemia, lymphoma, multiple myeloma)
SCID (severe combined immunodeficiency)
Cystic fibrosis
Gaucher's disease
Familial hypercholesterolemia
Hemophilia
Purine nucleoside phosphorylase deficiency
Alpha-1 antitrypsin deficiency
Fanconi's anemia
Hunter's syndrome
Chronic granulomatous disease
Rheumatoid arthritis
Peripheral vascular disease
AIDS

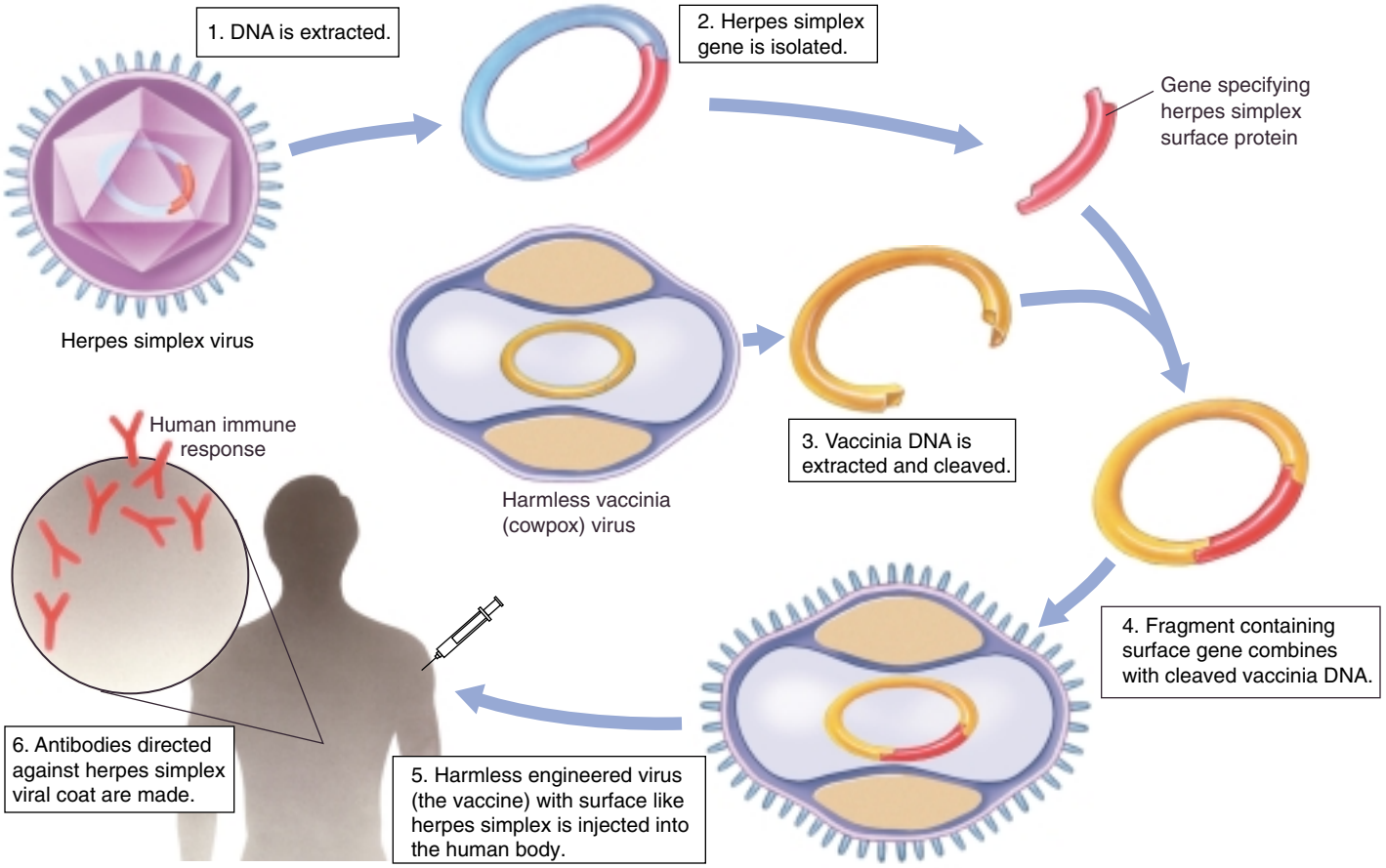


FIGURE 19.18
Strategy for constructing a subunit vaccine for herpes simplex.

Piggyback Vaccines

Another area of potential significance involves the use of genetic engineering to produce **subunit vaccines** against viruses such as those that cause herpes and hepatitis. Genes encoding part of the protein-polysaccharide coat of the herpes simplex virus or hepatitis B virus are spliced into a fragment of the vaccinia (cowpox) virus genome (figure 19.18). The vaccinia virus, which British physician Edward Jenner used almost 200 years ago in his pioneering vaccinations against smallpox, is now used as a vector to carry the herpes or hepatitis viral coat gene into cultured mammalian cells. These cells produce many copies of the recombinant virus, which has the outside coat of a herpes or hepatitis virus. When this recombinant virus is injected into a mouse or rabbit, the immune system of the infected animal produces antibodies directed against the coat of the recombinant virus. It therefore develops an immunity to herpes or hepatitis virus. Vaccines produced in this way are harmless because the vaccinia virus is benign and only a small fragment of the DNA from the disease-causing virus is introduced via the recombinant virus.

The great attraction of this approach is that it does not depend upon the nature of the viral disease. In the future, similar recombinant viruses may be injected into humans to confer resistance to a wide variety of viral diseases.

In 1995, the first clinical trials began of a novel new kind of **DNA vaccine,** one that depends not on antibodies but rather on the second arm of the body's immune defense, the so-called cellular immune response, in which blood cells known as killer T cells attack infected cells. The infected cells are attacked and destroyed when they stick fragments of foreign proteins onto their outer surfaces that the T cells detect (the discovery by Peter Doherty and Rolf Zinkernagel that infected cells do so led to their receiving the Nobel Prize in Physiology or Medicine in 1996). The first DNA vaccines spliced an influenza virus gene encoding an internal nucleoprotein into a plasmid, which was then injected into mice. The mice developed strong cellular immune responses to influenza. New and controversial, the approach offers great promise.

Genetic engineering has produced commercially valuable proteins, gene therapies, and, possibly, new and powerful vaccines.

Agricultural Applications

Another major area of genetic engineering activity is manipulation of the genes of key crop plants. In plants the primary experimental difficulty has been identifying a suitable vector for introducing recombinant DNA. Plant cells do not possess the many plasmids that bacteria do, so the choice of potential vectors is limited. The most successful results thus far have been obtained with the **Ti** (tumor-inducing) **plasmid** of the plant bacterium *Agrobacterium tumefaciens*, which infects broadleaf plants such as tomato, tobacco, and soybean. Part of the Ti plasmid integrates into the plant DNA, and researchers have succeeded in attaching other genes to this portion of the plasmid (figure 19.19). The characteristics of a number of plants have been altered using this technique, which should be valuable in improving crops and forests. Among the features scientists would like to affect are resistance to disease, frost, and other forms of stress; nutritional balance and protein content; and herbicide resistance. Unfortunately, *Agrobacterium* generally does not infect cereals such as corn, rice, and wheat, but alternative methods can be used to introduce new genes into them.

A recent advance in genetically manipulated fruit is Calgene's "Flavr Savr" tomato, which has been approved for sale by the USDA. The tomato has been engineered to inhibit genes that cause cells to produce ethylene. In tomatoes and other plants, ethylene acts as a hormone to speed fruit ripening. In Flavr Savr tomatoes, inhibition of ethylene production delays ripening. The result is a tomato that can stay on the vine longer and that resists overripening and rotting during transport to market.

Herbicide Resistance

Recently, broadleaf plants have been genetically engineered to be resistant to **glyphosate,** the active ingredient in Roundup, a powerful, biodegradable herbicide that kills most actively growing plants (figure 19.20). Glyphosate works by inhibiting an enzyme called EPSP synthetase, which plants require to produce aromatic amino acids. Humans do not make aromatic amino acids; they get them from their diet, so they are unaffected by glyphosate. To make glyphosate-resistant plants, agricultural scientists used a Ti plasmid to insert extra copies of the EPSP synthetase genes into plants. These engineered plants produce 20 times the normal level of EPSP synthetase, enabling them to synthesize proteins and grow despite glyphosate's suppression of the enzyme. In later experiments, a bacterial form of the EPSP synthetase gene that differs from the

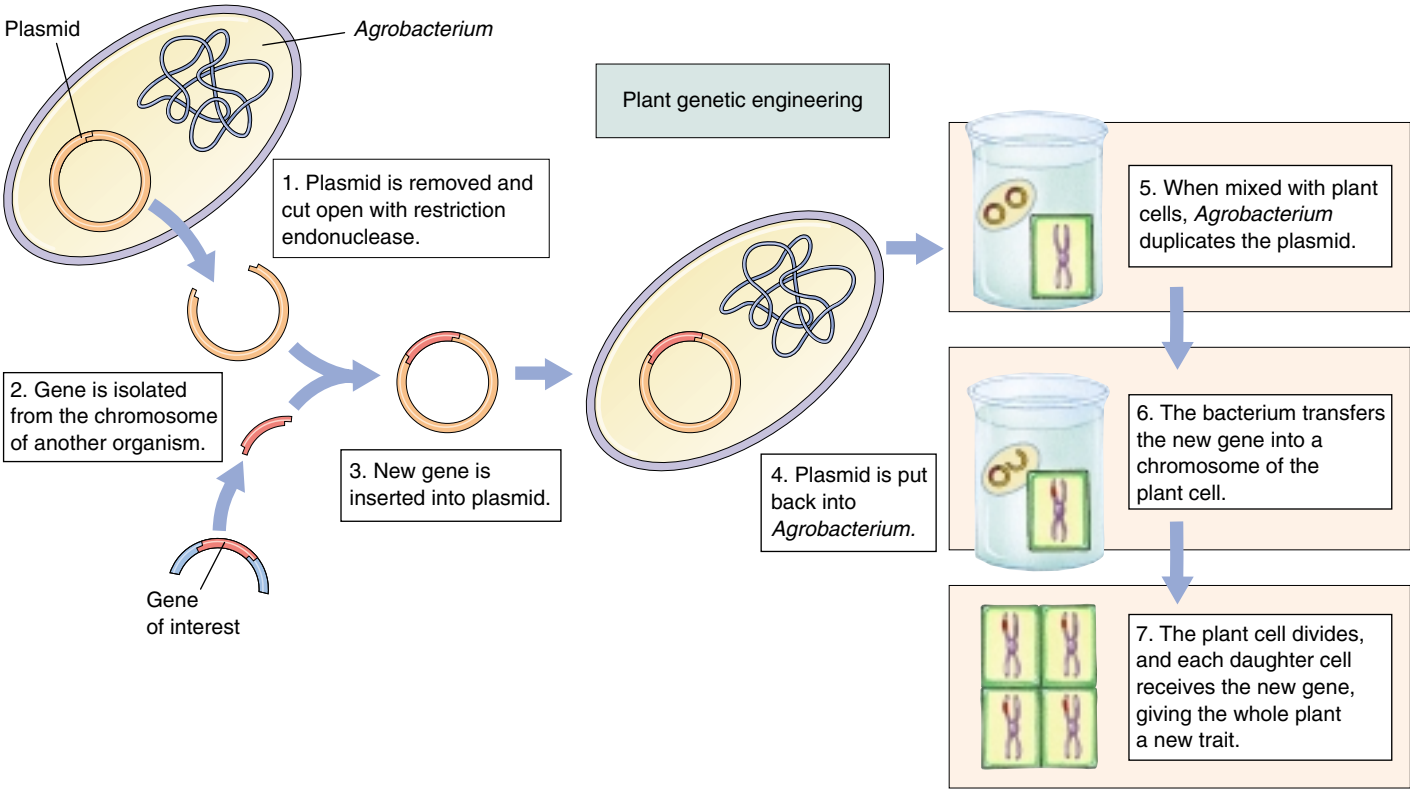


FIGURE 19.19
The Ti plasmid. This *Agrobacterium tumefaciens* plasmid is used in plant genetic engineering.

plant form by a single nucleotide was introduced into plants via Ti plasmids; the bacterial enzyme in these plants is not inhibited by glyphosate.

These advances are of great interest to farmers because a crop resistant to Roundup would never have to be weeded if the field were simply treated with the herbicide. Because Roundup is a broad-spectrum herbicide, farmers would no longer need to employ a variety of different herbicides, most of which kill only a few kinds of weeds. Furthermore, glyphosate breaks down readily in the environment, unlike many other herbicides commonly used in agriculture. A plasmid is actively being sought for the introduction of the EPSP synthetase gene into cereal plants, making them also glyphosate-resistant.

FIGURE 19.20
Genetically engineered herbicide resistance. All four of these petunia plants were exposed to equal doses of the herbicide Roundup. The two on top were genetically engineered to be resistant to glyphosate, the active ingredient of Roundup, while the two on the bottom were not.

Nitrogen Fixation

A long-range goal of agricultural genetic engineering is to introduce the genes that allow soybeans and other legume plants to "fix" nitrogen into key crop plants. These so-called ***nif* genes** are found in certain symbiotic root-colonizing bacteria. Living in the root nodules of legumes, these bacteria break the powerful triple bond of atmospheric nitrogen gas, converting N_2 into NH_3 (ammonia). The plants then use the ammonia to make amino acids and other nitrogen-containing molecules. Other plants lack these bacteria and cannot fix nitrogen, so they must obtain their nitrogen from the soil. Farmland where these crops are grown soon becomes depleted of nitrogen, unless nitrogenous fertilizers are applied. Worldwide, farmers applied over 60 million metric tons of such fertilizers in 1987, an expensive undertaking. Farming costs would be much lower if major crops like wheat and corn could be engineered to carry out biological nitrogen fixation. However, introducing the nitrogen-fixing genes from bacteria into plants has proved difficult because these genes do not seem to function properly in eukaryotic cells. Researchers are actively experimenting with other species of nitrogen-fixing bacteria whose genes might function better in plant cells.

Insect Resistance

Many commercially important plants are attacked by insects, and the traditional defense against such attacks is to apply insecticides. Over 40% of the chemical insecticides used today are targeted against boll weevils, bollworms, and other insects that eat cotton plants. Genetic engineers are now attempting to produce plants that are resistant to insect pests, removing the need to use many externally applied insecticides.

The approach is to insert into crop plants genes encoding proteins that are harmful to the insects that feed on the plants but harmless to other organisms. One such insecticidal protein has been identified in *Bacillus thuringiensis*, a soil bacterium. When the tomato hornworm caterpillar ingests this protein, enzymes in the caterpillar's stomach convert it into an insect-specific toxin, causing paralysis and death. Because these enzymes are not found in other animals, the protein is harmless to them. Using the Ti plasmid, scientists have transferred the gene encoding this protein into tomato and tobacco plants. They have found that these **transgenic** plants are indeed protected from attack by the insects that would normally feed on them. In 1995, the EPA approved altered forms of potato, cotton, and corn. The genetically altered potato can kill the Colorado potato beetle, a common pest. The altered cotton is resistant to cotton bollworm, budworm, and pink bollworm. The corn has been altered to resist the European corn borer and other mothlike insects.

Monsanto scientists screening natural compounds extracted from plant and soil samples have recently isolated a new insect-killing compound from a fungus, the enzyme cholesterol oxidase. Apparently, the enzyme disrupts membranes in the insect gut. The fungus gene, called the Bollgard gene after its discoverer, has been successfully inserted into a variety of crops. It kills a wide range of insects, including the cotton boll weevil and the Colorado potato beetle, both serious agricultural pests. Field tests began in 1996.

Some insect pests attack plant roots, and *B. thuringiensis* is being employed to counter that threat as well. This bacterium does not normally colonize plant roots, so biologists have introduced the *B. thuringiensis* insecticidal protein gene into root-colonizing bacteria, especially strains of *Pseudomonas.* Field testing of this promising procedure has been approved by the Environmental Protection Agency.

The Real Promise of Plant Genetic Engineering

In the last decade the cultivation of genetically modified crops of corn, cotton, and soybeans has become commonplace in the United States—in 1999, over half of the 72 million acres planted with soybeans in the United States were planted with seeds genetically modified to be herbicide resistant, with the result that less tillage has been needed, and as a consequence soil erosion has been greatly lessened. These benefits, while significant, have been largely confined to farmers, making their cultivation of crops cheaper and more efficient. The food that the public gets is the same, it just costs less to get it to the table.

Like the first act of a play, these developments have served mainly to set the stage for the real action, which is only now beginning to happen. The real promise of plant genetic engineering is to produce genetically modified plants with desirable traits that directly benefit the consumer.

One recent advance, nutritionally improved rice, gives us a hint of what is to come. In developing countries large numbers of people live on simple diets that are poor sources of vitamins and minerals (what botanists called "micronutrients"). Worldwide, the two major micronutrient deficiencies are iron, which affects 1.4 billion women, 24% of the world population, and vitamin A, affecting 40 million children, 7% of the world population. The deficiencies are especially severe in developing countries where the major staple food is rice. In recent research, Swiss bioengineer Ingo Potrykus and his team at the Institute of Plant Sciences, Zurich, have gone a long way towards solving this problem. Supported by the Rockefeller Foundation and with results to be made free to developing countries, the work is a model of what plant genetic engineering can achieve.

To solve the problem of dietary iron deficiency among rice eaters, Potrykus first asked why rice is such a poor source of dietary iron. The problem, and the answer, proved to have three parts:

1. *Too little iron.* The proteins of rice endosperm have unusually low amounts of iron. To solve this problem, a ferritin gene was transferred into rice from beans (figure 19.21). Ferritin is a protein with an extraordinarily high iron content, and so greatly increased the iron content of the rice.
2. *Inhibition of iron absorption by the intestine.* Rice contains an unusually high concentration of a chemical called phytate, which inhibits iron reabsorption in the intestine—it stops your body from taking up the iron in the rice. To solve this problem, a gene encoding an enzyme that destroys phytate was transferred into rice from a fungus.
3. *Too little sulfur for efficient iron absorption.* Sulfur is required for iron uptake, and rice has very little of it. To solve this problem, a gene encoding a particularly sulfur-rich metallothionin protein was transferred into rice from wild rice.

To solve the problem of vitamin A deficiency, the same approach was taken. First, the problem was identified. It turns out rice only goes part way toward making beta-carotene (provitamin A); there are no enzymes in rice to catalyze the last four steps. To solve the problem, genes encoding these four enzymes were added to rice from a familiar flower, the daffodil.

Potrykus's development of transgenic rice to combat dietary deficiencies involved no subtle tricks, just straightforward bioengineering and the will to get the job done. The transgenic rice he has developed will directly improve the lives of millions of people. His work is rep-

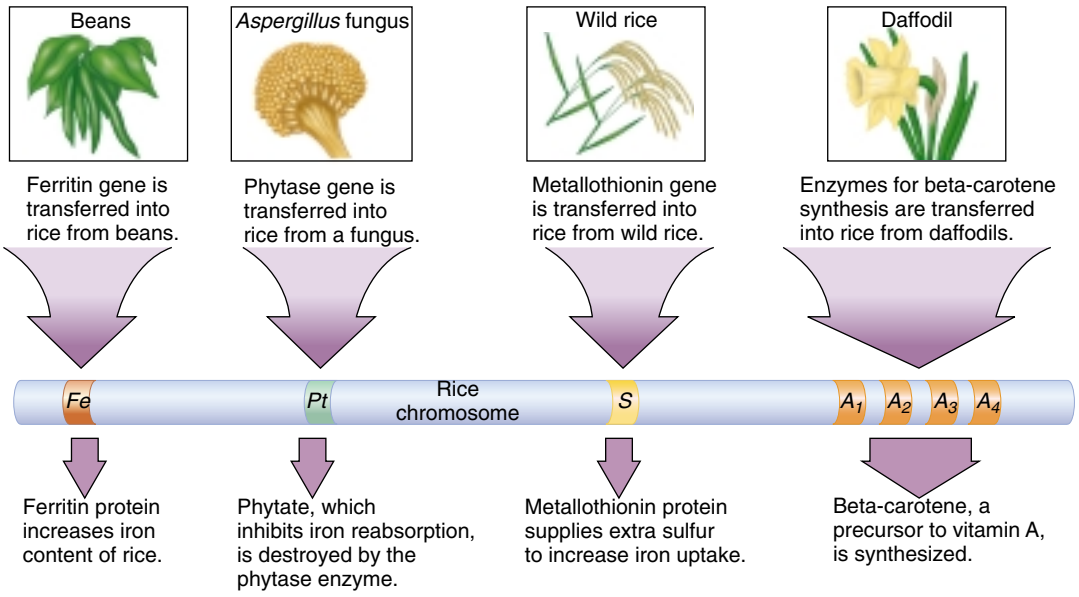


FIGURE 19.21
Transgenic rice. Developed by Swiss bioengineer Ingo Potrykus, transgenic rice offers the promise of improving the diets of people in rice-consuming developing countries, where iron and vitamin A deficiencies are a serious problem.

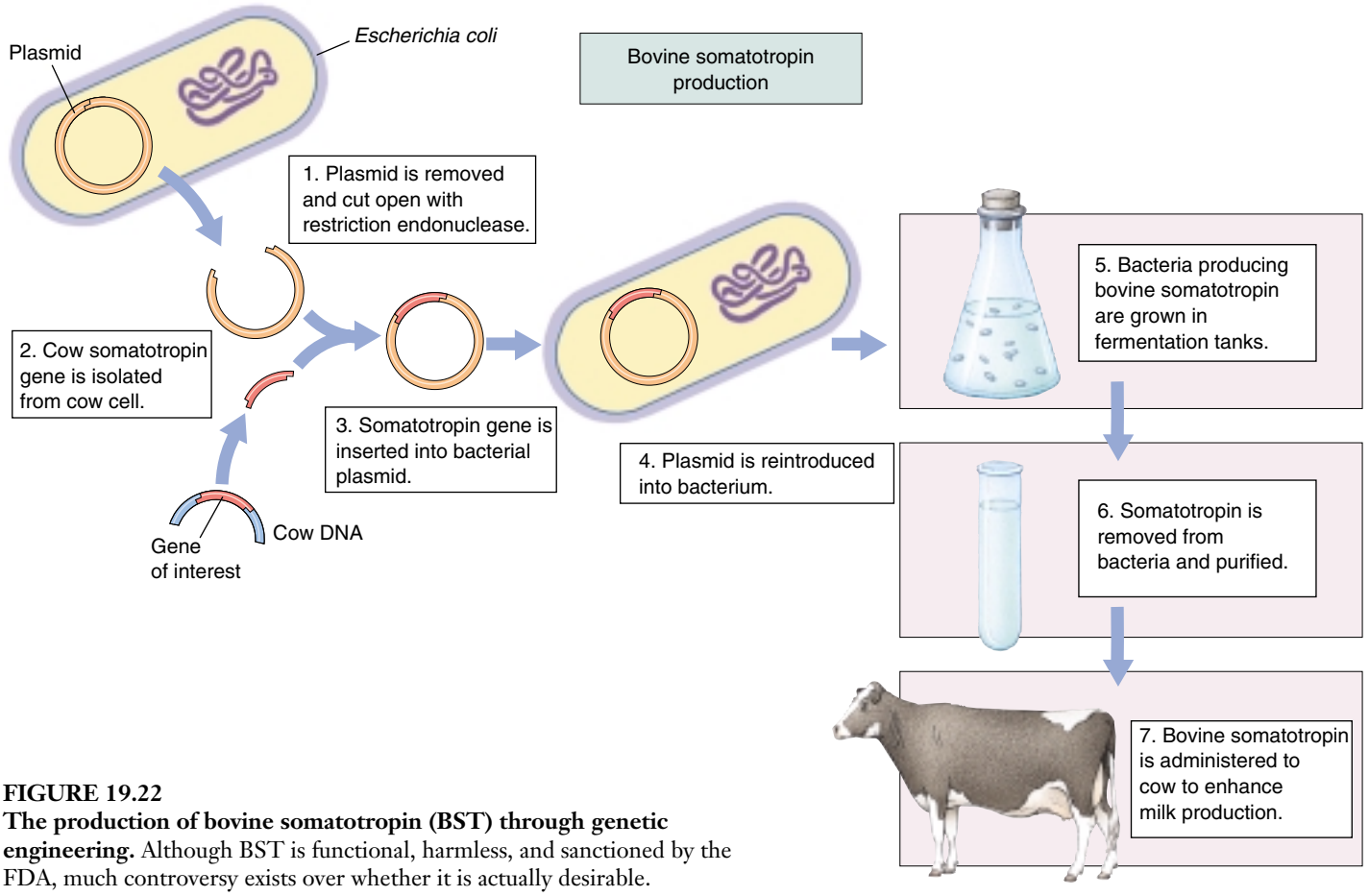


FIGURE 19.22
The production of bovine somatotropin (BST) through genetic engineering. Although BST is functional, harmless, and sanctioned by the FDA, much controversy exists over whether it is actually desirable.

resentative of the very real promise of genetic engineering to help meet the challenges of the coming new millennium.

The list of gene modifications that directly aid consumers will only grow. In Holland, Dutch bioengineers announced last month that they are genetically engineering plants to act as vaccine-producing factories! To petunias they have added a gene for a vaccine against dog parvovirus, hiding the gene within the petunia genes that direct nectar production. The drug is produced in the nectar, collected by bees, and extracted from the honey. It is hard to believe this isn't science fiction. Clearly, the real promise of plant genetic engineering lies ahead, and not very far.

Farm Animals

The gene encoding the growth hormone somatotropin was one of the first to be cloned successfully. In 1994, Monsanto received federal approval to make its recombinant bovine somatotropin (BST) commercially available, and dairy farmers worldwide began to add the hormone as a supplement to their cows' diets, increasing the animals' milk production (figure 19.22). Genetically engineered somatotropin is also being tested to see if it increases the muscle weight of cattle and pigs, and as a treatment for human disorders in which the pituitary gland fails to make adequate levels of somatotropin, producing dwarfism. BST ingested in milk or meat has no effect on humans, because it is a protein and is digested in the stomach. Nevertheless, BST has met with some public resistance, due primarily to generalized fears of gene technology. Some people mistrust milk produced through genetic engineering, even though the milk itself is identical to other milk. Problems concerning public perception are not uncommon as gene technology makes an even greater impact on our lives.

Transgenic animals engineered to have specific desirable genes are becoming increasingly available to breeders. Now, instead of selectively breeding for several generations to produce a racehorse or a stud bull with desirable qualities, the process can be shortened by simply engineering such an animal right at the start.

Gene technology is revolutionizing agriculture, increasing yields and resistance to pests, and producing animals with desirable traits.

Cloning

The difficulty in using transgenic animals to improve livestock is in getting enough of them. Breeding produces offspring only slowly, and recombination acts to undo the painstaking work of the genetic engineer. Ideally, one would like to "Xerox" many exact genetic copies of the transgenic strain—but until 1997 it was commonly accepted that adult animals can't be cloned. Now the holy grail of agricultural genetic engineers seems within reach. In 1997, scientists announced the first successful cloning of differentiated vertebrate tissue, a lamb grown from a cell taken from an adult sheep. This startling result promises to revolutionize agricultural science.

Spemann's "Fantastical Experiment"

The idea of cloning animals was first suggested in 1938 by German embryologist Hans Spemann (called the "father of modern embryology"), who proposed what he called a "fantastical experiment": remove the nucleus from an egg cell, and put in its place a nucleus from another cell.

It was 14 years before technology advanced far enough for anyone to take up Spemann's challenge. In 1952, two American scientists, Robert Briggs and T. J. King, used very fine pipettes to suck the nucleus from a frog egg (frog eggs are unusually large, making the experiment feasible) and transfer a nucleus sucked from a body cell of an adult frog into its place. The experiment did not work when done this way, but partial success was achieved 18 years later by the British developmental biologist John Gurdon, who in 1970 inserted nuclei from advanced frog embryos rather than adult tissue. The frog eggs developed into tadpoles, but died before becoming adults.

The Path to Success

For 14 years, nuclear transplant experiments were attempted without success. Technology continued to advance however, until finally in 1984, Steen Willadsen, a Danish embryologist working in Texas, succeeded in cloning a sheep using a nucleus from a cell of an early embryo. This exciting result was soon replicated by others in a host of other organisms, including cattle, pigs, and monkeys.

Only early embryo cells seemed to work, however. Researchers became convinced that animal embryo cells become irreversibly "committed" after the first few cell divisions. After that, nuclei from differentiated animal cells cannot be used to clone entire organisms.

We now know this conclusion to have been unwarranted. The key advance for unraveling this puzzle was made in Scotland by geneticist Keith Campbell, a specialist in studying the cell cycle of agricultural animals. By the early 1990s, knowledge of how the cell cycle is controlled, advanced by cancer research, had led to an understanding that cells don't divide until conditions are appropriate. Just as a washing machine checks that the water has completely emptied before initiating the spin cycle, so the cell checks that everything needed is on hand before initiating cell division. Campbell reasoned: "Maybe the egg and the donated nucleus need to be at the same stage in the cell cycle."

This proved to be a key insight. In 1994 researcher Neil First, and in 1995 Campbell himself working with reproductive biologist Ian Wilmut, succeeded in cloning farm animals from advanced embryos by first starving the cells, so that they paused at the beginning of the cell cycle at the G_1 checkpoint. Two starved cells are thus synchronized at the same point in the cell cycle.

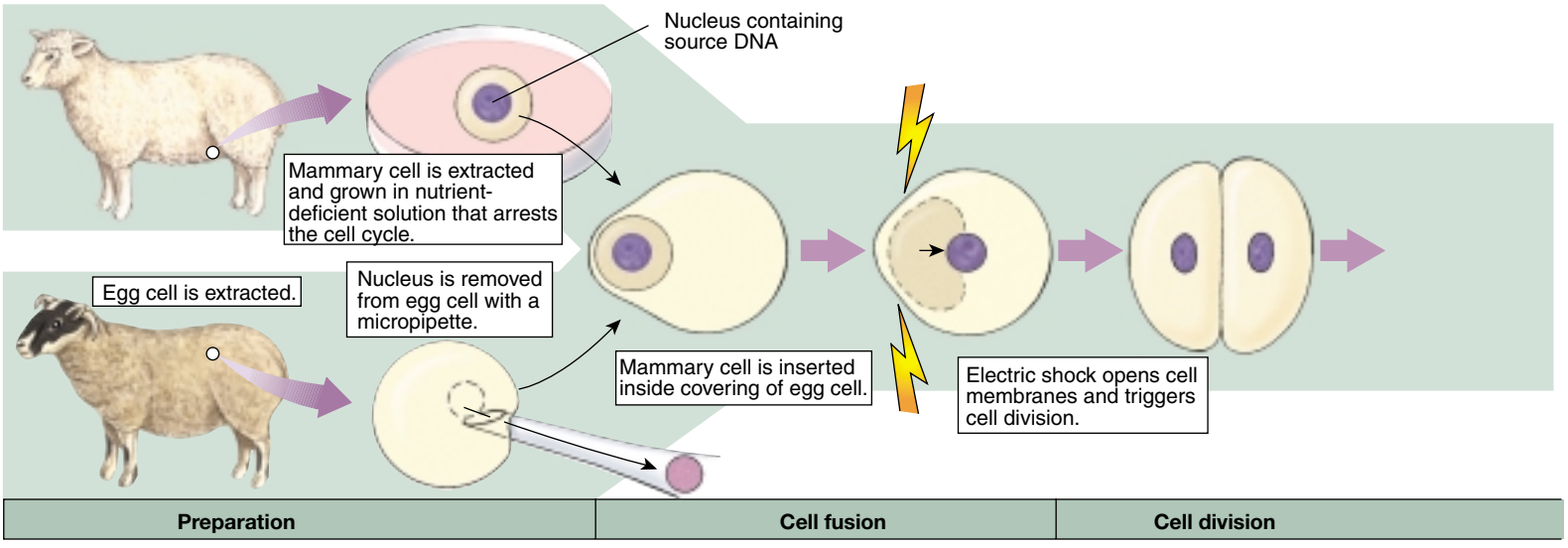


FIGURE 19.23
Wilmut's animal cloning experiment. Wilmut combined a nucleus from a mammary cell and an egg cell (with its nucleus removed) to successfully clone a sheep.

Wilmut's Lamb

Wilmut then set out to attempt the key breakthrough, the experiment that had eluded researchers since Spemann proposed it 59 years before: to transfer the nucleus from an adult differentiated cell into an enucleated egg, and allow the resulting embryo to grow and develop in a surrogate mother, hopefully producing a healthy animal.

Wilmut removed mammary cells from the udder of a six-year-old sheep (figure 19.23). The origin of these cells, gave the clone its name, "Dolly" after the country singer Dolly Parton. The cells were grown in tissue culture, and some frozen so that in the future it would be possible with genetic fingerprinting to prove that a clone was indeed genetically identical with the six-year-old sheep.

In preparation for cloning, Wilmut's team reduced for five days the concentration of serum on which the sheep mammary cells were subsisting. In parallel preparation, eggs obtained from a ewe were enucleated, the nucleus of each egg carefully removed with a micropipette.

Mammary cells and egg cells were then surgically combined in January of 1996, the mammary cells inserted inside the covering around the egg cell. Wilmut then applied a brief electrical shock. A neat trick, this causes the plasma membranes surrounding the two cells to become leaky, so that the contents of the mammary cell passes into the egg cell. The shock also kick-starts the cell cycle, causing the cell to begin to divide.

After six days, in 30 of 277 tries, the dividing embryo reached the hollow-ball "blastula" stage, and 29 of these were transplanted into surrogate mother sheep. A little over five months later, on July 5, 1997, one sheep gave birth to a lamb. This lamb, "Dolly," was the first successful clone generated from a differentiated animal cell.

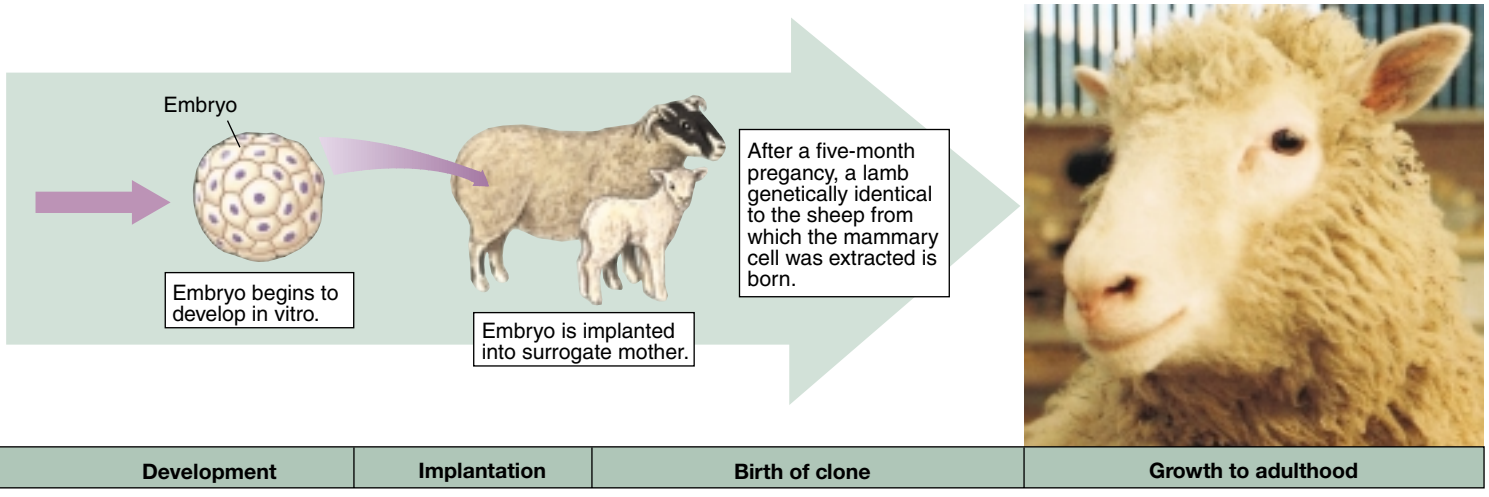
The Future of Cloning

Wilmut's successful cloning of fully differentiated sheep cells is a milestone event in gene technology. Even though his procedure proved inefficient (only one of 277 trials succeeded), it established the point beyond all doubt that cloning of adult animal cells *can* be done. In the following four years researchers succeeded in greatly improving the efficiency of cloning. Seizing upon the key idea in Wilmut's experiment, to clone a resting-stage cell, they have returned to the nuclear transplant procedure pioneered by Briggs and King. It works well. Many different mammals have been successfully cloned including mice, pigs, and cattle.

Transgenic cloning can be expected to have a major impact on medicine as well as agriculture. Animals with human genes can be used to produce rare hormones. For example, sheep that have recently been genetically engineered to secrete a protein called alpha-1 antitrypsin (helpful in relieving the symptoms of cystic fibrosis) into their milk may be cloned, greatly cheapening the production of this expensive drug.

It is impossible not to speculate on the possibility of cloning a human. There is no reason to believe such an experiment would not work, but many reasons to question whether it should be done. Because much of Western thought is based on the concept of human individuality, we can expect the possibility of human cloning to engender considerable controversy.

Recent experiments have demonstrated the possibility of cloning differentiated mammalian tissue, opening the door for the first time to practical transgenic cloning of farm animals.



Stem Cells

Since the isolation of embryonic stem cells in 1998, labs all over the world have been exploring the possibility of using stem cells to restore damaged or lost tissue. Exciting results are now starting to come in.

What is a stem cell? At the dawn of a human life, a sperm fertilizes an egg to create a single cell destined to become a child. As development commences, that cell begins to divide, producing a small ball of a few dozen cells. At this very early point, each of these cells is identical. We call these cells *embryonic stem cells*. Each one of them is capable by itself of developing into a healthy individual. In cattle breeding, for example, these cells are frequently separated by the breeder and used to produce multiple clones of valuable offspring.

The exciting promise of these embryonic stem cells is that, because they can develop into any tissue, they may give us the ability to restore damaged heart or spine tissue (figure 19.24). Experiments have already been tried successfully in mice. Heart muscle cells have been grown from mouse embryonic stem cells and successfully integrated with the heart tissue of a living mouse. This suggests that the damaged heart muscle of heart attack victims might be reparable with stem cells, and that injured spinal cords might be repairable. These very promising experiments are being pursued aggressively. They are, however, quite controversial, as embryonic stem cells are typically isolated from tissue of discarded or aborted embryos, raising serious ethical issues.

Tissue-Specific Stem Cells

New results promise a neat way around the ethical maze presented by stem cells derived from embryos. Go back for a moment to what we were saying about how a human child develops. What happens next to the embryonic stem cells? They start to take different developmental paths. Some become destined to form nerve tissue and, after this decision is taken, cannot ever produce any other kind of cell. They are then called nerve stem cells. Others become specialized to produce blood, still others muscle. Each major tissue is represented by its own kind of *tissue-specific stem cell*. Now here's the key point: as development proceeds, these tissue-specific stem cells persist. Even in adults. So why not use these adult cells, rather than embryonic stem cells?

Transplanted Tissue-Specific Stem Cells Cure MS in Mice

In pathfinding 1999 laboratory experiments by Dr. Evan Snyder of Harvard Medical School, tissue-specific stem cells were able to restore lost brain tissue. He and his coworkers injected neural stem cells (immediate descendants of embryonic stem cells able to become any kind of neural cell) into the brains of newborn mice with a disease resembling multiple sclerosis (MS). These mice lacked the cells that maintain the layers of myelin insulation around signal-conducting nerves. The injected stem cells migrated all over the brain, and were able to convert themselves into the missing type of cell. The new cells then proceeded to repair the ravages of the disease by replacing the lost insulation of signal-conducting nerve cells. Many of the treated mice fully recovered. In mice at least, tissue-specific stem cells offer a treatment for MS.

The approach seems very straightforward, and should apply to humans. Indeed, blood stem cells are already routinely used in humans to replenish the bone marrow of cancer patients after marrow-destroying therapy. The problem

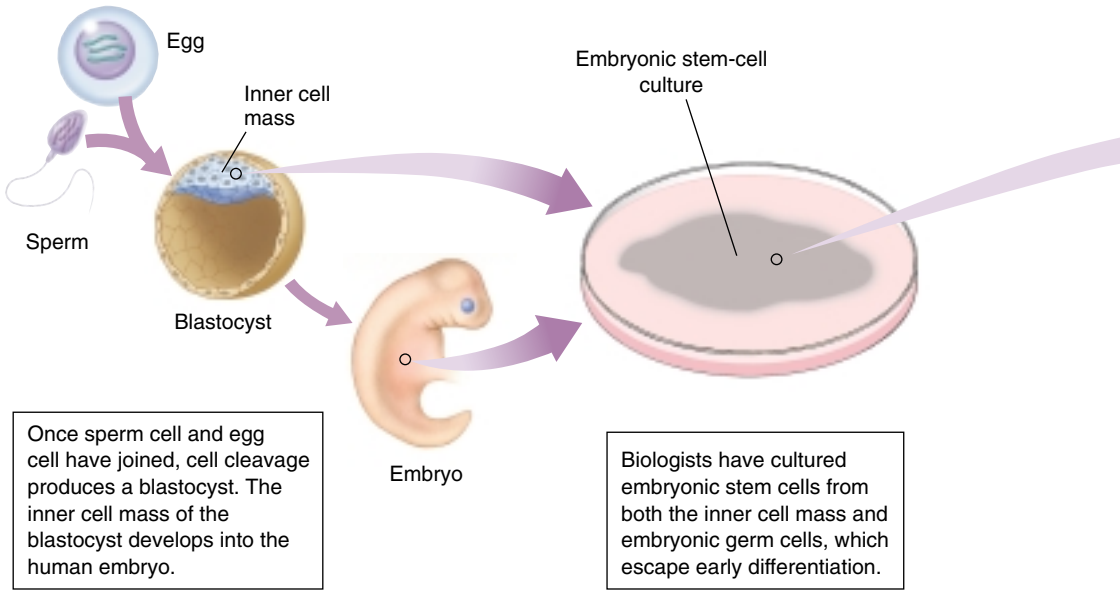


FIGURE 19.24
Using embryonic stem cells to restore damaged tissue. Embryonic stem cells can develop into any body tissue. Methods for growing the tissue and using it to repair damaged tissue in adults, such as the brain cells of multiple sclerosis patients, heart muscle, and spinal nerves, are being developed.

with extending the approach to other kinds of tissue-specific stem cells is that it has not always been easy to find the kind of tissue-specific stem cell you want.

Transplanted Stem Cells Reverse Juvenile Diabetes in Mice

Very promising experiments carried out in 2000 by Dr. Ammon Peck and a team of researchers at the University of Florida concern a particularly vexing problem, that of type 1 or juvenile diabetes. A person with juvenile diabetes lacks insulin-producing pancreas cells, because their immune system has mistakenly turned against them and destroyed them. They are no longer able to produce enough insulin to control their blood sugar levels and must take insulin daily. Adding back new insulin-producing cells called islet cells has been tried many times, but doesn't work well. Immune cells continue to destroy them.

Peck and his team reasoned, why not add instead the stem cells that produce islet cells? They would be able to produce a continuous supply of new islet cells, replacing those lost to immune attack. Because there would always be cells to make insulin, the diabetes would be cured.

No one knew just what such a stem cell looked like, but the researchers knew they come from the epithelial cells that line the pancreas ducts. Surely some must still lurk there unseen. So the research team took a bunch of these epithelial cells from mice and grew them in tissue culture until they had lots of them.

Were the stem cells they sought present in the cell culture they had prepared? Yes. In laboratory dishes the cell culture produced insulin in response to sugar, indicating islet cells had developed in the growing culture, islet cells that must have been produced from stem cells.

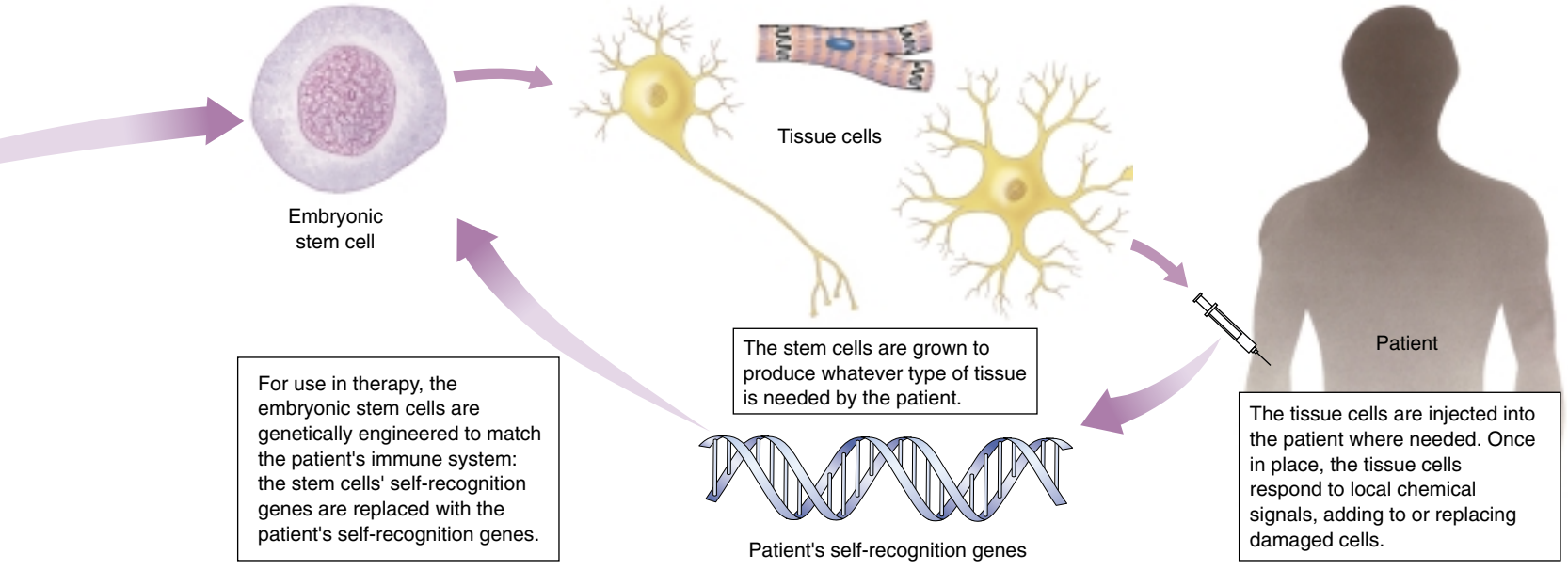
Now on to juvenile diabetes. The scientists injected their cell culture into the pancreas of mice specially bred to develop juvenile diabetes. Unable to manufacture their own insulin because they had no islet cells, these diabetic mice could not survive without daily insulin. What happened? The diabetes was reversed! The mice no longer required insulin.

Impatient to see in more detail what had happened, the researchers sacrificed the mice and examined the cells of their pancreas. The mice appeared to have perfectly normal islet cells.

One might have wished the researchers waited a little longer before terminating the experiment. It is not clear whether the cure was transitory or long term. Still, there is no escaping the conclusion that injection of a culture of adult stem cells cured their juvenile diabetes.

While certainly encouraging, a mouse is not a human, and there is no guarantee the approach will work in humans. But there is every reason to believe it might. The experiment is being repeated now with humans. People suffering from juvenile diabetes are being treated with human pancreatic duct cells obtained from people who have died and donated their organs for research. No ethical issues arise from using cells of adult organ donors, and initial results look promising.

Transplanted stem cells may allow us to replace damaged or lost tissue, offering cures for many disorders that cannot now be treated. Current work focuses on tissue-specific stem cells, which do not present the ethical problems that embryonic stem cells do.



Ethics and Regulation

The advantages afforded by genetic engineering are revolutionizing our lives. But what are the disadvantages, the potential costs and dangers of genetic engineering? Many people, including influential activists and members of the scientific community, have expressed concern that genetic engineers are "playing God" by tampering with genetic material. For instance, what would happen if one fragmented the DNA of a cancer cell, and then incorporated the fragments at random into vectors that were propagated within bacterial cells? Might there not be a danger that some of the resulting bacteria would transmit an infective form of cancer? Could genetically engineered products administered to plants or animals turn out to be dangerous for consumers after several generations? What kind of unforeseen impact on the ecosystem might "improved" crops have? Is it ethical to create "genetically superior" organisms, including humans?

How Do We Measure the Potential Risks of Genetically Modified Crops?

While the promise of genetic engineering is very much in evidence, this same genetic engineering has this summer been the cause of outright war between researchers and protesters in England. In June 1999, British protesters attacked an experimental plot of genetically modified (GM) sugar beets; the following August they destroyed a test field of GM canola (used for cooking oil and animal feed). The contrast could not be more marked between American acceptance of genetically modified crops on the one hand, and European distrust of genetically modified foods, on the other. The intense feelings generated by this dispute point to the need to understand how we measure the risks associated with the genetic engineering of plants.

Two sets of risks need to be considered. The first stems from eating genetically modified foods, the other concerns potential ecological effects.

Is Eating Genetically Modified Food Dangerous? Protesters worry that genetically modified food may have been rendered somehow dangerous. To sort this out, it is useful to bear in mind that bioengineers modify crops in two quite different ways. One class of gene modification makes the crop easier to grow; a second class of modification is intended to improve the food itself.

The introduction of Roundup-resistant soybeans to Europe is an example of the first class of modification. This modification has been very popular with farmers in the United States, who planted half their crop with these soybeans in 1999. They like GM soybeans because the beans can be raised without intense cultivation (weeds are killed with Roundup herbicide instead), which both saves money and lessens soil erosion. But is the soybean that results nutritionally different? No. The gene that confers Roundup resistance in soybeans does so by protecting the plant's ability to manufacture so-called "aromatic" amino acids. In unprotected weeds, by contrast, Roundup blocks this manufacturing process, killing the weed. Because humans don't make any aromatic amino acids anyway (we get them in our diets), Roundup doesn't hurt us. The GM soybean we eat is nutritionally the same as an "organic" one, just cheaper to produce.

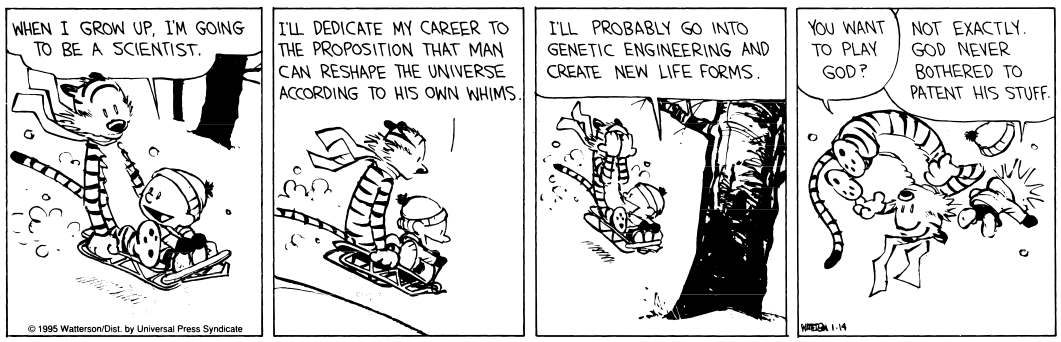
In the second class of modification, where a gene is added to improve the nutritional character of some food, the food will be nutritionally different. In each of these instances, it is necessary to examine the possibility that consumers may prove allergic to the product of the introduced gene. In one instance, for example, addition of a methionine-enhancing gene from Brazil nut into soybeans (which are deficient in this amino acid) was discontinued when six of eight individuals allergic to Brazil nuts produced antibodies to the GM soybeans, suggesting the possibility of a reverse reaction. Instead, methionine levels in GM crops are being increased with genes from sunflowers. Screening for allergy problems is now routine.

On both scores, then, the risk of bioengineering to the food supply seems to be very slight. GM foods to date seem completely safe.

Are GM Crops Harmful to the Environment? What are we to make of the much-publicized report that Monarch butterflies might be killed by eating pollen blowing out of fields planted with GM corn? First, it should come as no surprise. The GM corn (so-called Bt corn) was engineered to contain an insect-killing toxin (harmless to people) in order to combat corn borer pests. Of course it will kill any butterflies or other insects in the immediate vicinity of the field. However, focus on the fact that the GM corn fields do not need to be sprayed with pesticide to control the corn borer. An estimated $9 billion in damage is caused annually by the application of pesticides in the United States, and billions of insects and other animals, including an estimated 67 million birds, are killed each year. This pesticide-induced murder of wildlife is far more damaging ecologically than any possible effects of GM crops on butterflies.

Will pests become resistant to the GM toxin? Not nearly as fast as they now become resistant to the far higher levels of chemical pesticide we spray on crops.

How about the possibility that introduced genes will pass from GM crops to their wild or weedy relatives? This sort of gene flow happens naturally all the time, and so this is a legitimate question. But so what if genes for resistance to Roundup herbicide spread from cultivated sugar beets to wild populations of sugar beets in Europe? Why would that be a problem? Besides, there is almost never a potential relative around to receive the modified gene from the GM crop. There are no wild relatives of soybeans in Europe, for example. Thus, there can be no gene escape from GM soybeans in Europe, any more than genes can flow from you to other kinds of animals.



On either score, then, the risk of bioengineering to the environment seems to be very slight. Indeed, in some cases it lessens the serious environmental damage produced by cultivation and agricultural pesticides.

Should We Label Genetically Modified Foods?

While there seems little tangible risk in the genetic modification of crops, public assurance that these risks are being carefully assessed is important. Few issues manage to raise the temperature of discussions about plant genetic engineering more than labeling of genetically modified (GM) crops. Agricultural producers have argued that there are no demonstrable risks, so that a GM label can only have the function of scaring off wary consumers. Consumer advocates respond that consumers have every right to make that decision, and to the information necessary to make it.

In considering this matter, it is important to separate two quite different issues, the *need* for a label, and the *right* of the public to have one. Every serious scientific investigation of the risks of GM foods has concluded that they are safe—indeed, in the case of soybeans and many other crops modified to improve cultivation, the foods themselves are not altered in any detectable way, and no nutritional test could distinguish them from "organic" varieties. So there seems to be little if any health need for a GM label for genetically engineered foods.

The right of the public to know what it is eating is a very different issue. There is widespread fear of genetic manipulation in Europe, because it is unfamiliar. People there don't trust their regulatory agencies as we do here, because their agencies have a poor track record of protecting them. When they look at genetically modified foods, they are haunted by past experiences of regulatory ineptitude. In England they remember British regulators' failure to protect consumers from meat infected with mad cow disease.

It does no good whatsoever to tell a fearful European that there is no evidence to warrant fear, no trace of data supporting danger from GM crops. A European consumer will simply respond that the harm is not yet evident, that we don't know enough to see the danger lurking around the corner. "Slow down," the European consumers say. "Give research a chance to look around all the corners. Lets be sure." No one can argue against caution, but it is difficult to imagine what else researchers can look into—safety has been explored very thoroughly. The fear remains, though, for the simple reason that no amount of information can remove it. Like a child scared of a monster under the bed, looking under the bed again doesn't help—the monster still might be there next time. And that means we are going to have to have GM labels, for people have every right to be informed about something they fear.

What should these labels be like? A label that only says "GM FOOD" simply acts as a brand—like a POISON label, it shouts a warning to the public of lurking danger. Why not instead have a GM label that provides information to the consumer, that tells the consumer what regulators know about that product?

> (*For Bt corn*): The production of this food was made more efficient by the addition of genes that made plants resistant to pests so that less pesticides were required to grow the crop.
>
> *(For Roundup-ready soybeans)*: Genes have been added to this crop to render it resistant to herbicides—this reduces soil erosion by lessening the need for weed-removing cultivation.
>
> (*For high beta-carotene rice*): Genes have been added to this food to enhance its beta-carotene content and so combat vitamin A deficiency.

GM food labels that in each instance actually tell consumers what has been done to the gene-modified crop would go a long way toward hastening public acceptance of gene technology in the kitchen.

Genetic engineering affords great opportunities for progress in medicine and food production, although many are concerned about possible risks. On balance, the risks appear slight, and the potential benefits substantial.

Chapter 19

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Summary

Questions

Media Resources

19.1 The ability to manipulate DNA has led to a new genetics.

- Genetic engineering involves the isolation of specific genes and their transfer to new genomes.
- An important component of genetic engineering technology is a special class of enzymes called restriction endonucleases, which cleave DNA molecules into fragments.
- The first such recombinant DNA was made by Cohen and Boyer in 1973, when they inserted a frog ribosomal RNA gene into a bacterial plasmid.

1. Why do the ends of the DNA fragments created by restriction endonucleases enable fragments from different genomes to be spliced together?

- Experiment: Cohen/Boyer/Berg-The first Genetically Engineered Organism
- Student Research: Homeobox Genes in the Medicinal Leech

19.2 Genetic engineering involves easily understood procedures.

- Genetic engineering experiments consist of four stages: isolation of DNA, production of recombinant DNA, cloning, and screening for the gene(s) of interest.
- Preliminary screening can be accomplished by making the desired clones resistant to an antibiotic; hybridization can then be employed to identify the gene of interest.
- Gene technologies, including PCR, Southern blotting, RFLP analysis, and the Sanger method, enable researchers to isolate genes and produce them in large quantities.

2. Describe the procedure used to eliminate clones that have not incorporated a vector in a genetic engineering experiment.

3. What is used as a probe in a Southern blot? With what does the probe hybridize? How are the regions of hybridization visualized?

- Polymerase Chain Reaction

- Recombinant

- On Science Article: How Genetic Engineering is Done

19.3 Biotechnology is producing a scientific revolution.

- Extensive research on the human genome has yielded important information about the location of genes, such as those that may be involved in dyslexia, obesity, and resistance to high blood cholesterol levels.
- Gene splicing holds great promise as a clinical tool, particularly in the prevention of disease with bioengineered vaccines.
- A major focus of genetic engineering activity has been agriculture, where genes conferring resistance to herbicides or insect pests have been incorporated into crop plants.
- Recent experiments open the way for cloning of genetically altered animals and suggest that human cloning is feasible.
- The impact of genetic engineering has skyrocketed over the past decade, providing many useful innovations for society; its moral and ethical aspects still provide a topic for heated debates.

4. What is the primary vector used to introduce genes into plant cells? What types of plants are generally infected by this vector? Describe three examples of how this vector has been used for genetic engineering, and explain the agricultural significance of each example.

5. How is the genetic engineering of bovine somatotropin (BST) used to increase milk production in the dairy industry? What effect would BST in milk have on persons who drink it?

- Exploration: DNA from Real Court Cases

On Science Articles:
- The Real Promise of Plant Genetic Engineering
- Should We Label Genetically Modified Foods?
- Measuring Risks of Genetically Modified Crops
- The Search for the Natural Relatives of Cassava
- Renouncing the Terminator
- Frankenstein Grass is Poised to Invade my Back Yard
- The Road to Dolly
- Should a Clone Have Rights?
- Who Should Own the Secrets of Your Genes?