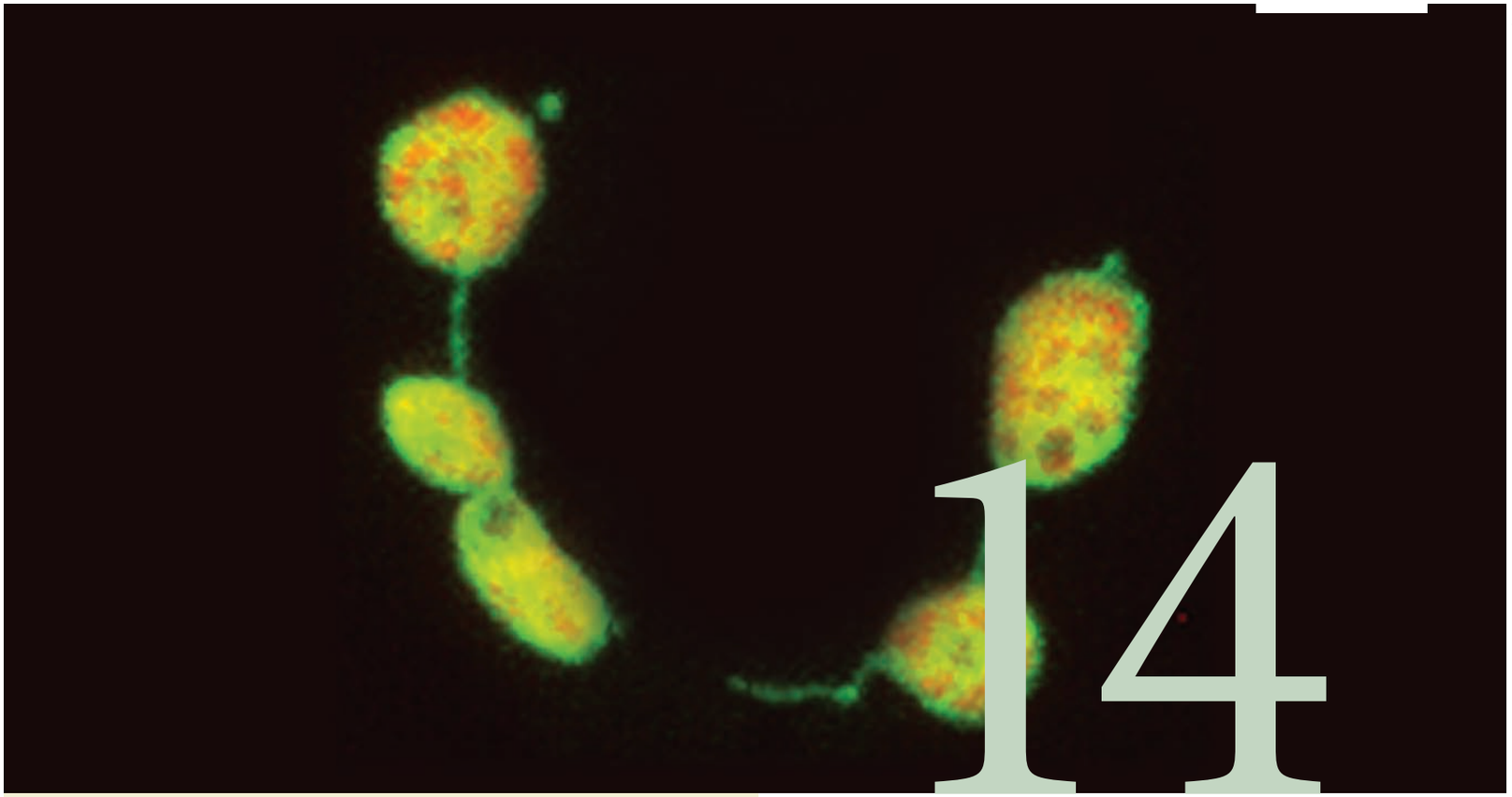

14

Genetic Technology

A jellyfish's green fluorescent protein revealed that chloroplasts can connect.

Biotechnology: Unlocking the Secrets of Chloroplasts

In the first century AD, Roman scholar Pliny the Elder noted an eerie glow in the Bay of Naples and identified its source—jellyfish. Today, a glowing protein from the jellyfish *Aequorea Victoria* is used in cell biology to light up any protein.

"Green fluorescent protein," or GFP, has illuminated such diverse cellular processes as protein production and secretion, contraction of microtubules and sliding of microfilaments, and calcium transport.

Douglas Prasher and Martin Chalfie at the Woods Hole Marine Biological Laboratory discovered GFP and its gene in 1992. Realizing the potential to hook the GFP gene onto any other gene, they attached it to genes from a bacterium and a type of worm—and these organisms glowed!

In 1997, plant molecular biologist Maureen Hanson and her colleagues at Cornell University confirmed that chloroplasts, long thought to be independent organelles, may in fact interact with one another. As early as 1962, researcher Sam Wildman had filmed what looked like projections forming on one chloroplast and leading to one another—but no one had been able to repeat the finding.

Hanson attached GFP to a very small protein that would carry it into the watery region of chloroplasts. Her team was able to view strands of fluorescent green extending from one chloroplast to another. These strands are now termed "stromules," and they are dynamic structures that extend, contract, move around, and change shape.

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- Antisense Technology Stops Expression
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Chapter Preview

1. Genes from one organism can be used accurately by any other organism.
2. Molecular tools allow us to excise specific DNA fragments and paste them to others, making recombinant DNA.
3. Plasmids maintain genes inside a bacterial cell and can be easily manipulated.
4. DNA cut by a restriction enzyme yields specific patterns that can identify the source of the DNA.
5. DNA sequences can be accurately determined by either of two methods.
6. Genes may be placed into entire organisms to form transgenic species that manufacture a protein of interest or replace a missing protein via gene therapy.
7. Using recombinant technology, gene expression can be blocked to study the role of genes in metabolism or the development of multicellular species.

14.1 Molecular Tools to Manipulate and Modify DNA

Watson and Crick, discoverers of the structure of DNA, suggested that they had discovered the secret of life. In a way, that statement became reality when scientists learned how to manipulate the molecule itself to reshape genes and move genes from one cell to another. With the deciphering of the universal genetic code, it became apparent that a gene from one organism would be interpreted by any other in exactly the same way. Recall that genes are just recipes for building proteins. Place a frog gene in a plant cell and the plant cell would make the frog protein, exactly as a frog would. But how does one find and extract one correct gene from billions of bases in a genome? How does one even identify the correct gene from thousands of possibilities? How can you move a gene from one cell to another cell?

Restriction Enzymes and Ligase Are Used to Cut and Paste

The answers came in contributions from different directions. Paul Berg was studying a group of enzymes found in bacteria that could destroy the DNA of invading viruses (see chapter 20). He found that these enzymes restricted the growth of the viruses and made highly specific cuts within a DNA strand. Consequently, these enzymes are called **restriction endonucleases** (or just "restriction enzymes"). Prior to this discovery, the enzymes known to cut DNA made only random cuts in the sugar-phosphate backbone, destroying the DNA sequences in the process. By contrast, restriction endonucleases recognize very specific sequences of bases (**figure 14.1**), usually in the form of **palindromes**—sequences that read the same in both forward and reverse directions, like the word "radar." No matter how long the DNA strand, the enzyme would not cut unless its specific recognition sequence was present. Bacteria had evolved these enzymes as a means to defend themselves. By chemically modifying their own DNA, they could ensure their own genes were unharmed, but the virus would be destroyed. Hundreds of such enzymes have been discovered and characterized.

Berg realized these enzymes could be used as a kind of "molecular scalpel" to surgically remove a segment of DNA containing a gene of interest without harming the gene itself. Another advantage became apparent as well—the cuts made by restriction enzymes. The fragments contain short single-stranded segments at their ends. When combined with fragments cut by the same enzyme, these single-stranded ends recognize each other and anneal (form double stranded molecules) through base pairing. Because of this base pairing, these ends are often referred to as **sticky ends** (figure 14.1).

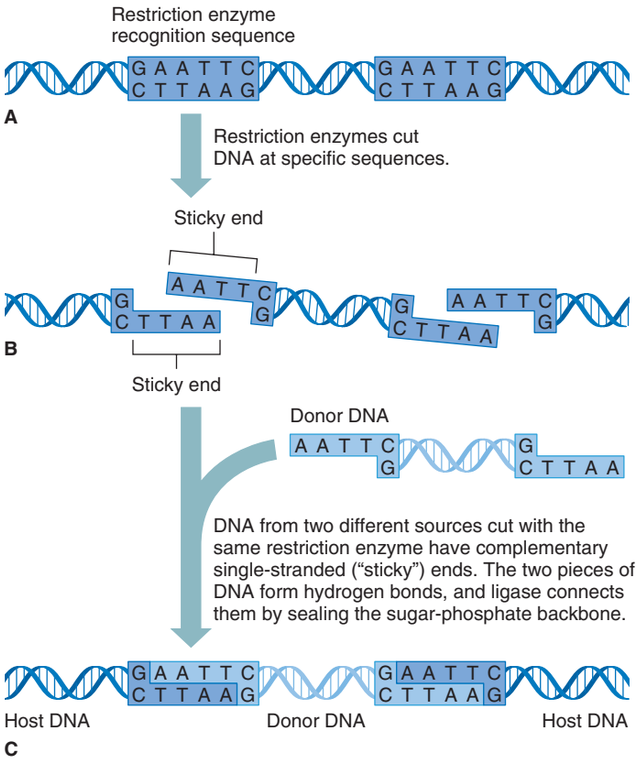


FIGURE 14.1 Recombining DNA. A restriction enzyme makes "sticky ends" in DNA by cutting it at specific sequences. (**A**) The enzyme *Eco*RI cuts the sequence GAATTC between G and the A. (**B**) This staggered cutting pattern produces "sticky ends" of sequence AATT. The ends attract through complementary base pairing. (**C**) DNA from two sources is cut with the same restriction enzyme. Pieces join, forming recombinant DNA molecules.

When fragments are combined in solution, they find one another and bind, leaving a small "nick" in the sugar-phosphate backbone. They are only held together by the base-pairing of the four base sticky overhangs. This is similar to the nicks left in the DNA molecule after replication of Okazaki fragments (see figure 13.14). Fortunately, nature has provided an enzyme to fix this very problem. **Ligase** can be added to the solution to seal the nicks. Berg realized he now had the tools to cut and paste DNA! Nowadays, several biotechnology companies provide hundreds of purified restriction enzymes, ligase, and dozens of other enzymes for use in the lab. With these tools, virtually any piece of DNA can be excised and pasted to any other piece.

Reverse Transcriptase Makes DNA from mRNA

Most experiments on DNA are done in a test tube, not under a microscope. But the DNA must be purified to remove any other molecules or enzymes that might destroy the DNA or interfere with the experiment. Genes can be excised from the entire genome of any organism. But identifying the region of interest takes a bit more work, which we will discuss shortly.

Another route to acquiring the DNA for a protein of interest makes use of a very unusual enzyme found in certain viruses known as **retroviruses** (see chapter 20). These viruses contain a genome of single-stranded RNA rather than the double-stranded DNA employed by most species. To make use of the enzyme machinery of a cell, these viruses carry an enzyme that makes DNA from an RNA template. Known as **reverse transcriptase,** this enzyme produces a double-stranded DNA molecule that precisely matches the bases in the single-stranded RNA template, using a process much like DNA replication.

If we purify the mRNA for a given protein instead of the entire DNA genome, we can place it in a test tube with reverse transcriptase and produce a DNA fragment of the gene for that protein. This approach has the additional advantage that the introns are already removed if we are working with a eukaryotic gene. The resulting DNA is called **complementary DNA** (**cDNA,**) to distinguish it from genomic DNA.

Vectors Are Molecules That Accomplish Cloning and Expression

Now that we have obtained a DNA fragment of interest, we need a way to carry it into a cell and have the cell maintain the DNA as though it were one of its own chromosomes. Molecules that can do this are called **vectors.** The most common type of vector is used to produce millions of duplicate copies—**clones**—of the DNA molecule and is therefore a **cloning vector.** To manipulate genes and DNA, scientists usually use the bacteria *E. coli* because it is relatively easy to grow in large quantities and much is already known about its biology and genetics. If we can place a gene into *E. coli,* we can grow large quantities of the DNA for study or even make the protein itself.

FIGURE 14.2 Plasmids. Plasmids are small circles of DNA found naturally in the cells of some organisms. A plasmid, along with any other DNA inserted into it, can replicate independently of the chromosomal DNA. For this reason, plasmids make excellent cloning vectors—structures that carry DNA from cells of one species into the cells of another.

In the early 1970s, researchers discovered that bacteria that became resistant to antibiotics contained small circular pieces of DNA called **plasmids.** Plasmids function like mini-chromosomes (**figure 14.2**), containing only a few genes and a signal used by the bacteria to replicate its own chromosomes. This signal, called the **origin of replication** (see figure 14.13), tells the bacteria to manufacture dozens, even hundreds of copies of the plasmid whenever it replicates its own DNA. Being made of DNA, plasmids can be cut by restriction enzymes, producing sticky ends. We can now put all of the pieces together to make a **recombinant DNA** molecule: the fragment containing our gene of interest, a plasmid cut with the same enzyme, and ligase (**figure 14.3**). Ligase will join the pieces, producing a new plasmid with our gene. When this recombinant plasmid is placed in a host cell, it will be duplicated into hundreds of clones of our gene!

The choice of vector is determined by the host cell into which we need to place our DNA. For human cells, which lack plasmids, we use retroviruses because they insert their DNA into the genome of the host cell. They are altered so that they transport DNA but cannot cause disease. Some vectors are designed with very powerful promoters in just the right location, so the host cell expresses the gene we have inserted. These **expression vectors** can produce huge quantities of whatever protein we choose.

Designing a Cloning Experiment

A recombinant DNA experiment is planned so that recombinant DNA molecules can be separated from molecules consisting of just donor DNA or just plasmid DNA. One way to do this is to use a plasmid containing two genes that each enable a cell to grow

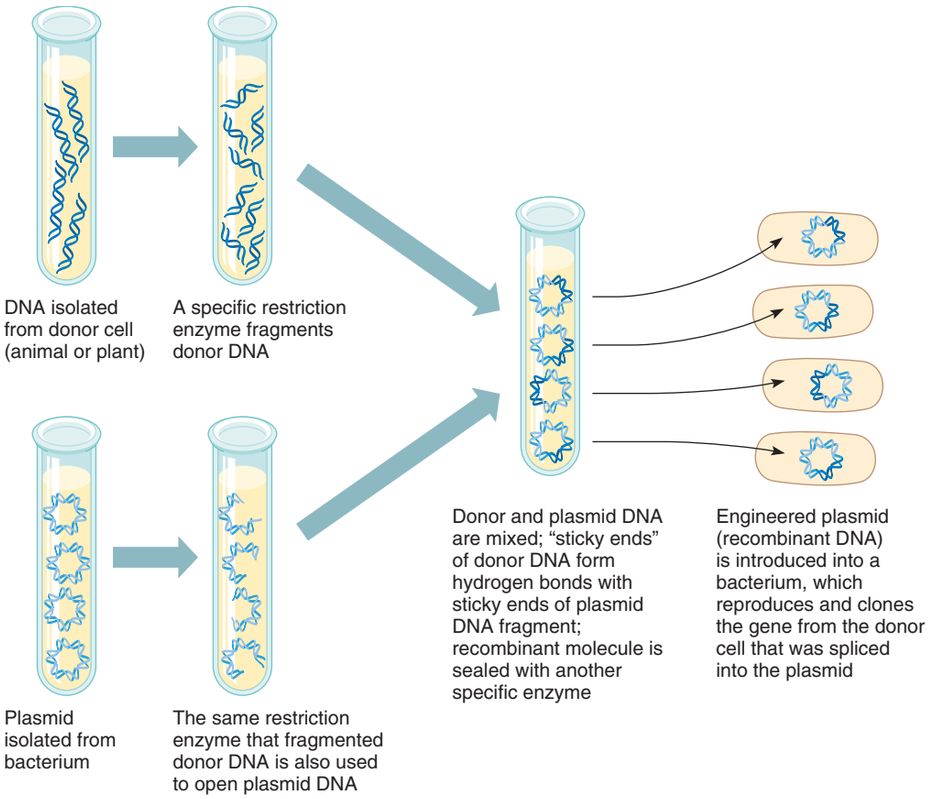


FIGURE 14.3 Recombinant DNA. To construct a recombinant DNA molecule, DNA isolated from a donor cell and a plasmid are cut with the same restriction enzyme and mixed. Some of the sticky ends from the donor DNA hydrogen bond with the sticky ends of the plasmid DNA, forming recombinant DNA molecules. When the plasmid is sent into a bacterium, it is mass-produced as the bacterium divides.

in the presence of a different antibiotic drug. By inserting our gene of interest into the plasmid in the middle of one of these antibiotic-resistance genes, the bacteria carrying the recombinant plasmid will be resistant to only one of the antibiotics. A cell that dies in the presence of one antibiotic but not the other is known to harbor the foreign gene. (The researcher keeps some of the cells aside so as not to kill off the desired ones.) Cells that grow in the presence of both antibiotics carry plasmids that have not incorporated the foreign gene; cells killed by both antibiotics lack plasmids.

The fact that all species use the same genetic code means that one type of organism can express a gene from another. Recombinant DNA technology takes advantage of this fact by using bacteria or other cells growing in culture to mass-produce a gene of interest and its protein product. Drugs made in this way are free of viral contamination that can contaminate drugs derived from donated tissue or cadavers. Another advantage is that recombinant DNA-derived proteins are the human versions and therefore are less likely to provoke allergic reactions than are drugs derived from other animals. Several dozen drugs are produced using this technology, including insulin, blood clotting factors, immune system biochemicals, and fertility hormones. But applications aren't all medical. The indigo dye used to make blue jeans blue, for example, comes from *E. coli* given another bacterial gene, rather than using the endangered plant that once supplied the dye.

Reviewing Concepts

- Restriction enzymes make very specific cuts in DNA.
- Ligase can paste DNA fragments together.
- Reverse transcriptase is another way to get a specific DNA copy of a gene of interest by starting with the mRNA.
- Plasmids are vectors that can carry and maintain a gene of interest in a host cell.
- Plasmids can also be used to express large quantities of a protein of interest.

14.2 The Polymerase Chain Reaction and DNA Amplification

Each cell has the enzymatic machinery needed to accurately replicate its entire DNA in a relatively short period of time. These enzymes have been purified and mass-produced for use in

the lab. Using the tremendous potential of this machinery, the **polymerase chain reaction** (**PCR**) can rapidly produce millions of copies of a DNA sequence of interest. The technique also takes advantage of advanced lab equipment that can artificially manufacture short pieces of DNA of any sequence it is programmed to produce. The **DNA synthesizer** cannot easily make entire genes, but it can make small fragments that can act as **primers** to DNA replication. If one primer is made for each end of the region of interest, they act to bracket the region to be amplified, ensuring only that region will be replicated. These are usually 15 or more bases in length, which ensures there will be no other such sequences in the entire genome.

PCR was born in Kary Mullis's mind on a moonlit night in northern California in 1983. As he drove up and down the hills, Mullis, a molecular biologist, was thinking about the incredible precision and power of DNA replication. Suddenly, a way to tap into that power popped into his mind. He excitedly explained his idea to his girlfriend and then went home to think it through further. "It was difficult for me to sleep with deoxyribonuclear bombs exploding in my brain," he wrote much later.

The idea behind PCR was so simple that Mullis had trouble convincing his superiors at Cetus Corporation that he was onto something. He spent the next year using the technique to amplify a well-studied gene. After he convinced his colleagues at Cetus, Mullis published his landmark 1985 paper and filed patent applications, launching the field of gene amplification. Cetus gave him a $10,000 bonus and then sold the technology for $300 million. Mullis did have the consolation of winning a Nobel Prize.

PCR Uses Rapid Cycling to Produce DNA Copies

PCR rapidly replicates a selected sequence of DNA in a test tube (**figure 14.4**). The requirements include the following:

1. a small, purified amount of the species' DNA—as little as that found in a single cell will work;
2. knowing parts of a target DNA sequence;
3. large molecular quantities of the two primers that bracket the target sequence;
4. a hefty supply of the four types of DNA nucleotide building blocks;
5. *Taq*1, a DNA polymerase produced by *Thermus aquaticus,* a bacterium that inhabits hot springs. This enzyme is adapted to its natural host's hot surroundings and makes PCR easy because it does not fall apart when DNA is heated. (Other heat-tolerant polymerases have also been identified and used.)

All of the components are mixed together in a small test tube and placed in a device that can rapidly change temperatures in a highly controlled manner—a thermal cycle. In the first step of PCR, heat is used to separate the two strands of the target DNA. Next, the temperature is lowered, allowing the two short DNA primers to anneal to the separated target strands. The temperature is then lowered to the ideal working temperature for the enzyme *Taq*1. The DNA polymerase adds the bases to the primers

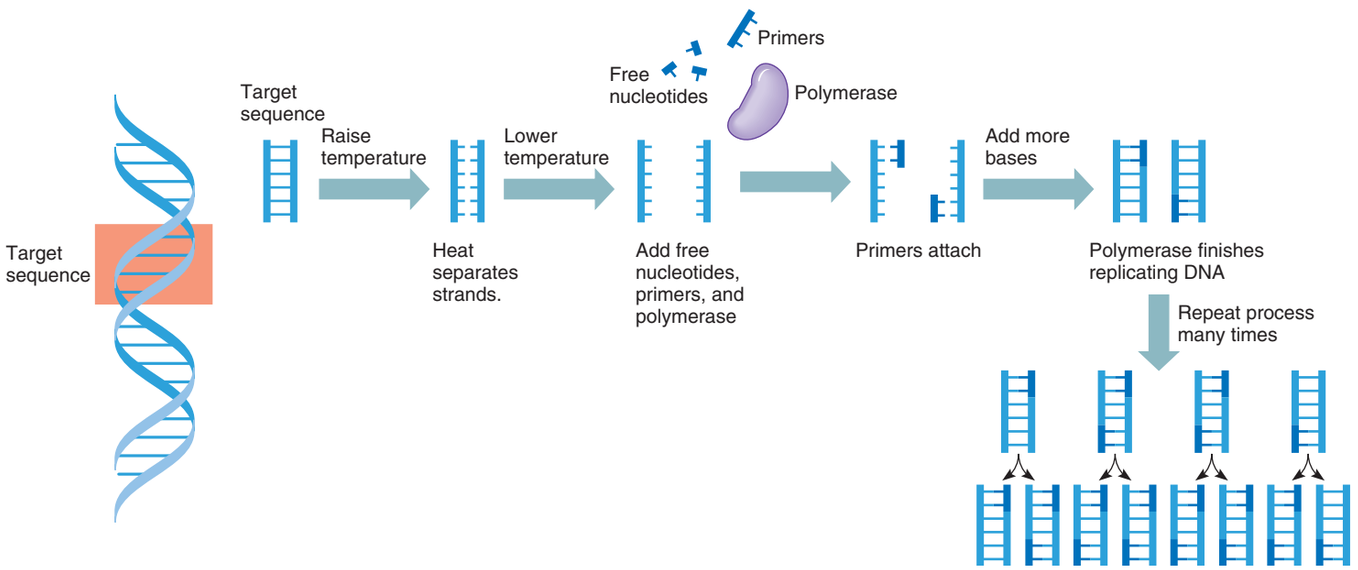


FIGURE 14.4 Amplifying a Specific DNA Sequence. In the polymerase chain reaction, specific primers are used to bracket a DNA sequence of interest. By using these primers, a thermostable DNA polymerase, and plenty of free nucleotides, the reaction rapidly builds up millions of copies of the target sequence.

and builds sequences complementary to the target sequence. The newly synthesized strands then act as templates in the next round of replication, which is initiated by raising the temperature to separate the strands. Since there are large amounts of both primers and the four nucleotides, the process can continue through many cycles.

The pieces of DNA accumulate geometrically. The number of amplified pieces of DNA equals 2^n, where n equals the number of temperature cycles. After just 20 cycles, a 1 millionfold increase in the number of copies of the original sequence accumulates.

Even a Tiny Amount of DNA Can Be Amplified and Matched

PCR is useful in any situation where a small amount of DNA or RNA would provide information if it was mass-produced (**table 14.1**). Applications are varied. In forensics, PCR is used routinely to establish genetic relationships, identify remains, and to help convict criminals or exonerate the falsely accused. When used to amplify the nucleic acids of microorganisms, viruses, and other parasites, PCR is important in agriculture, veterinary medicine, environmental science, and human health care. In genetics, PCR is both a crucial basic research tool and a way to identify disease-causing genes.

PCR's greatest strength is that it works on crude samples, such as a tiny droplet of blood in a white Ford Bronco, which led to victim identification in a highly celebrated murder case. PCR's greatest weakness, ironically, is its exquisite sensitivity. Any left-over DNA from a previous analysis or something as simple as a stray eyelash dropped from the person running the reaction can yield a false result. Even breathing into the tube can often leave enough DNA to be amplified.

Reviewing Concepts

- PCR has proven to be a powerful tool for analyzing DNA.
- A specific region of DNA can be detected from just a single cell.
- DNA can be amplified for study or cloning.

14.3 Separating and Analyzing DNA

Even with the specificity of restriction enzyme cutting, the billions of base pairs of a human genome would yield thousands, or millions, of fragments when cut by one. We need a method for separating one fragment from all of the others. This will also allow us to analyze the DNA patterns made by different species and make comparisons. Fortunately, DNA, with all of its negatively charged phosphates, will move in an electric field toward the positive terminal. This is the basis for a process known as **electrophoresis.**

TABLE 14.1

Eclectic PCR Applications

PCR Has Been Used to Amplify
A bit of DNA in a preserved quagga (a relative of the zebra) and a marsupial wolf, which are recently extinct animals.
Human remains from the World Trade Center, identifying most of the victims.
Similar genes from several species. Comparing the extent of similarity reveals evolutionary relationships among species.
Genetic material from saliva, hair, skin, and excrement of organisms that we cannot catch to study. The prevalence of a rare DNA sequence among all of the bird droppings from a certain species in an area can be extrapolated to estimate the population size.
DNA in products illegally made from endangered species, such as powdered rhinoceros horn, sold as an aphrodisiac.
DNA from genetically altered bacteria that are released in field tests, to follow their dispersion.
DNA from one cell of an eight-celled human embryo to diagnose cystic fibrosis.
DNA from poached moose in ground meat.
DNA from human remains in Jesse James's grave, to make a positive identification.
DNA from semen on a blue dress of a White House intern, which helped identify the person with whom she had a sexual encounter.
DNA from cat hairs on a murder victim, matched to DNA from a suspect's cat, Fluffy.
DNA from remains in Afghanistan thought to be from Osama bin Laden.

Gel Electrophoresis Separates DNA Fragments of Different Sizes

If we apply a direct electrical current across a container filled with buffer and water, DNA will move quickly toward the positive terminal. But as soon as the current is turned off, the DNA will rapidly diffuse away. To hold the DNA in place, and to separate fragments on the basis of their size, we use a semiliquid matrix called **agarose gel.** Samples of DNA are loaded at the negative end, and a current is applied (**figure 14.5**). Due to the pores created in the matrix by the agarose, larger fragments are impeded and move slower than short fragments. When the current is switched off, the fragments stay locked into place as discrete bands, each containing one size of fragment. Genomic DNA would contain so many such bands that the pattern would look more like a continuous smear. Smaller DNA samples produce highly reproducible patterns of bands. To understand how this process works, imagine a series of screens with increasingly small openings. If we apply a mixture of rocks and gravel, we can sort them into different sizes, trapped at each screen. Different mixtures of agarose produce different filtering effects.

Other compounds, such as **polyacrylamide,** are also used to separate DNA fragments under different types of analysis. Polyacrylamide is used when fragments need to be separated on the differences of just a single base.

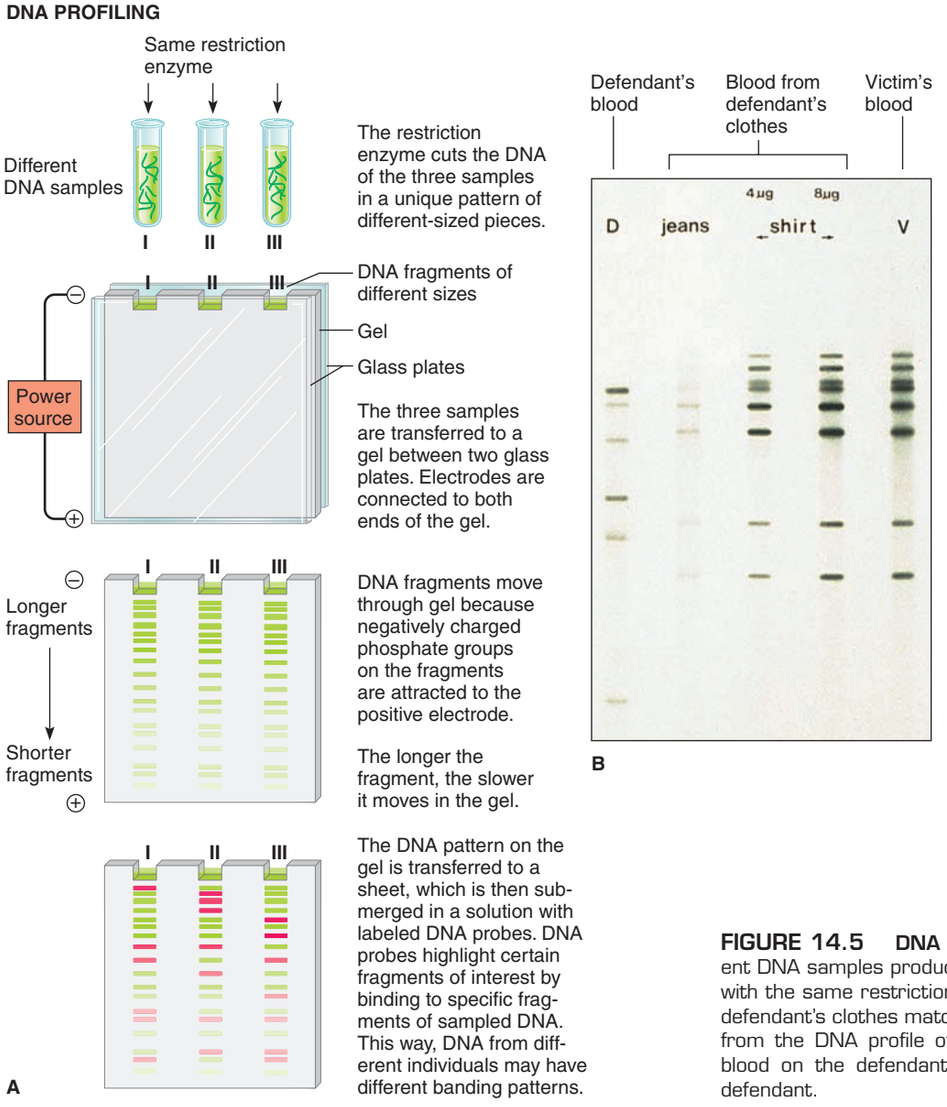


FIGURE 14.5 DNA Profiles from a Murder Case. (**A**) Different DNA samples produce different patterns of fragments when cut with the same restriction enzyme. (**B**) DNA from bloodstains on the defendant's clothes matches the DNA profile of the victim but differs from the DNA profile of the defendant. This is evidence that the blood on the defendant's clothes came from the victim, not the defendant.

DNA Fingerprinting Can Yield Precise Identification

So specific are the patterns produced by DNA cut by a restriction enzyme that they can be used to distinguish one human from another, even though they share better than 99% of the genes. **DNA fingerprinting,** or **profiling,** identifies genetic differences between individuals, with applications ranging from solving crimes to breeding wine grapes. It is based on the fact that the characteristic measured—DNA sequences—can vary in more ways than there are people.

Rather than sequencing and comparing entire genomes, DNA profiling considers highly variable parts of genomes. These are of two general types—single bases and repeats. **Single nucleotide polymorphisms** (**SNPs,** pronounced "snips") are single-base sites that tend to vary in a population. If a SNP alters a cutting site for a restriction enzyme, the number and sizes of pieces resulting from exposure to that restriction enzyme change. Researchers use gel electrophoresis to track such changes in DNA cutting patterns that reveal underlying sequence differences. Using restriction enzymes can also show whether stretches of DNA are missing or extra (figure 14.5).

The second approach to DNA profiling compares the numbers of certain short repeated DNA sequences. For example, one person might have 17 copies of the sequence ACT at a particular site on a particular chromosome; another individual might have 35 copies. Using restriction enzymes that cut on either side of this region generates different-sized pieces in these individuals.

DNA profiling results are given in terms of probability, that is, how likely two DNA patterns match because they come from the same person—such as semen in a rape victim's body and white blood cells from a suspect—rather than because two individuals resemble each other by chance. To make this distinction, the investigator consults population databases on allele frequencies to calculate the likelihood of a particular combination of DNA sequences occurring in an individual from a certain population. If the allele combination being examined is fairly common, a DNA match could happen by coincidence, just like two tall people with blond hair and blue eyes are not necessarily related just because they share some traits. To generate the statistics that make DNA fingerprinting results meaningful, allele frequencies are multiplied, which is an application of the product rule (see figure 11.13).

DNA profiling became a forensic tool in the mid-1980s, pioneered in the United Kingdom on short repeated sequences. Today, it is routine to take DNA samples from convicted felons, and many older criminal cases have been reopened thanks to improved DNA tests. In the United States and the United Kingdom, a third of all rape suspects are released soon after arrest, vindicated by DNA evidence.

Until September 11, 2001, the most challenging application was identifying plane-crash victims, a grim task eased by having lists of passengers. The terrorist attacks on the World Trade Center provided a staggeringly more complex situation, for several reasons: the high number of casualties, the condition of the remains, and the lack of a list of who was actually in the buildings. The scale of the DNA profiling task that followed the September 11, 2001, attack was unprecedented. DNA profiling of the 230 victims of a plane crash in 1996 took a year; the World Trade Center disaster yielded more than a million DNA samples. It was a very distressing experience for the technicians and researchers whose jobs had suddenly shifted from detecting breast cancer and sequencing genomes to helping in recovery. Said J. Craig Venter, former president of Celera, "I never, ever thought we would have to do DNA forensics at this level, and for this reason."

Hybridization Identifies DNA Segments of Interest

If there are so many bands in a human genome run through gel electrophoresis, how does one find the band containing the gene of interest? Scientists use a method known as **Southern blotting.** The DNA fragments in an agarose gel are transferred to a thin membrane that looks very much like a sheet of very fine paper, but is made of nylon. The DNA pieces stick to the surface of the membrane very tightly, allowing scientists to conduct experiments on the DNA without it moving. A probe is made from a known piece of DNA, such as a cDNA from the mRNA of interest. Under the right conditions, the probe will form base pairs with its complementary sequence on the membrane, a process known as **hybridization.** If we make the probe radioactive, we can tell where our fragment of interest is by Geiger counter or by exposing the membrane to film. We can then use this information to go back to the gel and extract the DNA from the agarose. The fragment can then be inserted into a plasmid for further study. Probes can even be made artificially using a DNA synthesizer. All that is needed is a short bit of the actual sequence of the gene.

Reviewing Concepts

- DNA can be cut into identifiable fragments using restriction enzymes.
- Gel electrophoresis then separates these fragments by size.
- The patterns produced are unique to each individual and can be used for identification.
- Southern blotting can help identify fragments for cloning.

14.4 DNA Sequencing

We have discussed how to cut and paste DNA to make virtually any kind of recombinant DNA molecule. But we can gain much more information if we know, base by base, the entire sequence of a gene or even the entire genome of an organism. The technique for reading the sequence of a DNA molecule is called **DNA sequencing.** Two major methods are currently employed by sophisticated instruments known as DNA sequencers. They

are approaching the state where a technician can simply add the DNA and the machine reads it.

The Sanger Method Creates Fragments During Replication

Modern DNA sequencing instruments utilize a basic technique Frederick Sanger developed in 1977. The overall goal is to generate a series of DNA fragments of identical sequence that are complementary to the sequence of interest. These fragments differ in length from each other by one end base.

Note that the entire complementary sequence appears in the sequence of end bases of each fragment. If the complement of the gene of interest can be cut into a collection of such pieces, and the end bases distinguished with a radioactive or fluorescent label, then polyacrylamide gel electrophoresis can be used to separate the fragments by size. Once the areas of overlap are aligned, reading the labeled end bases in size order reveals the sequence of the complement. Replacing A with T, G with C, T with A, and C with G establishes the sequence of the DNA in question.

Sanger invented a clever way to generate the DNA pieces by stopping DNA replication instead of cutting the DNA. In a test tube, he included the unknown DNA fragment and all of the biochemicals needed to replicate it, including supplies of the four nucleotide bases. A separate sample of each of the four types of bases were chemically modified at a specific location on the base sugar to contain no oxygen atoms instead of one—in the language of chemistry, they were dideoxyribonucleotides rather than deoxyribonucleotides. DNA synthesis halts when DNA polymerase encounters a "dideoxy" base, leaving only a piece of the newly replicated strand. A radioactive nucleotide, usually "C," is also included in each reaction to visualize the bands.

Sanger repeated the experiment four times, each time using a dideoxy version of A, T, C, then G. The four experiments were run in four lanes of a gel (**figure 14.6**). Today, fluorescent labels are used, one for each of the four base types, allowing a single experiment to reveal the sequence. A laser is used to scan the gel as the fragments separate. The data appear as a sequential readout of the wavelengths of the fluorescence from the labels.

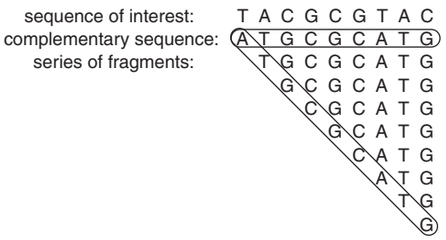
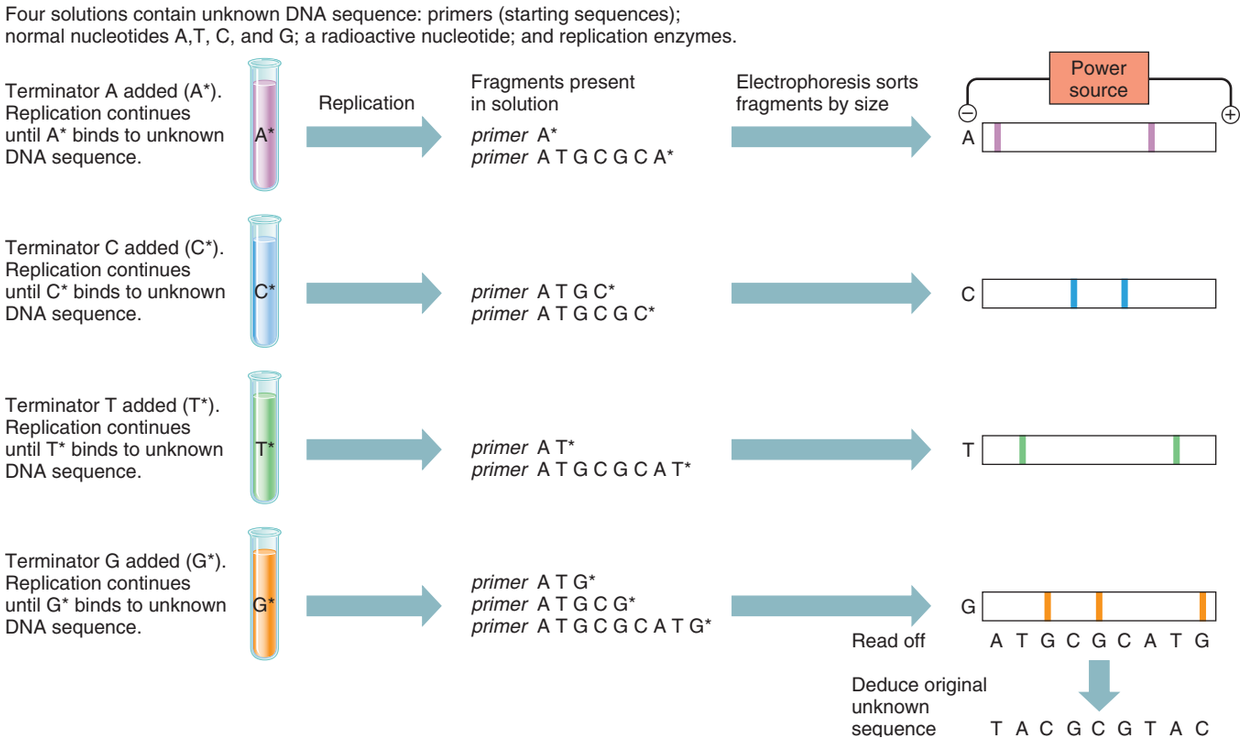


FIGURE 14.6 Determining the Sequence of DNA. In the Sanger method of DNA sequencing, complementary copies of an unknown DNA sequence are terminated early because of the incorporation of dideoxynucleotide terminators. A researcher or computer deduces the sequence by placing the fragments in size order. Radioactive labels are used to visualize the sparse quantities of each fragment.



DNA Chips with Known Sequences Facilitate Analysis

Sequencing by hybridization is another way to analyze DNA. Short DNA fragments of known sequence are immobilized on a small glass square called a DNA microchip or a DNA microarray (**figure 14.7**). These chips are carefully manufactured to allow hybridization to known sequences. In one version, the 4,096 possible six-base combinations (hexamers) of DNA are placed onto a 1-centimeter-by-1-centimeter microchip. Copies of an unknown DNA segment containing a fluorescent label are then used to probe the microchip. The copies stick (hybridize) to immobilized hexamers whose sequences are complementary to the DNA segment's sequences. Under laser light, the bound hexamers fluoresce. Because the researcher (or computer) knows which hexamers occupy which positions on the microchip, a scan of the chip reveals which six-base sequences comprise the unknown sequence. Then, software aligns the identified hexamers by their overlaps. This reconstructs the complement of the entire unknown sequence.

Reviewing Concepts

- The specific sequence of a DNA molecule can be determined chemically.
- The Sanger method generates fragments with known bases at their ends.
- Modern approaches use hybridization on chips and computer technology to assemble sequence data based on complementary base pairing to known short sequences on a chip.

14.5 Applying Recombinant DNA Technology

With our constantly growing understanding of the nature and functions of genes, scientists have been able to use recombinant DNA technology to address new problems of biology. Since a gene

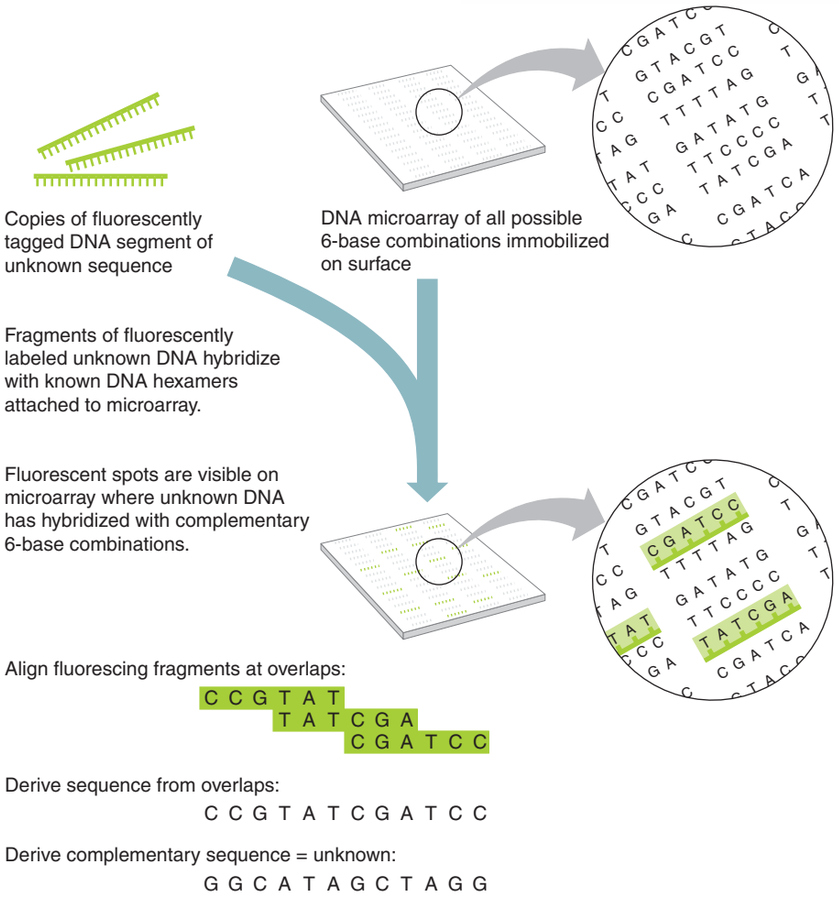


FIGURE 14.7 Sequencing by Hybridization. A labeled DNA segment of unknown sequence base pairs to short, known DNA sequences immobilized on a small glass microchip. Identifying the small, bound sequences; overlapping them; and then reading off their sequences reveals the unknown DNA sequence.

is read the same way in every organism, we can use plants to make needed human proteins. We can alter the genetic makeup of an organism to produce new foods or new drugs or to repair errors in existing genes. While simple in concept, these techniques are often very difficult to achieve. Here we discuss a few of the possibilities.

Transgenic Technology Alters the Genes of an Organism

Bacteria are relatively easy to work with due to their single chromosome and their ability to carry and express plasmids. More complex cells and organisms require different approaches to introducing DNA into a target cell or organism. Recombinant DNA technology in a multicellular organism is termed **transgenic technology.** Genes of interest are introduced into a gamete or fertilized ovum, and the organism that develops carries the foreign gene, or "transgene," in every cell and expresses it in appropriate tissues. **Figure 14.8** shows some ways that researchers introduce transgenes: zapping the recipient cell with electricity to open temporary holes that admit "naked" DNA; shooting in or injecting DNA; sending it in inside a fatty bubble called a liposome; or hitching DNA to an infecting virus. In many flowering plants, transgenes are delivered in a *Ti* plasmid, which is a ring of DNA that normally resides in certain bacteria.

Transgenic organisms have diverse applications. A mouse "model" transgenic for a human gene can reveal how a disease begins, enabling researchers to develop drugs that treat the early stages of diseases. Transgenic farm animals can secrete human proteins in their milk or semen, yielding abundant, pure supplies of otherwise rare substances useful as drugs, such as clotting factors. **Figure 14.9** illustrates a combination of transgenes from several organisms that encodes a protein, calcitonin, which is used as a drug.

Transgenic crops resist pests, survive harsh environmental conditions, or contain nutrients they otherwise wouldn't. Tobacco plants can be altered to express the glow of a firefly's luciferase gene or to produce human hemoglobin. Transgenic fish given growth hormone reach market size very quickly, and those given antifreeze genes can live in colder climes. "Golden rice" contains genes from petunia and bacteria that enable it to produce beta-carotene (a vitamin A precursor) and extra iron, making it more nutritious.

Gene Therapy Seeks to Fix Inborn Genetic Errors

Gene therapy replaces a nonfunctioning gene in somatic cells. The 1990s began with a gene therapy success but ended with a tragic failure.

In 1990, 4-year-old Ashanti DaSilva received her own white blood cells bolstered with a gene whose absence caused her inherited immune deficiency. She and another child treated soon after did well but had to receive booster treatments as their altered white blood cells naturally died off. Others treated as babies received genetically altered umbilical-cord stem cells, which have provided a longer-lasting cure, with healthy cells gradually replacing their deficient ones.

Since then, gene therapy has had limited clinical success. The therapeutic gene must be isolated and delivered to the cell type that needs correction, then be expressed long enough to improve health—without alerting the immune system. Unfortunately, this is what happened to 18-year-old Jesse Gelsinger. In September 1999, Gelsinger received a massive infusion of viruses carrying a gene to correct an inborn error of metabolism. He

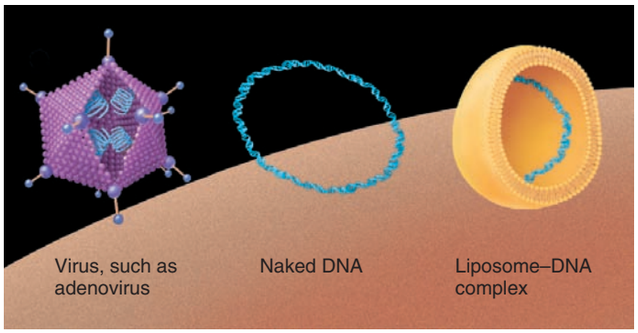


FIGURE 14.8 Adding DNA to a Cell. DNA can be sent into cells in viruses, alone (naked) or in liposomes.

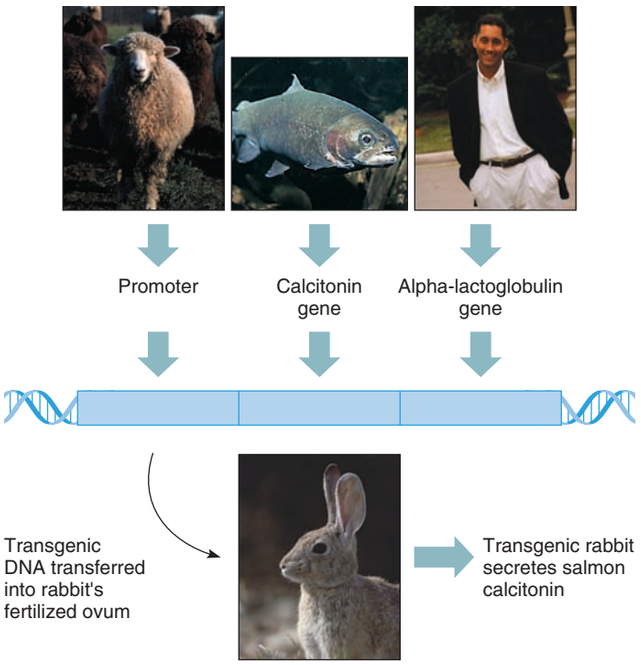


FIGURE 14.9 Combining Genes. Transgenic technology can combine gene segments from different organisms to produce a new gene that codes for a useful substance. DNA from a sheep, a salmon, and a human is combined and transferred to a rabbit's fertilized ovum to make a transgenic rabbit that produces calcitonin, used for treating bone disorders. The human alpha-lactoglobulin gene enables the drug to be secreted in the rabbit's milk.

died in days from an overwhelming immune system reaction. If more extensive genetic tests had been run, a significant susceptibility might have been identified, and Gelsinger not allowed to participate. Jesse's death led to a temporary halt to several gene therapy protocols and stricter rules for conducting experiments. But gene therapy research and clinical trials continue.

DNA Microarrays Monitor Gene Expression

A **DNA microarray,** also known as a "DNA chip," is a collection of genes or gene pieces placed in defined positions on a small square of glass, nylon, silicon (an element), or silica (sand). A microarray is used to monitor gene expression in different cell types or in cells under different conditions. Researchers first make DNA copies, called complementary DNAs, or cDNAs, of the mRNAs in the cells of interest and bind them to a chip. When fluorescently tagged DNA from a tissue sample, such as cells scraped from the inside of the cheek, is applied to the chip, dots of light fluoresce where the sample DNA matches the chip's cDNAs. A computer then analyzes the significance of the pattern of colored dots on the square.

Overall, a DNA microarray provides a peek at gene expression. By creatively choosing genes to target, researchers can ask and answer a seemingly endless list of questions, from basic biology to clinical applications.

Reviewing Concepts

- The tools of recombinant technology can be applied to modify the genes of an entire organism or to reprogram the genetics of an organism to produce new or missing proteins.
- Other techniques can monitor the expression of genes in a group of cells.

14.6 Gene Silencing: Antisense and Knockout Technologies

If we can block the expression of a gene, we can look for missing functions that correspond to that gene. In a kind of reverse logic, we can work out the steps of development and the roles of various genes in maintaining life by removing just that gene from the picture. Antisense and knockout technologies do this, with potential applications in agriculture and health care.

Antisense Technology Stops Expression

In **antisense technology**, an RNA sequence complementary to a messenger RNA (mRNA) blocks a gene's expression by binding to the mRNA, making protein synthesis impossible. The name derives from the fact that mRNA is sometimes called "sense" RNA. Its complement is therefore called "antisense" RNA (**figure 14.10**). Antisense technology theoretically can squelch activity of any gene if the RNA can persist long enough and if it can be delivered to the appropriate tissue. When a cell encounters the resulting double-stranded, or duplex, RNA, it destroys it as foreign. Usually, only viruses make use of dsRNA, so cells have evolved an enzyme called RNase that quickly responds to their presence.

As first conceived in the late 1970s, antisense technology used synthetic pieces of single-stranded nucleic acids, usually about 20 bases long, called **oligonucleotides.** But enzymes in cells quickly dismantled the "oligos." A more successful approach is to add a DNA sequence that encodes the antisense RNA sequence to organisms from the very beginning of development. In the "FlavrSavr" tomato, developed in the mid-1980s, for example, an antisense sequence silenced a ripening enzyme, and the tomatoes remained firm and red in the supermarket for days longer than their unaltered counterparts. However, FlavrSavr was doomed because it just didn't taste very good and consumers feared genetically modified foods. Today, a variation of antisense technology called RNA interference, or RNAi, uses small, double-

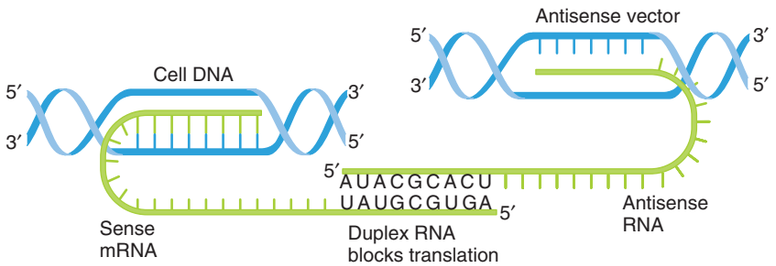


FIGURE 14.10 Silencing Gene Expression. Antisense technology uses a complementary nucleic acid sequence, or a similar molecule, to bond with mRNA, thereby preventing protein synthesis. RNase, found in most cells, dismantles the blocked mRNA. Antisense mechanisms exist naturally to fight viral infections.

stranded pieces of RNA to bind to and silence complementary mRNAs. RNA interference occurs naturally and is a way that organisms fight viral infections.

Another alternative to oligos is synthetic chemicals that retain the base sequence of interest but alter the sugar-phosphate backbone so that the molecules are more stable. Several of these antisense compounds are on the market or in clinical trials as drugs. The first has been available since 1998 to treat an eye condition caused by cytomegalovirus, which is severe in people with AIDS. The antisense molecule blocks synthesis of two proteins that the virus needs to replicate. Another antisense drug blocks production of a cellular adhesion molecule that is overproduced in Crohn disease, an intestinal inflammatory condition. Other antisense drugs target psoriasis, hepatitis C, multiple sclerosis, and cancers.

Knockout Technology Reveals Developmental Steps

In contrast to the practical applications of antisense technology, **knockout technology** is more of a basic research tool, used to reveal a gene's function by blocking it. Also called gene targeting, knockout technology uses a natural process called homologous recombination to swap a disabled form of a gene for its natural counterpart in a chromosome. The technology was developed in the 1980s, using mice, then was extended to include "knock-ins" that swap in a gene with altered function—from any species.

Gene targeting is technically difficult because unlike antisense manipulations, it does not work on fertilized ova. Instead, researchers perform the manipulation on isolated cells of a very early mouse embryo. These altered embryonic stem (ES) cells are then transferred to embryos to continue development in a female mouse. Breeding the resulting mice to each other then yields some individuals who have the knocked-out or knocked-in gene in every cell.

Researchers today routinely knock out mouse genes that have disease-causing counterparts in humans to better understand how the disease arises. Knockouts have also yielded surprises, when animals with genes thought to be vital do quite well without them. Such results suggest that gene functions can be redundant—that is, if one gene is disabled, another might substitute for it.

Reviewing Concepts

- Gene expression can be blocked to study the effect of the gene on the cell or on an entire organism.
- The involvement of different genes in each developmental step may be deciphered by removing them in the early embryo.

Connections

We have learned much about life by understanding how DNA functions within a cell. We have gained the ability to significantly modify organisms at the genetic level. There will be some very difficult ethical questions that accompany these developments. We should remember that we are dealing with life and have respect for its unique nature. As we explore the topic of evolution, starting in Chapter 15, we will see that Nature has been altering the genetic makeup of species for billions of years, in a process known as natural selection. The advent of recombinant DNA technologies has raised interesting questions about the process of natural selection and the formation of new species.

Student Study Guide

Key Terms

agarose gel *261*
antisense technology *266*
clone *257*
cloning vector *257*
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single nucleotide polymorphism (SNP) *262*
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vector *257*

Chapter Summary

14.1 Molecular Tools to Manipulate and Modify DNA

1. Naturally occurring enzymes, such as restriction enzymes, ligase, and reverse transcriptase, are used to modify and manipulate DNA molecules.
2. Plasmids are vectors that can carry and maintain a gene of interest in a host cell to express large quantities of a protein of interest.

14.2 The Polymerase Chain Reaction and DNA Amplification.

3. PCR can produce large quantities of a specific region of the DNA for study or cloning.
4. Each individual's DNA is unique and can be used in identification.

14.3 Separating and Analyzing DNA

5. DNA can be cut into identifiable fragments using restriction enzymes that make cuts in DNA molecules only at highly specific sequences.
6. Gel electrophoresis separates DNA fragments by size, producing unique patterns of bands.

14.4 DNA Sequencing

7. Several methods are available for determining the precise sequence of a DNA molecule.
8. Modern approaches use hybridization on chips and computer technology to assemble sequence data based on complementary base pairing to known short sequences on a chip.

14.5 Applying Recombinant DNA Technology

9. The tools of recombinant technology can be applied to modify the genes of an entire organism or to reprogram the genetics of an organism to produce new or missing proteins.

14.6 Gene Silencing: Antisense and Knockout Technologies

10. Gene expression can be blocked to study the effect of the gene on the cell or on an entire organism.
11. The involvement of different genes in each developmental step may be deciphered by removing them in the early embryo.

What Do I Remember?

1. Describe the uses of restriction enzymes in nature and in modifying DNA.
2. Describe the steps involved in identifying and excising a specific gene from a DNA sample and inserting it into a plasmid.
3. Compare and contrast the three different methods for determining the sequence of a DNA molecule.
4. Describe the features that must be present in a vector that is used to clone DNA.
5. Describe three rounds of amplification in PCR.

Fill-in-the-Blank

1. DNA fragments produced by a restriction enzyme have single-stranded regions known as _____.
2. A plasmid must contain a(n) _____ to maintain it within a dividing cell.
3. Reverse transcriptase makes a DNA copy of mRNA known as _____.
4. _____ uses an electric field to separate DNA molecules by their size.
5. A(n)_____ animal contains a gene from another organism.

Multiple Choice

1. The enzyme used to "glue" two pieces of recombinant DNA together is
   a. DNA polymerase.
   b. ligase.
   c. reverse transcriptase.
   d. restriction enzyme.
   e. RNA polymerase.
2. A gene may be "silenced" by the production within the cell of
   a. antisense RNA.
   b. restriction enzymes.
   c. reverse transcriptase.
   d. a new genetic code.
   e. an artificial chromosome.
3. If a gene for frog slime is placed into a cat embryo, what would be the result?
   a. The cat would make slime identical to the frog.
   b. The cat would make different slime from the frog gene.
   c. Nothing; cats cannot process frog genes.
   d. The genetic code of the frog gene would be altered by the cat.

4. The DNA regions used for DNA fingerprinting are called
   a. SNPs.
   b. RNPs.
   c. PCRs.
   d. RNAi.

5. Gene therapy should best be described as
   a. replacing a defective gene in an individual.
   b. modifying the genetic code of a person.
   c. removing all of the mutations from a person's genes.
   d. injecting a missing protein to provide a missing function.