Gene Cloning and Editing

12.1 Genes Can Be Cloned in Recombinant Plasmids (1 of 2)

- **Gene cloning** is one application of **biotechnology**, the manipulation of organisms or their components to make useful products.
- Researchers can manipulate bacterial **plasmids** so that they contain genes from other organisms.
  - These **recombinant DNA** plasmids can then be inserted into bacteria.
  - If the recombinant bacteria multiply into a **clone**, the foreign genes are also duplicated and copies of the gene or its protein product can be harvested.

12.2 Visualizing the Concept: Enzymes Are Used to "Cut and Paste" DNA

- **Restriction enzymes** cut DNA at specific sequences, forming restriction fragments.
- DNA ligase "pastes" DNA fragments together.

Checkpoint question What are "sticky ends"?

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

Figure 12.2_5

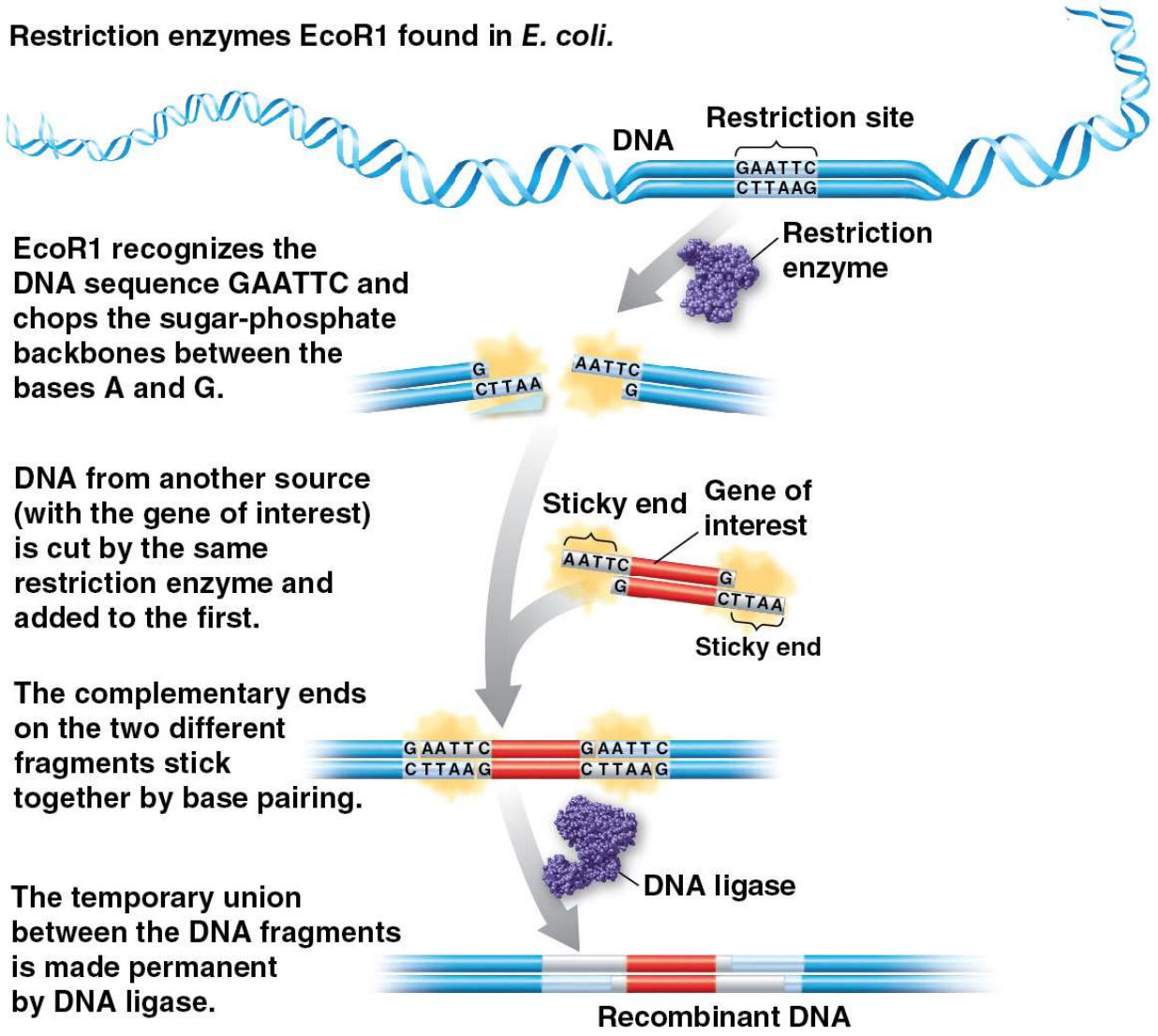


Figure 12.1b_1_4

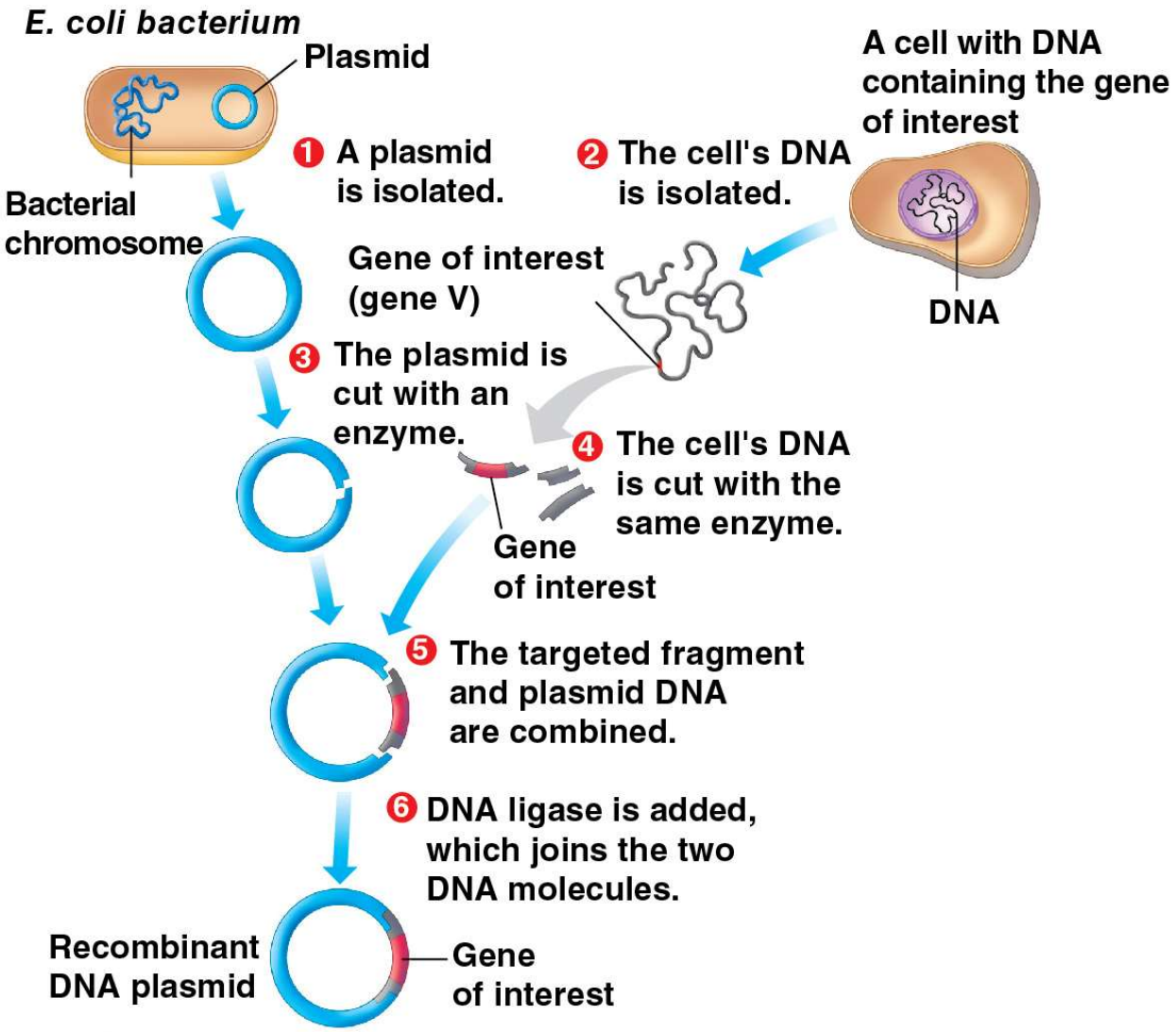
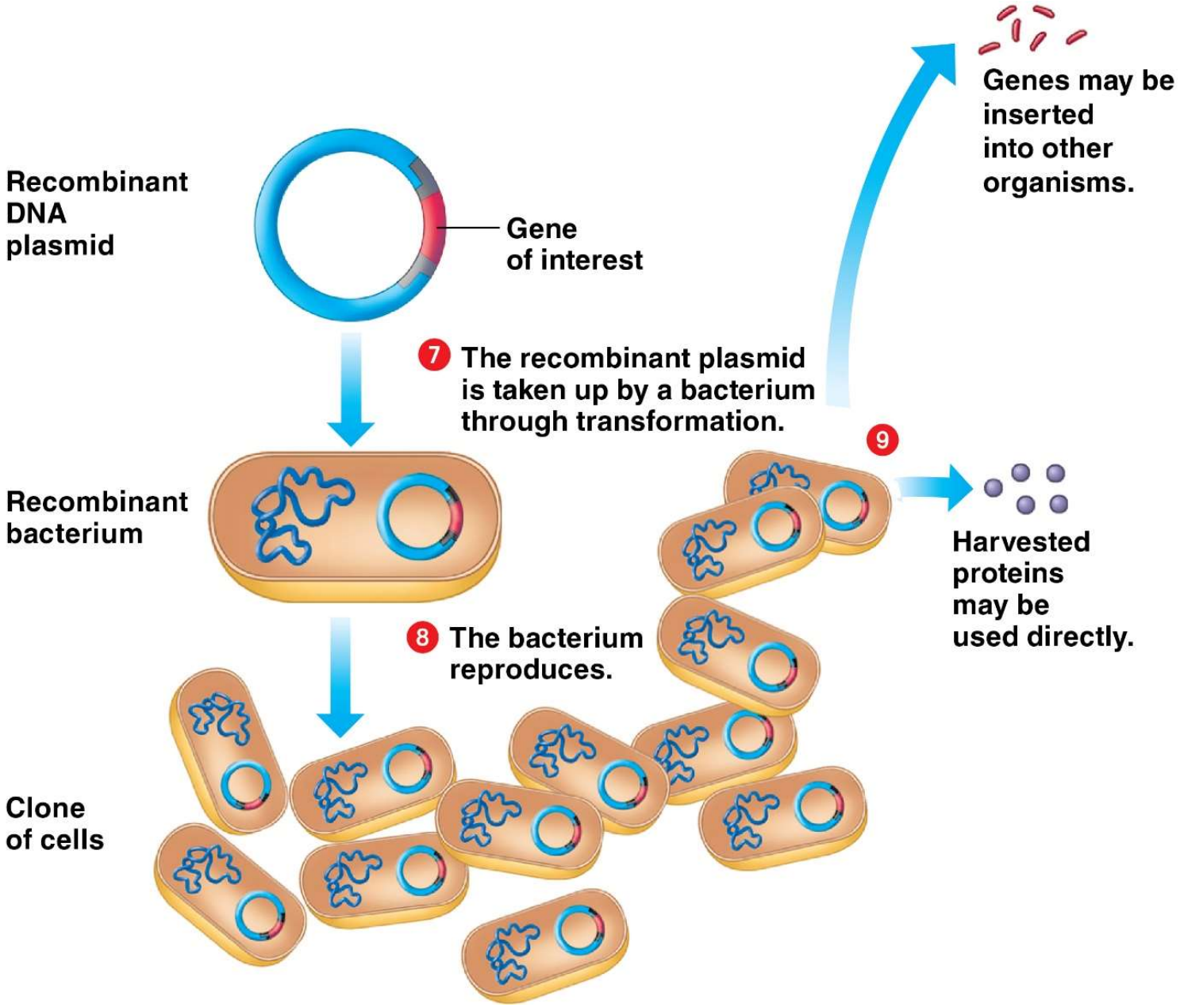


Figure 12.1b_2_3



12.1 Genes Can Be Cloned in Recombinant Plasmids (2 of 2)

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

A plasmid isolated from an *E. coli* bacterium

12.3 Nucleic Acid Probes Can Label Specific DNA Segments

- A short, single-stranded molecule of labeled DNA, called a **nucleic acid probe**, can tag a desired nucleotide sequence.

Checkpoint question 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.

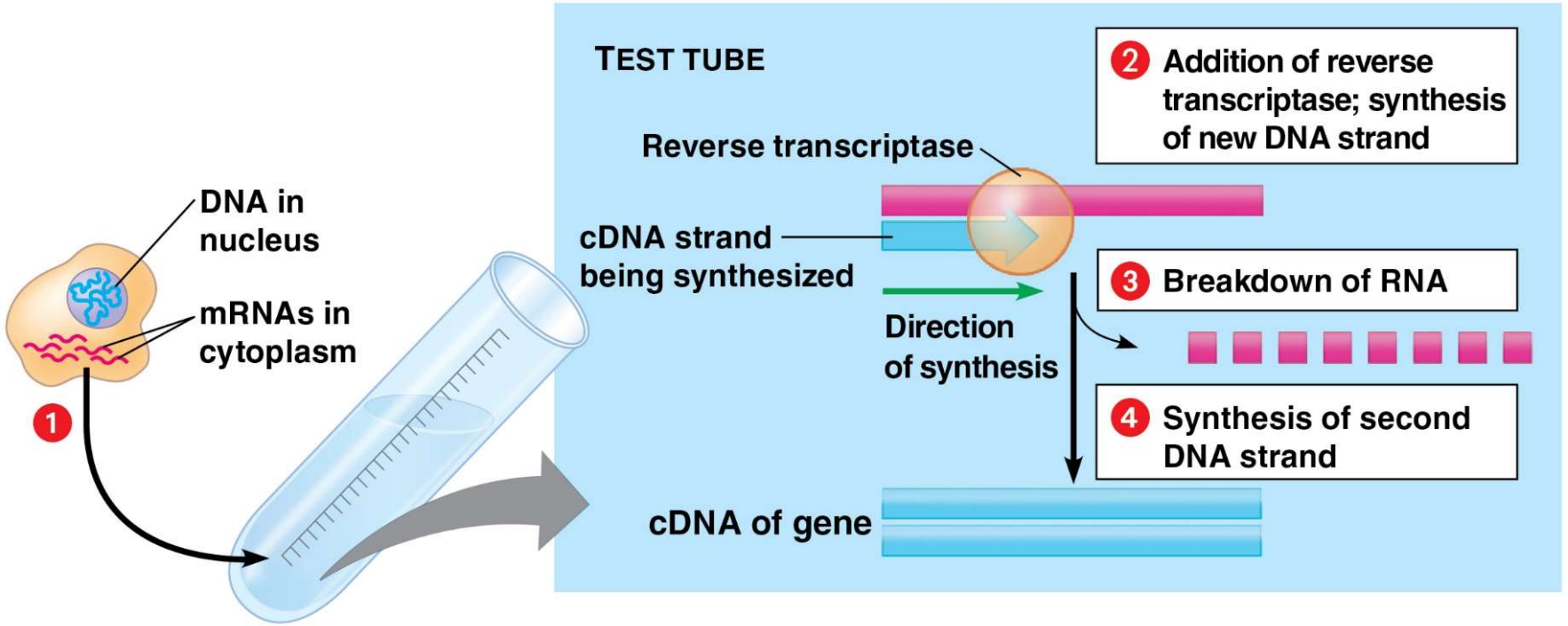
12.4 Reverse Transcriptase Can Help Make Genes for Cloning

- **Complementary DNA (cDNA)** can be used to identify the genes that are being transcribed by a particular cell at a given moment.

Checkpoint question 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.

Figure 12.4



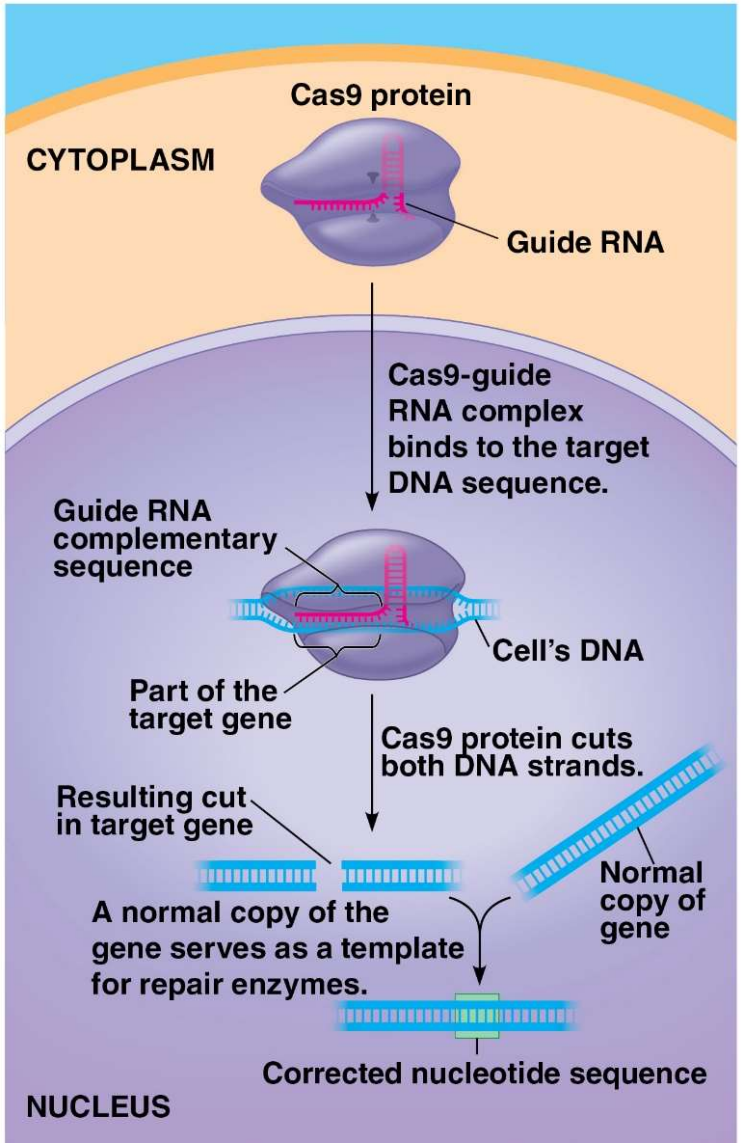
12.5 New Techniques Allow a Specific Gene to Be Edited

- The CRISPR-Cas9 system allows researchers to target a specific gene in a living cell for removal or editing.

Checkpoint question How does the CRISPR-Cas9 system differ from gene cloning?

Gene cloning exactly copies a gene, while the CRISPR-Cas9 system modifies the gene.

Figure 12.5_4



Genetically Modified Organisms

12.6 Recombinant Cells and Organisms Can Mass-Produce Gene Products

- Bacteria, yeast, cell cultures, and whole animals can be genetically modified to make products for medical and other uses.

Checkpoint question Why can't all human proteins be synthesized in *E. coli*?

Because bacteria cannot correctly produce some proteins, such as ones that require the attachment of sugar groups

12.7 Connection: DNA Technology Has Changed the Pharmaceutical Industry and Medicine

- Researchers use DNA technologies to
  - produce drugs,
  - diagnose diseases, and
  - produce **vaccines**, harmless variants (mutants) or derivatives of a pathogen.

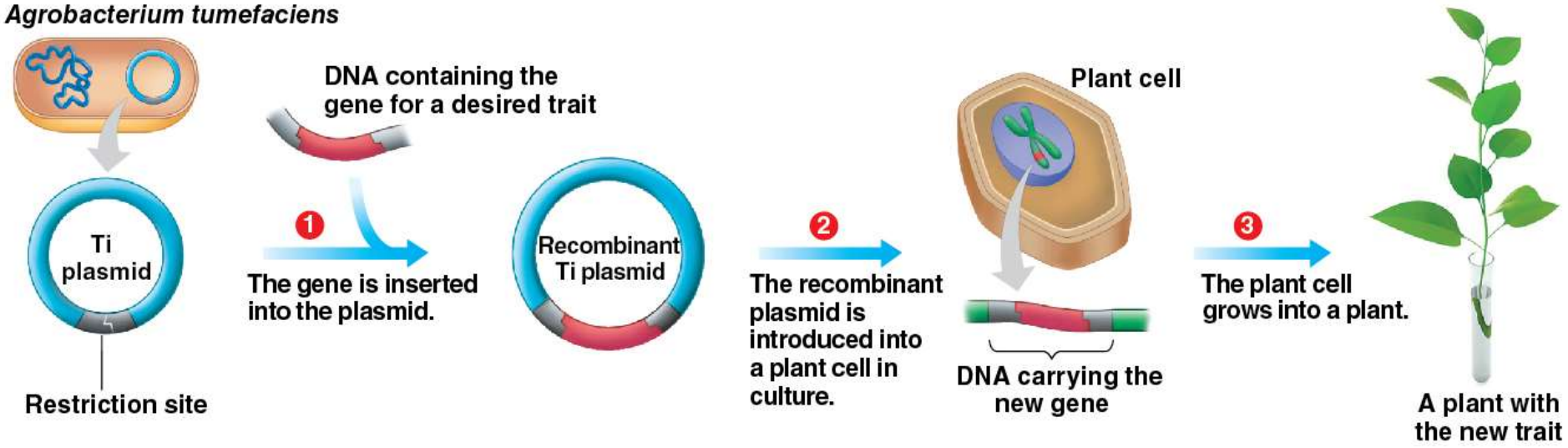
Checkpoint question If insulin and human growth hormone are both natural products, why use genetic engineering to make them?

DNA technology can provide these drugs in much larger quantities than can be naturally obtained.

12.8 Connection: Genetically Modified Organisms Are Transforming Agriculture

- Scientists have produced many different varieties of **genetically modified organisms (GMOs)**, organisms that have acquired one or more genes by artificial means.
- If a gene is transplanted from one organism into another, typically of another species, the recombinant organism is called a **transgenic organism**.
- A number of important crop plants are genetically modified.

Figure 12.8a_3



12.9 Scientific Thinking: The Use of Genetically Modified Organisms Raises Questions and Concerns

- Scientists are investigating the potential risks to human and environmental health posed by DNA technologies.

Checkpoint question Why is it often necessary to run both human and animal studies to learn about human health?

Animal diets and lifestyles can be closely controlled, but the results may not apply directly to humans.

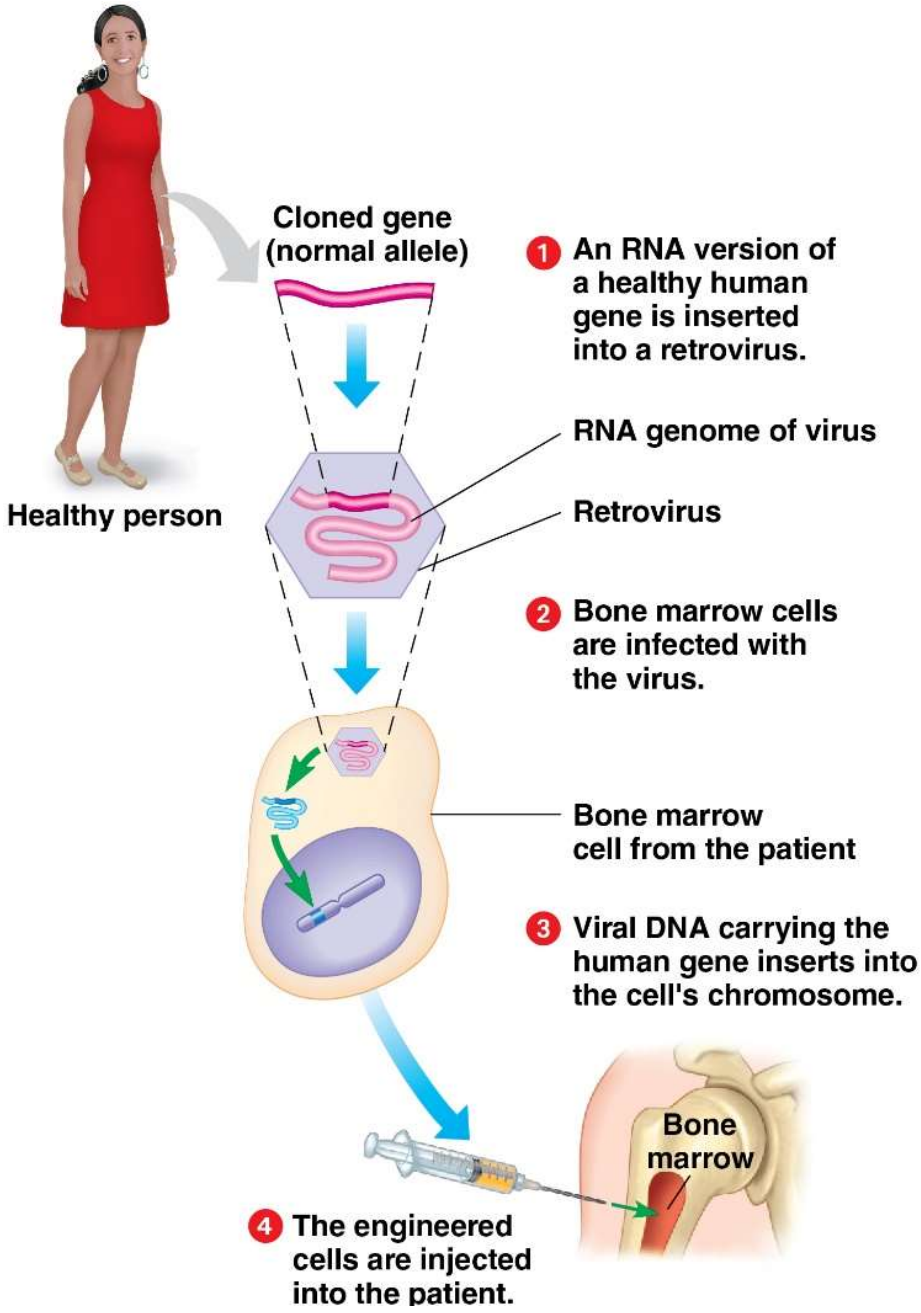
12.10 Connection: Gene Therapy May Someday Help Treat a Variety of Diseases

- **Gene therapy**, changing a defective gene to a normal one in a living human, shows promise for curing defective genes, but actual successes are rare.

Checkpoint question Why does bone marrow make a good target for gene therapy?

Bone marrow cells multiply throughout a person's life and contain stem cells that give rise to different kinds of blood cells.

Figure 12.10

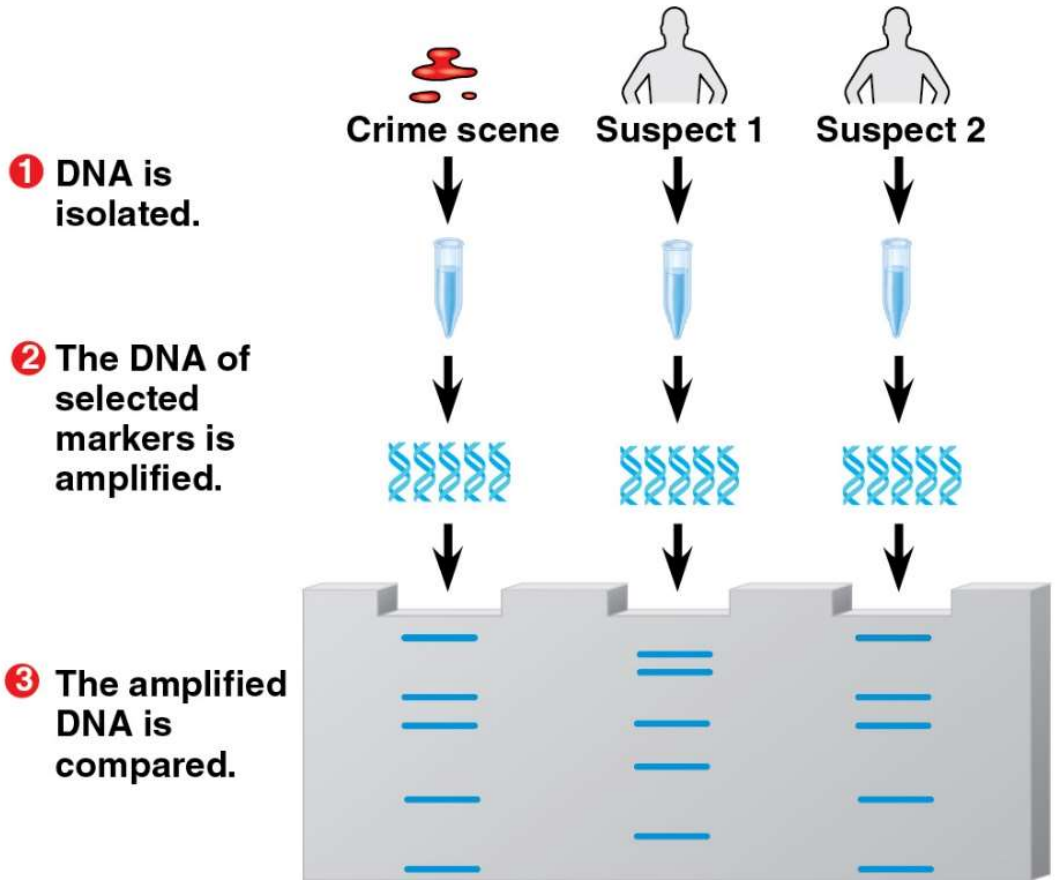


DNA Profiling

12.11 The Analysis of Genetic Markers Can Produce a DNA Profile (1 of 2)

- **DNA technology**—modern laboratory techniques for studying and manipulating genetic material—has rapidly transformed the field of forensics, the scientific analysis of evidence for crime scene investigations and other legal proceedings.
- **DNA profiling** can determine whether two samples of DNA came from the same individual.

12.11 The Analysis of Genetic Markers Can Produce a DNA Profile (2 of 2)



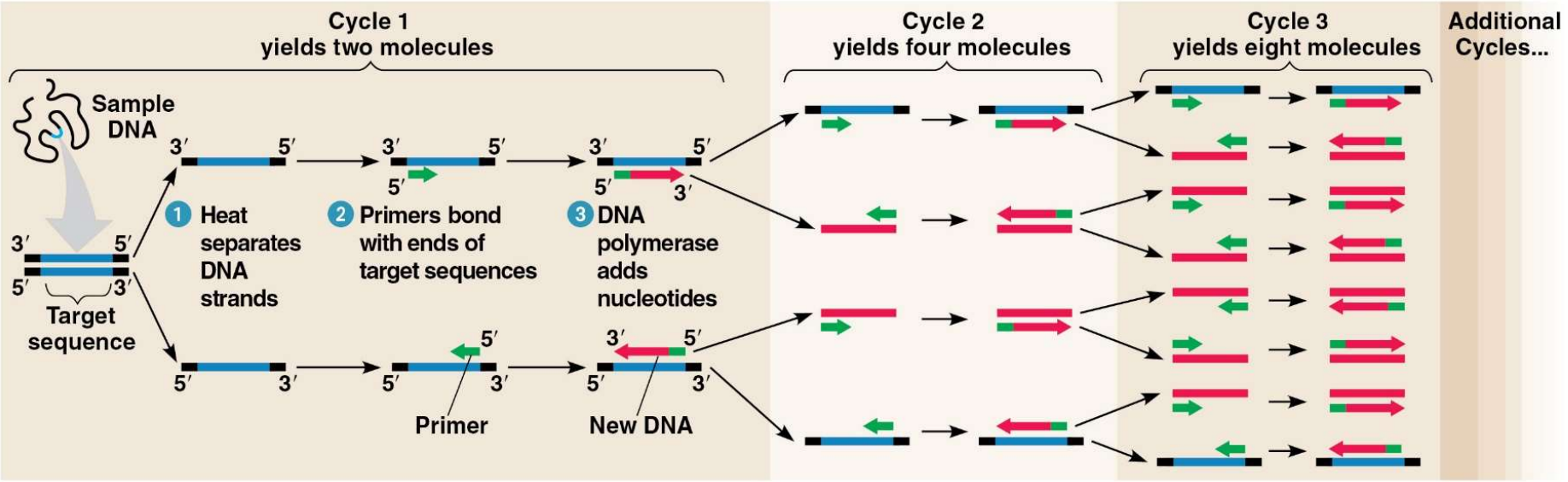
Suspect 2: Notice that the number and location of the DNA markers in suspect 2's DNA and the crime scene DNA match.

Checkpoint question According to the data presented in Figure 12.11, which suspect left DNA at the crime scene?

12.12 The PCR Method Is used to Amplify DNA Sequences

- The **polymerase chain reaction (PCR)** can be used to amplify a DNA sample.
- The use of specific **primers** that flank the desired sequence ensures that only a particular subset of the DNA sample will be copied.
- Starting with a minute sample, automated PCR can generate billions of copies of a DNA segment in just a few hours, producing enough DNA to allow a DNA profile to be constructed.

Figure 12.12



12.12 The PCR Method Is Used to Amplify DNA Sequences

Checkpoint question Why does PCR amplify only one specific region of DNA rather than all of it?

The primers mark the ends, ensuring that only the DNA within the region between is amplified.

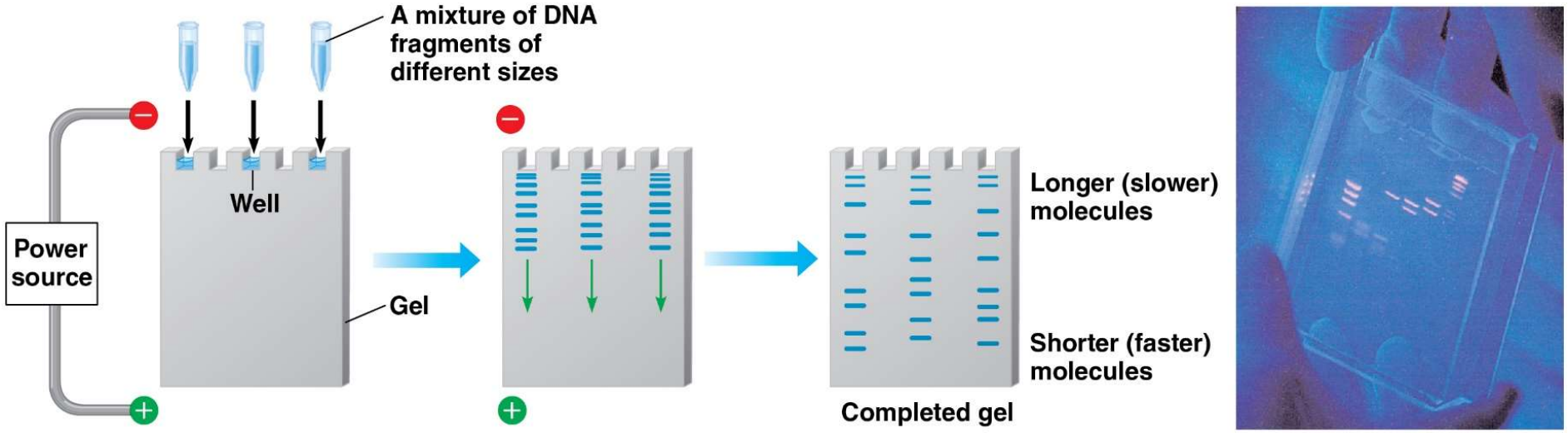
12.13 Gel Electrophoresis Sorts DNA Molecules by Size

- Many DNA technology applications rely on **gel electrophoresis**, a method that separates macromolecules, usually proteins or nucleic acids, on the basis of size, electrical charge, or other physical properties.

Checkpoint question What causes DNA molecules to move toward the positive pole during electrophoresis? Why do large molecules move more slowly than smaller ones?

The negatively charged phosphate groups of the DNA are attracted to the positive pole; longer fragments are more restricted by the tangle of fibers in the gel than are shorter fragments.

Figure 12.13



12.14 Short Tandem Repeat Analysis Is Used for DNA Profiling

- **Repetitive DNA** consists of nucleotide sequences that are present in multiple copies in the genome. Much of the DNA that lies between genes in humans is of this type.
- **Short tandem repeats (STRs)** are stretches of DNA that contain short nucleotide sequences repeated many times in a row.
- DNA profiling by **STR analysis** involves amplifying and quantifying 13 STRs.

Figure 12.14a

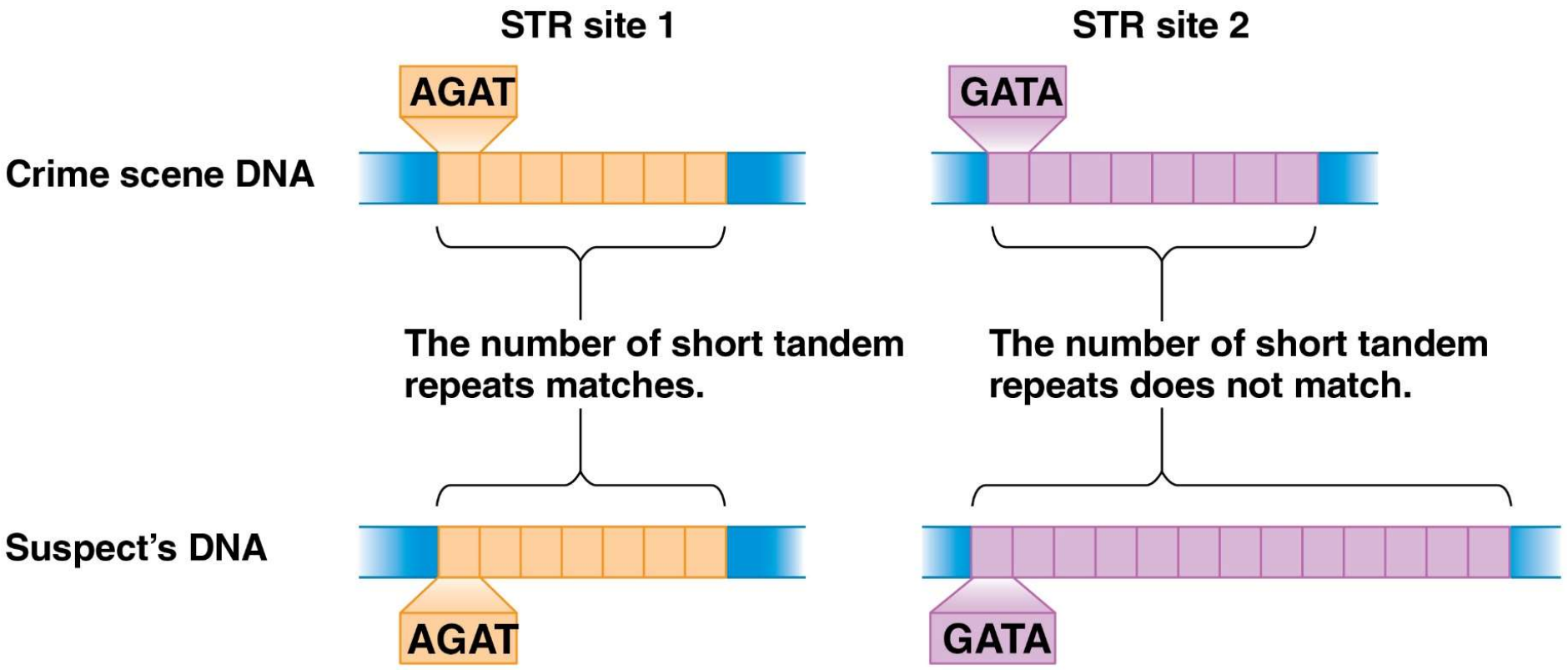
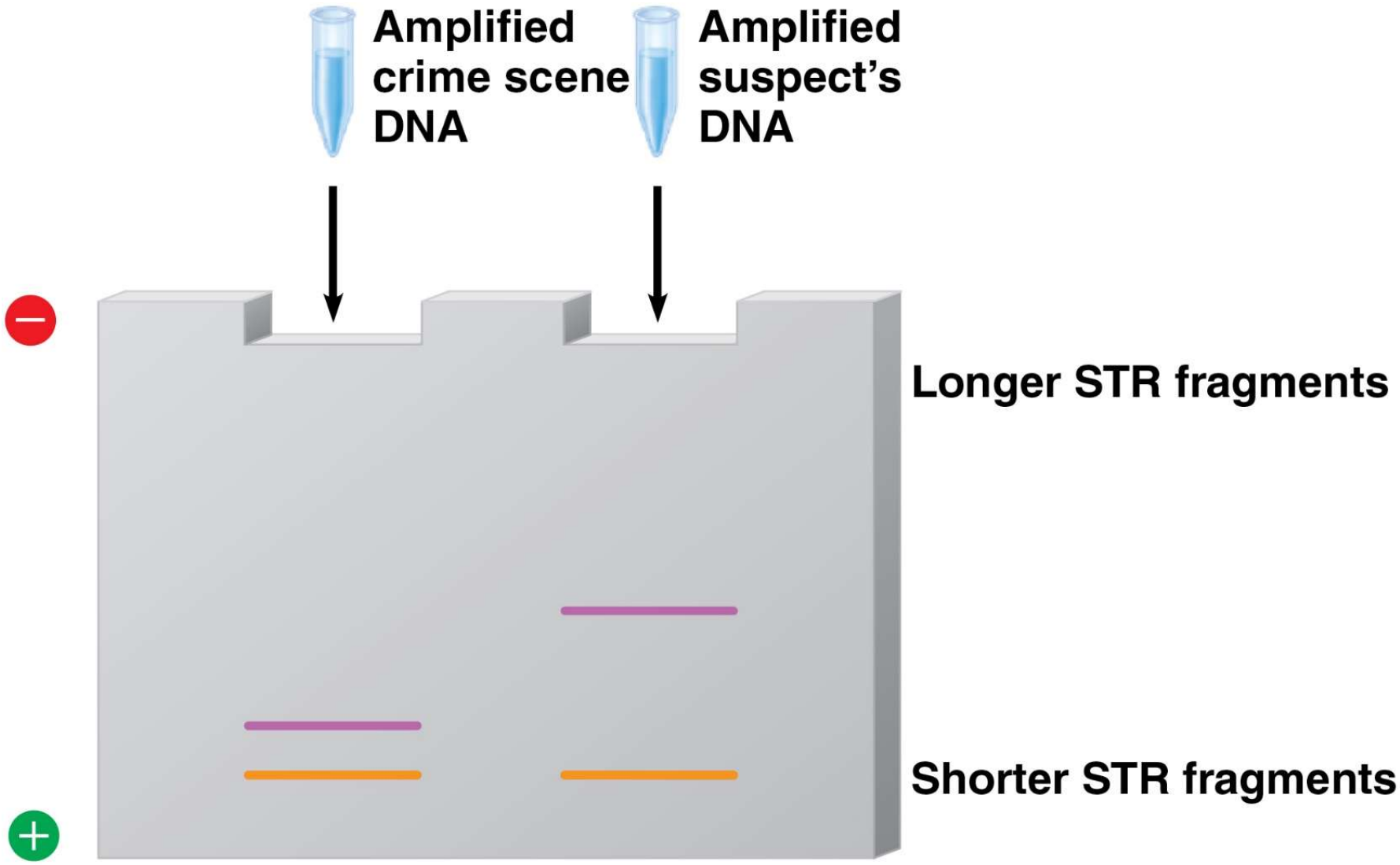


Figure 12.14b



12.15 Connection: DNA Profiling Has Provided Evidence in Many Forensic Investigations

- The applications of DNA profiling include helping to
  - solve crimes,
  - establish paternity, and
  - identify victims.

Checkpoint question In what way is DNA profiling valuable for determining innocence as well as guilt?

A DNA profile can prove with near certainty that a sample of DNA does or does not come from a particular individual. DNA profiling therefore can provide evidence in support of guilt or innocence.

Genomics and Bioinformatics

Researchers Can Monitor the Expression of Specific Genes (1 of 2)

- Scientists can use various techniques to study how genes work together.
  - **Nucleic acid hybridization** allows researchers to identify cells in which a target gene is expressed.
  - A **DNA microarray** can gather data about which genes are turned on or off in a particular cell.

11.9 Connection: Researchers Can Monitor the Expression of Specific Genes (2 of 2)

Checkpoint question What can be learned from a DNA microarray?

Which genes are active (transcribed) in a particular sample of cells

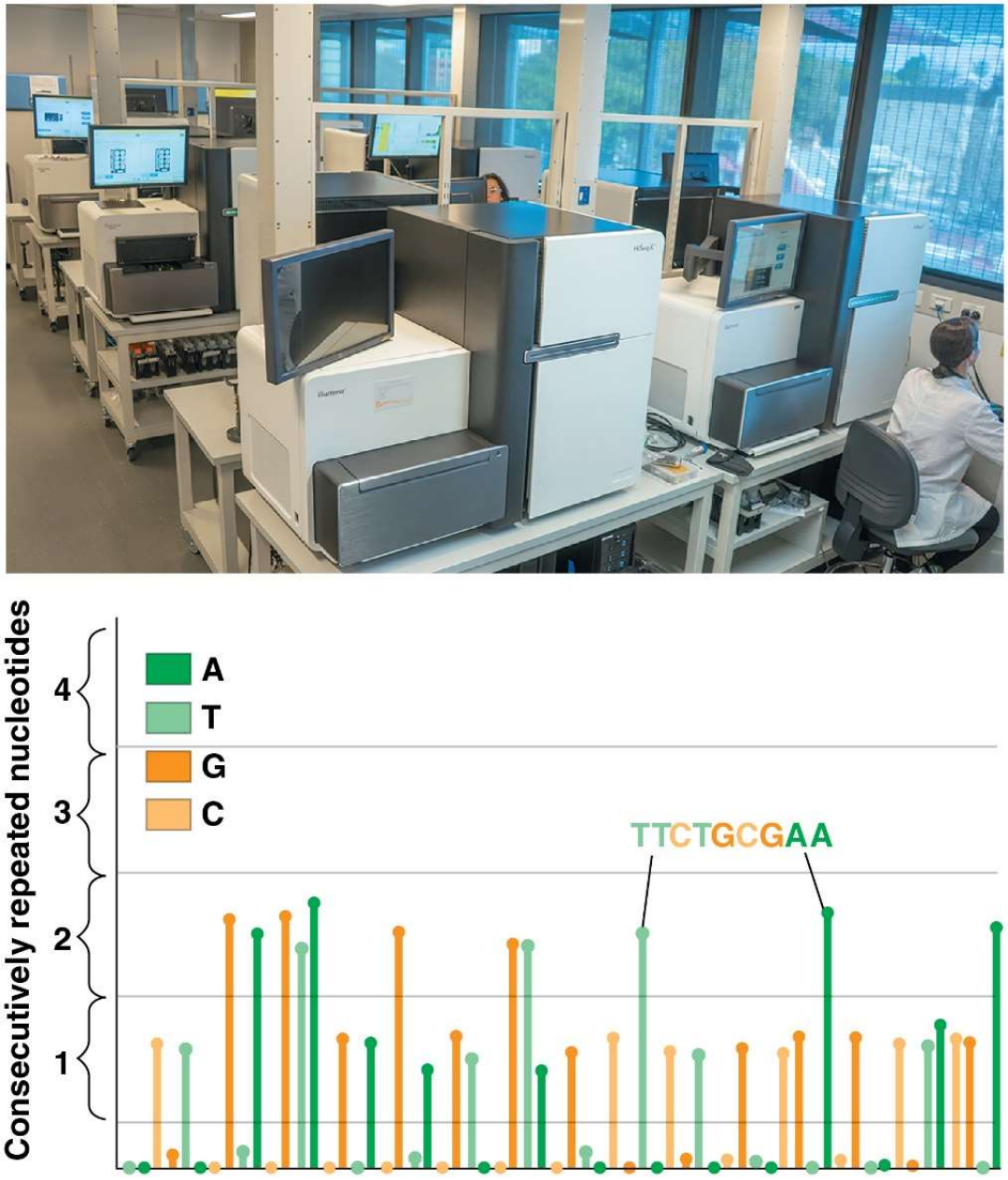
12.16 Small Segments of DNA Can Be Sequenced Directly

- Next- and third-generation sequencing machines can quickly determine the sequence of relatively short stretches of DNA

Checkpoint question Why is third-generation sequencing potentially more efficient than next-generation sequencing?

Third-generation sequencing works with a single long DNA molecule, while next-generation sequencing involves piecing together many smaller molecules.

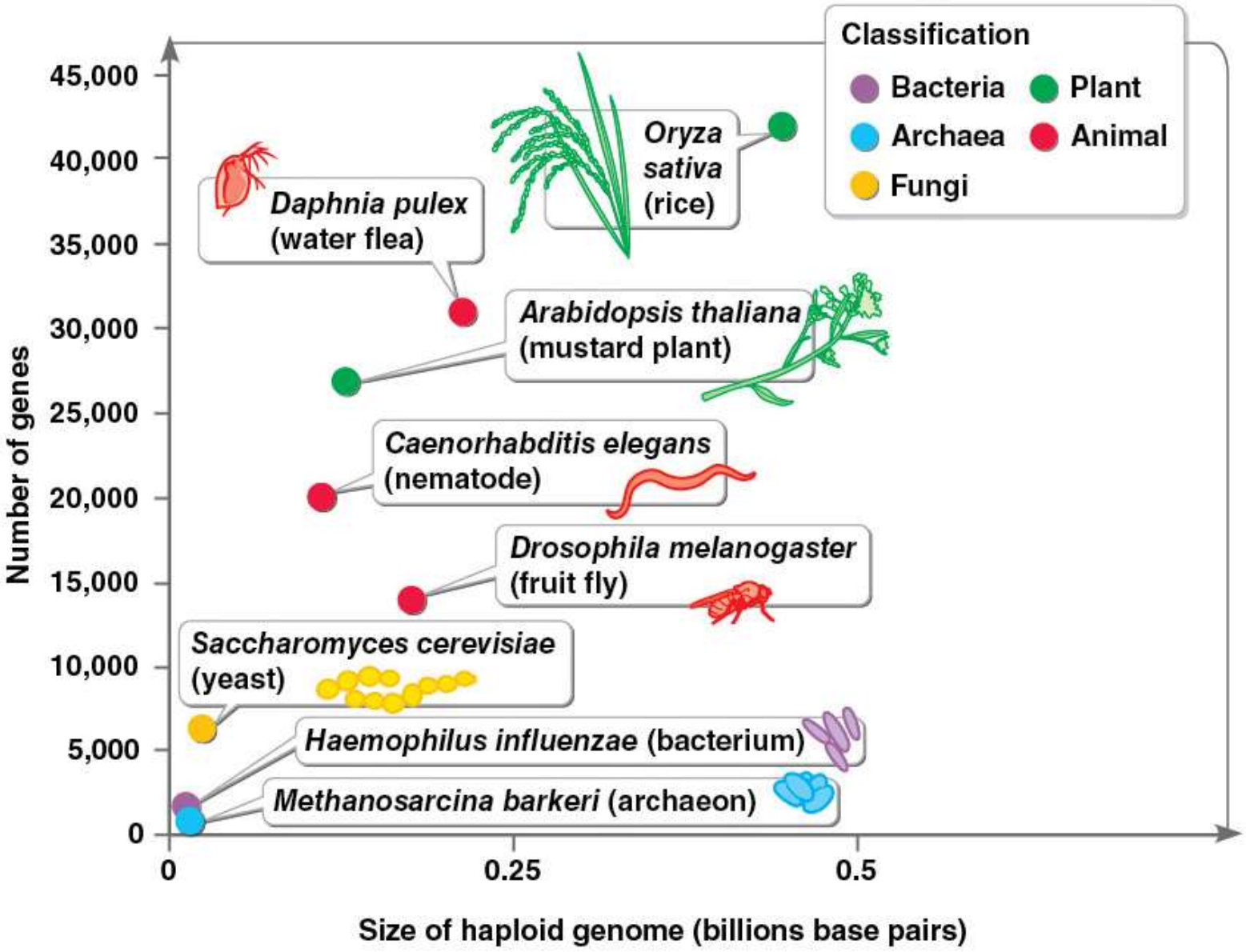
Figure 12.16



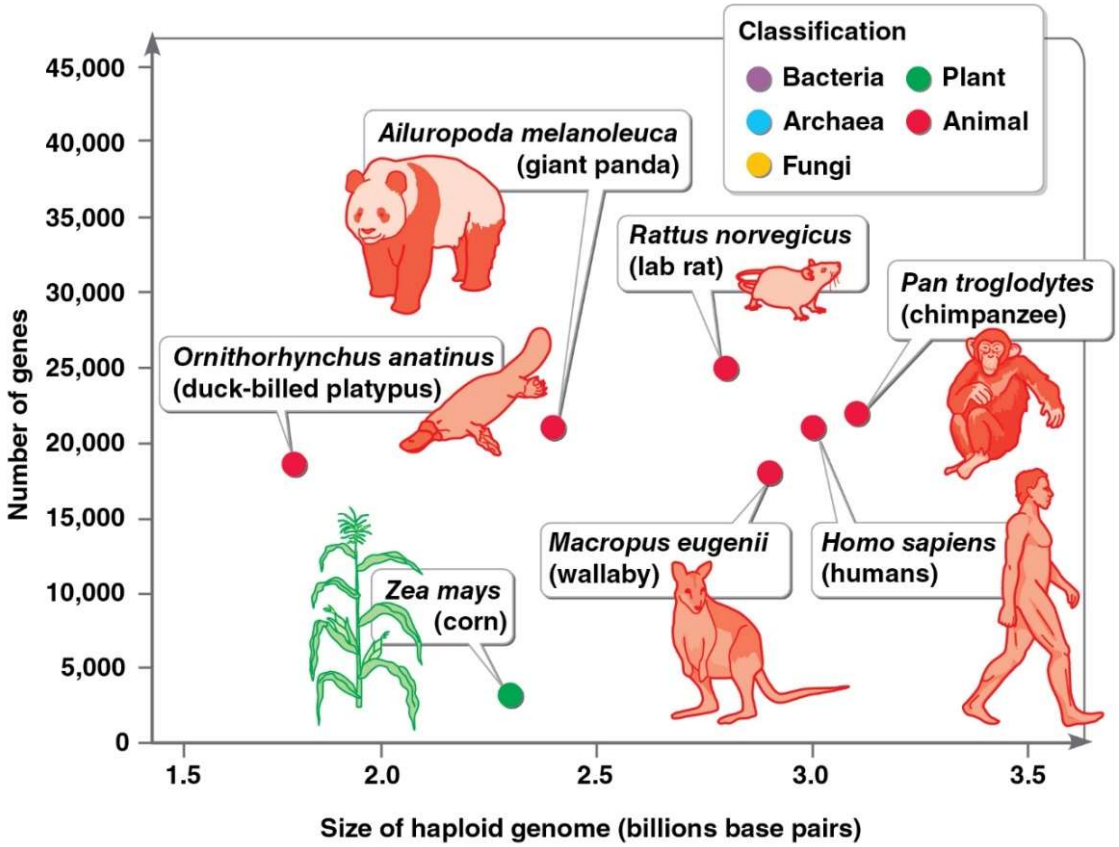
12.17 Genomics Is the Scientific Study of Whole Genomes (1 of 2)

- Genomics researchers have sequenced many prokaryotic and eukaryotic genomes.
- Besides being of interest in their own right, nonhuman genomes can be compared with the human genome.

Figure 12.17_1



12.17 Genomics Is the Scientific Study of Whole Genomes (2 of 2)



No. Compare, for example, the genomes of rice and mice.

Checkpoint question Does a larger genome always correlate with more genes?

Pearson

12.18 Connection: The Human Genome Project Revealed That Most of the Human Genome Does Not Consist of Genes

- The **Human Genome Project (HGP)** was a massive, publicly funded scientific endeavor to determine the nucleotide sequence of all DNA in the human genome and identify the location and sequence of every gene.
- Data from the HGP revealed that the human genome contains just under 21,000 genes and a huge amount of noncoding DNA, much of which consists of repetitive nucleotide sequences.

12.19 The Whole-Genome Shotgun Method of Sequencing a Genome Can Provide a Wealth of Data Quickly

- Modern genomic analysis depends upon the **whole-genome shotgun** method, which involves sequencing and arranging many small DNA fragments simultaneously.

Checkpoint question What are the primary advantages of the whole-genome shotgun method over previous methods?

12.20 The Field of Bioinformatics Is Expanding Our Understanding of Genomes

- **Bioinformatics**, the use of computational methods to analyze biological data, can be used to analyze large sets of data about DNA sequences and proteins.
- **Proteomics** involves similar systematic studies of the full protein sets (proteomes) encoded by genomes.

Checkpoint question What are the primary advantages of the whole-genome shotgun method over previous methods?

12.21 Evolution Connection: Genomes Hold Clues to Human Evolution

- Using databases like GenBank, researchers can now compare genome sequences from many species, allowing hypotheses about evolutionary relationships between those species to be tested.
- The comparison of genomic sequences between humans and our nearest evolutionary relatives provides insight into human evolution.

Checkpoint question How can cross-species comparisons of the nucleotide sequences of a gene provide insight into evolution? Similarities in gene sequences correlate with evolutionary relatedness; greater genetic similarities reflect a more recent shared ancestry.