Microbiology

with Diseases by Taxonomy

FIFTH EDITION

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Pearson

Invest in your future:

Microbiology Matters

Gene Libraries

LEARNING OUTCOME

8.11 Explain the significance of gene libraries.

Suppose you were a scientist investigating the effects of the genes for 24 different kinds of interleukins (proteins that mediate certain aspects of immunity). Having to isolate the specific genes for each type of interleukin would require much time, labor, and expense. Your task would be made much easier if you could obtain the genes you need from a **gene library,** a collection of bacterial or phage clones—identical descendants—each of which contains a portion of the genetic material of interest. In effect, each clone is like one book in a library in that it contains one fragment (typically a single gene) of an organism's entire genome. Alternatively, a gene library may contain clones with all the genes of a single chromosome or the full set of cDNA that is complementary to an organism's mRNA.

As depicted in **FIGURE 8.4**, genetic researchers can create each of the clones in a gene library by using restriction enzymes to generate fragments of the DNA of interest and then using ligase to synthesize recombinant vectors. They insert the vectors into bacterial cells, which are then grown on culture media. Once a scientist isolates a recombinant clone and places it in a gene library, the gene that the clone carries becomes available to other investigators, saving them the time and effort required to isolate that gene. Many gene libraries are now commercially available.

TELL ME WHY

Why did the discovery and development of restriction enzymes speed up the study of recombinant DNA technology?

Techniques of Recombinant DNA Technology

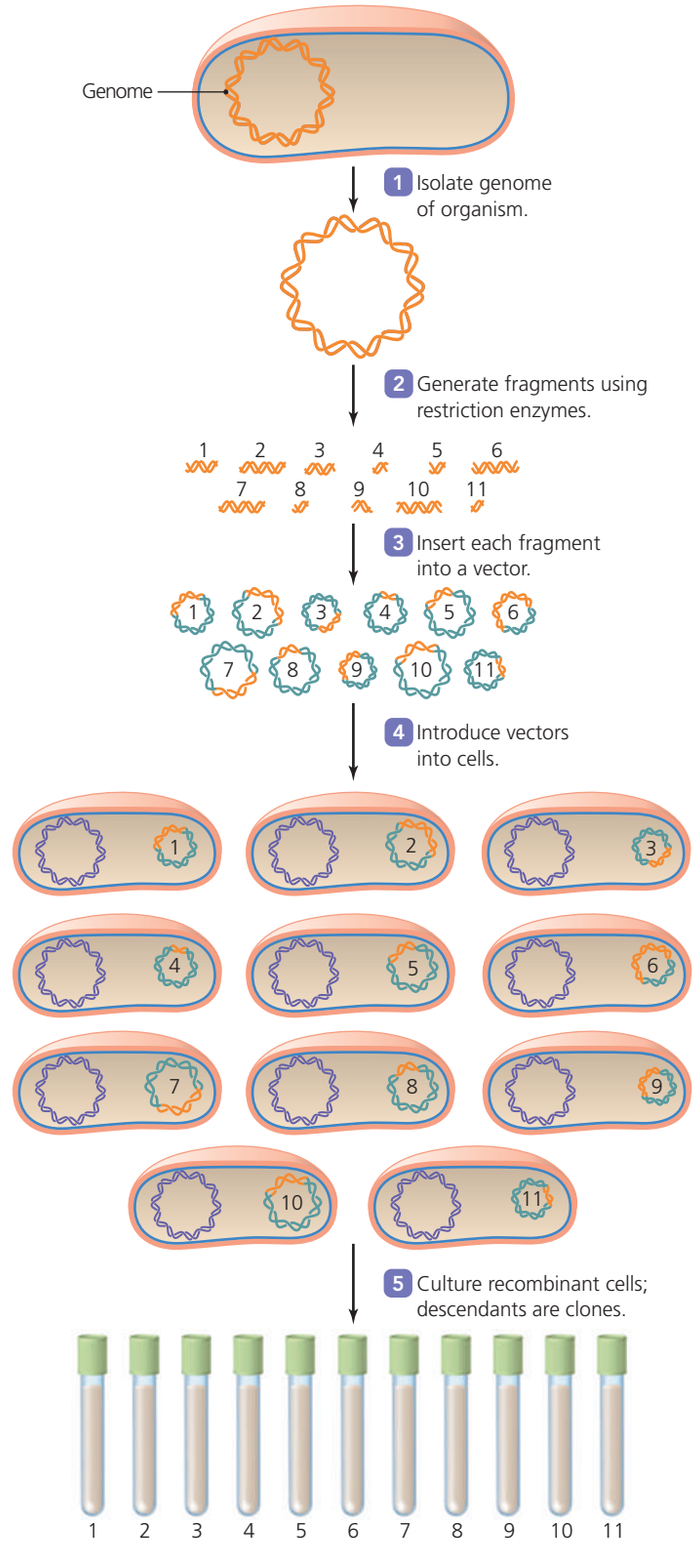
Scientists use the tools of recombinant DNA technology in a number of basic techniques to multiply, identify, manipulate, isolate, map, and sequence the nucleotides of genes.

Multiplying DNA *In Vitro*: The Polymerase Chain Reaction

LEARNING OUTCOME

8.12 Describe the purpose and application of the polymerase chain reaction.

The **polymerase chain reaction (PCR)** is a technique by which scientists produce a large number of identical molecules of DNA *in vitro.* Using PCR, researchers start with a single molecule of DNA and generate billions of exact replicas within hours. Such rapid amplification of DNA is critical in a variety of situations. For example, epidemiologists used PCR to amplify the genomes of Ebola viruses from both western and central



▲ **FIGURE 8.4 Production of a gene library.** A gene library is the population of all cells or phages that together contain all of the genetic material of interest. In this figure, each clone of cells carries a portion of a bacterium's genome.

Africa in 2014. The large number of identical DNA molecules produced by PCR allowed scientists to determine and compare the nucleotide sequences of the two viruses. Because the

two viruses differed significantly, epidemiologists were able to determine that the western epidemic had not escaped their quarantine procedures and spread to central Africa; instead, the central African outbreak was a separate epidemic.

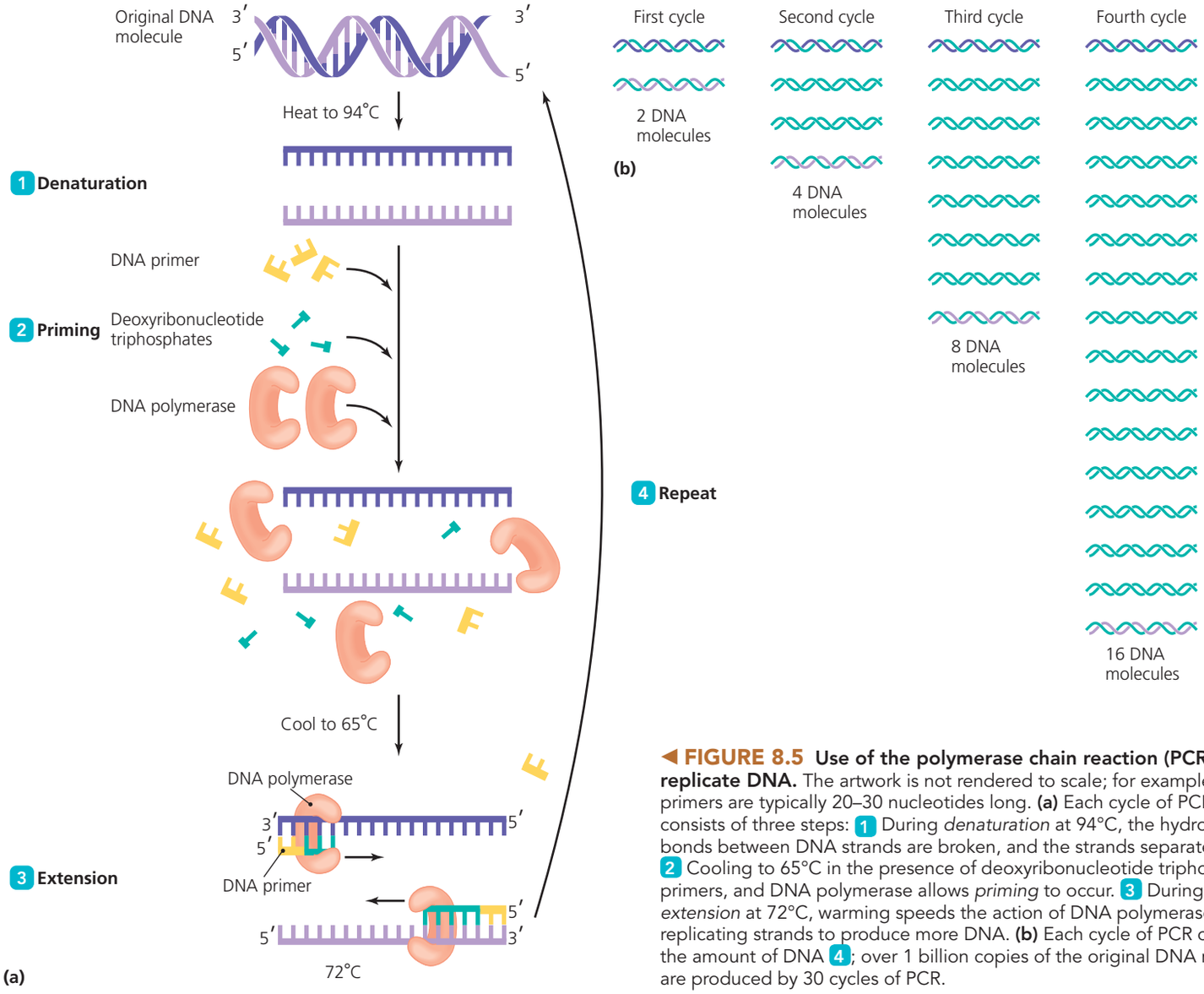
MM ANIMATIONS: *Polymerase Chain Reaction (PCR): Overview, Components*

PCR is a repetitive process that alternately separates and replicates the two strands of DNA. Each cycle of PCR consists of the following three steps **(FIGURE 8.5a)**:

1. **Denaturation.** Exposure to heat (about 94°C) separates the two strands of the target DNA by breaking the hydrogen bonds between base pairs but otherwise leaves the two strands unaltered.
2. **Priming.** A mixture containing an excess of DNA primers (synthesized such that they are complementary to nucleotide sequences near the ends of the target DNA), DNA polymerase, and an abundance of the four deoxyribonucleotide triphosphates (A, T, G, and C) is added to the target DNA. Because there is an excess of primers, single strands are more likely to bind to a primer than to one another. The primers provide DNA polymerase with the 3′ hydroxyl group it requires for DNA synthesis.
3. **Extension.** Raising the temperature to about 72°C increases the rate at which DNA polymerase replicates each strand to produce more DNA.

MM ANIMATIONS: *PCR: The Process*

These steps are repeated over and over 4, so the number of DNA molecules increases exponentially **(FIGURE 8.5b)**. After only 30 cycles—which requires only a few hours to complete—PCR produces over 1 billion identical copies of the original DNA molecule.



◀ **FIGURE 8.5 Use of the polymerase chain reaction (PCR) to replicate DNA.** The artwork is not rendered to scale; for example, primers are typically 20–30 nucleotides long. **(a)** Each cycle of PCR consists of three steps: 1 During *denaturation* at 94°C, the hydrogen bonds between DNA strands are broken, and the strands separate. 2 Cooling to 65°C in the presence of deoxyribonucleotide triphosphates, primers, and DNA polymerase allows *priming* to occur. 3 During *extension* at 72°C, warming speeds the action of DNA polymerase in replicating strands to produce more DNA. **(b)** Each cycle of PCR doubles the amount of DNA 4; over 1 billion copies of the original DNA molecule are produced by 30 cycles of PCR.

The process can be automated using a *thermocycler,* a device that automatically performs PCR by continuously cycling all the necessary reagents—DNA, DNA polymerase, primers, and triphosphate deoxynucleotides—through the three temperature regimes. A thermocycler uses DNA polymerase derived from hyperthermophilic archaea or bacteria, such as *Thermus aquaticus* (ther′mŭs a-kwa′ti-kŭs). This enzyme, called *Taq DNA polymerase* or simply *Taq,* is not denatured at 94°C, so the machine need not be replenished with DNA polymerase after each cycle.

Investigators use a variation of PCR—called **real-time PCR**—to accurately measure the number of DNA sequences present in a sample. For example, health care providers can use real-time PCR to determine the number of HIV genome copies in a patient's blood sample. This is important in monitoring the progression of disease and effectiveness of treatment of diseases such as AIDS.

Investigators perform real-time PCR by adding a dye that fluoresces when bound to double-stranded DNA (dsDNA). A photometer measures the amount of fluorescence—an amount that corresponds to the number of dsDNA molecules. As the amount of fluorescence increases with each cycle of PCR, the number of new molecules of dsDNA can be ascertained. The number of original molecules can then be calculated.

Combined with a step of reverse transcription, researchers can use real-time PCR to accurately determine the number of copies of a specific mRNA in a cell; with this information, they can determine the expression level of the corresponding gene.

Selecting a Clone of Recombinant Cells

LEARNING OUTCOME

8.13 Explain how researchers use DNA probes to identify recombinant cells.

Before recombinant DNA technology can have practical application, a scientist must be able to select and isolate recombinant cells that contain particular genes of interest. For example, once researchers have created a gene library, they must find the clone containing the DNA of interest. To do so, scientists use probes—which, as explained earlier in the chapter, bind specifically and exclusively to their complementary nucleotide sequences and have either radioactive or fluorescent markers. Researchers then isolate and culture cells that have the radioactive or fluorescent marker, which also aids in identifying the specific location of the genes of interest, as performed in a technique called *gel electrophoresis.*

Separating DNA Molecules: Gel Electrophoresis and the Southern Blot

LEARNING OUTCOME

8.14 Describe the process and use of gel electrophoresis, particularly as it is used in a Southern blot.

Electrophoresis (ē-lek-trō-fōr-ē′sis) is a technique that involves separating molecules based on their electrical charge, size, and shape. In recombinant DNA technology, scientists use **gel electrophoresis** to isolate fragments of DNA molecules that can then be inserted into vectors, multiplied by PCR, or preserved in a gene library.

In gel electrophoresis, DNA molecules, which have an overall negative charge, are drawn through a semisolid gel by an electric current toward the positive electrode within an electrophoresis chamber **(FIGURE 8.6)**. The gel is typically composed of a purified sugar component of agar, called *agarose,* which acts as a molecular sieve that retards the movement of DNA fragments down the chamber and separates the fragments by size. Smaller DNA fragments move faster and farther than larger ones. Scientists can determine the size of a fragment by comparing the distance it travels to the distances traveled by standard DNA fragments of known sizes.

As we have seen, DNA probes allow a researcher to find specific DNA sequences such as genes in a cell. Scientists could try to use probes in electrophoresis gels, but because gels are flimsy and easily broken, they are difficult to probe. In 1975, Ed Southern (1938–) devised a method, called the **Southern blot,** to transfer DNA from gels onto nitrocellulose membranes, which are less delicate. Fluorescently labeled probes are then added, and the probes are detected using a digital camera, which reveals the presence of DNA of interest. A *northern blot* is a similar technique used to detect specific RNA molecules.

DNA Microarrays

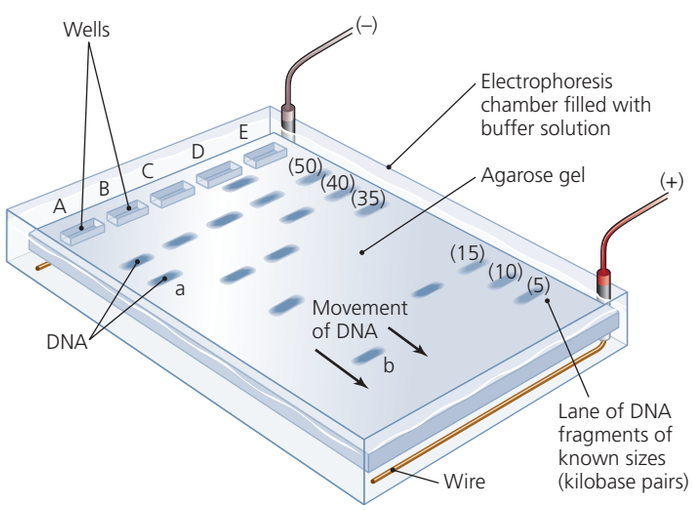
LEARNING OUTCOME

8.15 Describe the manufacture and use of DNA microarrays.

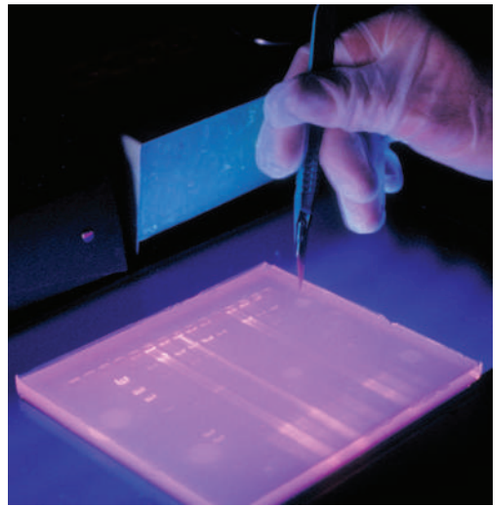
Another tool of biotechnology is a **DNA microarray.** An array consists of molecules of single-stranded DNA (ssDNA), either genomic DNA or cDNA, immobilized on glass slides, silicon chips, or nylon membranes. Robots, similar to those that construct computer chips, deposit PCR-derived copies of hundreds or thousands of different DNA sequences in precise locations on an array **(FIGURE 8.7)**. An array may consist of DNA from a single species (e.g., DNA microarrays containing sequences from all the genes of *E. coli* are available commercially), or an array may contain DNA sequences from numerous species. In any case, single strands of fluorescently labeled DNA in a sample washed over an array adhere only to locations on the array where there are complementary DNA sequences.

Scientists use DNA microarrays in a number of ways, including the following:

- **Monitoring gene expression.** One way organisms control metabolism is by controlling RNA transcription. Scientists use DNA microarrays to monitor which genes a cell is transcribing at a particular time by making fluorescently labeled, single-stranded cDNA from mRNA in the cell. These DNA strands bind to complementary DNA sequences on an array, and the location of fluorescence on



(a)



(b)

▲ **FIGURE 8.6 Gel electrophoresis. (a)** After DNA is cleaved into fragments by restriction enzymes, it is loaded into wells, which are small holes cut into the agarose gel. DNA fragments of known sizes, typically in thousands (kilo) of nucleotide base pairs, are often loaded into one well (in this case, E) to serve as standards. After the DNA fragments are drawn toward the positive electrode by an electric current, they are stained with a dye. **(b)** Ethidium bromide dye fluoresces under ultraviolet illumination to reveal the locations of DNA within a gel. *Compare the positions of the fragments in lanes A and B of the diagram to the positions of the fragments of known sizes. What sizes are the fragments labeled a and b?*

Figure 8.6 *a, 40 kilobase pairs. b, 10 kilobase pairs.*

the array at specific sites reveals which genes the cell was transcribing at the time. For example, activity of normal cells can be compared to activity in cancerous cells to determine how gene expression is related to oncogenesis.

Researchers using DNA microarrays can simultaneously monitor the expression of thousands of genes and compare and contrast genetic expression under different conditions. Different colors of fluorescent dye can be used to label DNA from microbes grown in each condition.

- **Diagnosing infection.** DNA microarrays made with DNA sequences of numerous pathogens reveal the presence of those pathogens in medical samples.
- **Identifying organisms in an environmental sample.** Microbial ecologists monitor the presence or absence of microbes in an environment by using microarrays of DNA from the organisms. Health care professionals can use microarrays to simultaneously detect and identify numerous bacterial and fungal species in clinical blood samples.

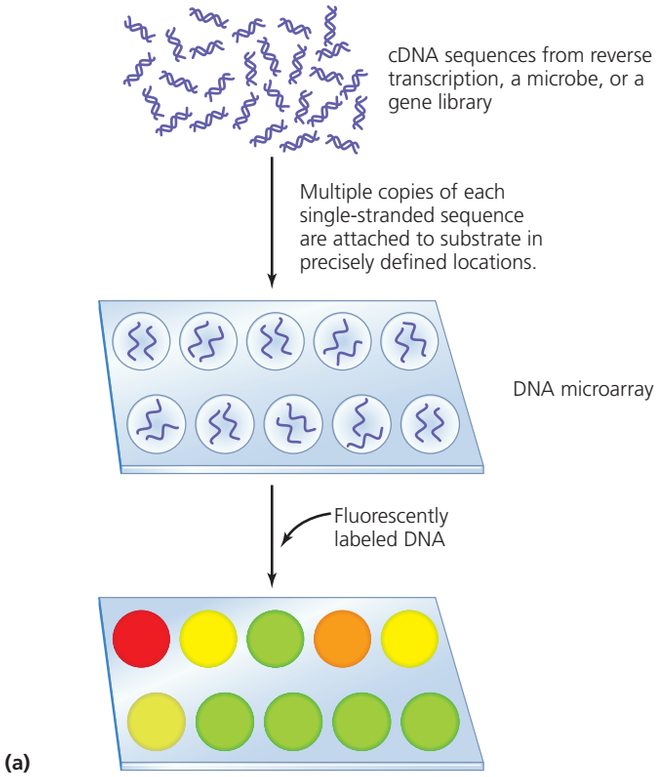
Inserting DNA into Cells

LEARNING OUTCOME

8.16 List and explain three artificial techniques for introducing DNA into cells.

A goal of recombinant DNA technology is the insertion of a gene into a cell. In addition to using vectors and the natural methods of transformation of competent cells, transduction, and conjugation, scientists have developed several artificial methods to introduce DNA into cells, including the following:

- **Electroporation (FIGURE 8.8a).** Electroporation involves using an electrical current to puncture microscopic holes through a cell's membrane so that DNA can enter the cell from the environment. Electroporation can be used on all types of cells, though the thick-walled cells of fungi and algae must first be converted to *protoplasts,* which are cells whose cell walls have been enzymatically removed. Cells treated by electroporation repair their membranes and cell walls after a time.
- **Protoplast fusion (FIGURE 8.8b).** When protoplasts encounter one another, their cytoplasmic membranes may fuse to form a single cell that contains the genomes of both "parent" cells. Exposure to polyethylene glycol increases the rate of fusion. The DNA from the two fused cells recombines to form a recombinant molecule. Scientists often use protoplast fusion for the genetic modification of plants.
- **Injection.** Two types of injection are used with larger eukaryotic cells. Researchers use a *gene gun* powered by a blank .22-caliber cartridge or compressed gas to fire tiny tungsten or gold beads coated with DNA into a target cell **(FIGURE 8.8c).** The cell eventually eliminates the inert metal



▲ **FIGURE 8.7 DNA microarray. (a)** Construction and use of a microarray. Multiple copies of single-stranded DNA with known sequences are affixed in precise locations on a glass slide, silicon chip, nylon membrane, or other substrate. Fluorescently labeled DNA washed over the microarray binds to complementary strands. **(b)** Photograph of a DNA microarray showing locations of differently labeled cDNA molecules.

beads. In *microinjection*, a geneticist inserts DNA into a target cell with a glass micropipette having a tip diameter smaller than that of the cell or nucleus **(FIGURE 8.8d)**. Unlike electroporation and protoplast fusion, injection can be used on intact tissues such as in plant seeds.

In every case, foreign DNA that enters a cell remains in a cell's progeny only if the DNA is self-replicating, as in the case of plasmid and viral vectors, or if the DNA integrates into a cellular chromosome by recombination.

TELL ME WHY

Why wasn't polymerase chain reaction (PCR) practical before the discovery of hyperthermophilic bacteria?

Applications of Recombinant DNA Technology

interactivemicrobiology
Human Microbiome

The importance of recombinant DNA technology lies not in the novelty, cleverness, or elegance of its procedures but in its wide range of applications. In this section we consider how recombinant DNA technology is used to solve various problems and create research, medical, and agricultural products.

Genetic Mapping

LEARNING OUTCOMES

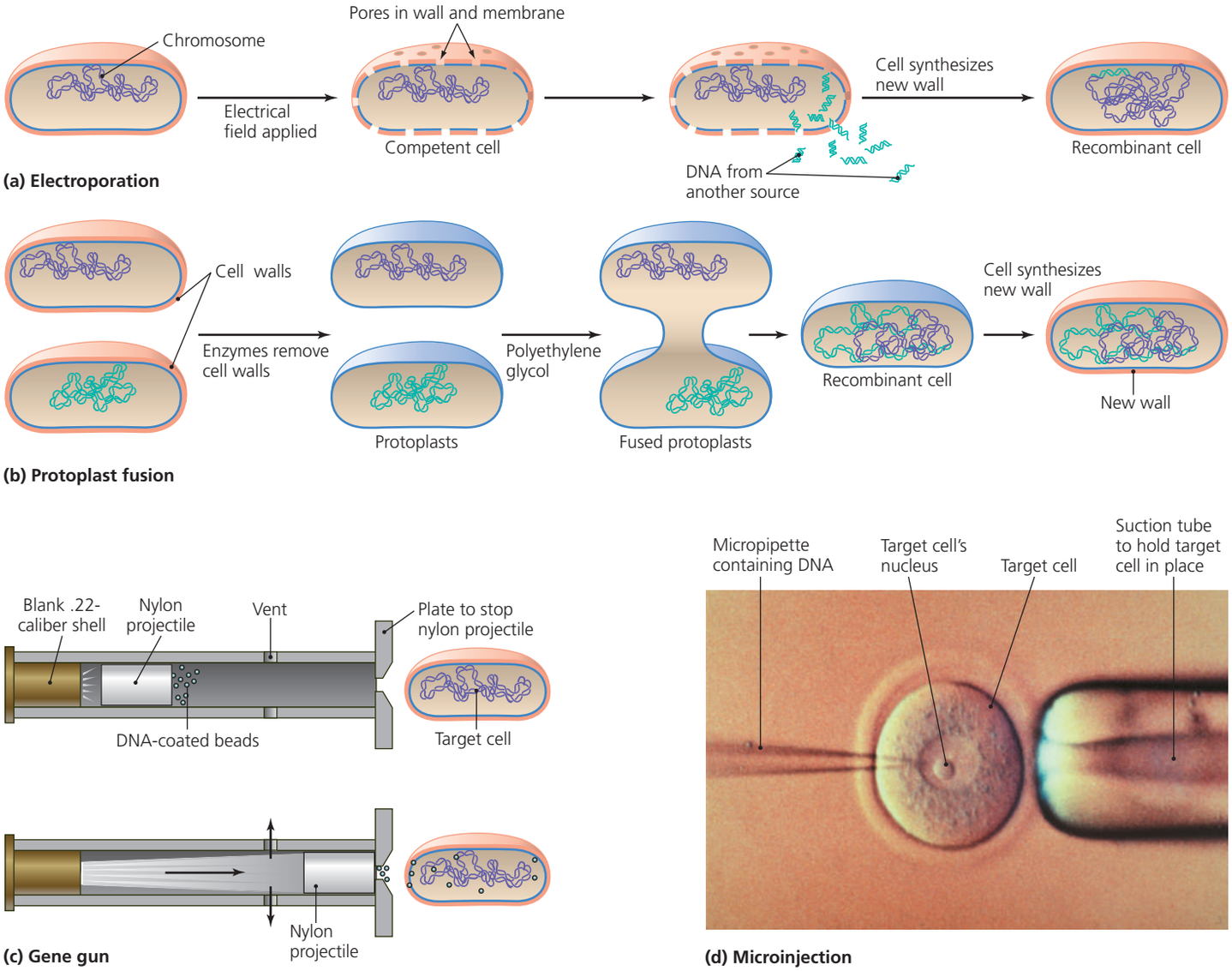
8.17 Describe genetic mapping and fluorescent *in situ* hybridization, and explain their usefulness.
8.18 Define genomics and functional genomics.
8.19 Contrast Sanger sequencing and next-generation sequencing.

One application of these tools and techniques is **genetic mapping,** which involves locating genes on a nucleic acid molecule. Genetic maps provide scientists with useful facts, including information concerning an organism's metabolism and growth characteristics, as well as its potential relatedness to other microbes. For example, scientists have discovered a virus with a genetic map similar to those of certain hepatitis viruses. They named the new discovery *hepatitis G virus* because it presumably causes hepatitis, though it has not been demonstrated that the virus actually causes the disease.

Locating Genes

Until about 1970, scientists identified the specific location of genes on chromosomes by cumbersome, time-consuming, labor-intensive methods. Recombinant DNA techniques provide simpler and universal methods for genetic mapping.

One technique for locating genes, called *restriction fragmentation*, was one of the earliest applications of restriction enzymes. In this technique, which is used for mapping the relative locations of genes in plasmids and viruses, researchers compare DNA fragments resulting from cleavages by several restriction enzymes to determine each fragment's location relative to the



▲ **FIGURE 8.8 Artificial methods of inserting DNA into cells. (a)** Electroporation, in which an electrical current applied to a cell makes it competent to take up DNA. **(b)** Protoplast fusion, in which enzymes digest cell walls to create protoplasts that fuse at a high rate when treated with polyethylene glycol. **(c)** A gene gun, which fires DNA-coated beads into a cell. **(d)** Microinjection, in which a solution of DNA is introduced into a cell through a micropipette.

others. If the researchers know the locations of specific genes on specific fragments, then elucidation of the correct arrangement of the fragments will reveal the relative locations of the genes on the entire DNA molecule.

Using this method, scientists first completed the entire gene map of a cellular microbe—the bacterium *H. influenzae*—in 1995. Since then, geneticists have elucidated complete gene maps of numerous viruses and prokaryotic and eukaryotic organisms.

Often a scientist wants to know where in the environment, clinical sample, or biofilm a particular microbial species is located. When researchers know of a particular gene exclusive to that organism, they can locate the gene and thereby the microbe using *fluorescent in situ hybridization* (*FISH*).

In this method, scientists use fluorescent DNA probes that hybridize with their complementary target. Scientists using fluorescent microscopes to view such probes can determine where the gene and its organism are located **(FIGURE 8.9)**. Using a number of different colors of fluorescent probes, researchers can locate numerous genes and the microbes that carry them simultaneously. FISH is used for a variety of purposes, including diagnosing disease, identifying microbes in environmental samples, and analyzing biofilms.

Genomics and Nucleotide Sequencing

Genomics is the sequencing and analysis of the nucleotide bases of genomes of organisms, viruses, and eukaryotic mitochondria and chloroplasts. At first, scientists sequenced DNA