GLOBAL EDITION

Campbell Biology

Concepts & Connections

TENTH EDITION

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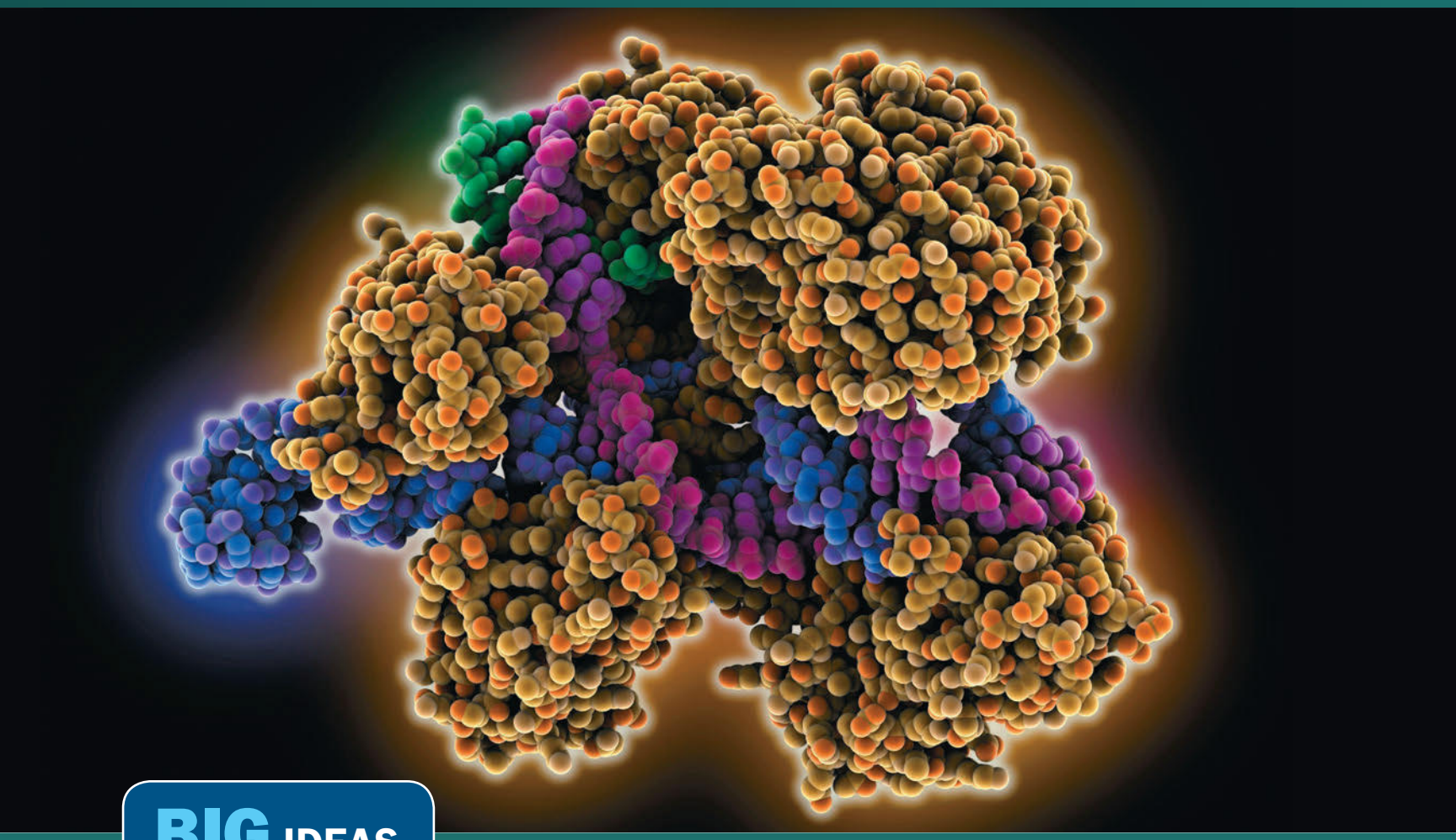
Brief Contents

CHAPTER 12

DNA Technology and Genomics



BIG IDEAS

Gene Cloning and Editing (12.1–12.5)

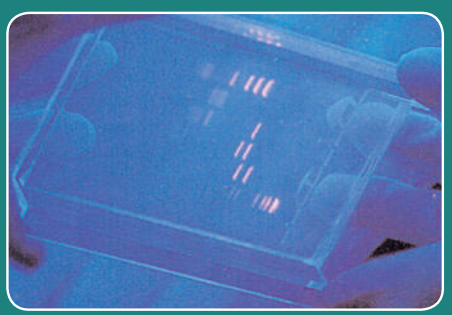
A variety of laboratory techniques can be used to copy, combine, and edit DNA molecules.

Genetically Modified Organisms (12.6–12.10)

Transgenic cells, plants, and animals are used in agriculture and medicine.

DNA Profiling (12.11–12.15)

Genetic markers can be used to definitively match a DNA sample to an individual.



12.0 DNA technology can save lives

Duchenne muscular dystrophy (DMD) is a fatal genetic disease caused by a mutation in the gene that encodes dystrophin, a protein vital to muscle function. Muscle weakness worsens over time, leaving a person unable to walk and, eventually, breathe. Although treatments can prolong life, the disease is fatal.

Hope may reside in recently developed gene-editing techniques (see Module 12.5). Researchers have used a harmless virus to deliver gene-editing components to the muscles of dogs. Within weeks, normal dystrophin levels were restored, proving that DMD could be halted in a large mammal. The researchers are monitoring the dogs for long-term effects and hope to offer the treatment in human clinical trials.

In addition to medical applications, DNA technologies affect our lives in many other ways: New varieties of genetically modified organisms are being introduced into our food supply; DNA profiling has changed the field of forensic science; bioinformatics provides data for biological research as well as historical and evolutionary investigations. In this chapter, we'll discuss each of these applications. We'll also consider the techniques used, how they are applied, and some of the social, legal, and ethical issues that are raised by DNA technologies.

Genomics and Bioinformatics
(12.16–12.21)

The study of biological sequence information provides valuable data.

PRE-TEST

1. Why are restriction enzymes so useful as genetic engineering tools?

a. They always cut DNA at the exact same sequence.
b. They cut all DNA at exactly the same place.
c. They cut all DNA in exactly the same way.
d. They cut all types of nucleic acids.
e. They can select specific proteins to clone.

2. What is the genetic basis of DNA profiling?

a. the number of repeats of noncoding DNA
b. the number of copies of a particular gene
c. the entire genome of each person
d. the specific mutations each person has
e. the mutations carried by each person

3. Plasmids are important genetic engineering tools. Where do they originate?

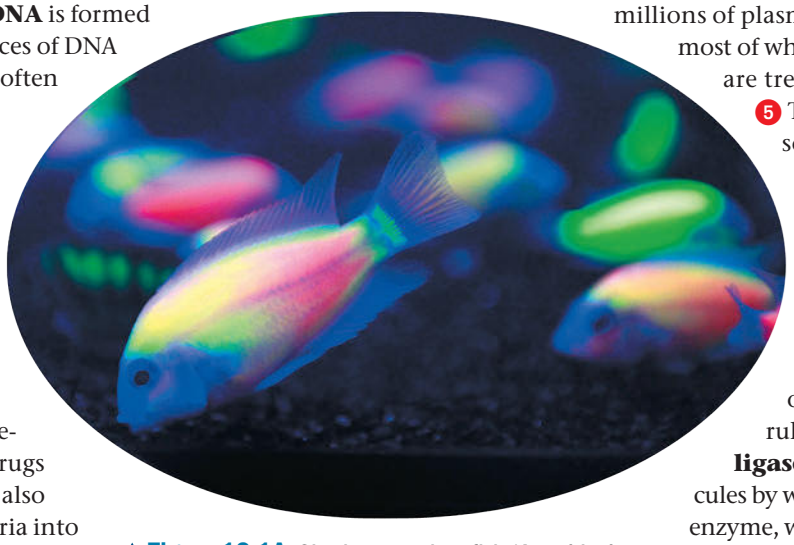
a. bacteria
b. viruses
c. plant cells
d. animal cells
e. mammalian cells

Gene Cloning and Editing

12.1 Genes can be cloned in recombinant plasmids

Although it may seem like a modern field, **biotechnology**, the manipulation of organisms or their components to make useful products, actually dates back to the dawn of civilization. Consider such ancient practices as the use of yeast to make beer and bread, and the selective breeding of livestock, dogs, and other animals. But when people use the term *biotechnology* today, they are usually referring to **DNA technology**, modern laboratory techniques for studying and manipulating genetic material. Using these methods, scientists can, for instance, extract genes from one organism and transfer them to another, effectively moving genes between species as different as *Escherichia coli* bacteria, papaya, and fish.

In the 1970s, the field of biotechnology was advanced by the invention of methods for making recombinant DNA in the lab. **Recombinant DNA** is formed when scientists combine pieces of DNA from two different sources—often different species—*in vitro* (in a test tube) to form a single DNA molecule. Today, recombinant DNA technology is widely used for **genetic engineering**, the direct manipulation of genes for practical purposes. Scientists have genetically engineered bacteria to mass-produce a variety of useful chemicals, from cancer drugs to pesticides. Scientists have also transferred genes from bacteria into plants and from one animal species into another **(Figure 12.1A)**.



▲ **Figure 12.1A** Glowing aquarium fish (*Amatitlania nigrofasciatus*, a type of cichlid) produced by transferring a gene originally obtained from a jellyfish (cnidarian)

To manipulate genes in the laboratory, biologists often use bacterial **plasmids**, small, circular DNA molecules that replicate (duplicate) separately from the much larger bacterial chromosome (see Module 10.23). Plasmids typically carry only a few genes, can easily be transferred into bacteria, and are passed from one generation to the next. Because plasmids are easily manipulated to carry virtually any genes, they are key tools for **DNA cloning**, the production of many identical copies of a target segment of DNA. Through DNA cloning, scientists can mass produce many useful products.

Consider a typical genetic engineering challenge: A molecular biologist at a pharmaceutical company has identified a gene that codes for a valuable product, a hypothetical substance called protein V. The biologist wants to manufacture the protein on a large scale. The biggest challenge in such an effort is of the "needle in a haystack" variety: The gene of interest is one relatively tiny segment embedded in a much longer DNA molecule. **Figure 12.1B** illustrates how the techniques of gene cloning can be used to mass produce a desired gene.

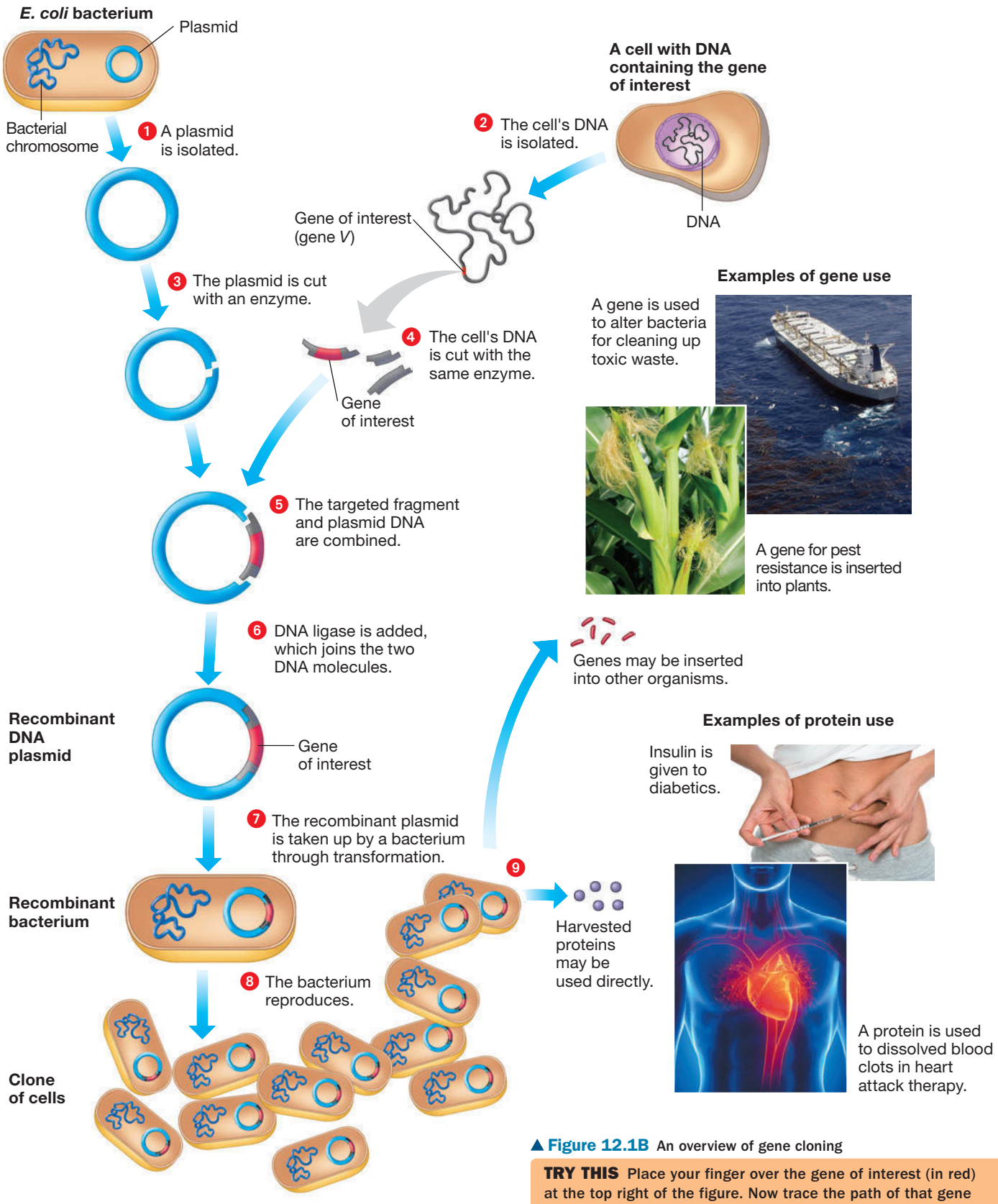
To begin, the biologist isolates two kinds of DNA: (1) a bacterial plasmid (usually from the bacterium *E. coli*) that will serve as the **vector**, or gene carrier, and (2) the DNA from another organism ("foreign" DNA) that includes the gene that codes for protein V (gene *V*) along with other, unwanted genes. The DNA containing gene *V* could come from a variety of sources, such as a different bacterium, a plant, a nonhuman animal, or even human tissue cells growing in laboratory culture.

(3) The researcher treats both the plasmid and the gene *V* source DNA with an enzyme that cuts DNA. An enzyme is chosen that cleaves the plasmid in only one place. (4) The source DNA, which is usually much longer in sequence than the plasmid, may be cut into many fragments, only one of which carries gene *V*. The figure shows the processing of just one DNA fragment and one plasmid, but actually, millions of plasmids and DNA fragments, most of which do not contain gene *V*, are treated simultaneously.

(5) The cut DNA from both sources—the plasmid and target gene—are mixed. The single-stranded ends of the plasmid base-pair with the complementary ends of the target DNA fragment (see Module 10.3 if you need a refresher on the DNA base-pairing rules). (6) The enzyme **DNA ligase** joins the two DNA molecules by way of covalent bonds. This enzyme, which the cell normally uses in DNA replication (see Module 10.4), is a "DNA pasting" enzyme that catalyzes the formation of covalent bonds between adjacent nucleotides, joining the strands. The resulting plasmid is a recombinant DNA molecule.

(7) The recombinant plasmid containing the targeted gene is mixed with bacteria. Under the right conditions, a bacterium takes up the plasmid DNA by transformation (see Module 10.22). (8) The recombinant bacterium then reproduces through repeated cell cycles to form a **clone** of cells, a population of genetically identical cells. In this clone, each bacterium carries a copy of gene *V*. When DNA cloning involves a gene-carrying segment of DNA (as it does here), it is called **gene cloning**. In our example, the biologist will eventually grow a cell clone large enough to produce protein *V* in marketable quantities.

(9) Gene cloning can be used for two basic purposes. Copies of the gene itself can be the immediate product, to be used in additional genetic engineering projects. For example, a pest-resistance gene present in one plant species might be cloned and transferred into plants of another species. Other times, the protein product of the cloned gene is harvested



▲ **Figure 12.1B** An overview of gene cloning

TRY THIS Place your finger over the gene of interest (in red) at the top right of the figure. Now trace the path of that gene throughout the entire process shown.

and used. For example, a protein with medical uses, such as insulin, can be harvested in large quantities using recombinant bacteria.

In the next four modules, we discuss the methods outlined in Figure 12.1B. You may find it useful to turn back to this summary figure as each technique is discussed.

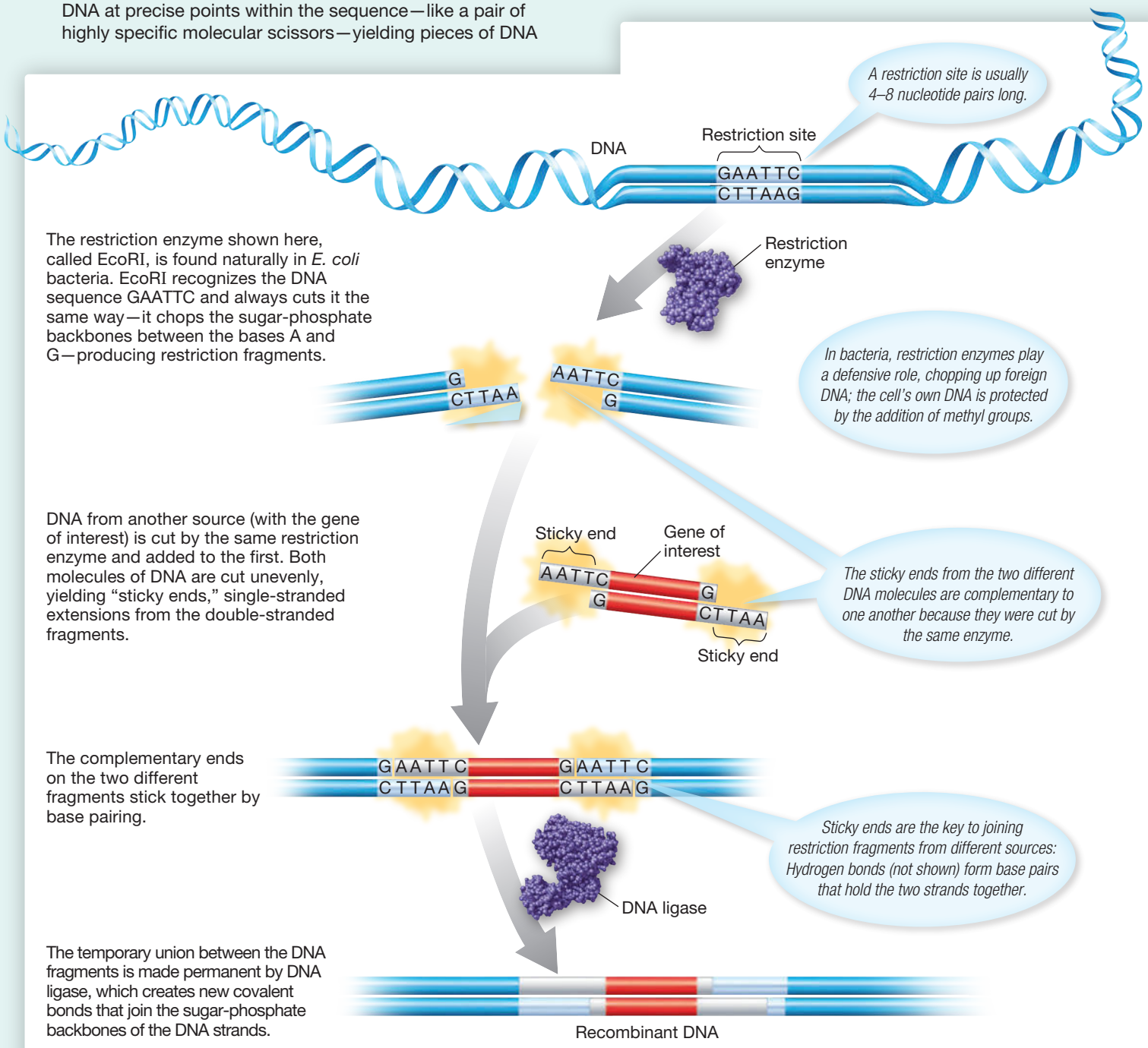
? In the example shown in Figure 12.1B, what is the vector?

■ A plasmid isolated from an *E. coli* bacterium

VISUALIZING THE CONCEPT

12.2 Enzymes are used to "cut and paste" DNA

To understand how DNA is manipulated in the laboratory, you need to learn how enzymes cut and paste DNA. The cutting tools are bacterial enzymes called **restriction enzymes**. Biologists have identified hundreds of different restriction enzymes, each of which recognizes a particular short DNA sequence, which is called a **restriction site**. After a restriction enzyme binds to its restriction site, it cuts both strands of the DNA at precise points within the sequence—like a pair of highly specific molecular scissors—yielding pieces of DNA called **restriction fragments**. The same restriction enzyme will always cut all copies of a particular DNA molecule at the same place. Once cut, restriction fragments of DNA can be pasted together by the enzyme DNA ligase. The techniques outlined here form the basis of many genetic engineering procedures that involve combining DNA from different sources.



TRY THIS The restriction enzyme Hind III recognizes the sequence AAGCTT, cutting between the two A's. Draw the double-stranded sequence before and after the enzyme cuts.

? What are "sticky ends"?

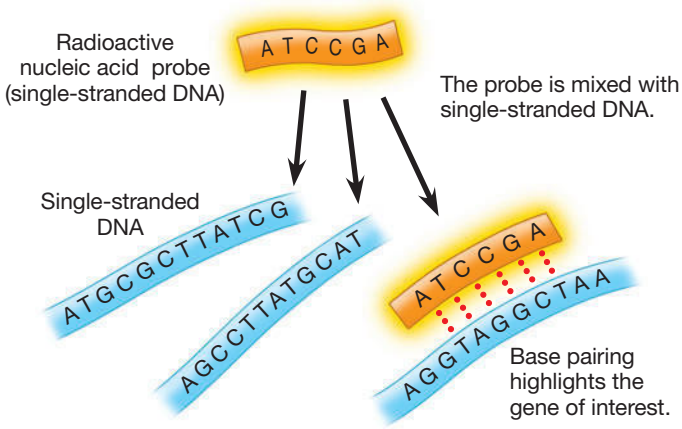
■ Single-stranded regions of a DNA fragment whose unpaired bases can hydrogen-bond to complementary single-stranded regions of another fragment

12.3 Nucleic acid probes can label specific DNA segments

Often, a researcher wishes to find a specific segment of DNA among a much larger collection (for example, just the segment containing the gene colored red in step 4 of Figure 12.1B). When at least part of the nucleotide sequence of a gene is known, this information can be used to a researcher's advantage. For example, if we know that a gene contains the sequence TAGGCT, a biochemist can synthesize a short single strand of DNA with the complementary sequence (ATCCGA) and label it with a radioactive isotope or fluorescent tag. This labeled, complementary molecule is called a **nucleic acid probe** because it is used to find a specific gene or other nucleotide sequence within a mass of DNA. (In actual practice, probe molecules are considerably longer than six nucleotides.)

Figure 12.3 shows how a probe works. When a radioactive DNA probe is added to a collection of DNA strands, it tags the correct molecules by hydrogen-bonding to the complementary sequence in the gene of interest. Such a probe can be simultaneously applied to many DNA molecules to screen all of them at once for a desired gene.

Nucleic acid probes have several practical applications. For example, they can be used in DNA microarrays to test the expression of many genes at once (see Module 11.9). In another technique, a piece of filter paper is pressed against bacterial colonies (clones) growing on a petri dish. The filter paper picks up cells from each colony. A chemical treatment is used to break open the cells and separate the DNA strands. The DNA strands are then soaked in probe solution.



▲ **Figure 12.3** How a DNA probe tags a gene by base pairing

Any bacterial colonies carrying the gene of interest will be tagged on the filter paper, marking them for easy identification. Once the researcher identifies a colony carrying the desired gene, the cells of that colony can be grown further, and the gene of interest, or its protein product, can be collected in large amounts.

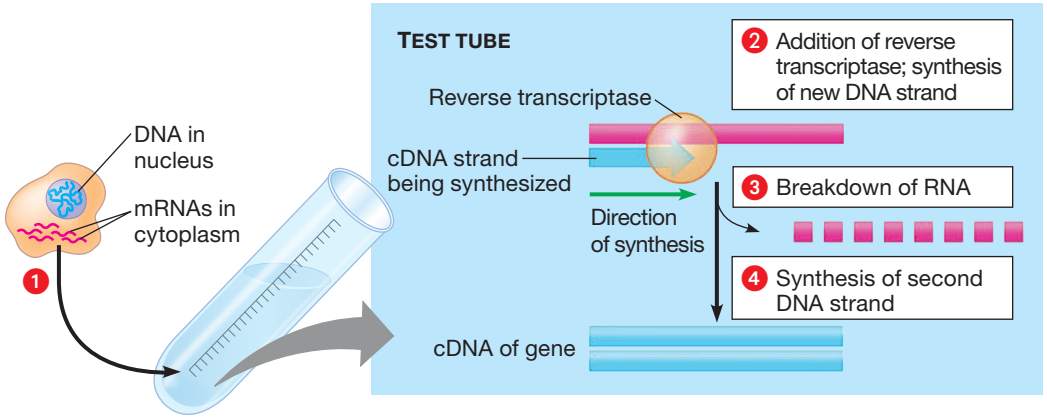
? How does a probe consisting of radioactive DNA enable a researcher to find the bacterial clones carrying a particular gene?

■ The probe molecules bind to and label DNA only from the cells containing the gene of interest, which has a complementary DNA sequence.

12.4 Reverse transcriptase can help make genes for cloning

Researchers often wish to clone eukaryotic genes—which usually contain stretches of non-coding introns—in prokaryotic cells, which lack the machinery to remove introns. To overcome this problem, a researcher can use mRNA as the starting material for cloning **(Figure 12.4)**. ❶ The chosen cells transcribe their genes within the nucleus, producing mRNA. The researcher isolates the mRNA in a test tube. ❷ Single-stranded DNA transcripts are made from the mRNA using **reverse transcriptase**, a viral enzyme that can synthesize DNA from an RNA template (gold in the figure; see Module 10.20). ❸ Another enzyme is added to break down the mRNA, and ❹ DNA polymerase (the enzyme that replicates DNA; see Module 10.5) is used to synthesize a second DNA strand.

The DNA that results from such a procedure, called **complementary DNA (cDNA)**, represents only the subset of genes that had been transcribed into mRNA in the starting cells. Among other purposes, cDNA is useful for studying the genes responsible for the specialized functions of a particular cell type, such as brain or liver cells (see Module 11.9).



▲ **Figure 12.4** Making complimentary DNA (cDNA) from eukaryotic mRNA

? Why is the use of a viral enzyme critical to producing cDNAs?

■ The viral enzyme reverse transcriptase produces DNA from RNA; most cells lack such an enzyme.