# 15.2 Recombinant DNA

**THINK ABOUT IT** Suppose you have an electronic game you want to change. Knowing that the game depends on a coded program in a computer microchip, how would you set about rewriting the program? First you'd need a way to get the existing program out of the microchip. Then you'd have to read the program, make the changes you want, and put the modified code back into the microchip. What does this scenario have to do with genetic engineering? Just about everything.

## Copying DNA

***How do scientists copy the DNA of living organisms?***

Until recently plant and animal breeders could only work with variations that already exist in nature. Even when breeders tried to add variation by introducing mutations, the changes they produced were unpredictable. Today genetic engineers can transfer certain genes at will from one organism to another, designing new living things to meet specific needs.

Recall from Chapter 14 that it is relatively easy to extract DNA from cells and tissues. The extracted DNA can be cut into fragments of manageable size using restriction enzymes. These restriction fragments can then be separated according to size using gel electrophoresis or another similar technique. That's the easy part. The tough part comes next: How do you find a specific gene?

The problem is huge. If we were to cut DNA from a bacterium like *E. coli* into restriction fragments averaging 1000 base pairs in length, we would have 4000 restriction fragments. In the human genome, we would have 3 million restriction fragments. How do we find the DNA of a single gene among millions of fragments? In some respects, it's the classic problem of finding a needle in a haystack—we have an enormous pile of hay and just one needle.

Actually, there is a way to find a needle in a haystack. We can toss the hay in front of a powerful magnet until something sticks. The hay won't stick, but a needle made of iron or steel will. Believe it or not, similar techniques can help scientists identify specific genes.

### Key Questions

- ***How do scientists copy the DNA of living organisms?***
- ***How is recombinant DNA used?***
- ***How can genes from one organism be inserted into another organism?***

### Vocabulary

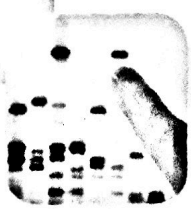
polymerase chain reaction
recombinant DNA
plasmid
genetic marker
transgenic
clone

### Taking Notes

**Preview Visuals** Before you read, preview **Figure 15–7** and write down any questions you may have about the figure. As you read, find answers to your questions.

**MYSTERY CLUE**

**How could restriction enzymes be used to analyze the DNA evidence found on the suspect?**



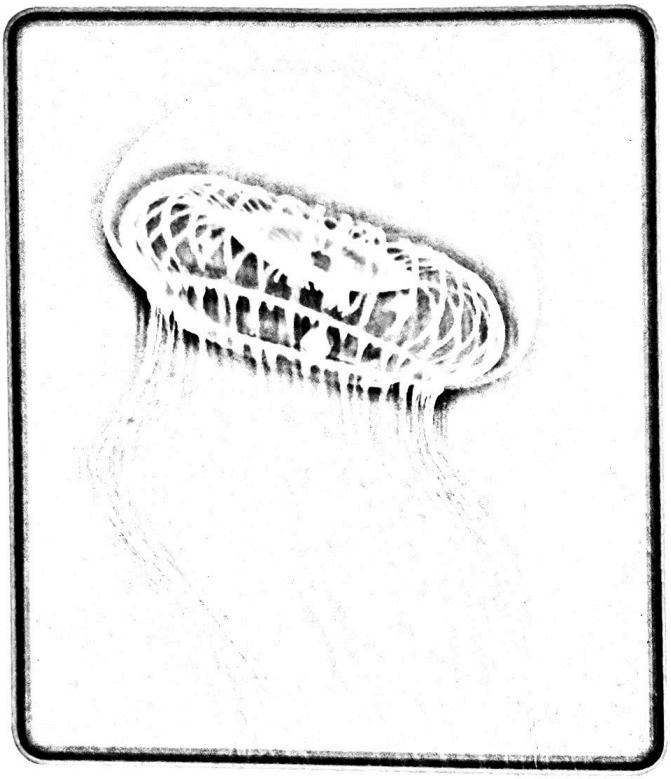


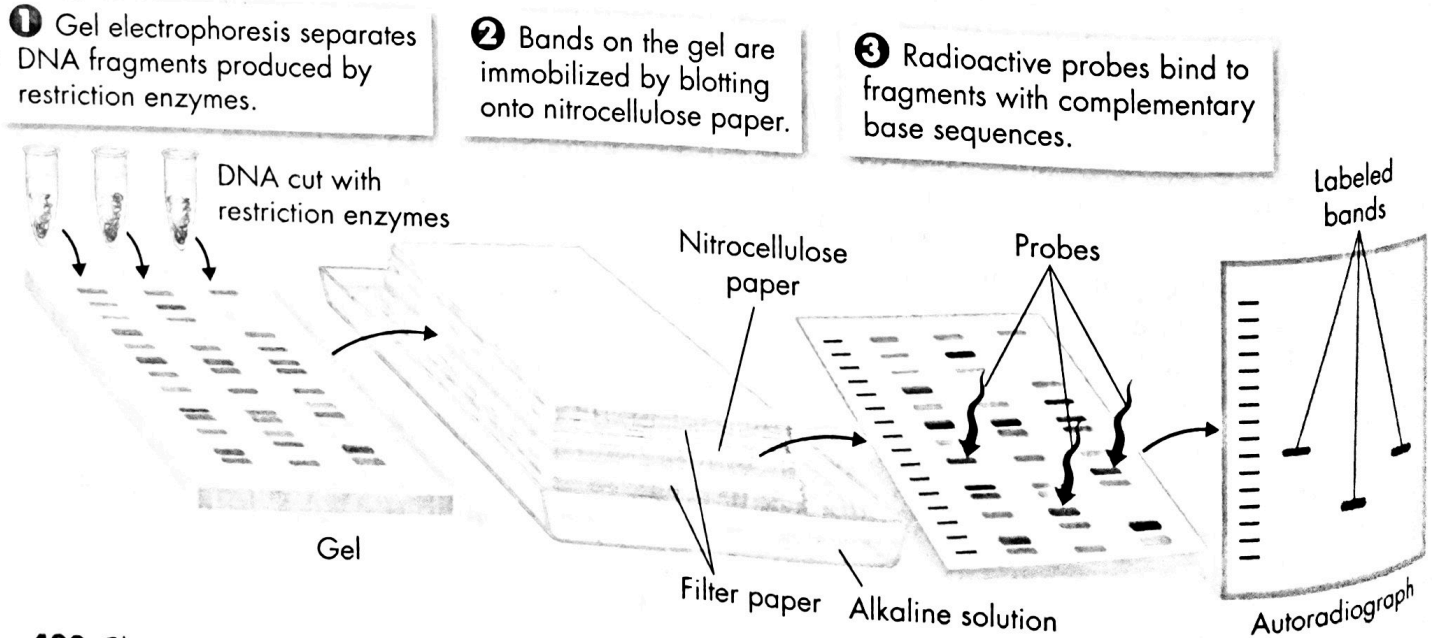
**FIGURE 15–5 A Fluorescent Gene** The Pacific Ocean jellyfish, *Aequoria victoria,* emits a bluish glow. A protein in the jellyfish absorbs the blue light and produces green fluorescence. This protein, called GFP, is now widely used in genetic engineering.

**Finding Genes** In 1987, Douglas Prasher, a biologist at Woods Hole Oceanographic Institute in Massachusetts, wanted to find a specific gene in a jellyfish. The gene he hoped to identify is the one that codes for a molecule called green fluorescent protein, or GFP. This natural protein, found in the jellyfish shown in **Figure 15–5,** absorbs energy from light and makes parts of the jellyfish glow. Prasher thought that GFP from the jellyfish could be used to report when a protein was being made in a cell. If he could somehow link GFP to a specific protein, it would be a bit like attaching a light bulb to that molecule.

To find the GFP gene, Prasher studied the amino acid sequence of part of the GFP protein. By comparing this sequence to a genetic code table, he was able to predict a probable mRNA base sequence that would have coded for this sequence of amino acids. Next, Prasher used a complementary base sequence to "attract" an mRNA that matched his prediction and would bind to that sequence by base pairing. After screening a genetic "library" with thousands of different mRNA sequences from the jellyfish, he found one that bound perfectly.

After Prasher located the mRNA that produced GFP, he set out to find the actual gene. Taking a gel in which restriction fragments from the jellyfish genome had been separated, he found that one of the fragments bound tightly to the mRNA. That fragment contained the actual gene for GFP, which is now widely used to label proteins in living cells. The method he used, shown in **Figure 15–6,** is called Southern blotting. Today it is often quicker and less expensive for scientists to search for genes in computer databases where the complete genomes of many organisms are available.

**FIGURE 15–6 Southern Blotting** Southern blot analysis, named after its inventor Edwin Southern, is a research technique for finding specific DNA sequences, among dozens. A labeled piece of nucleic acid serves as a probe among the DNA fragments.



**Polymerase Chain Reaction** Once they find a gene, biologists often need to make many copies of it. A technique known as **polymerase chain reaction** (PCR) allows them to do exactly that. At one end of the original piece of DNA, a biologist adds a short piece of DNA that complements a portion of the sequence. At the other end, the biologist adds another short piece of complementary DNA. These short pieces are known as primers because they prepare, or prime, a place for DNA polymerase to start working.

As **Figure 15–7** suggests, the idea behind the use of PCR primers is surprisingly simple. **The first step in using the polymerase chain reaction method to copy a gene is to heat a piece of DNA, which separates its two strands. Then, as the DNA cools, primers bind to the single strands. Next, DNA polymerase starts copying the region between the primers. These copies can serve as templates to make still more copies.** In this way, just a few dozen cycles of replication can produce billions of copies of the DNA between the primers.

Where did Kary Mullis, the American scientist who invented PCR, find a DNA polymerase enzyme that could stand repeated cycles of heating and cooling? Mullis found it in bacteria from the hot springs of Yellowstone National Park in the northwestern United States—a powerful example of the importance of biodiversity to biotechnology!

**In Your Notebook** *List the steps in the PCR method.*

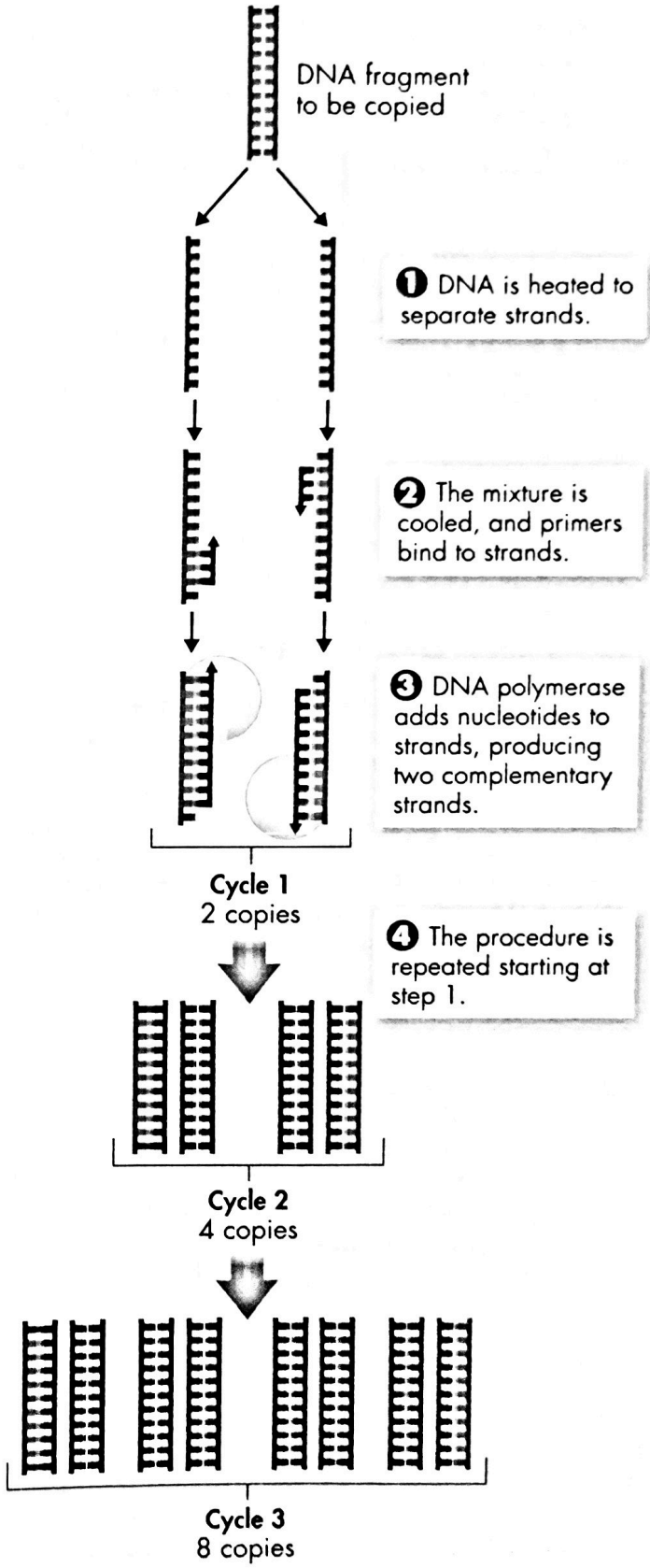



**FIGURE 15–7 The PCR Method** Polymerase chain reaction is used to make multiple copies of a gene. This method is particularly useful when only tiny amounts of DNA are available. **Calculate** ***How many copies of the DNA fragment will there be after six PCR cycles?***

# Changing DNA

***How is recombinant DNA used?***

Just as they were beginning to learn how to read and analyze DNA sequences, scientists began wondering if it might be possible to change the DNA of a living cell. As many of them realized, this feat had already been accomplished decades earlier. Do you remember Griffith's experiments on bacterial transformation? During transformation, a cell takes in DNA from outside the cell, and that added DNA becomes a component of the cell's own genome. Today biologists understand that Griffith's extract of heat-killed bacteria contained DNA fragments. When he mixed those fragments with live bacteria, a few of them took up the DNA molecules, transforming them and changing their characteristics. Griffith, of course, could only do this with DNA extracted from other bacteria.

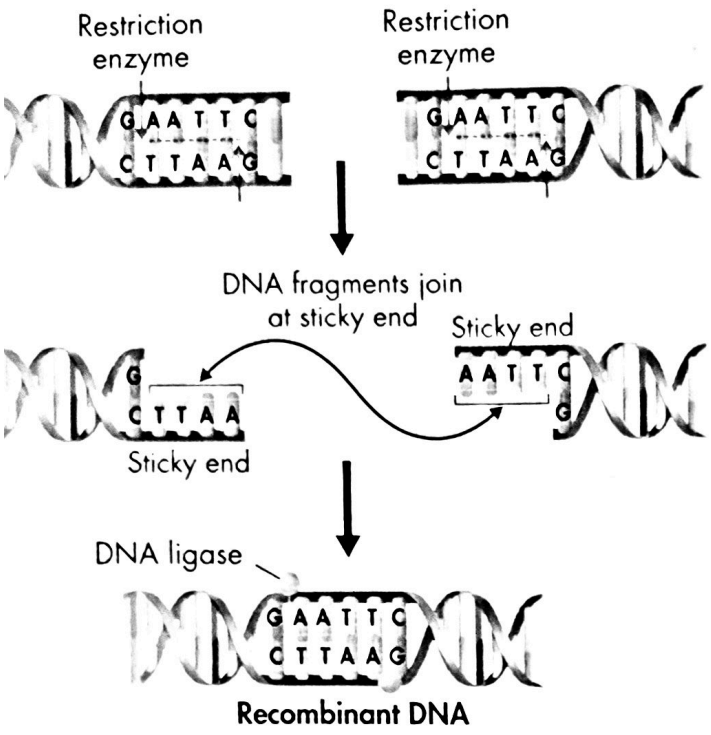



**FIGURE 15–8 Joining DNA Pieces Together** Recombinant DNA molecules are made up of DNA from different sources. Restriction enzymes cut DNA at specific sequences, producing "sticky ends," which are single-stranded overhangs of DNA. If two DNA molecules are cut with the same restriction enzyme, their sticky ends will bond to a fragment of DNA that has the complementary sequence of bases. An enzyme known as DNA ligase can then be used to join the two fragments.

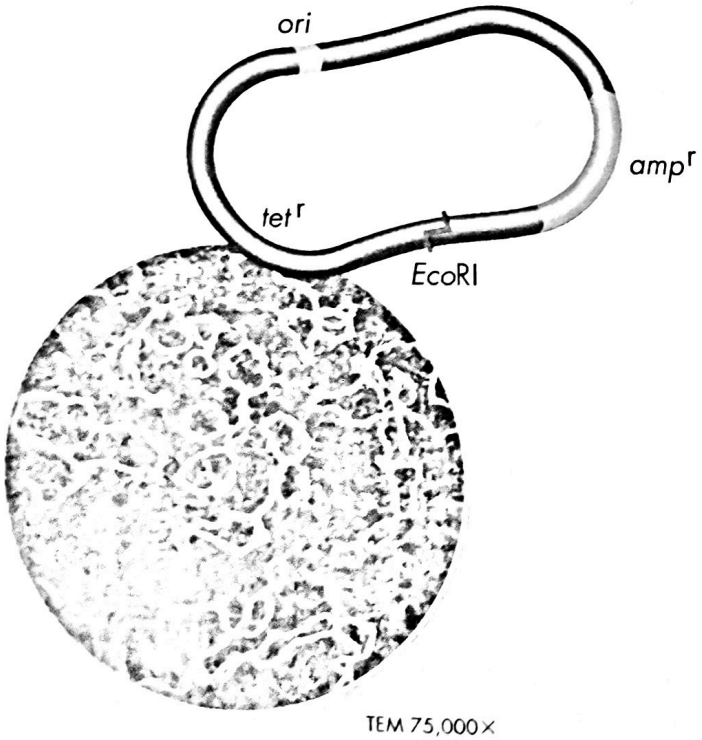



**FIGURE 15–9 A Plasmid Map** Plasmids used for genetic engineering typically contain a replication start signal, called the origin of replication (*ori*), and a restriction enzyme cutting site, such as *Eco*RI. They also contain genetic markers, like the antibiotic resistance genes *tet*$^r$ and *amp*$^r$ shown here.

**Combining DNA Fragments** With today's technologies, scientists can produce custom-built DNA molecules in the lab and then insert those molecules—along with the genes they carry—into living cells. The first step in this sort of genetic engineering is to build a DNA sequence with the gene or genes you'd like to insert into a cell. Machines known as DNA synthesizers can produce short pieces of DNA, up to several hundred bases in length. These synthetic sequences can then be joined to natural sequences using DNA ligase or other enzymes that splice DNA together. These same enzymes make it possible to take a gene from one organism and attach it to the DNA of another organism, as shown in **Figure 15–8.** The resulting molecules are called **recombinant DNA.** This technology relies on the fact that any pair of complementary sequences tends to bond, even if each sequence comes from a different organism.

**Recombinant-DNA technology—joining together DNA from two or more sources—makes it possible to change the genetic composition of living organisms.** By manipulating DNA in this way, scientists can investigate the structure and functions of genes.

**Plasmids and Genetic Markers** Scientists working with recombinant DNA soon discovered that many of the DNA molecules they tried to insert into host cells simply vanished because the cells often did not copy, or replicate, the added DNA. Today scientists join recombinant DNA to another piece of DNA containing a replication "start" signal. This way, whenever the cell copies its own DNA, it copies the recombinant DNA too.

In addition to their own chromosomes, some bacteria contain small circular DNA molecules known as **plasmids.** Plasmids, like those shown in **Figure 15–9,** are widely used in recombinant DNA studies. Joining DNA to a plasmid, and then using the recombinant plasmid to transform bacteria, results in the replication of the newly added DNA along with the rest of the cell's genome.

Plasmids are also found in yeasts, which are single-celled eukaryotes that can be transformed with recombinant DNA as well. Biologists working with yeasts can construct artificial chromosomes containing centromeres, telomeres, and replication start sites. These artificial chromosomes greatly simplify the process of introducing recombinant DNA into the yeast genome.

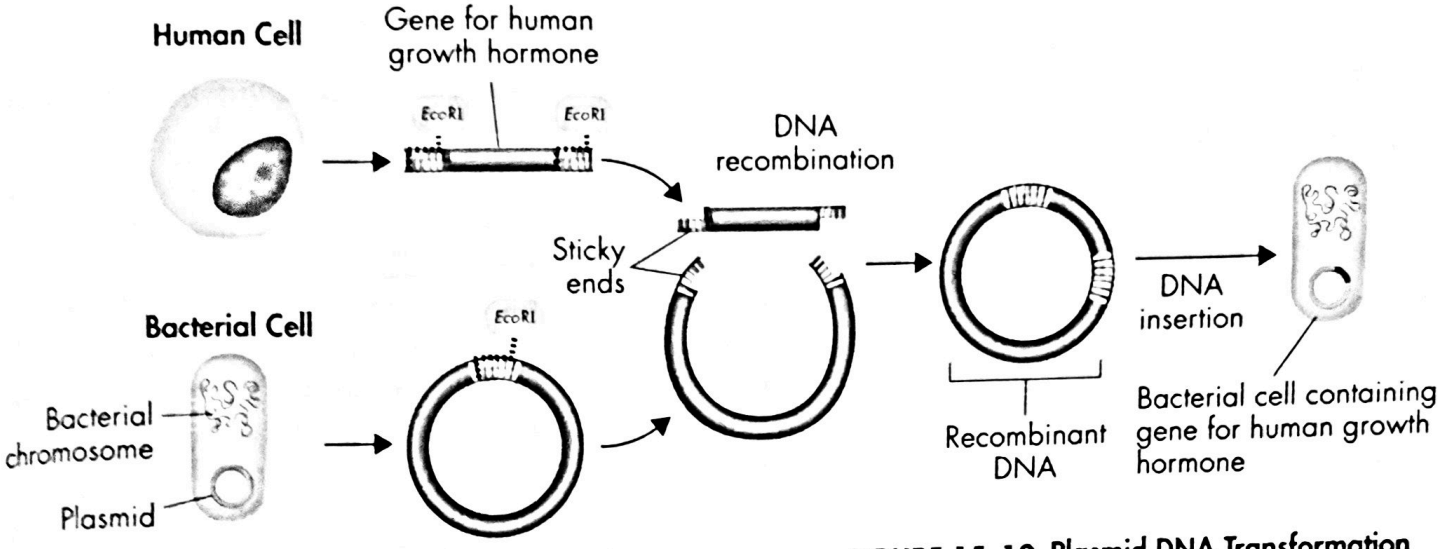



**FIGURE 15–10 Plasmid DNA Transformation** Scientists can insert a piece of DNA into a plasmid if both the plasmid and the target DNA have been cut by the same restriction enzymes to create sticky ends. With this method, bacteria can be used to produce human growth hormone. First, a human gene is inserted into bacterial DNA. Then, the new combination of genes is returned to a bacterial cell, which replicates the recombinant DNA over and over again. **Infer** ***Why might scientists want to copy the gene for human growth hormone?***

**Figure 15–10** shows how bacteria can be transformed using recombinant plasmids. First, the DNA being used for transformation is joined to a plasmid. The plasmid DNA contains a signal for replication, helping to ensure that if the DNA does get inside a bacterial cell, it will be replicated. In addition, the plasmid also has a genetic marker, such as a gene for antibiotic resistance. A **genetic marker** is a gene that makes it possible to distinguish bacteria that carry the plasmid from those that don't. Using genetic markers, researchers can mix recombinant plasmids with a culture of bacteria, add enough DNA to transform just one cell in a million, and still locate that one cell. After transformation, the culture is treated with an antibiotic. Only those rare cells that have been transformed survive, because only they carry the resistance gene.

**In Your Notebook** *Write a summary of the process of plasmid DNA transformation.*

## Quick Lab
GUIDED INQUIRY

### Inserting Genetic Markers

❶ Write a random DNA sequence on a long strip of paper to represent an organism's genome.

❷ Have your partner write a short DNA sequence on a short strip of paper to represent a marker gene.

❸ Using the chart your teacher gives you, work with your partner to figure out how to insert the marker gene into the genome.

**Analyze and Conclude**

**1. Apply Concepts** Which restriction enzyme did you use? Why?

**2. Use Models** What kind of molecule did you and your partner develop?

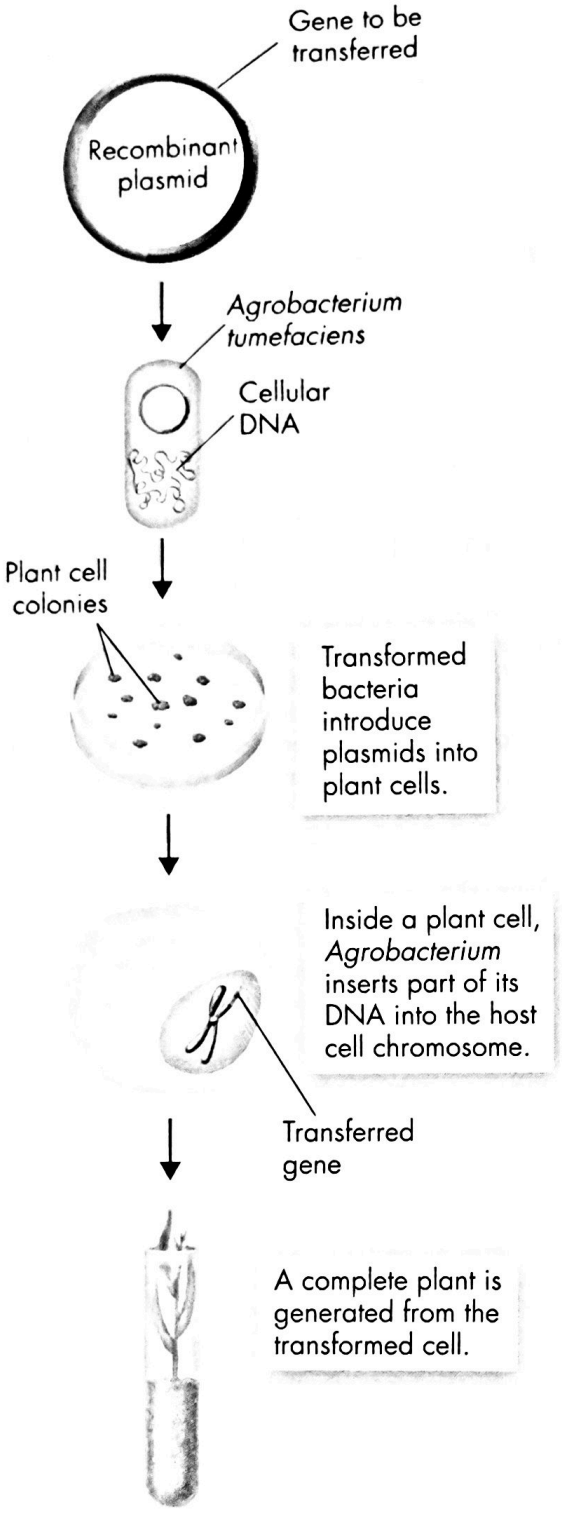



**FIGURE 15–11 Transforming a Plant Cell** *Agrobacterium* can be used to introduce bacterial DNA into a plant cell. The transformed cells can be cultured to produce adult plants.

# Transgenic Organisms

***How can genes from one organism be inserted into another organism?***

The universal nature of the genetic code makes it possible to construct organisms that are **transgenic,** containing genes from other species. **Transgenic organisms can be produced by the insertion of recombinant DNA into the genome of a host organism.** Like bacterial plasmids, the DNA molecules used for transformation of plant and animal cells contain genetic markers that help scientists identify which cells have been transformed.

Transgenic technology was perfected using mice in the 1980s. Genetic engineers can now produce transgenic plants, animals, and microorganisms. By examining the traits of a genetically modified organism, it is possible to learn about the function of the transferred gene. This ability has contributed greatly to our understanding of gene regulation and expression.

**Transgenic Plants** Many plant cells can be transformed using *Agrobacterium.* In nature this bacterium inserts a small DNA plasmid that produces tumors in a plant's cells. Scientists can deactivate the plasmid's tumor-producing gene and replace it with a piece of recombinant DNA. The recombinant plasmid can then be used to infect and transform plant cells, as shown in **Figure 15–11.**

There are other ways to produce transgenic plants as well. When their cell walls are removed, plant cells in culture will sometimes take up DNA on their own. DNA can also be injected directly into some cells. If transformation is successful, the recombinant DNA is integrated into one of the plant cell's chromosomes.

**Transgenic Animals** Scientists can transform animal cells using some of the same techniques used for plant cells. The egg cells of many animals are large enough that DNA can be injected directly into the nucleus. Once the DNA is in the nucleus, enzymes that are normally responsible for DNA repair and recombination may help insert the foreign DNA into the chromosomes of the injected cell.

Recently it has become possible to eliminate particular genes by carefully engineering the DNA molecules that are used for transformation. The DNA molecules can be constructed with two ends that will sometimes recombine with specific sequences in the host chromosome. Once they do, the host gene normally found between those two sequences may be lost or specifically replaced with a new gene. This kind of gene replacement has made it possible to pinpoint the specific functions of genes in many organisms, including mice.

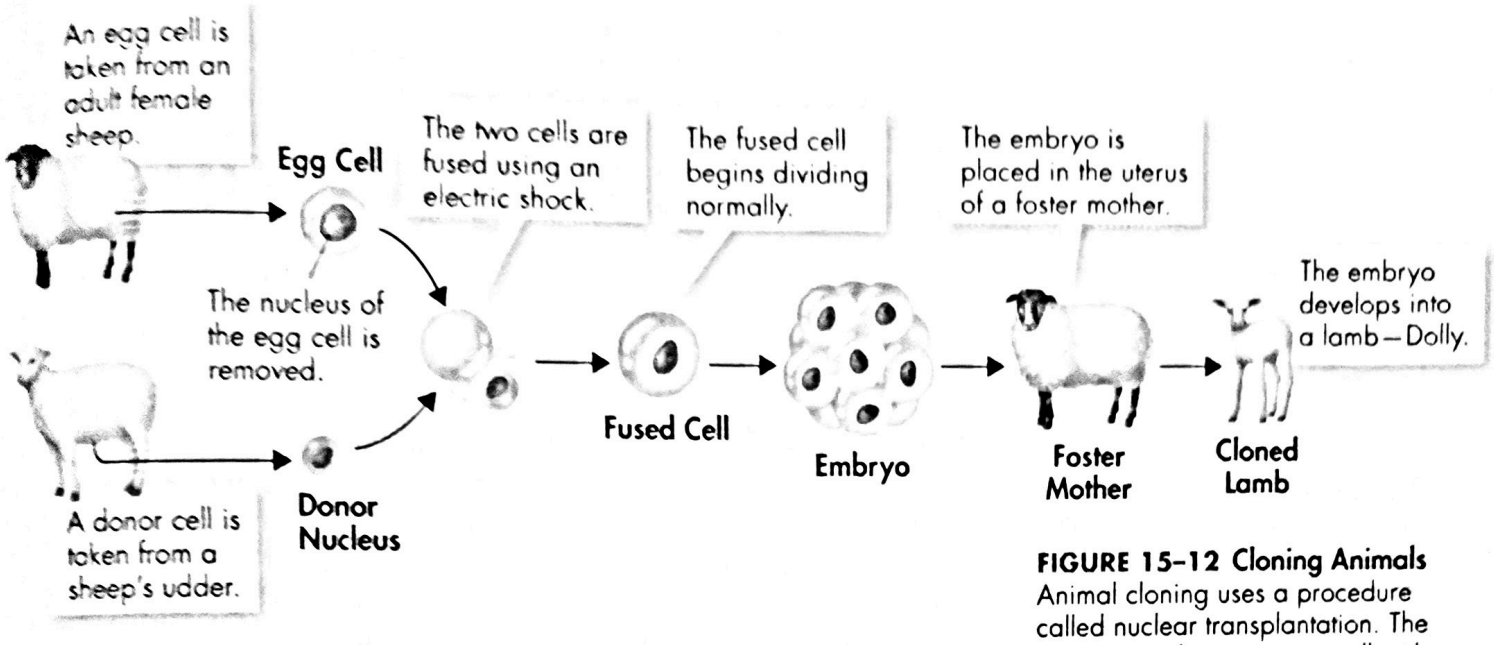



**FIGURE 15–12 Cloning Animals** Animal cloning uses a procedure called nuclear transplantation. The process combines an egg cell with a donor nucleus to produce an embryo. **Apply Concepts** ***Why won't the cloned lamb resemble its foster mother?***

**Cloning** A **clone** is a member of a population of genetically identical cells produced from a single cell. The technique of cloning uses a single cell from an adult organism to grow an entirely new individual that is genetically identical to the organism from which the cell was taken.

Cloned colonies of bacteria and other microorganisms are easy to grow, but this is not always true of multicellular organisms, especially animals. Clones of animals were first produced in 1952 using amphibian tadpoles. In 1997, Scottish scientist Ian Wilmut stunned biologists by announcing that he had produced a sheep, called Dolly, by cloning.

**Figure 15–12** shows the basic steps by which an animal can be cloned. First, the nucleus of an unfertilized egg cell is removed. Next, the egg cell is fused with a donor cell that contains a nucleus, taken from an adult. The resulting diploid egg develops into an embryo, which is then implanted in the uterine wall of a foster mother, where it develops until birth. Cloned cows, pigs, mice, and even cats have since been produced using similar techniques.

## 15.2 Assessment

### Review Key Concepts

**1. a. Review** Describe the process scientists use to copy DNA.

**b. Infer** Why would a scientist want to know the sequence of a DNA molecule?

**2. a. Review** How do scientists use recombinant DNA?

**b. Use Analogies** How is genetic engineering like computer programming?

**3. a. Review** What is a transgenic organism?

**b. Compare and Contrast** Compare the transformation of a plant cell with the transformation of an animal cell.

### PRACTICE PROBLEM

**4.** Design an experiment to find a way to treat disorders caused by a single gene. State your hypothesis and list the steps you would follow. (*Hint*: Think about the uses of recombinant DNA.)