LECTURE PRESENTATIONS
For CAMPBELL BIOLOGY, NINTH EDITION
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Chapter 20

Biotechnology

Lectures by
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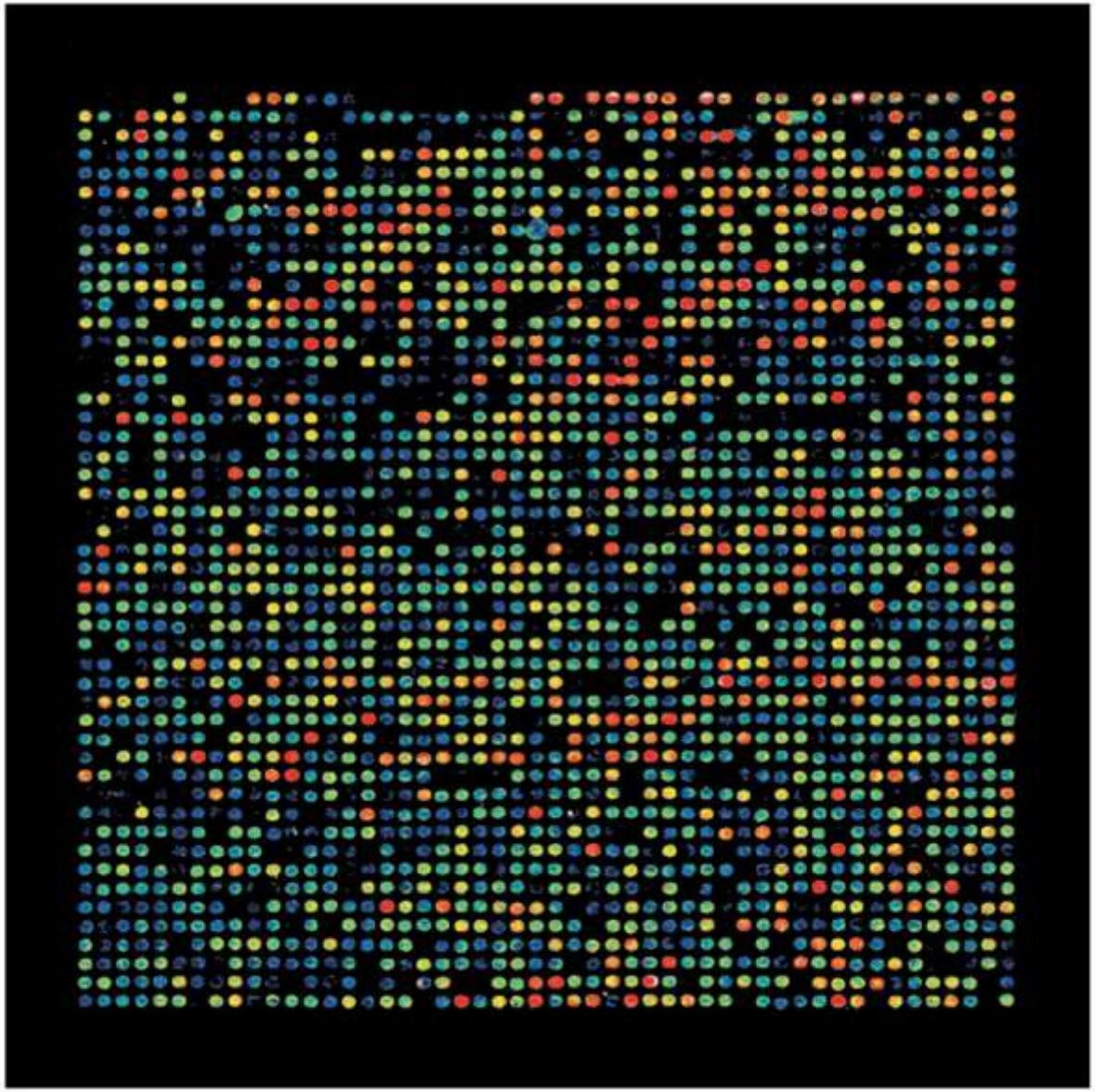
Kathleen Fitzpatrick

Overview: The DNA Toolbox

- Sequencing of the genomes of more than 7,000 species was under way in 2010
- DNA sequencing has depended on advances in technology, starting with making recombinant DNA
- In **recombinant DNA**, **nucleotide sequences from two different sources, often two species, are combined *in vitro* into the same DNA molecule**

- Methods for making recombinant DNA are central to **genetic engineering**, the direct manipulation of genes for practical purposes
- DNA technology has revolutionized **biotechnology**, the manipulation of organisms or their genetic components to make useful products
- An example of DNA technology is the microarray, a measurement of gene expression of thousands of different genes

Figure 20.1



Concept 20.1: DNA cloning yields multiple copies of a gene or other DNA segment

- To work directly with specific genes, scientists prepare well-defined segments of DNA in identical copies, a process called *DNA cloning*

DNA Cloning and Its Applications: *A Preview*

- Most methods for cloning pieces of DNA in the laboratory share general features, such as the use of bacteria and their plasmids
- **Plasmids** are small circular DNA molecules that replicate separately from the bacterial chromosome
- Cloned genes are useful for making copies of a particular gene and producing a protein product

- **Gene cloning** involves using bacteria to make multiple copies of a gene
- Foreign DNA is inserted into a plasmid, and the recombinant plasmid is inserted into a bacterial cell
- Reproduction in the bacterial cell results in cloning of the plasmid including the foreign DNA
- This results in the production of multiple copies of a single gene

Figure 20.2

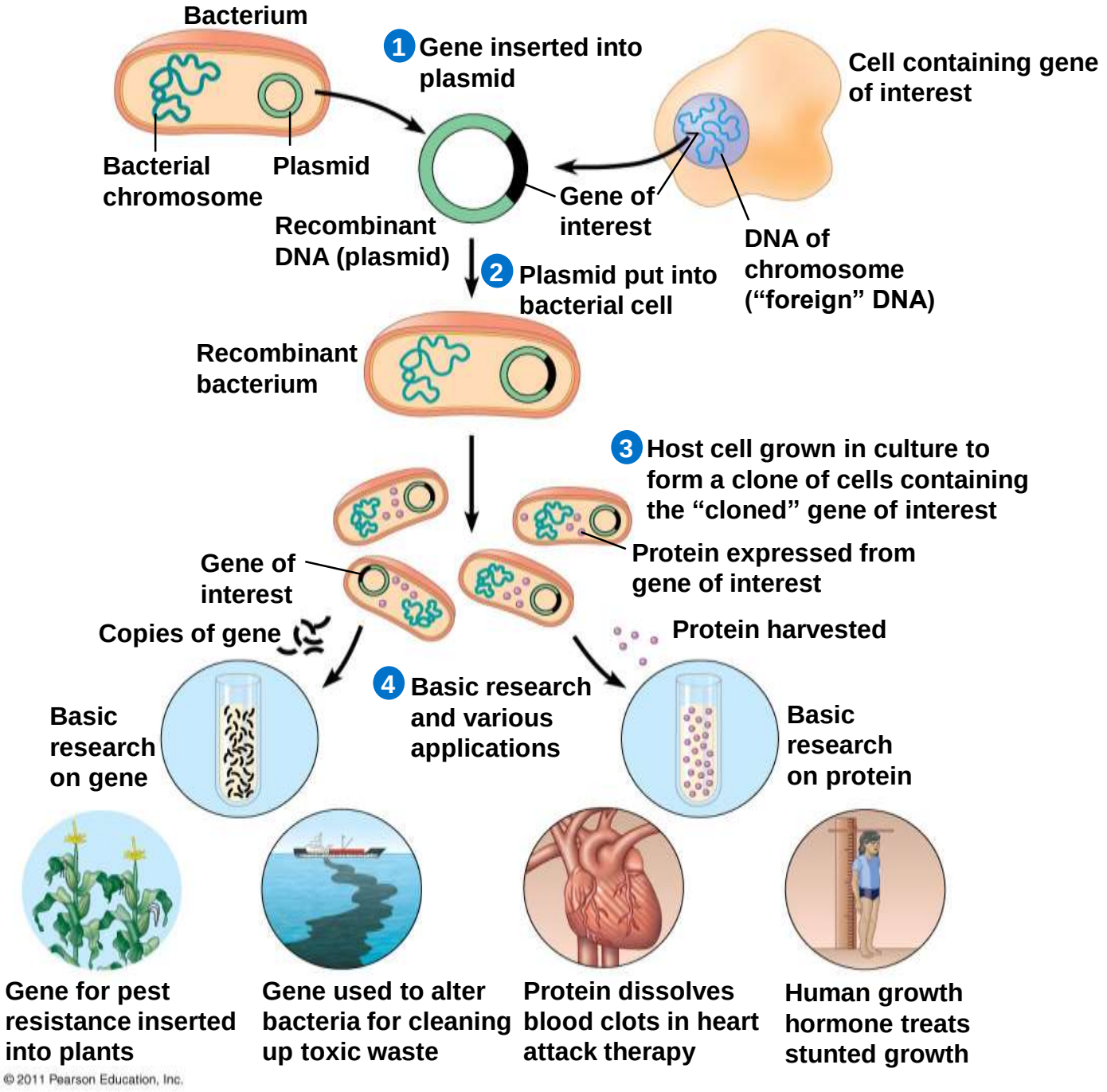


Figure 20.2a

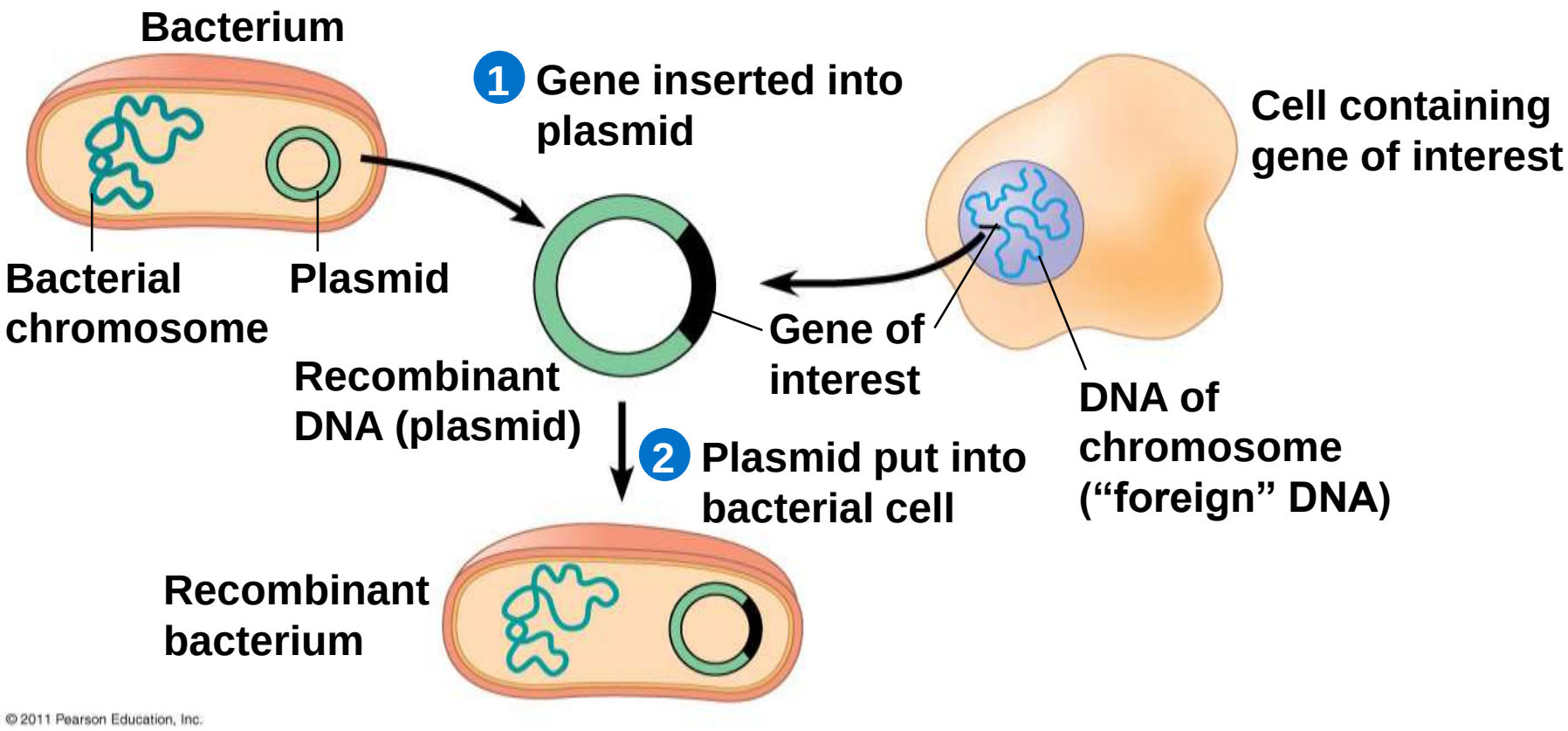
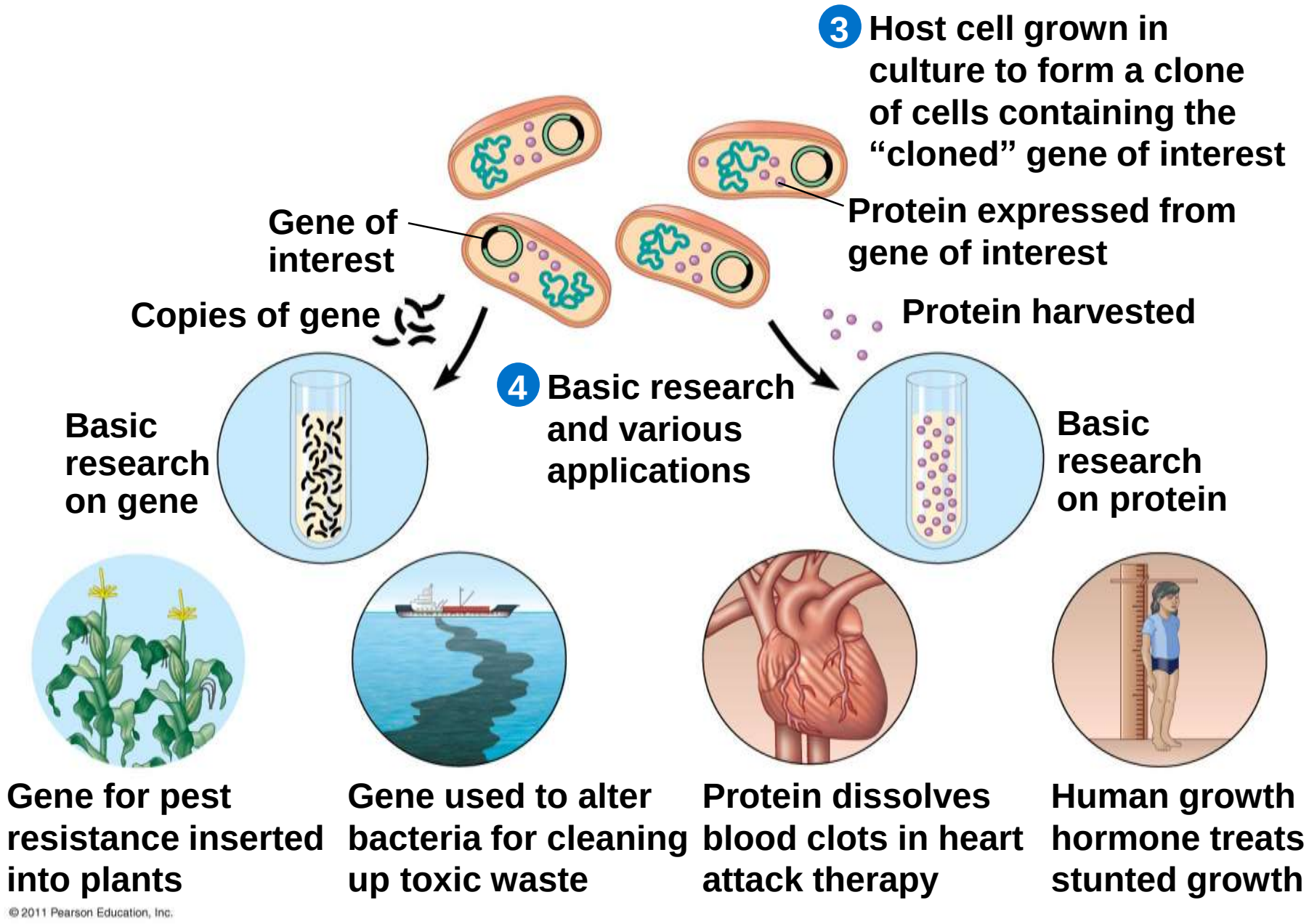
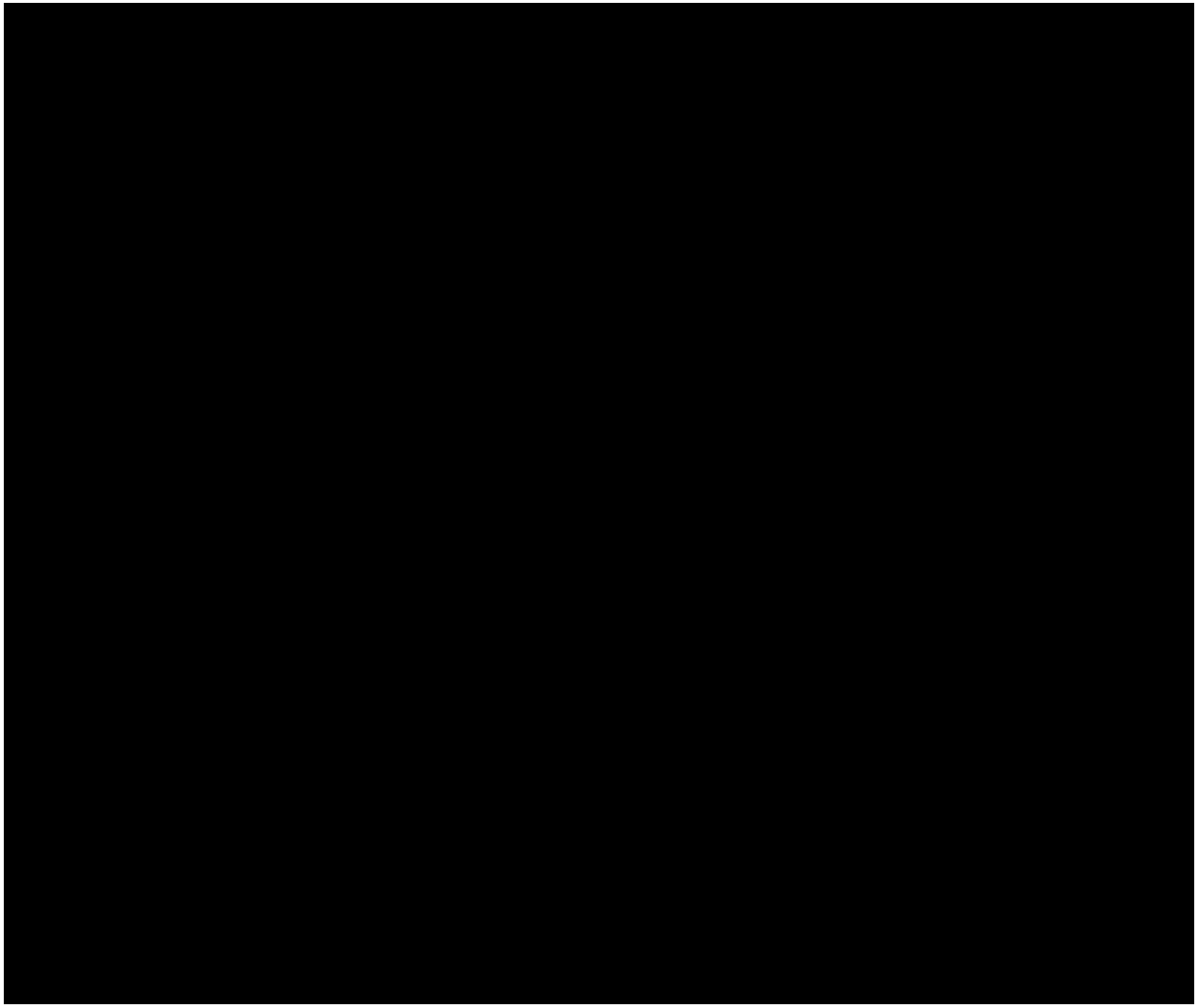


Figure 20.2b



Using Restriction Enzymes to Make Recombinant DNA

- Bacterial **restriction enzymes** cut DNA molecules at specific DNA sequences called **restriction sites**
- A restriction enzyme usually makes many cuts, yielding **restriction fragments**
- The most useful restriction enzymes cut DNA in a staggered way, producing fragments with "**sticky ends**."



Animation: Restriction Enzymes

Right-click slide / select “Play”

- Sticky ends can bond with complementary sticky ends of other fragments
- **DNA ligase** is an enzyme that seals the bonds between restriction fragments

Figure 20.3-1

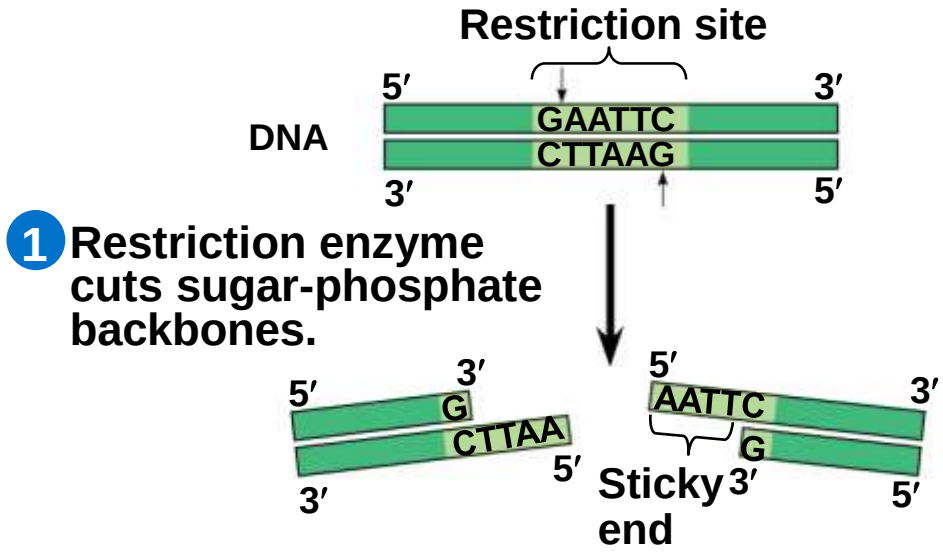


Figure 20.3-2

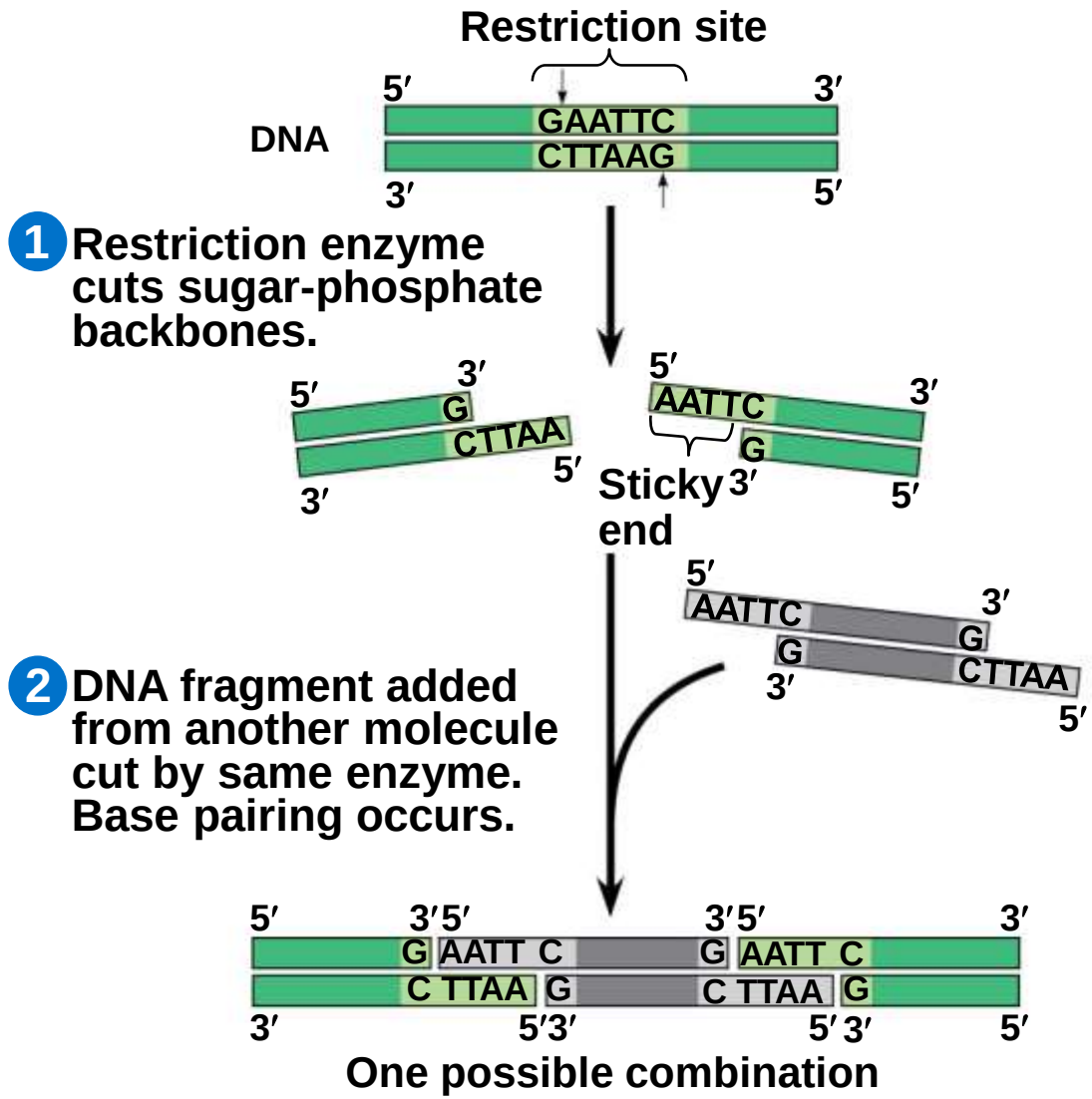
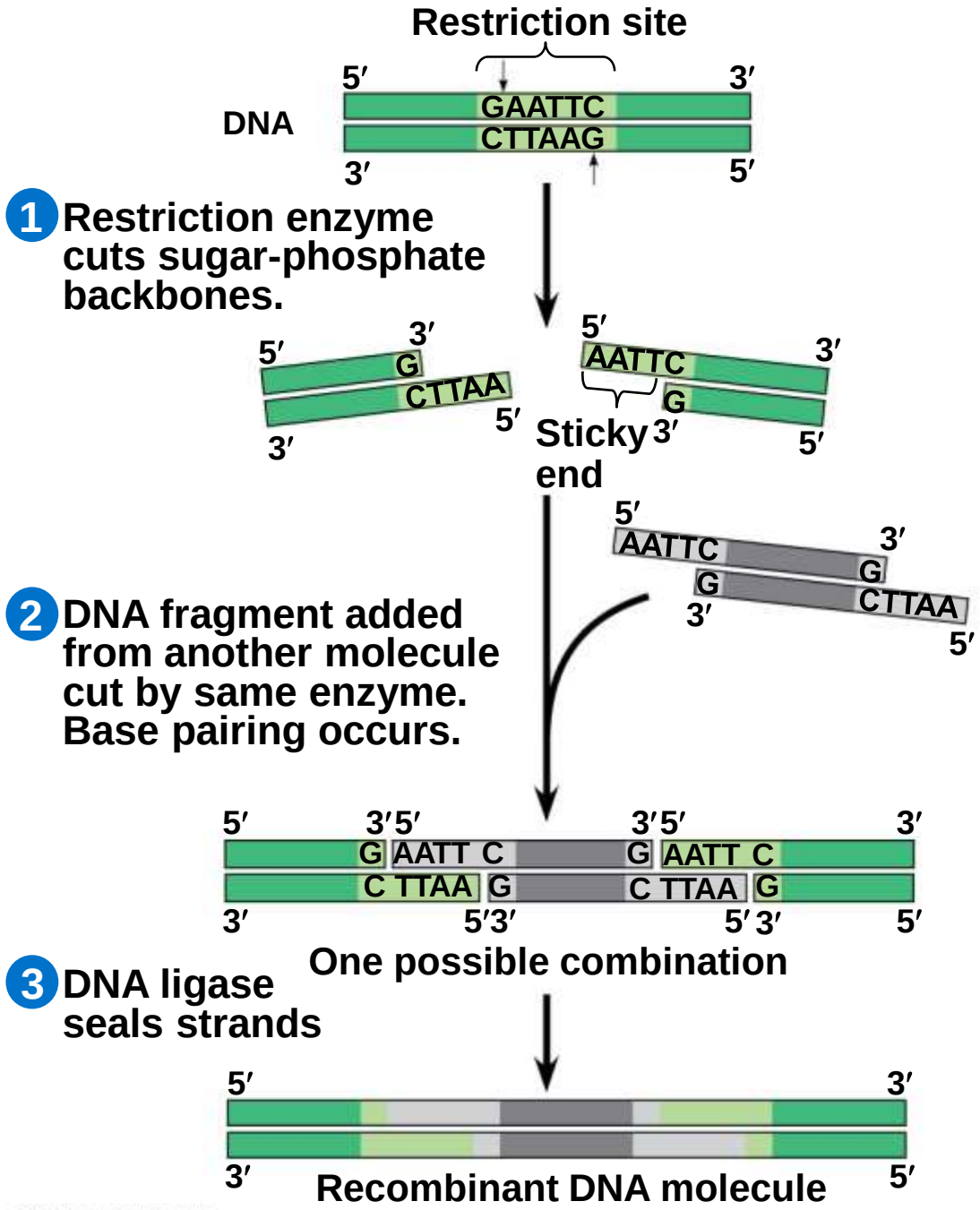


Figure 20.3-3



Cloning a Eukaryotic Gene in a Bacterial Plasmid

- In gene cloning, the original plasmid is called a **cloning vector**
- A cloning vector is a DNA molecule that can carry foreign DNA into a host cell and replicate there

Producing Clones of Cells Carrying Recombinant Plasmids

- Several steps are required to clone the hummingbird *β*-globin gene in a bacterial plasmid
  - The hummingbird genomic DNA and a bacterial plasmid are isolated
  - Both are cut with the same restriction enzyme
  - The fragments are mixed, and DNA ligase is added to bond the fragment sticky ends

Animation: Cloning a Gene

Right-click slide / select “Play”

- Some recombinant plasmids now contain hummingbird DNA
- The DNA mixture is added to bacteria that have been genetically engineered to accept it
- The bacteria are plated on a type of agar that selects for the bacteria with recombinant plasmids
- This results in the cloning of many hummingbird DNA fragments, including the *β*-globin gene

Figure 20.4

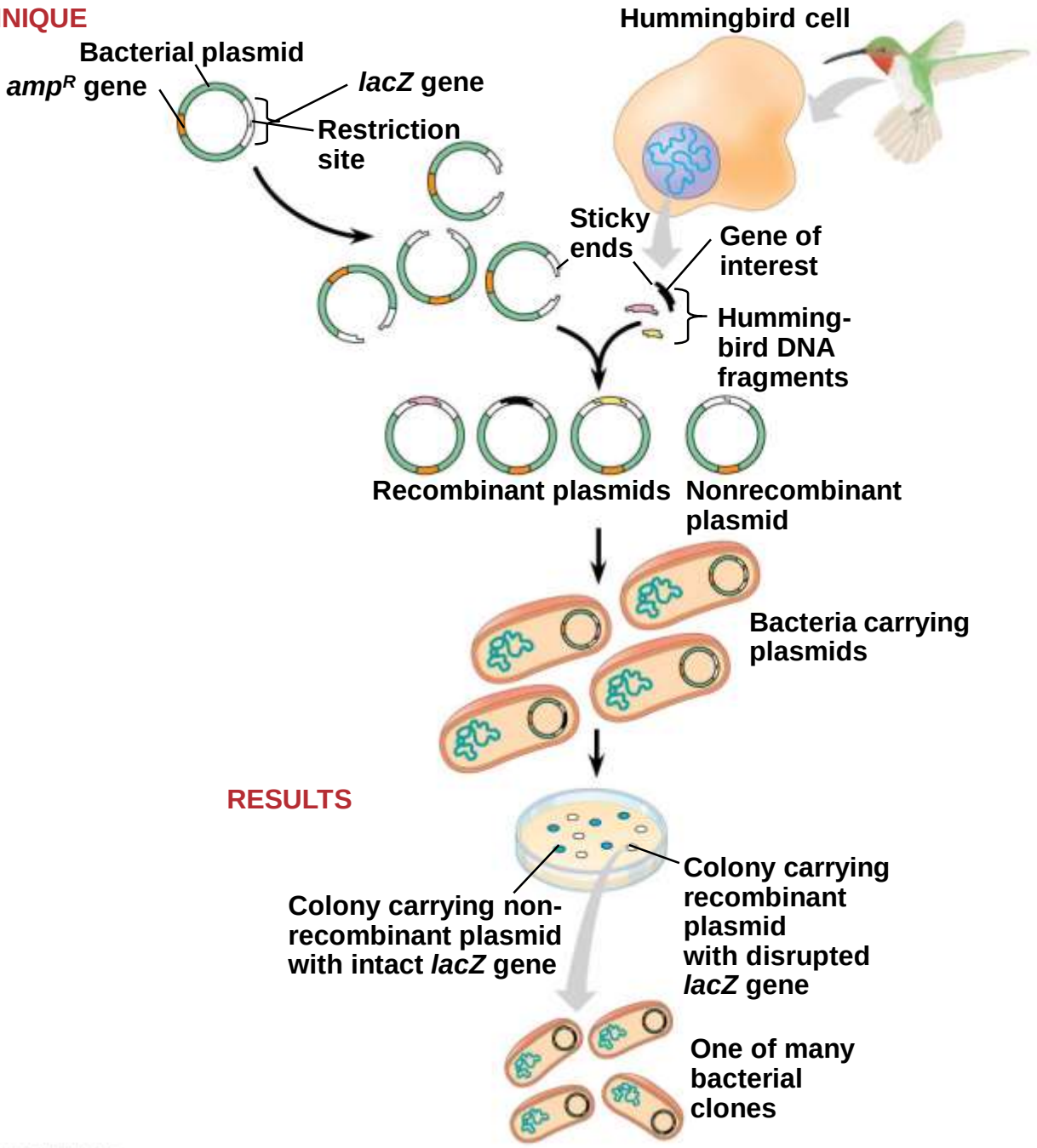


Figure 20.4a-1

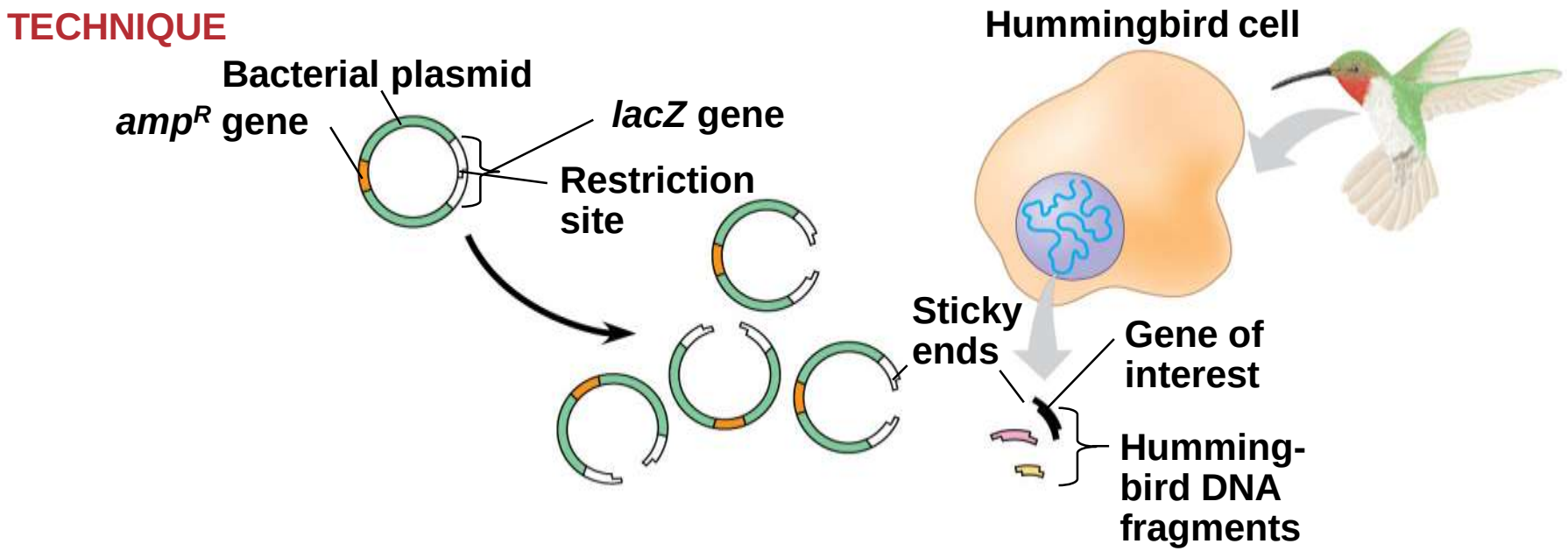


Figure 20.4a-2

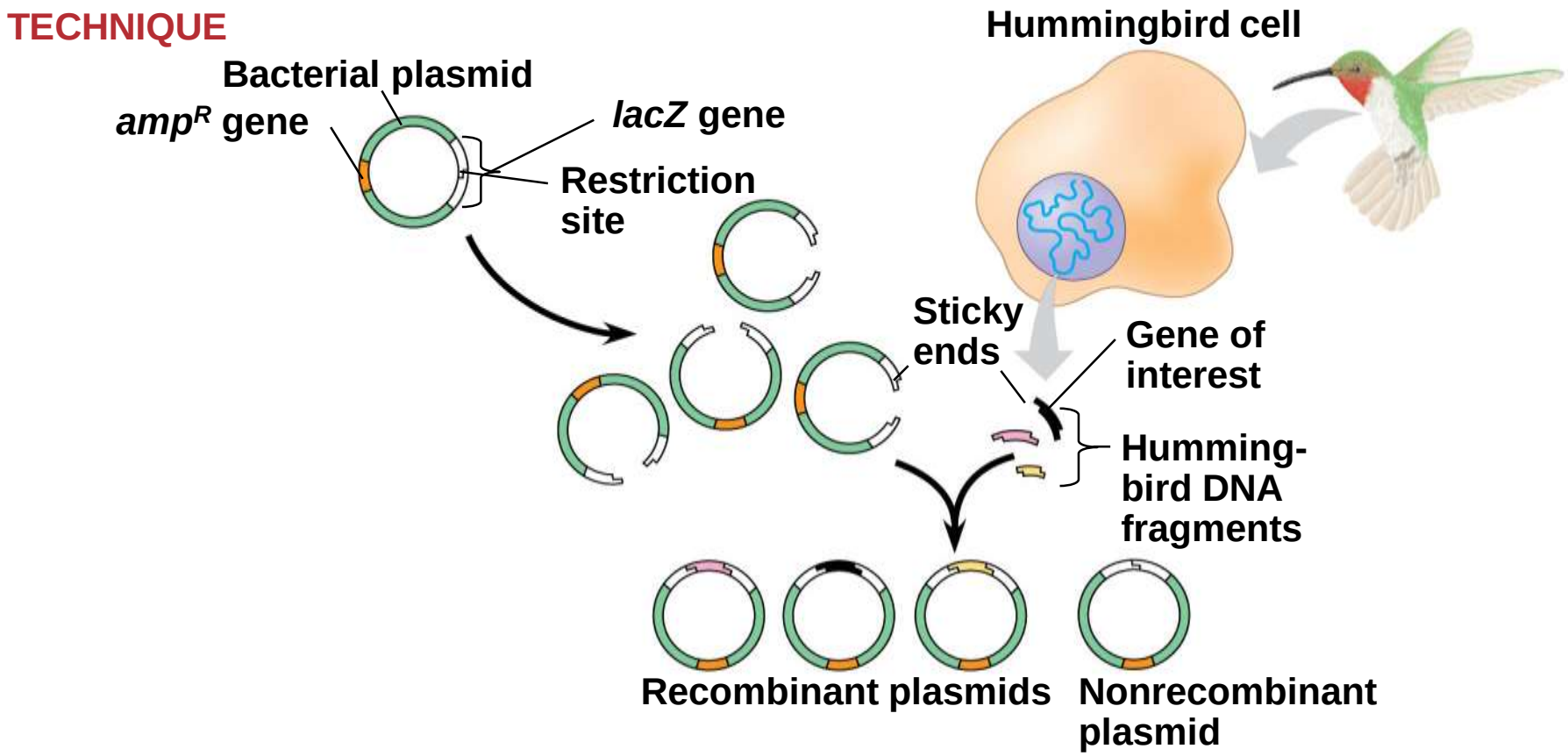


Figure 20.4a-3

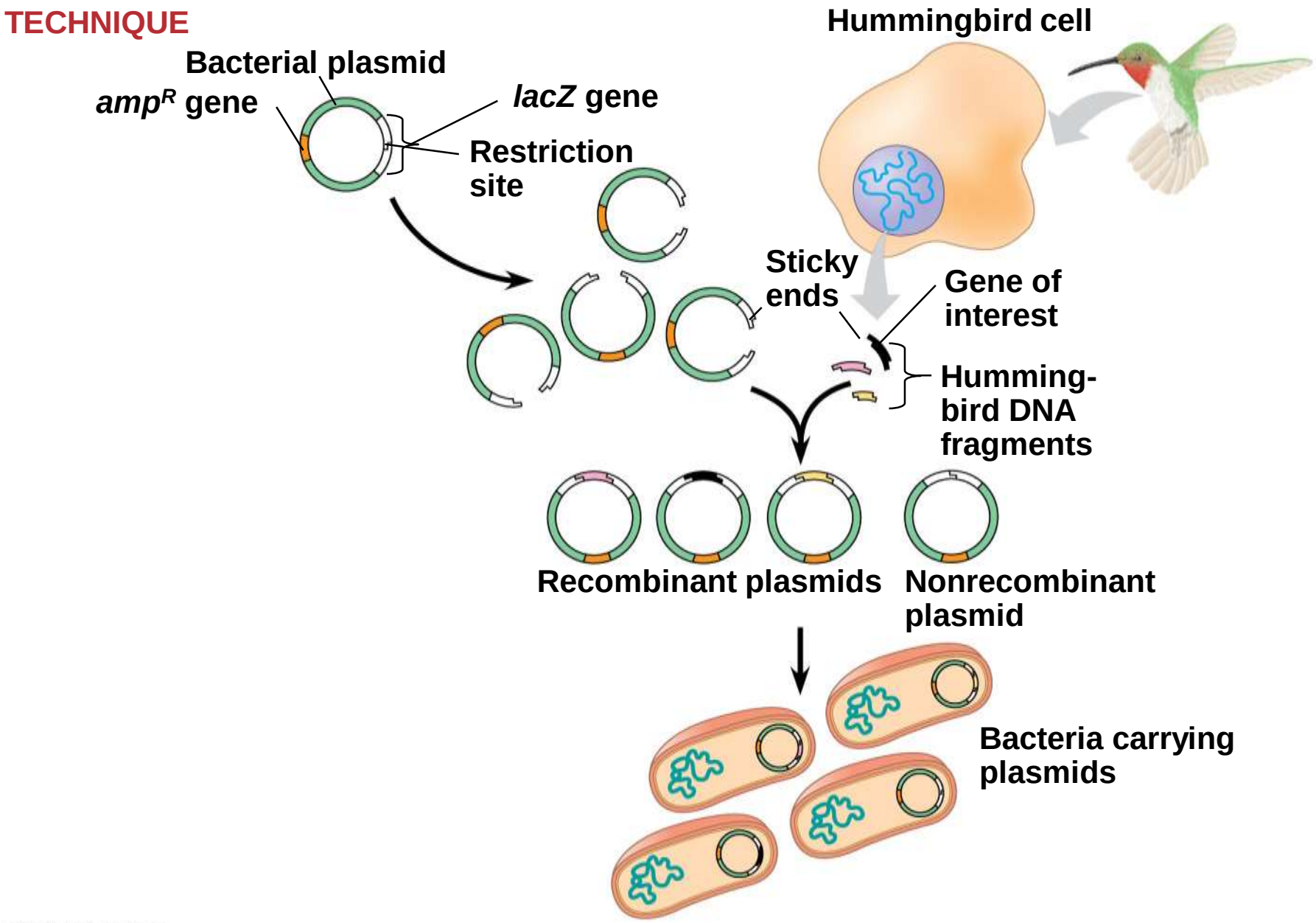
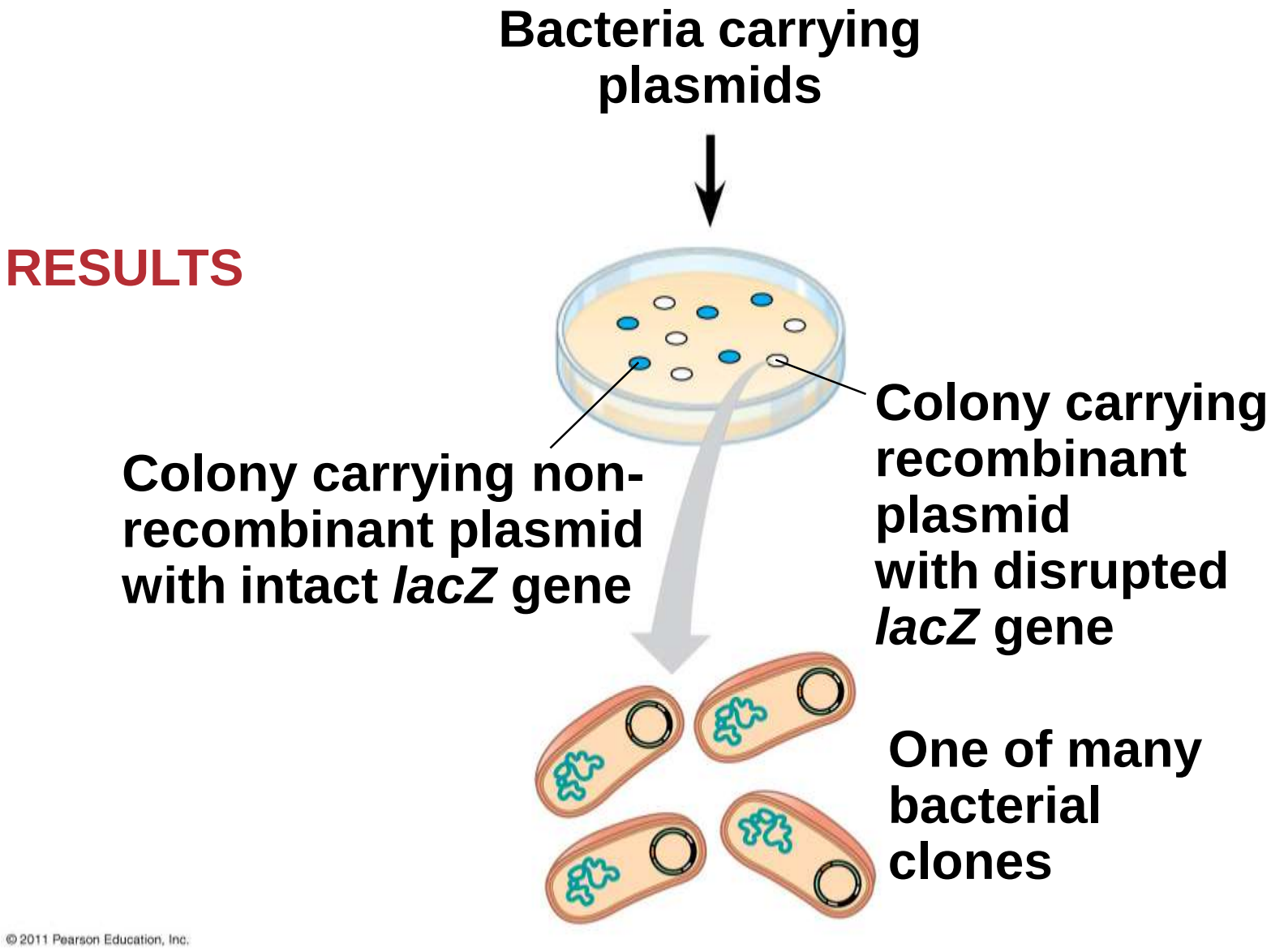


Figure 20.4b



Storing Cloned Genes in DNA Libraries

- A **genomic library** that is made using bacteria is the collection of recombinant vector clones produced by cloning DNA fragments from an entire genome
- A genomic library that is made using bacteriophages is stored as a collection of phage clones

Figure 20.5

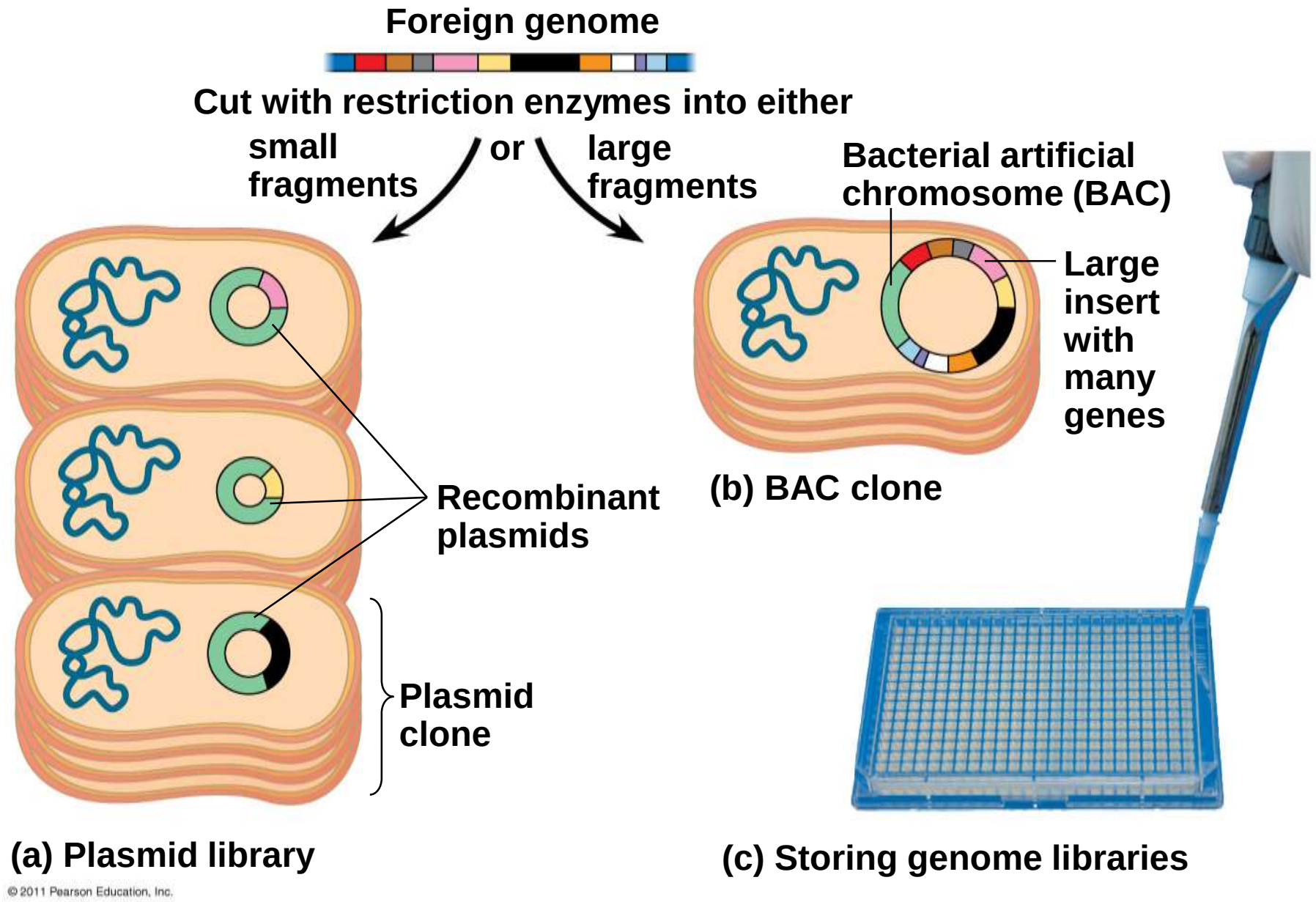


Figure 20.5a

(c) Storing genome libraries

- A **bacterial artificial chromosome (BAC)** is a large plasmid that has been trimmed down and can carry a large DNA insert
- BACs are another type of vector used in DNA library construction

- A **complementary DNA (cDNA)** library is made by cloning DNA made *in vitro* by reverse transcription of all the mRNA produced by a particular cell
- A **cDNA library** represents only part of the genome—only the subset of genes transcribed into mRNA in the original cells

Figure 20.6-1

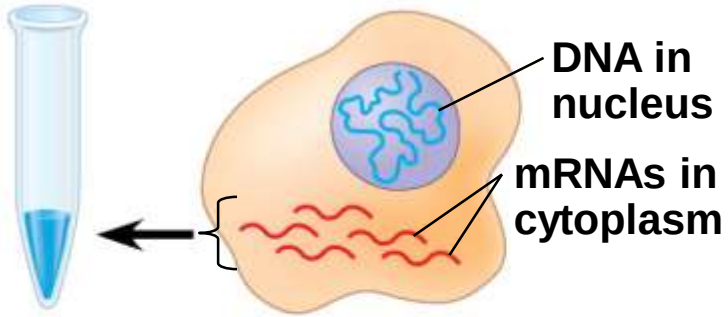


Figure 20.6-2

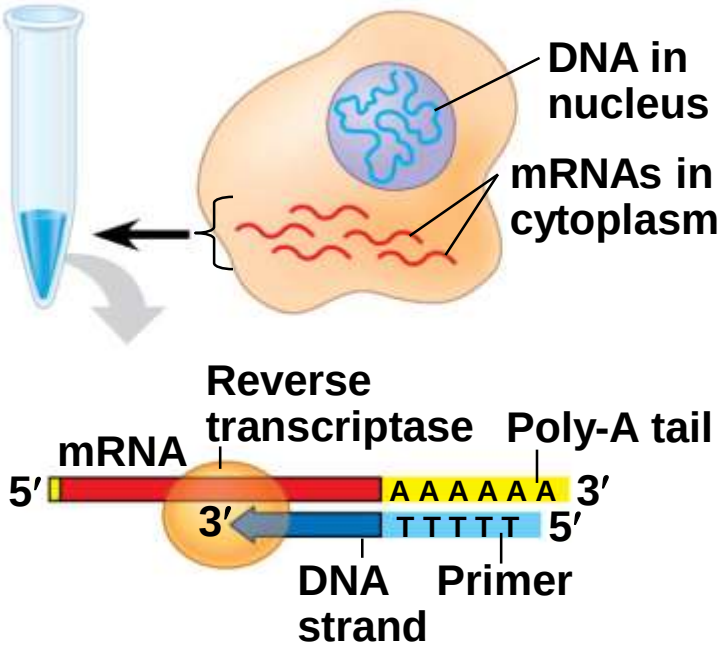


Figure 20.6-3

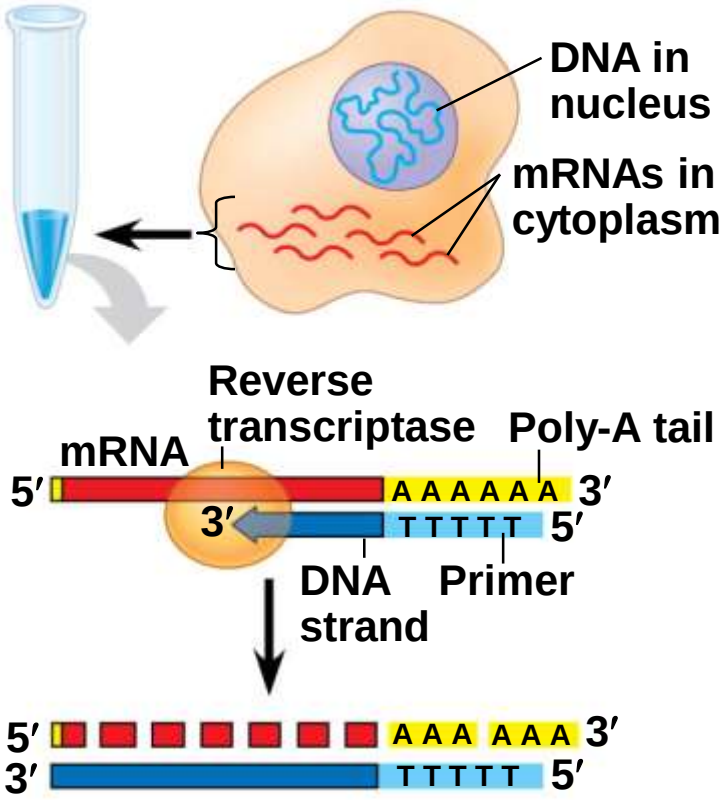


Figure 20.6-4

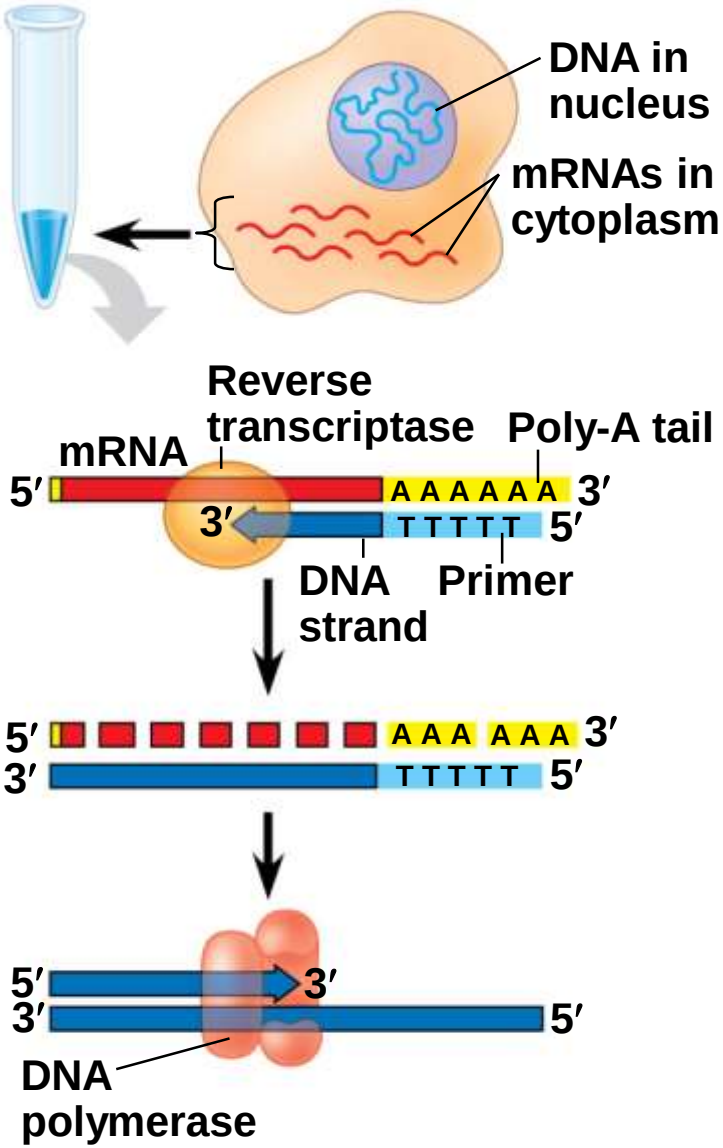
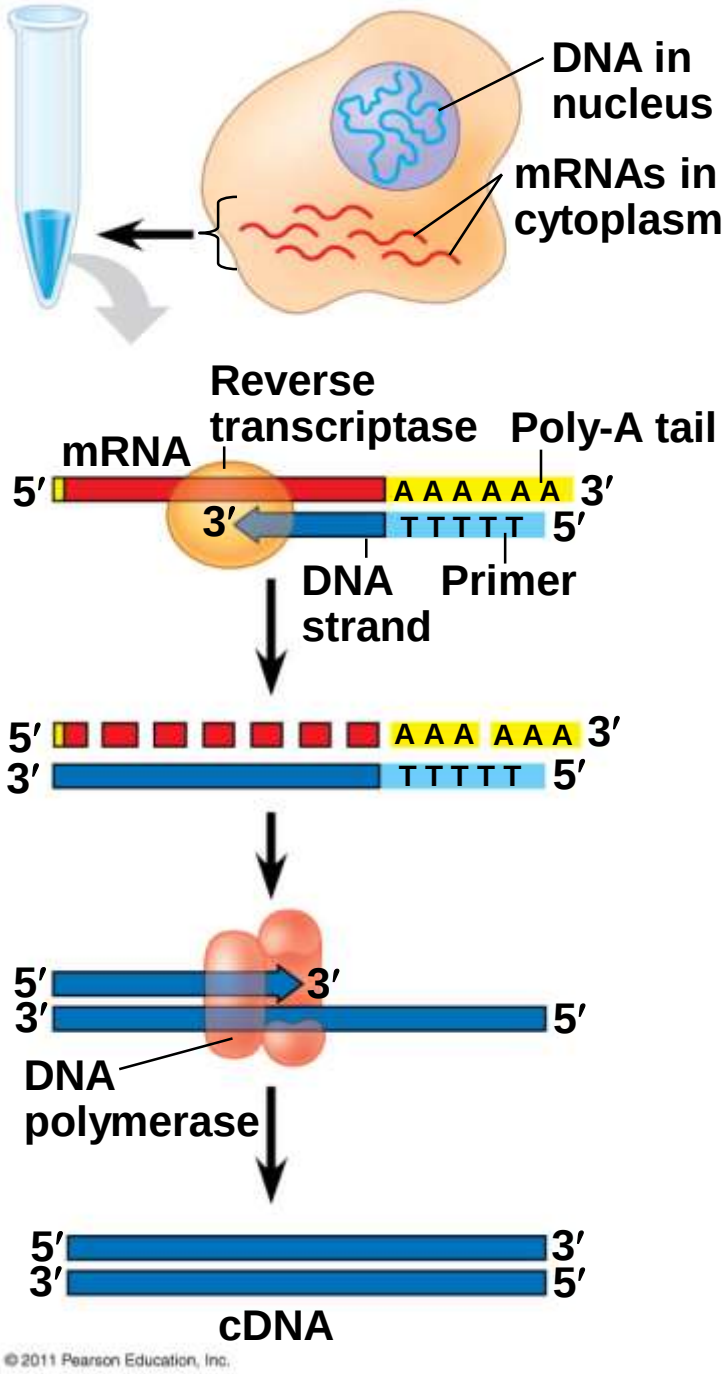


Figure 20.6-5



Screening a Library for Clones Carrying a Gene of Interest

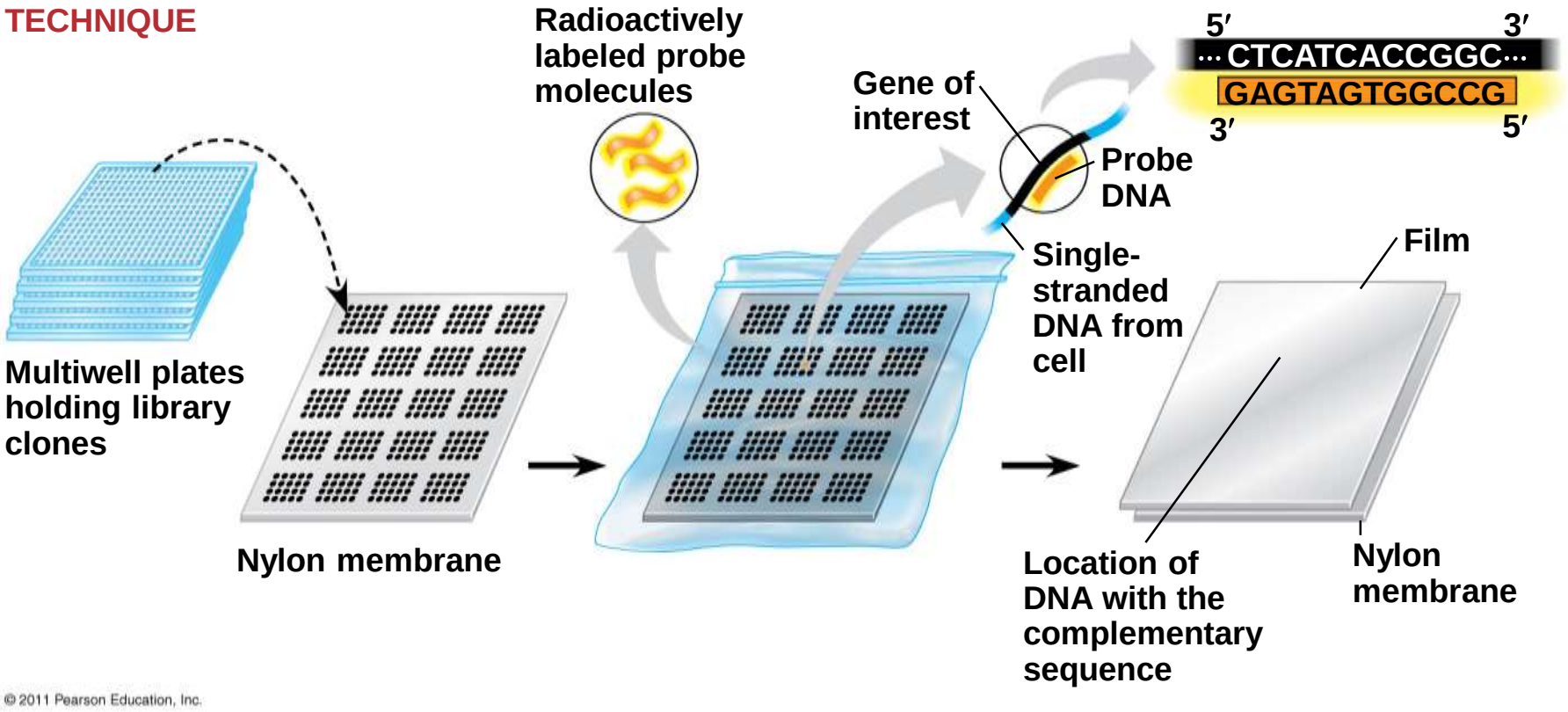
- A clone carrying the gene of interest can be identified with a **nucleic acid probe** having a sequence complementary to the gene
- This process is called **nucleic acid hybridization**

- A probe can be synthesized that is complementary to the gene of interest
- For example, if the desired gene is

  – Then we would synthesize this probe

- The DNA probe can be used to screen a large number of clones simultaneously for the gene of interest
- Once identified, the clone carrying the gene of interest can be cultured

Figure 20.7



Expressing Cloned Eukaryotic Genes

- After a gene has been cloned, its protein product can be produced in larger amounts for research
- Cloned genes can be expressed as protein in either bacterial or eukaryotic cells

Bacterial Expression Systems

- Several technical difficulties hinder expression of cloned eukaryotic genes in bacterial host cells
- To overcome differences in promoters and other DNA control sequences, scientists usually employ an **expression vector**, a cloning vector that contains a highly active bacterial promoter

Eukaryotic Cloning and Expression Systems

- Molecular biologists can avoid eukaryote-bacterial incompatibility issues by using eukaryotic cells, such as yeasts, as hosts for cloning and expressing genes
- Even yeasts may not possess the proteins required to modify expressed mammalian proteins properly
- In such cases, cultured mammalian or insect cells may be used to express and study proteins

- One method of introducing recombinant DNA into eukaryotic cells is **electroporation**, applying a brief electrical pulse to create temporary holes in plasma membranes
- Alternatively, scientists can inject DNA into cells using microscopically thin needles
- Once inside the cell, the DNA is incorporated into the cell's DNA by natural genetic recombination

Cross-Species Gene Expression and Evolutionary Ancestry

- The remarkable ability of bacteria to express some eukaryotic proteins underscores the shared evolutionary ancestry of living species
- For example, *Pax-6* is a gene that directs formation of a vertebrate eye; the same gene in flies directs the formation of an insect eye (which is quite different from the vertebrate eye)
- The *Pax-6* genes in flies and vertebrates can substitute for each other

Amplifying DNA *in Vitro*: The Polymerase Chain Reaction (PCR)

- The **polymerase chain reaction**, **PCR**, can produce many copies of a specific target segment of DNA
- A three-step cycle—heating, cooling, and replication—brings about a chain reaction that produces an exponentially growing population of identical DNA molecules
- The key to PCR is an unusual, heat-stable DNA polymerase called Taq polymerase.

Figure 20.8

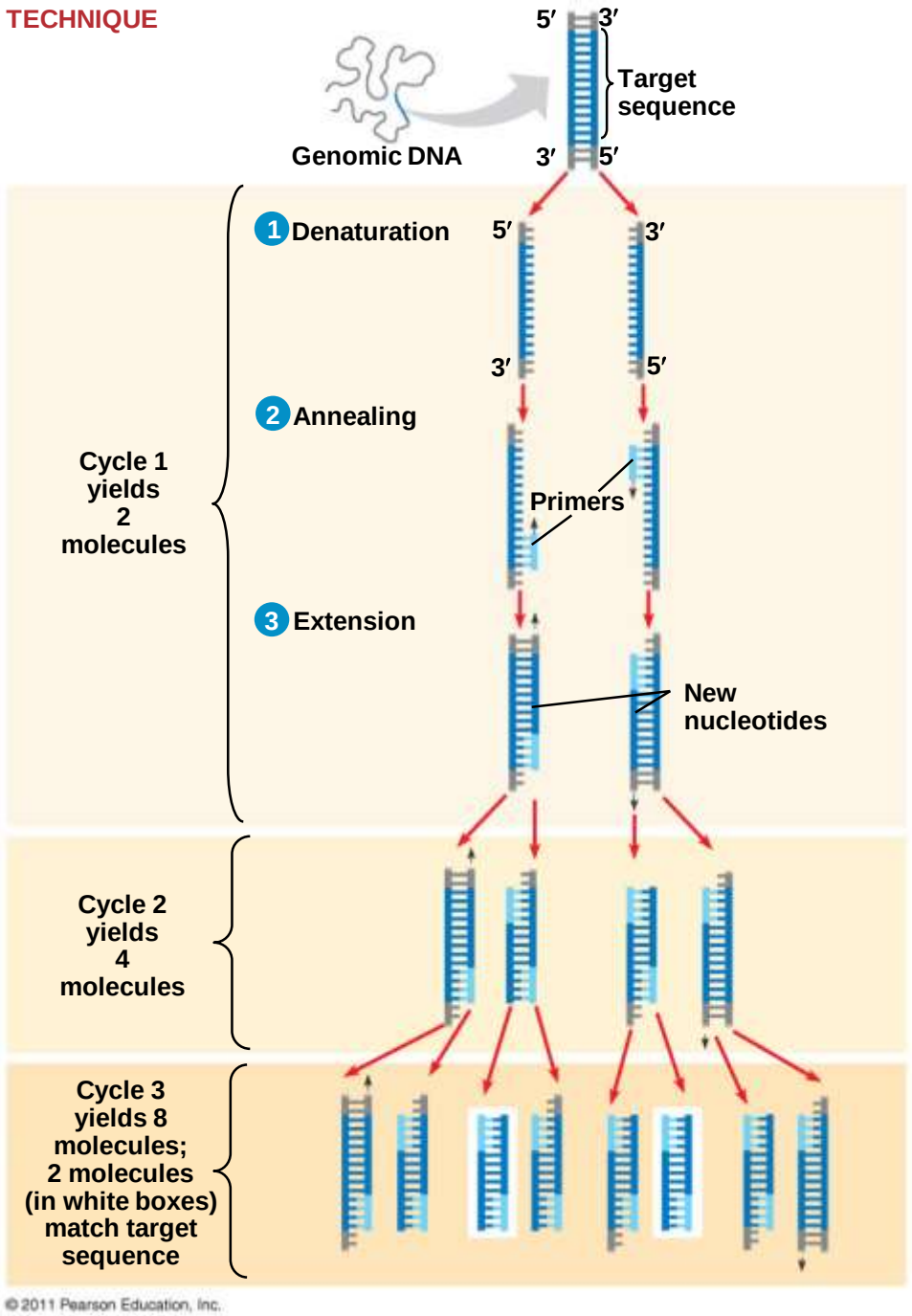


Figure 20.8a

TECHNIQUE

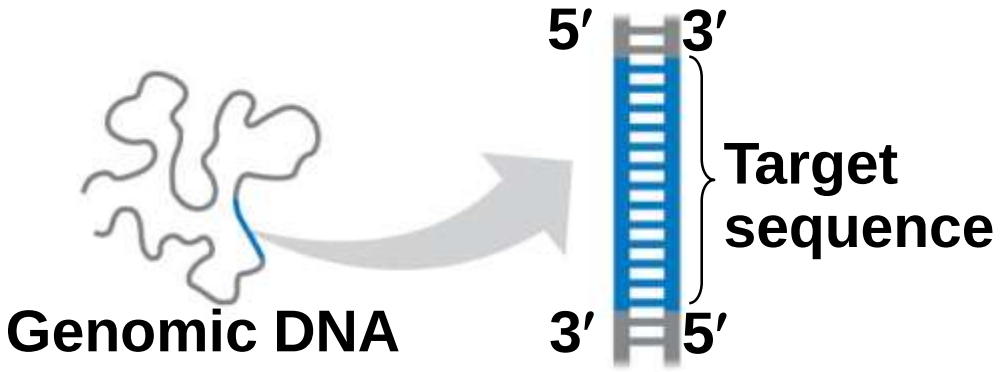


Figure 20.8b

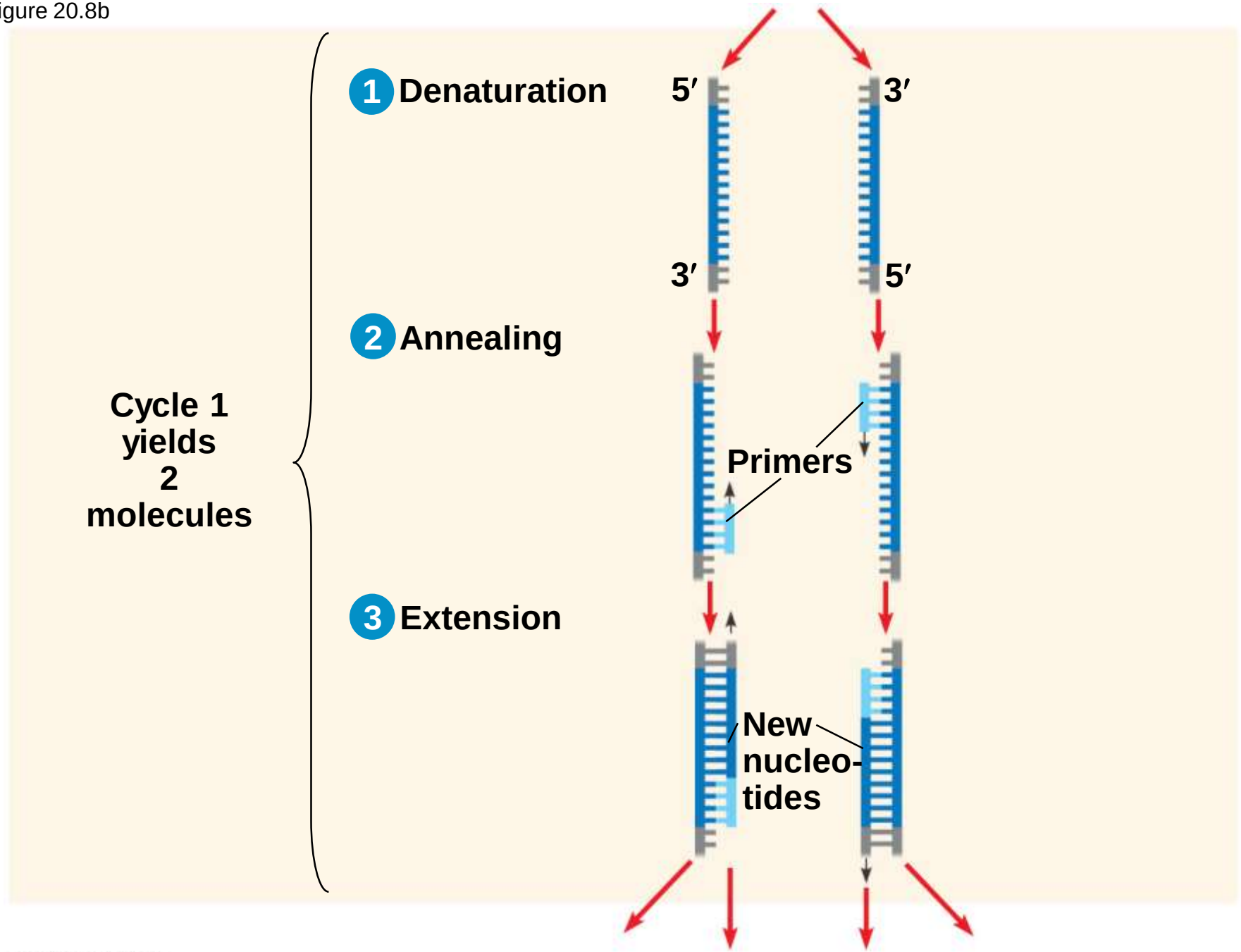


Figure 20.8c

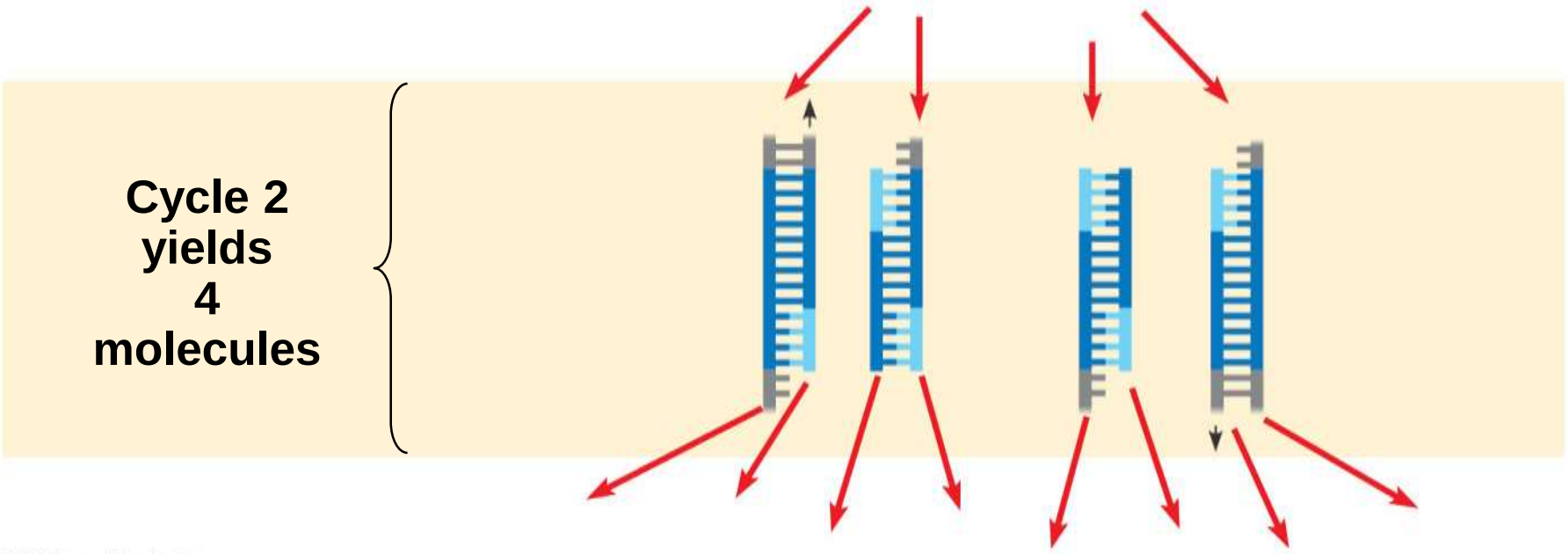
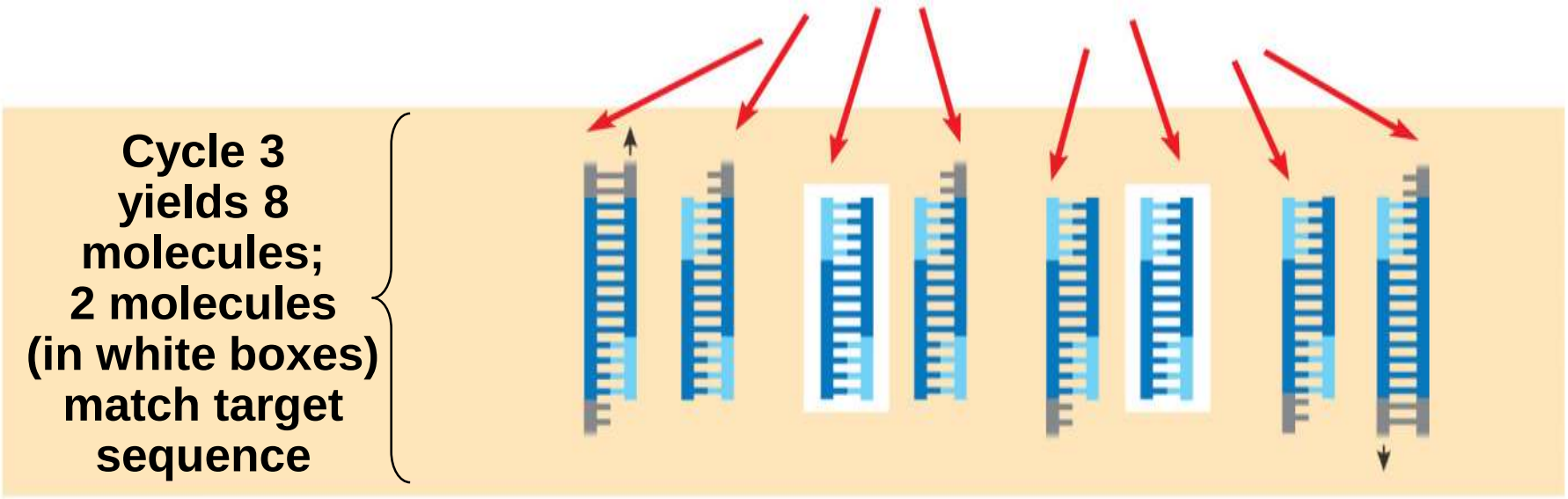


Figure 20.8d

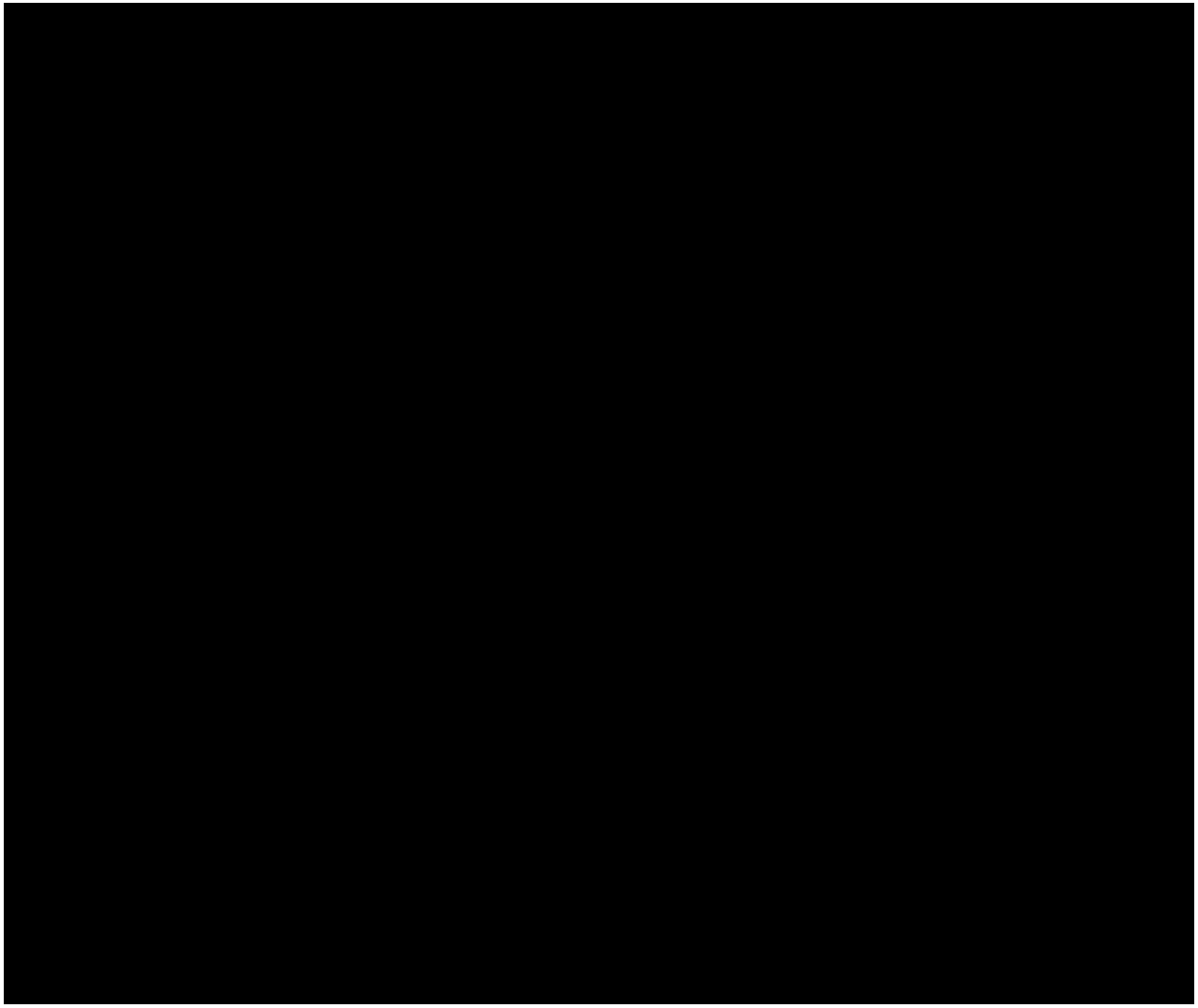


Concept 20.2: DNA technology allows us to study the sequence, expression, and function of a gene

- DNA cloning allows researchers to
  - Compare genes and alleles between individuals
  - Locate gene expression in a body
  - Determine the role of a gene in an organism
- Several techniques are used to analyze the DNA of genes

Gel Electrophoresis and Southern Blotting

- One indirect method of rapidly analyzing and comparing genomes is **gel electrophoresis**
- This technique uses a gel as a molecular sieve to separate nucleic acids or proteins by size, electrical charge, and other properties
- A current is applied that causes charged molecules to move through the gel
- Molecules are sorted into “bands” by their size



Animation: Biotechnology Lab

Right-click slide / select “Play”

Figure 20.9

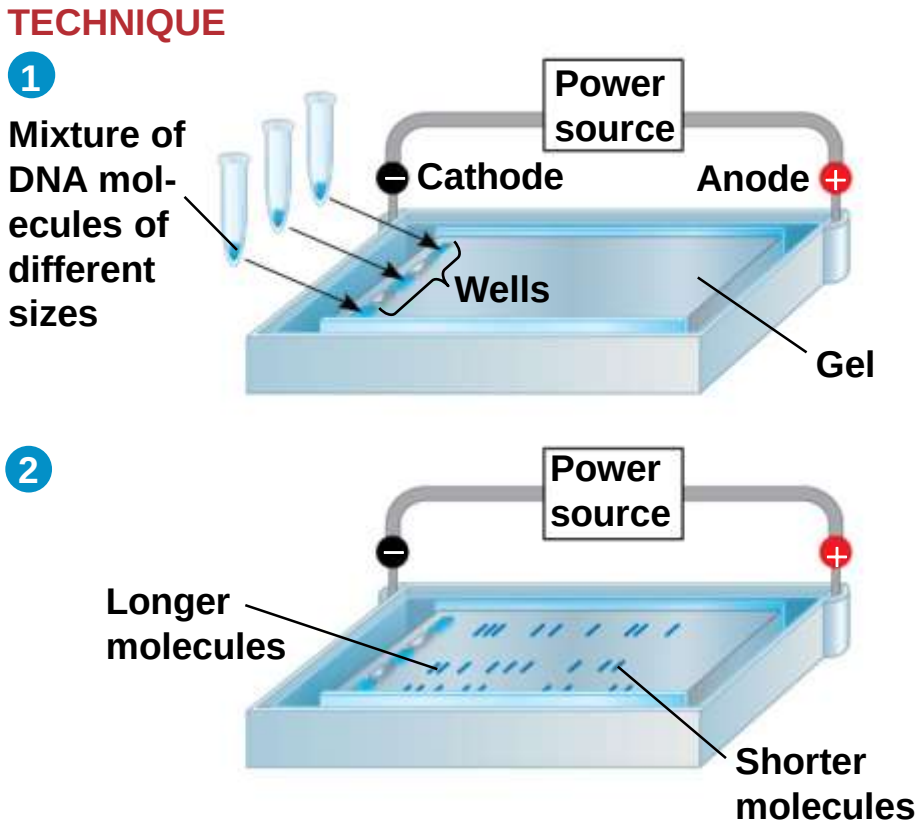


Figure 20.9a

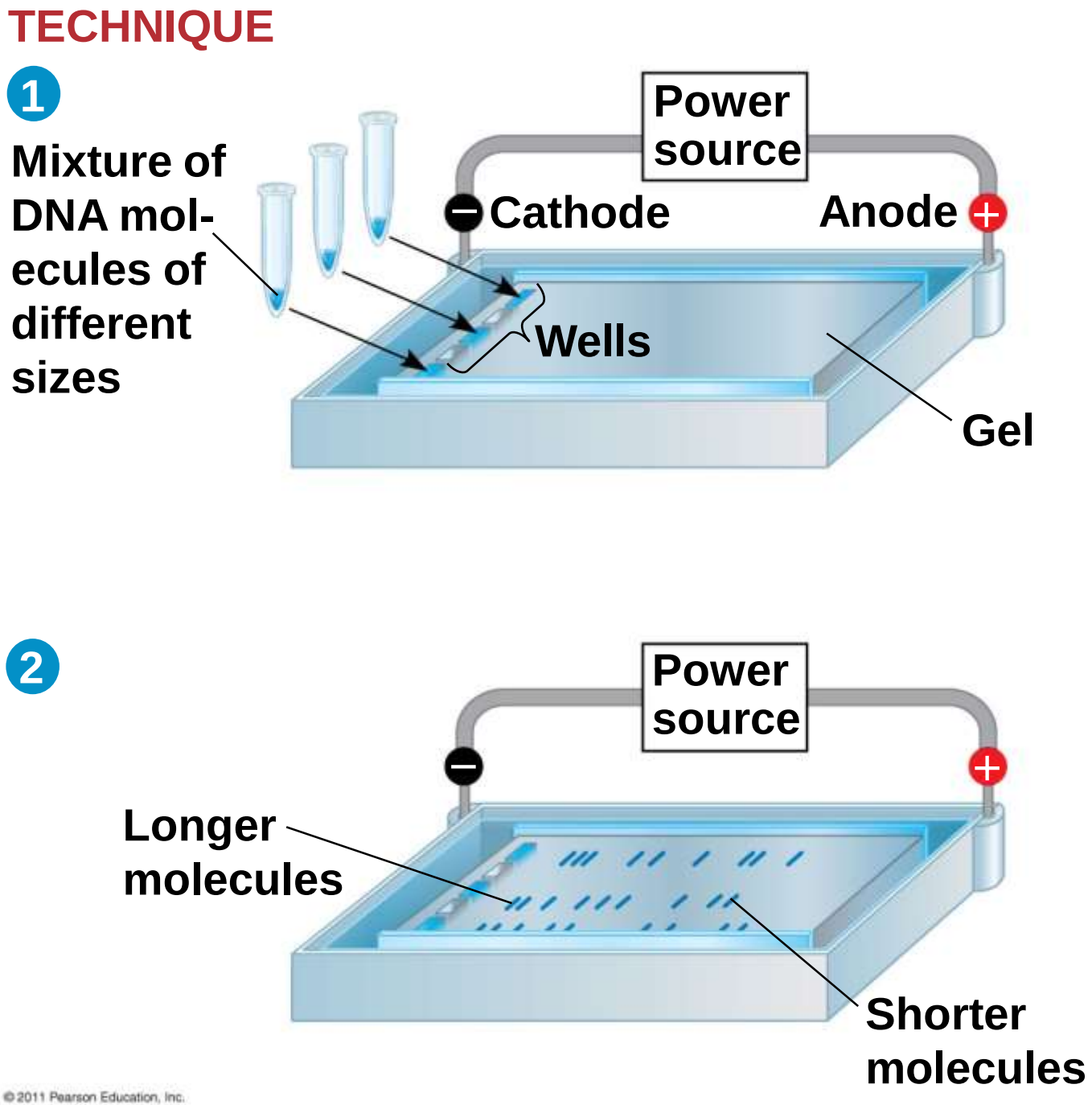
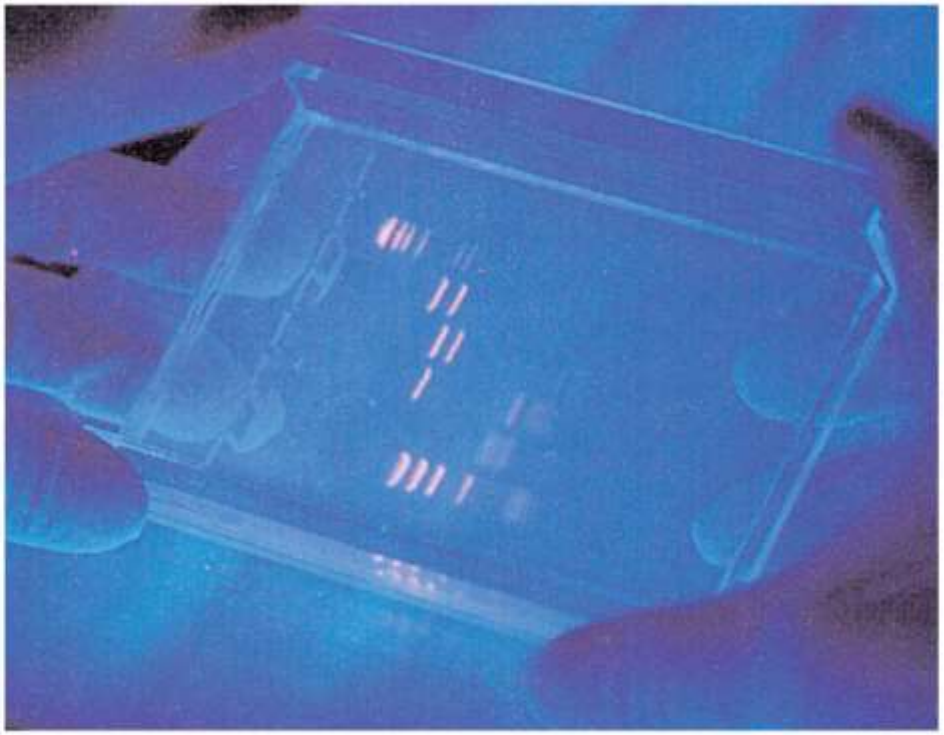


Figure 20.9b

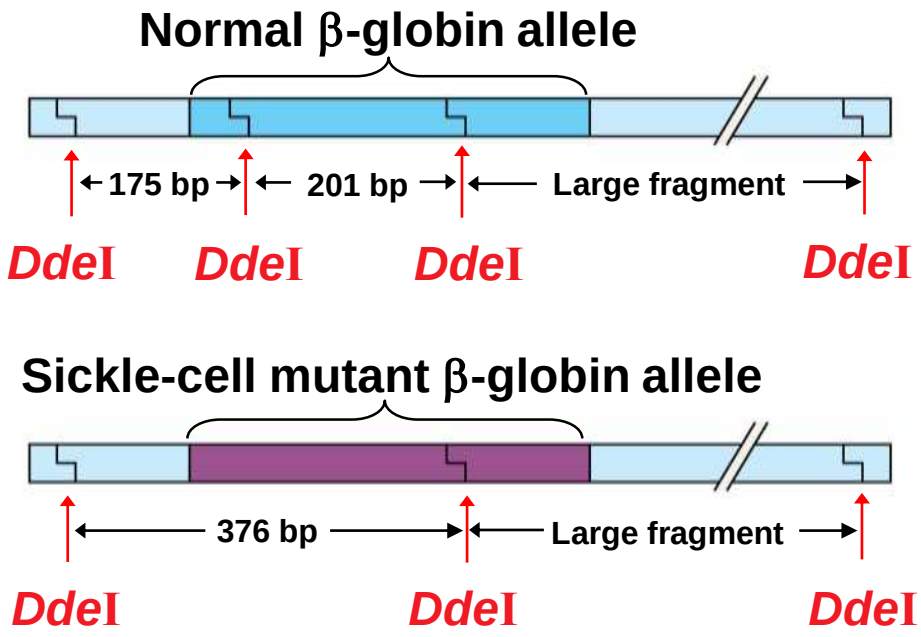
RESULTS



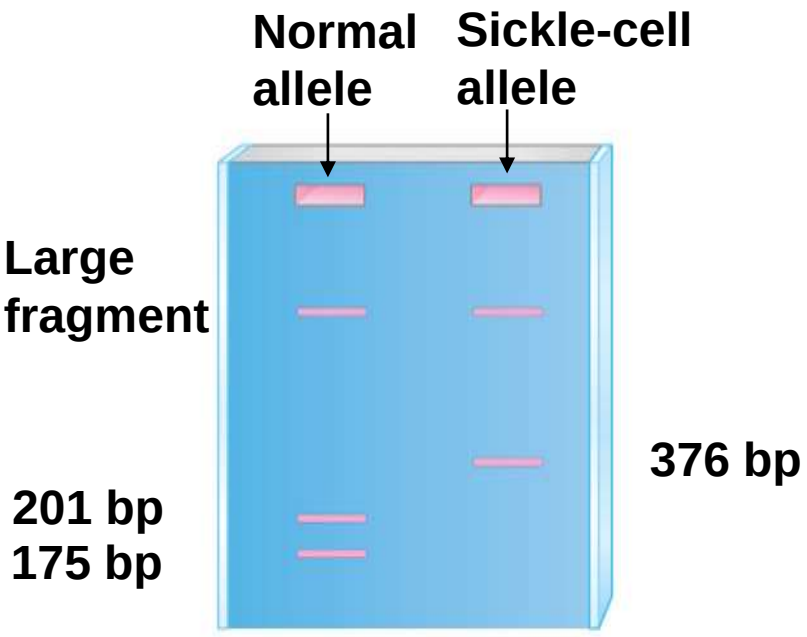
- In restriction fragment analysis, DNA fragments produced by restriction enzyme digestion of a DNA molecule are sorted by gel electrophoresis
- Restriction fragment analysis can be used to compare two different DNA molecules, such as two alleles for a gene, if the nucleotide difference alters a restriction site

- Variations in DNA sequence are called polymorphisms
- Sequence changes that alter restriction sites are called **RFLPs** (**restriction fragment length polymorphisms**)

Figure 20.10

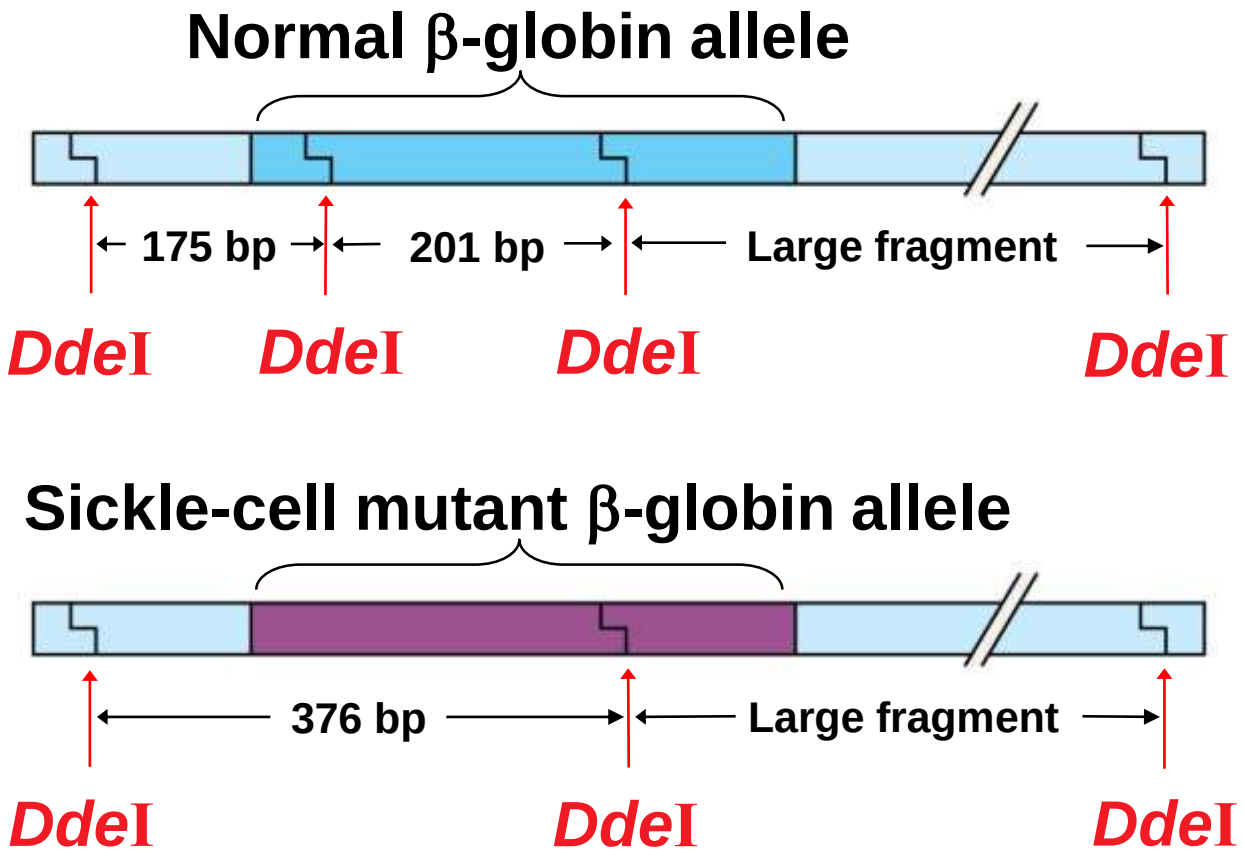


(a) *DdeI* restriction sites in normal and sickle-cell alleles of the β-globin gene



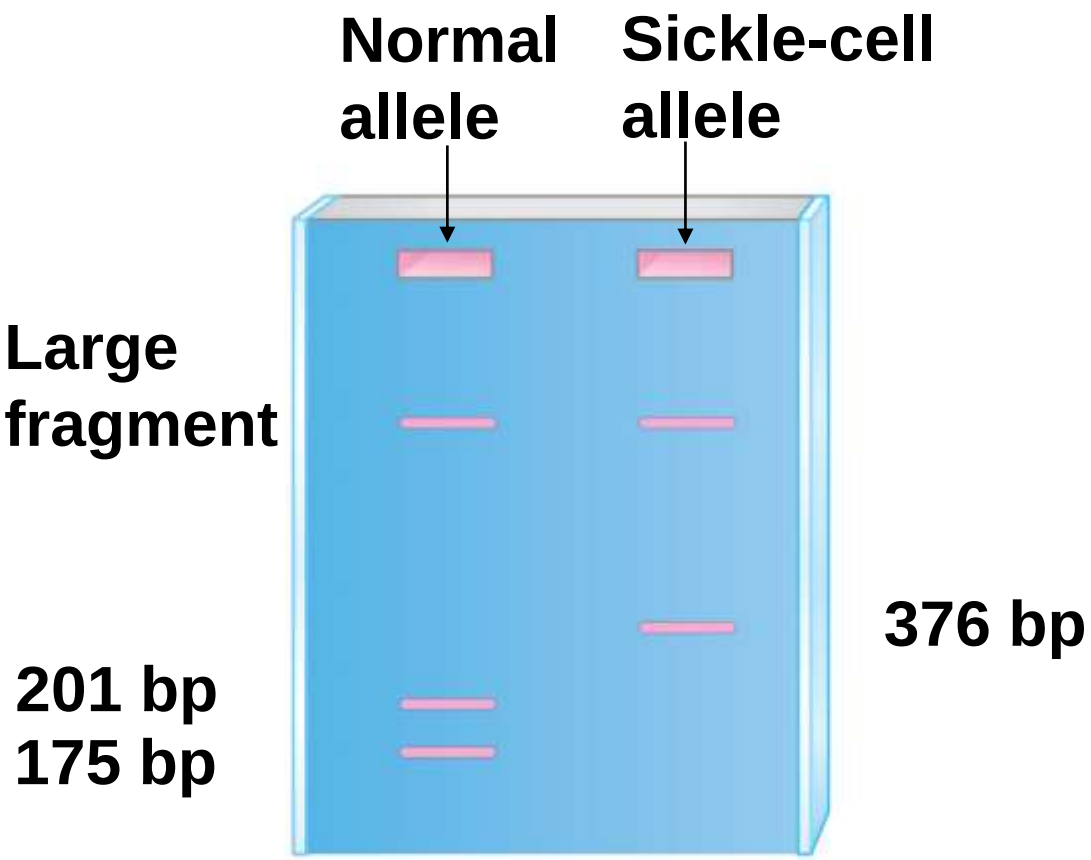
(b) Electrophoresis of restriction fragments from normal and sickle-cell alleles

Figure 20.10a



(a) *Dde*I restriction sites in normal and sickle-cell alleles of the β-globin gene

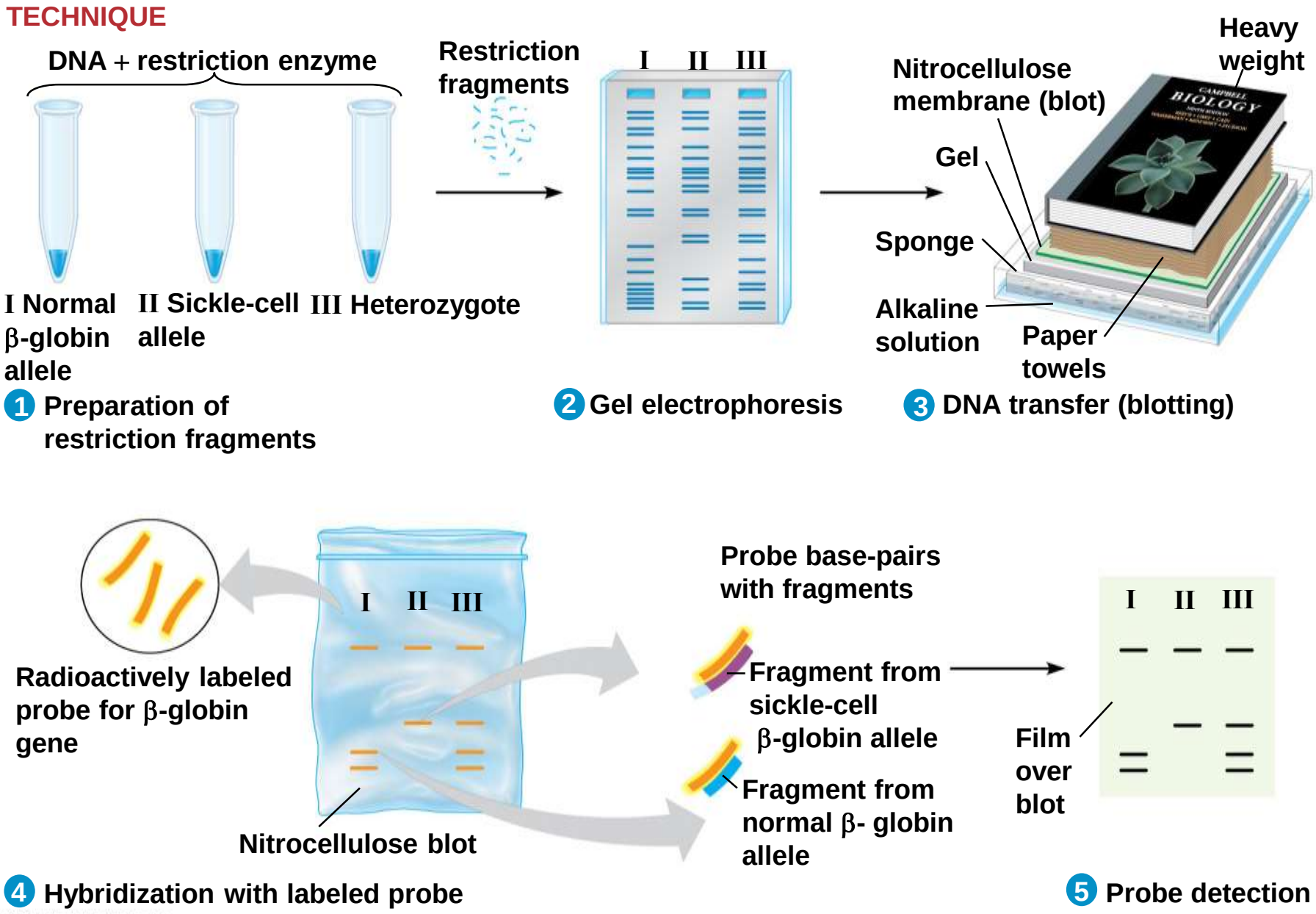
Figure 20.10b



(b) Electrophoresis of restriction fragments from normal and sickle-cell alleles

- A technique called **Southern blotting** combines gel electrophoresis of DNA fragments with nucleic acid hybridization
- Specific DNA fragments can be identified by Southern blotting, using labeled probes that hybridize to the DNA immobilized on a “blot” of gel

Figure 20.11



DNA Sequencing

- Relatively short DNA fragments can be sequenced by the dideoxy chain termination method, the first automated method to be employed
- Modified nucleotides called dideoxyribonucleotides (ddNTP) attach to synthesized DNA strands of different lengths
- Each type of ddNTP is tagged with a distinct fluorescent label that identifies the nucleotide at the end of each DNA fragment
- The DNA sequence can be read from the resulting spectrogram

Figure 20.12

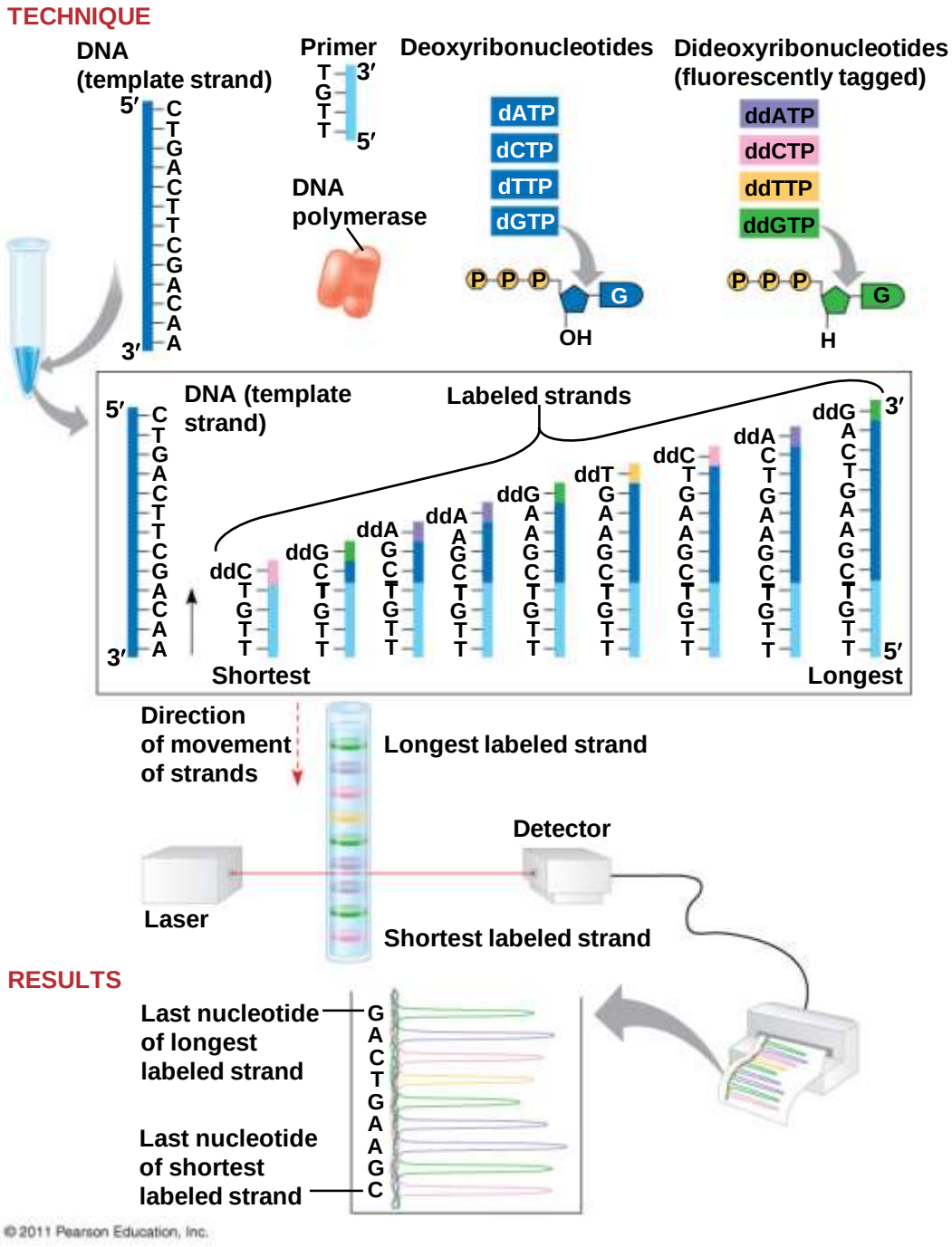


Figure 20.12a

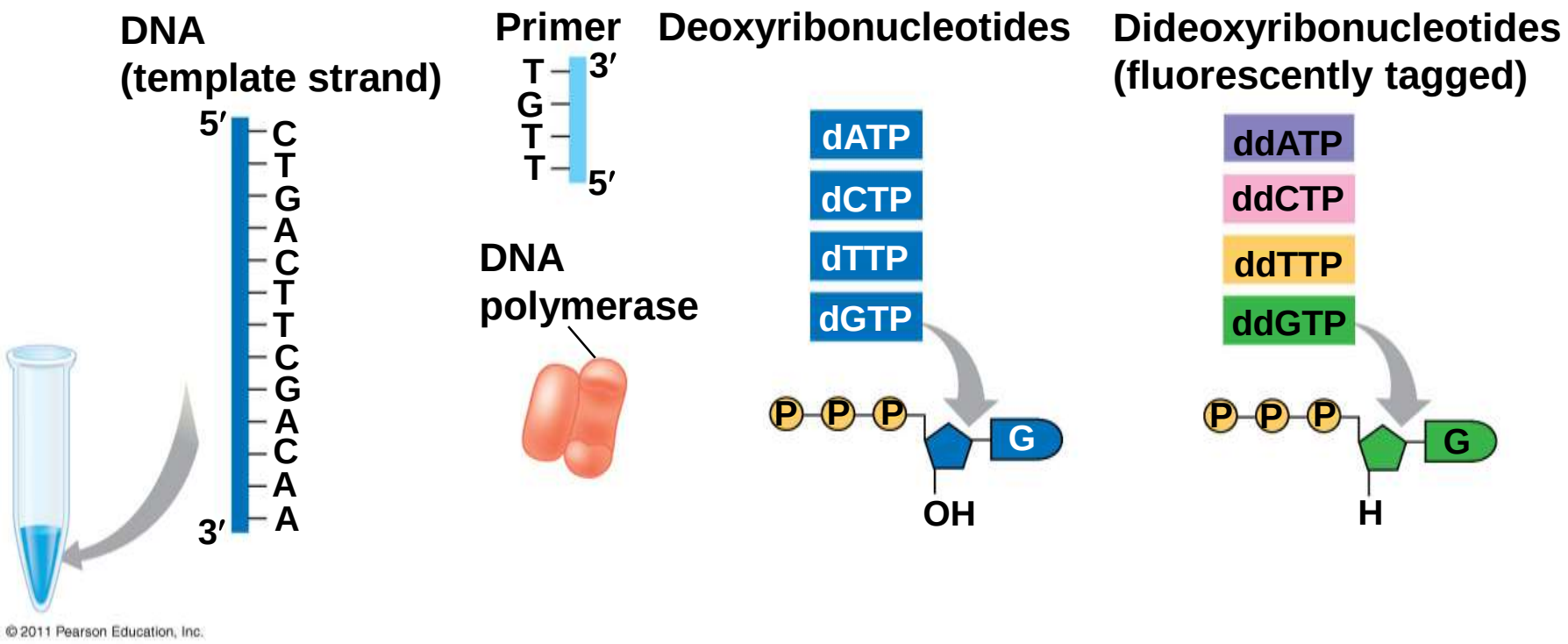


Figure 20.12b

TECHNIQUE (continued)

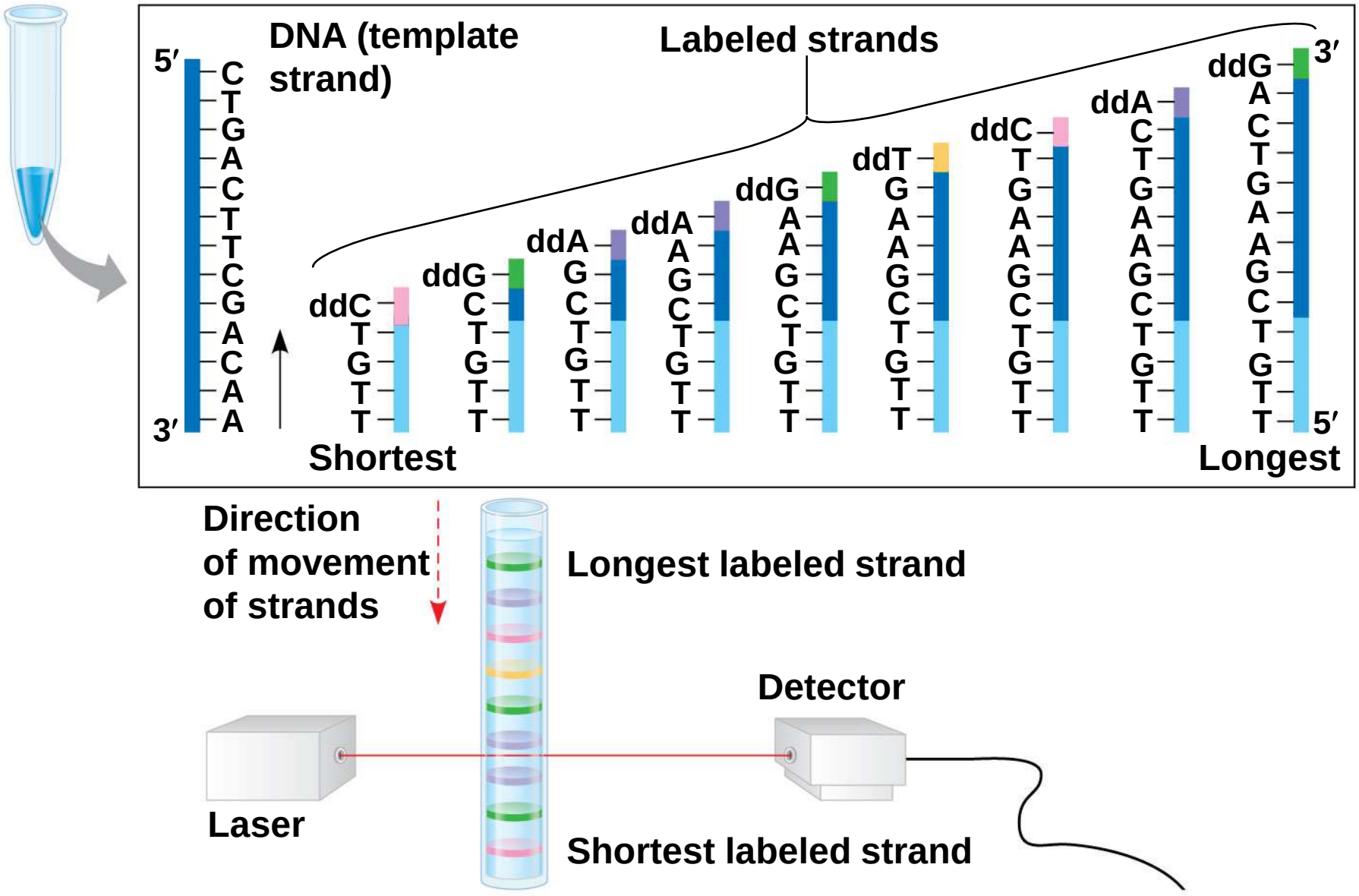
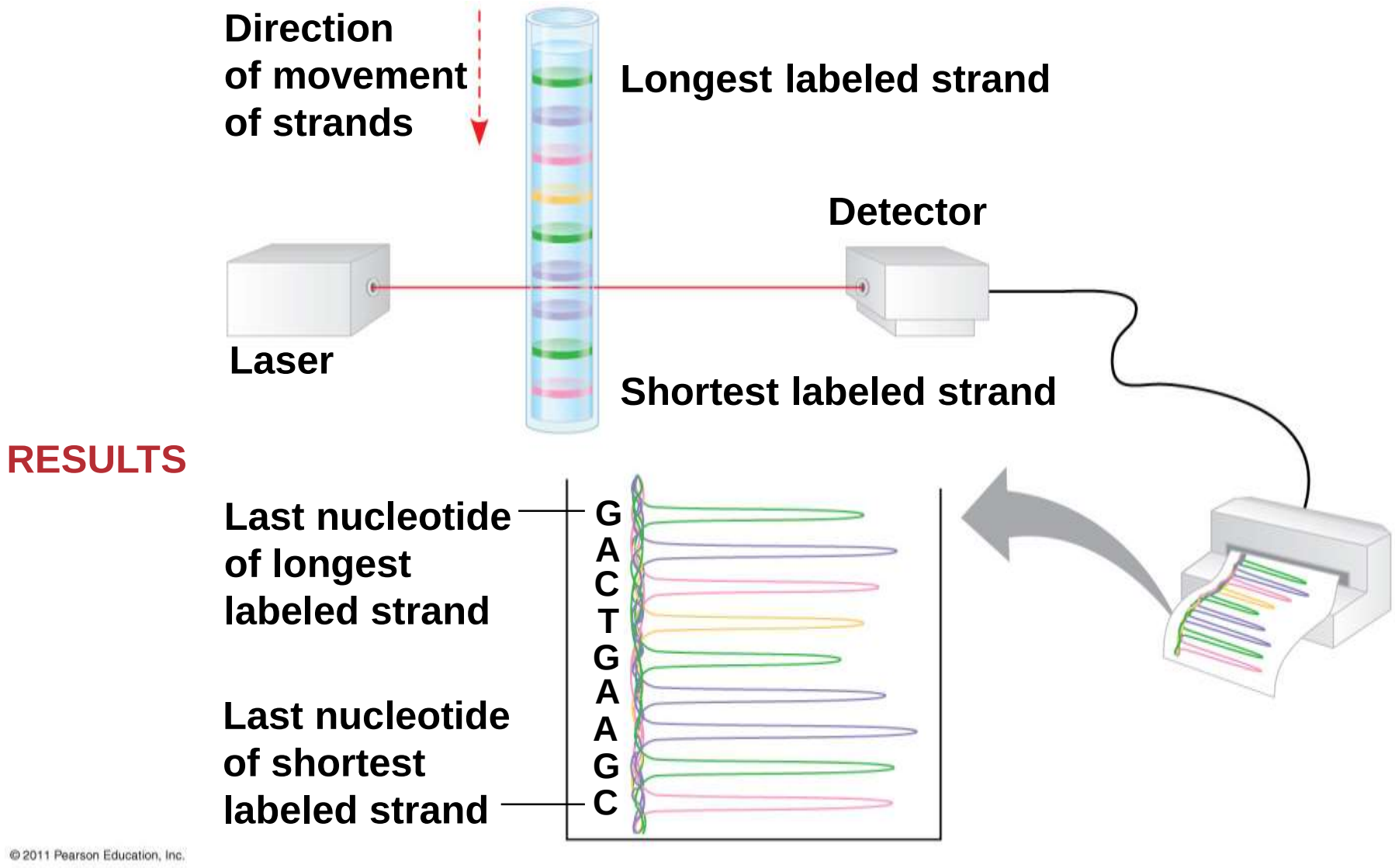


Figure 20.12c



Analyzing Gene Expression

- Nucleic acid probes can hybridize with mRNAs transcribed from a gene
- Probes can be used to identify where or when a gene is transcribed in an organism

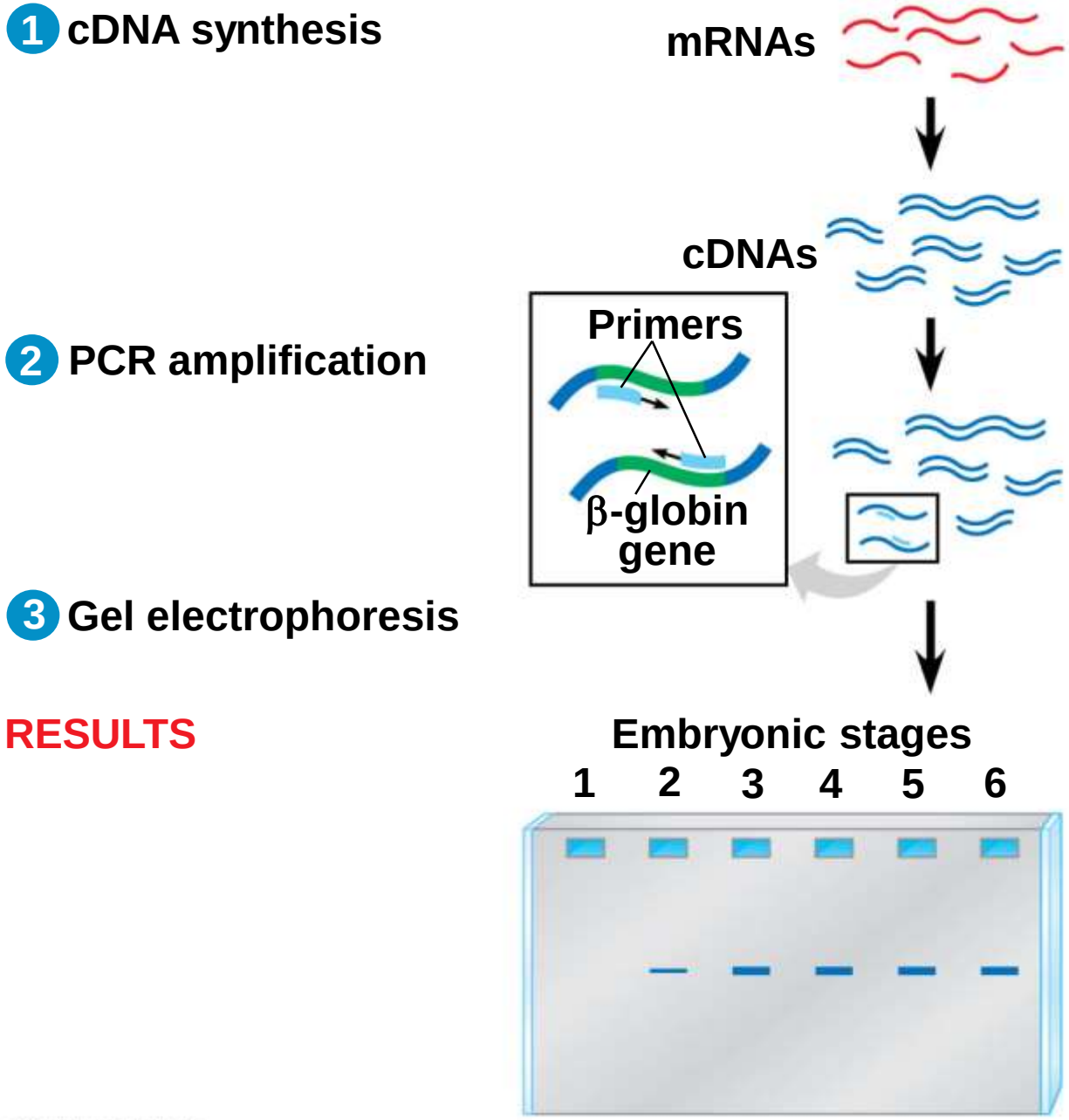
Studying the Expression of Single Genes

- Changes in the expression of a gene during embryonic development can be tested using
  - Northern blotting
  - Reverse transcriptase-polymerase chain reaction
- Both methods are used to compare mRNA from different developmental stages

- **Northern blotting** combines gel electrophoresis of mRNA followed by hybridization with a probe on a membrane
- Identification of mRNA at a particular developmental stage suggests protein function at that stage

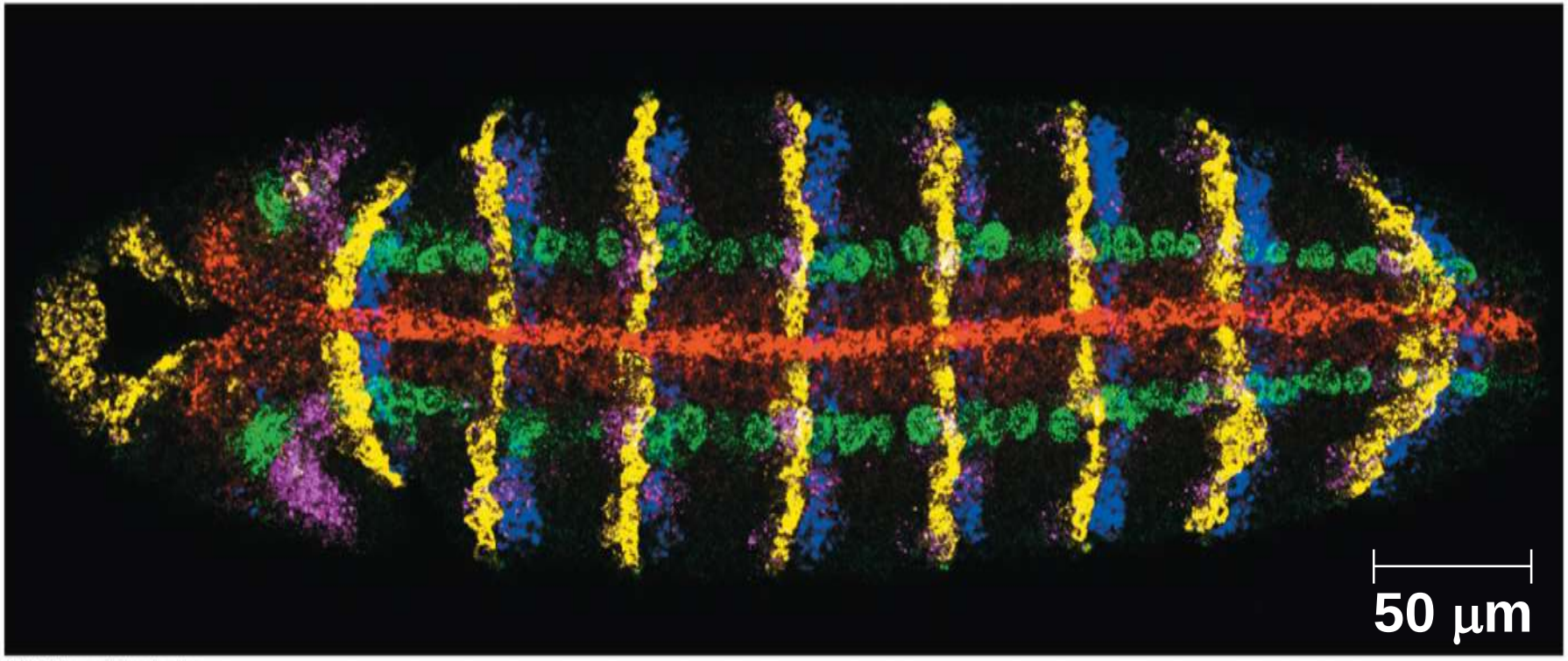
- **Reverse transcriptase-polymerase chain reaction (RT-PCR)** is quicker and more sensitive because it requires less mRNA than Northern blotting
- Reverse transcriptase is added to mRNA to make cDNA, which serves as a template for PCR amplification of the gene of interest
- The products are run on a gel and the mRNA of interest is identified

Figure 20.13



- ***In situ* hybridization** uses fluorescent dyes attached to probes to identify the location of specific mRNAs in place in the intact organism

Figure 20.14



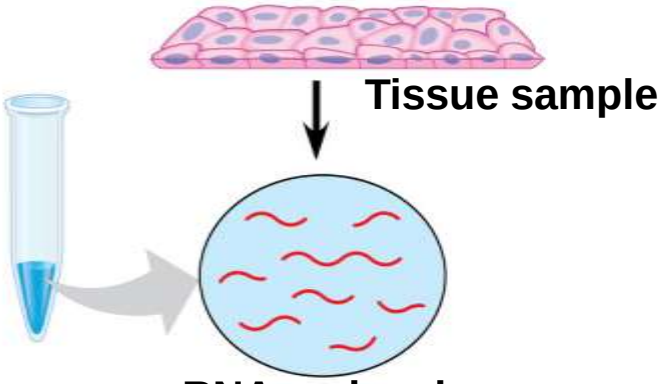
Studying the Expression of Interacting Groups of Genes

- Automation has allowed scientists to measure the expression of thousands of genes at one time using DNA microarray assays
- **DNA microarray assays** compare patterns of gene expression in different tissues, at different times, or under different conditions

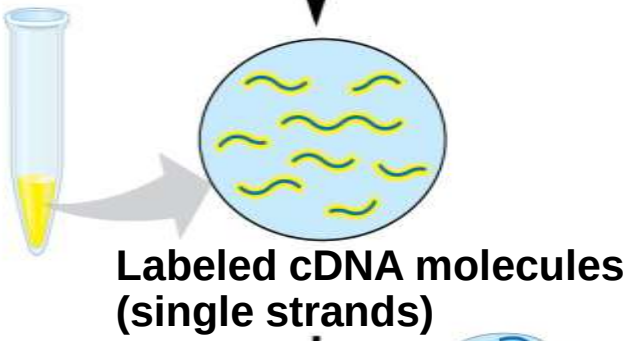
Figure 20.15

TECHNIQUE

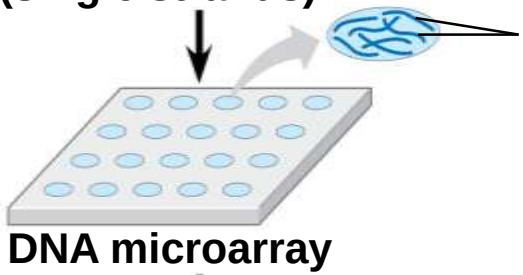
1. Isolate mRNA.



2. Make cDNA by reverse transcription, using fluorescently labeled nucleotides.



3. Apply the cDNA mixture to a microarray, a different gene in each spot. The cDNA hybridizes with any complementary DNA on the microarray.



4. Rinse off excess cDNA; scan microarray for fluorescence. Each fluorescent spot (yellow) represents a gene expressed in the tissue sample.

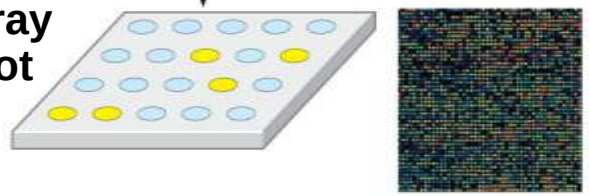
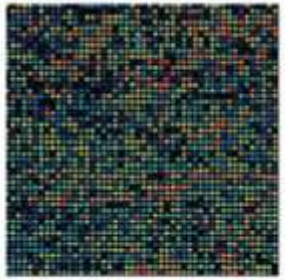


Figure 20.15a



DNA microarray with 2,400 human genes

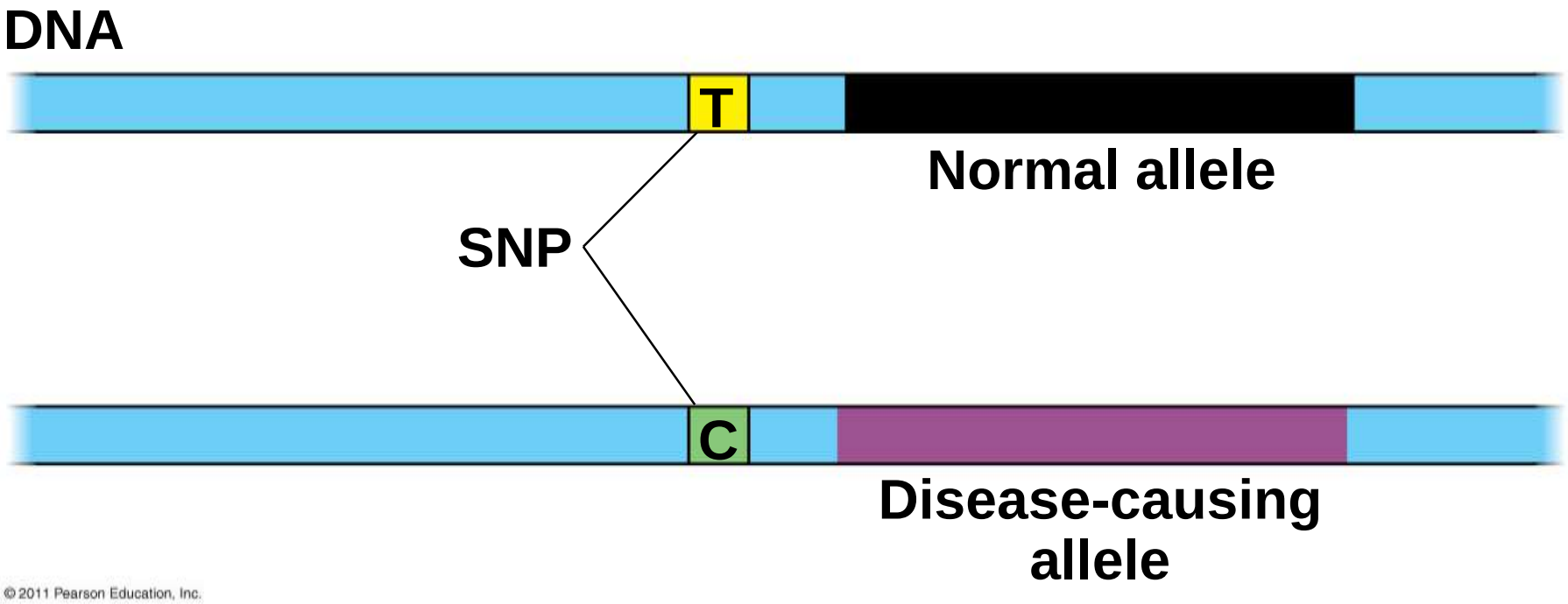
Determining Gene Function

- One way to determine function is to disable the gene and observe the consequences
- Using ***in vitro* mutagenesis**, mutations are introduced into a cloned gene, altering or destroying its function
- When the mutated gene is returned to the cell, the normal gene's function might be determined by examining the mutant's phenotype

- Gene expression can also be silenced using **RNA interference (RNAi)**
- Synthetic double-stranded RNA molecules matching the sequence of a particular gene are used to break down or block the gene's mRNA

- In humans, researchers analyze the genomes of many people with a certain genetic condition to try to find nucleotide changes specific to the condition
- Genetic markers called **SNPs** (**single nucleotide polymorphisms**) occur on average every 100–300 base pairs
- SNPs can be detected by PCR, and any SNP shared by people affected with a disorder but not among unaffected people may pinpoint the location of the disease-causing gene

Figure 20.16



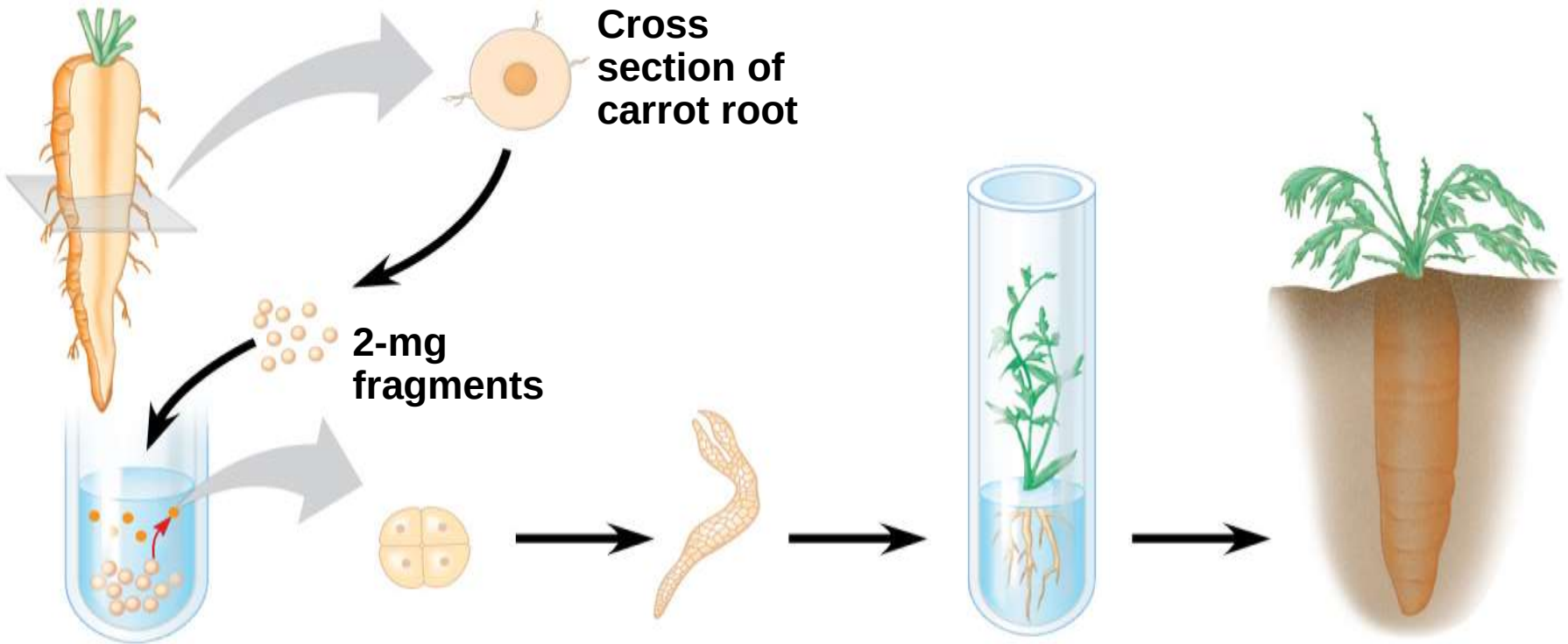
Concept 20.3: Cloning organisms may lead to production of stem cells for research and other applications

- Organismal cloning produces one or more organisms genetically identical to the “parent” that donated the single cell

Cloning Plants: Single-Cell Cultures

- One experimental approach for testing genomic equivalence is to see whether a differentiated cell can generate a whole organism
- A **totipotent** cell is one that can generate a complete new organism
- Plant cloning is used extensively in agriculture

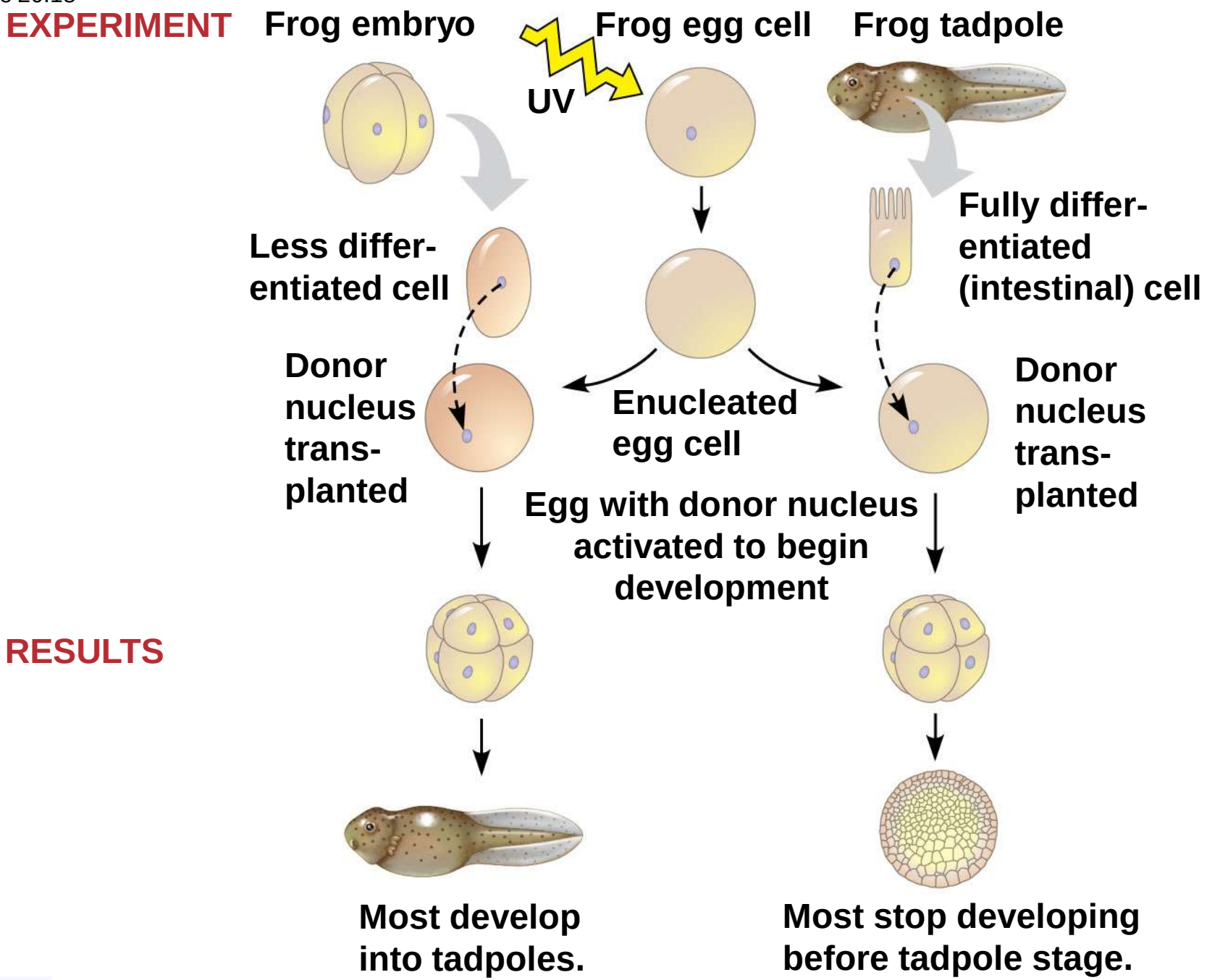
Figure 20.17



Cloning Animals: Nuclear Transplantation

- In nuclear transplantation, the nucleus of an unfertilized egg cell or zygote is replaced with the nucleus of a differentiated cell
- Experiments with frog embryos have shown that a transplanted nucleus can often support normal development of the egg
- However, the older the donor nucleus, the lower the percentage of normally developing tadpoles

Figure 20.18



Reproductive Cloning of Mammals

- In 1997, Scottish researchers announced the birth of Dolly, a lamb cloned from an adult sheep by nuclear transplantation from a differentiated mammary cell
- Dolly's premature death in 2003, as well as her arthritis, led to speculation that her cells were not as healthy as those of a normal sheep, possibly reflecting incomplete reprogramming of the original transplanted nucleus

Figure 20.19

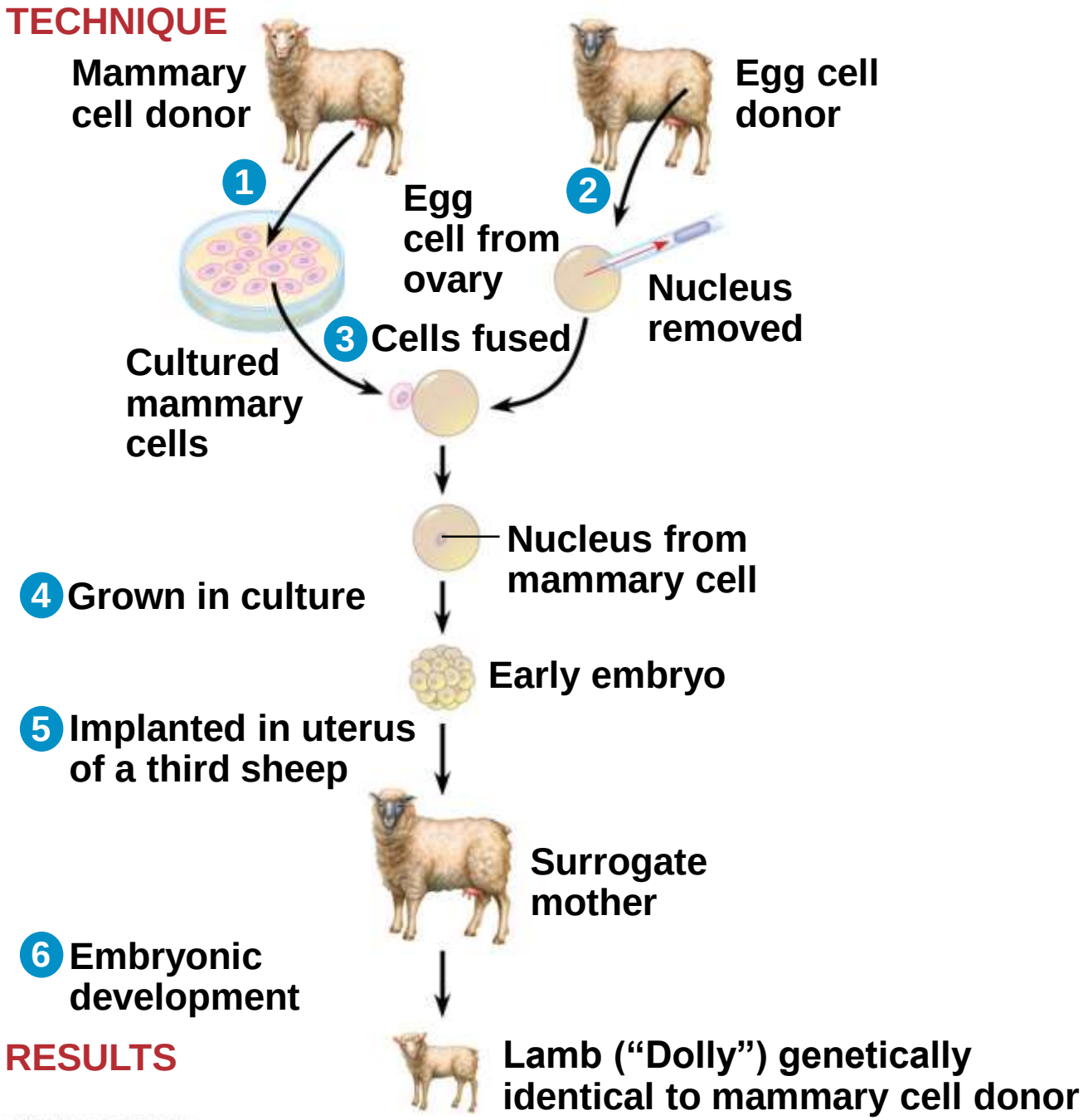


Figure 20.19a

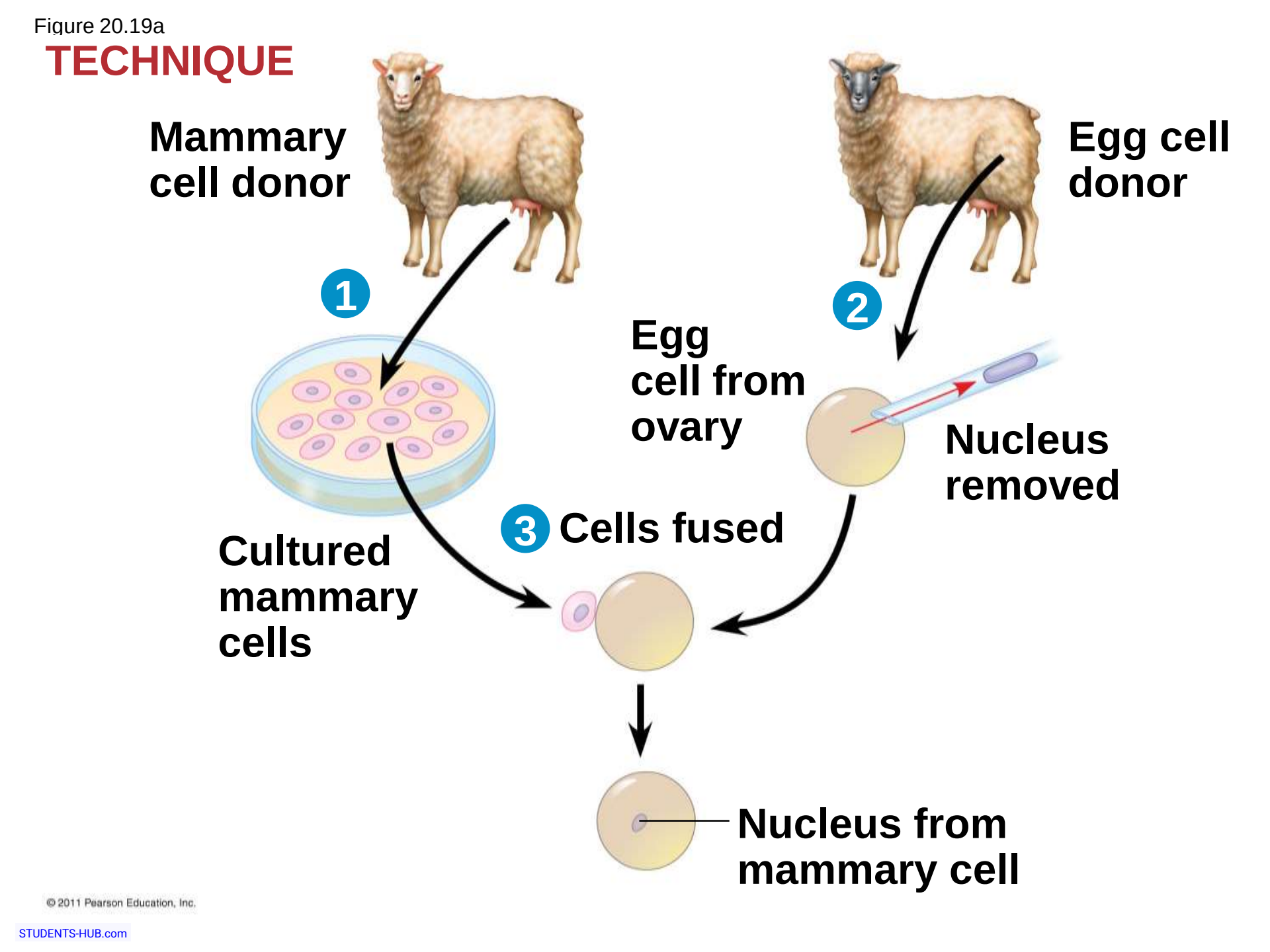
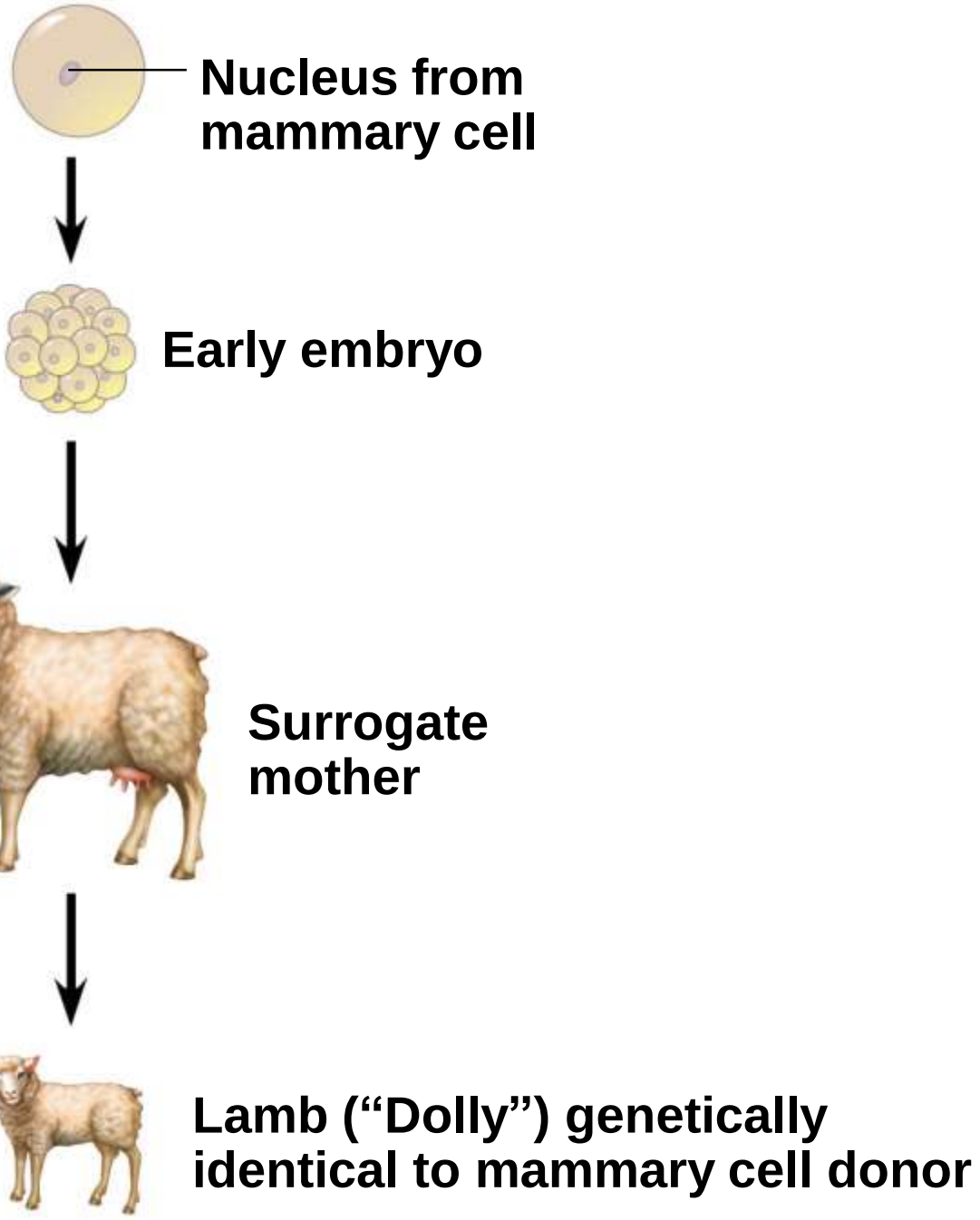
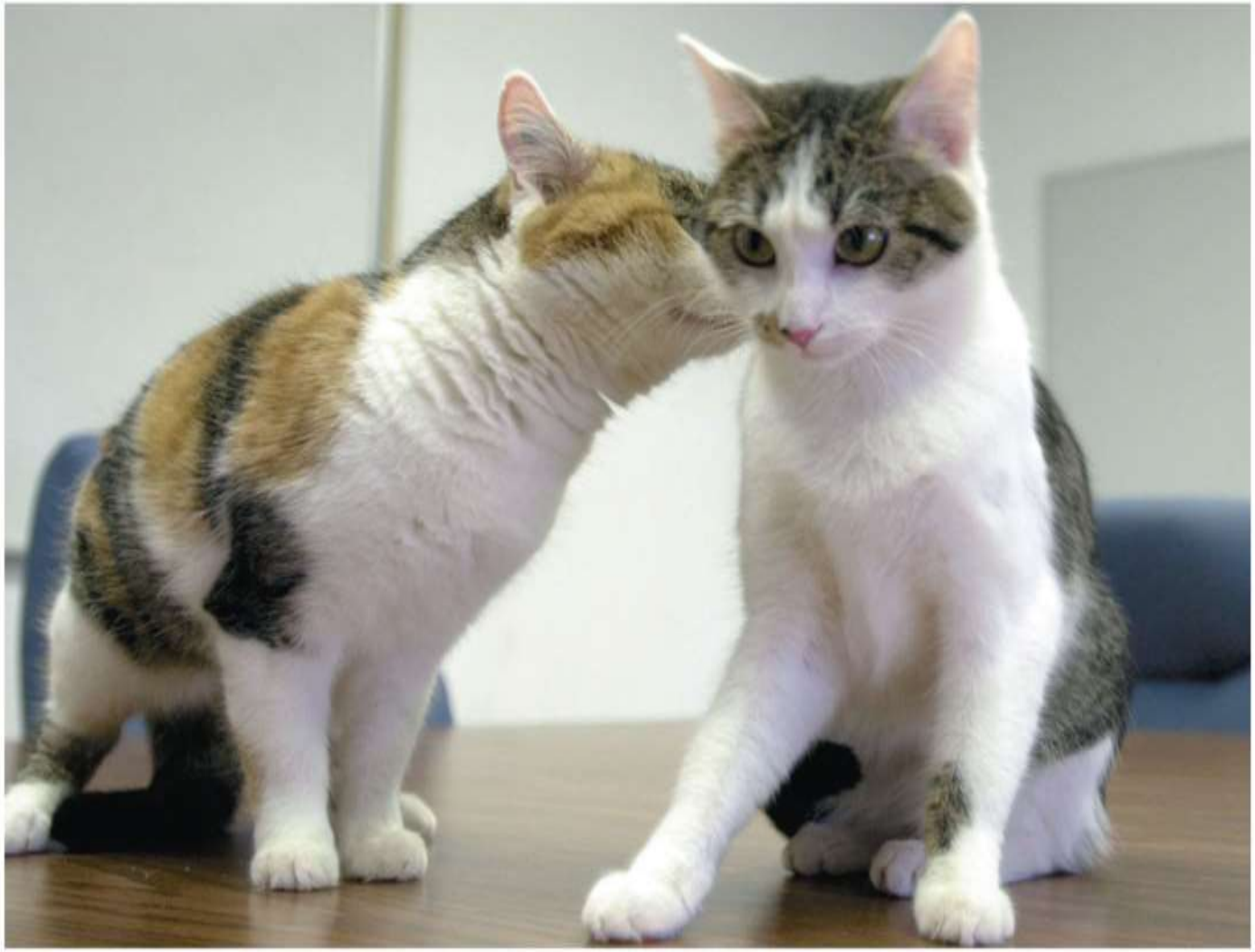


Figure 20.19b



- Since 1997, cloning has been demonstrated in many mammals, including mice, cats, cows, horses, mules, pigs, and dogs
- CC (for Carbon Copy) was the first cat cloned; however, CC differed somewhat from her female "parent"
- Cloned animals do not always look or behave exactly the same

Figure 20.20



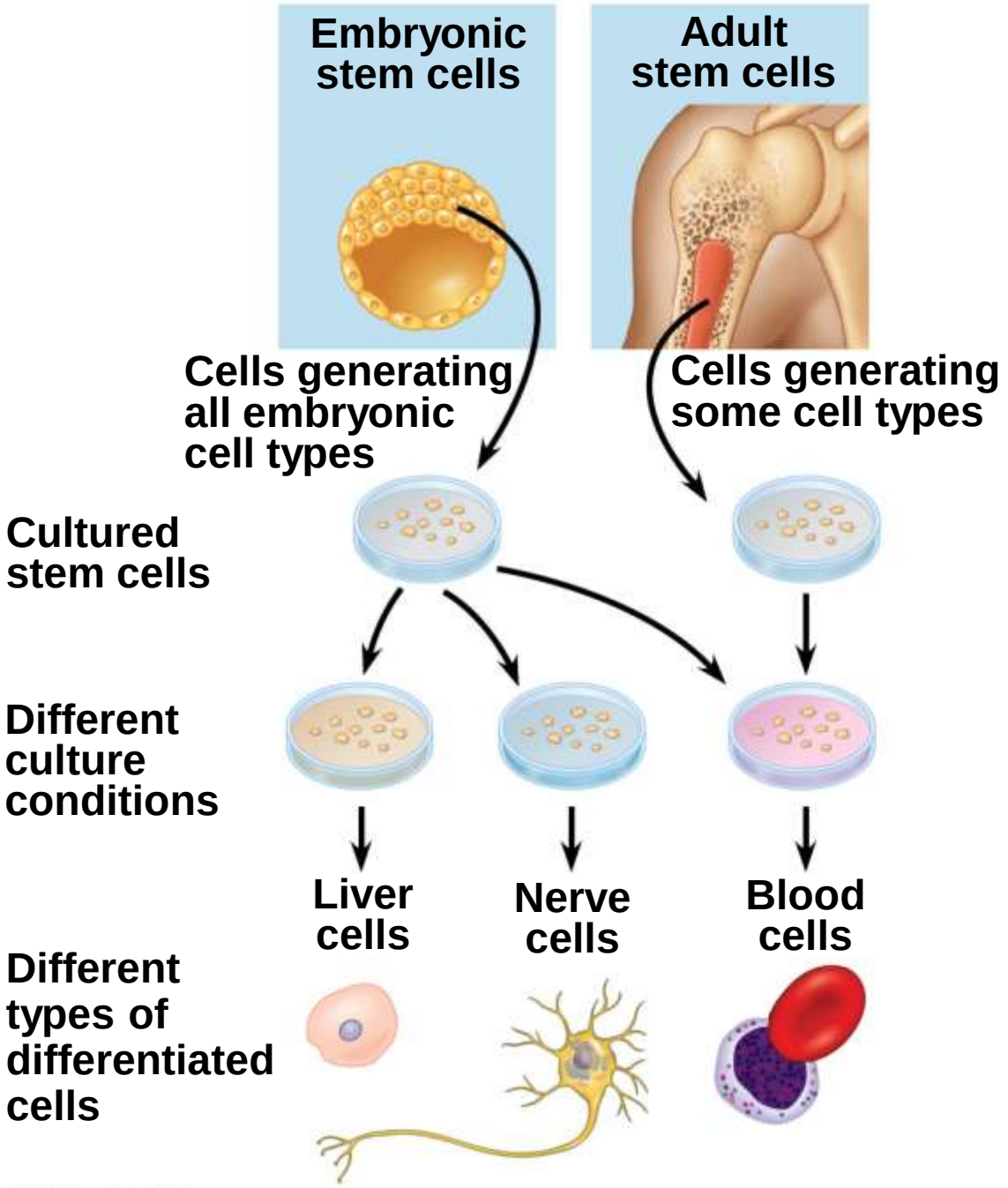
Problems Associated with Animal Cloning

- In most nuclear transplantation studies, only a small percentage of cloned embryos have developed normally to birth, and many cloned animals exhibit defects
- Many epigenetic changes, such as acetylation of histones or methylation of DNA, must be reversed in the nucleus from a donor animal in order for genes to be expressed or repressed appropriately for early stages of development

Stem Cells of Animals

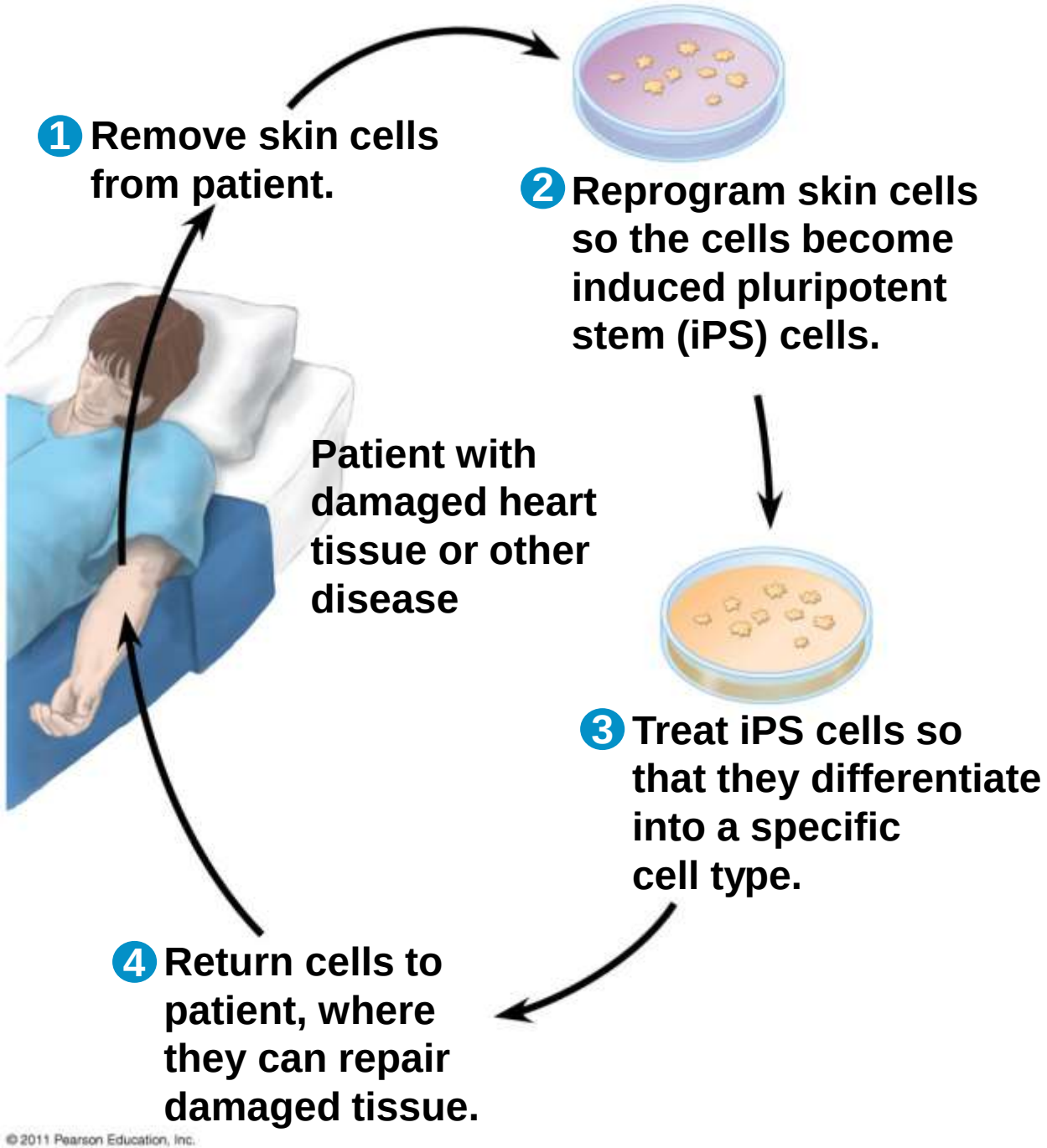
- A **stem cell** is a relatively unspecialized cell that can reproduce itself indefinitely and differentiate into specialized cells of one or more types
- Stem cells isolated from early embryos at the blastocyst stage are called embryonic stem (ES) cells; these are able to differentiate into all cell types
- The adult body also has stem cells, which replace nonreproducing specialized cells

Figure 20.21



- Researchers can transform skin cells into ES cells by using viruses to introduce stem cell master regulatory genes
- These transformed cells are called iPS cells (induced pluripotent cells)
- These cells can be used to treat some diseases and to replace nonfunctional tissues

Figure 20.22



Concept 20.4: The practical applications of DNA technology affect our lives in many ways

- Many fields benefit from DNA technology and genetic engineering

Medical Applications

- One benefit of DNA technology is identification of human genes in which mutation plays a role in genetic diseases

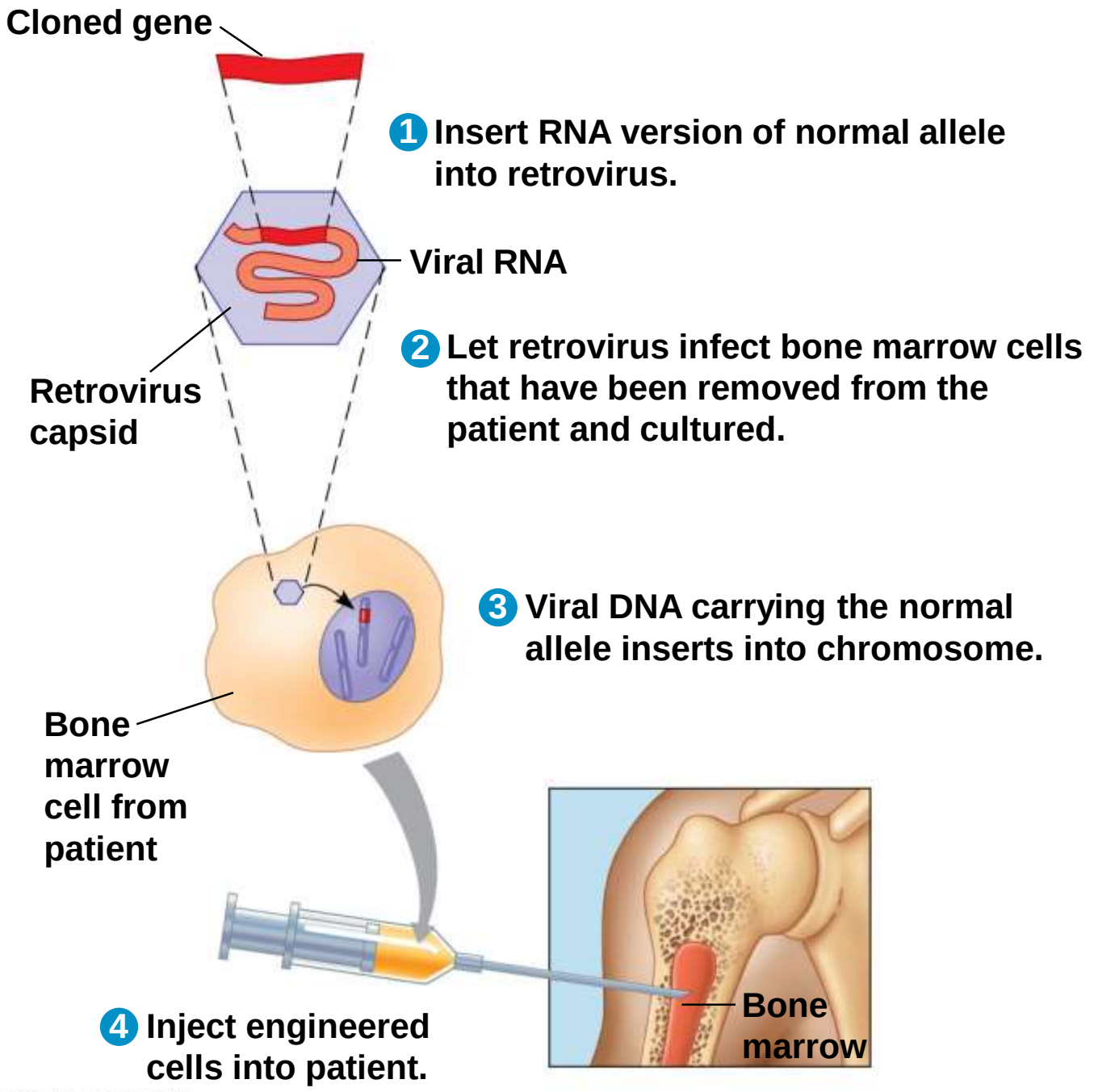
Diagnosis and Treatment of Diseases

- Scientists can diagnose many human genetic disorders using PCR and sequence-specific primers, then sequencing the amplified product to look for the disease-causing mutation
- SNPs may be associated with a disease-causing mutation
- SNPs may also be correlated with increased risks for conditions such as heart disease or certain types of cancer

Human Gene Therapy

- **Gene therapy** is the alteration of an afflicted individual's genes
- Gene therapy holds great potential for treating disorders traceable to a single defective gene
- Vectors are used for delivery of genes into specific types of cells, for example bone marrow
- Gene therapy provokes both technical and ethical questions

Figure 20.23



Pharmaceutical Products

- Advances in DNA technology and genetic research are important to the development of new drugs to treat diseases

Synthesis of Small Molecules for Use as Drugs

- The drug imatinib is a small molecule that inhibits overexpression of a specific leukemia-causing receptor
- Pharmaceutical products that are proteins can be synthesized on a large scale

Protein Production in Cell Cultures

- Host cells in culture can be engineered to secrete a protein as it is made, simplifying the task of purifying it
- This is useful for the production of insulin, human growth hormones, and vaccines

Protein Production by “Pharm” Animals

- **Transgenic** animals are made by introducing genes from one species into the genome of another animal
- Transgenic animals are pharmaceutical “factories,” producers of large amounts of otherwise rare substances for medical use

Figure 20.24

Figure 20.24a

Figure 20.24b

Forensic Evidence and Genetic Profiles

- An individual's unique DNA sequence, or **genetic profile**, can be obtained by analysis of tissue or body fluids
- DNA testing can identify individuals with a high degree of certainty
- Genetic profiles can be analyzed using RFLP analysis by Southern blotting

- Even more sensitive is the use of genetic markers called **short tandem repeats (STRs)**, which are variations in the number of repeats of specific DNA sequences
- PCR and gel electrophoresis are used to amplify and then identify STRs of different lengths
- The probability that two people who are not identical twins have the same STR markers is exceptionally small

Figure 20.25

(a) This photo shows Washington just before his release in 2001, after 17 years in prison.

Source of sample	STR marker 1	STR marker 2	STR marker 3
Semen on victim	17,19	13,16	12,12
Earl Washington	16,18	14,15	11,12
Kenneth Tinsley	17,19	13,16	12,12

(b) These and other STR data exonerated Washington and led Tinsley to plead guilty to the murder.

Figure 20.25a

(a) This photo shows Washington just before his release in 2001, after 17 years in prison.

Environmental Cleanup

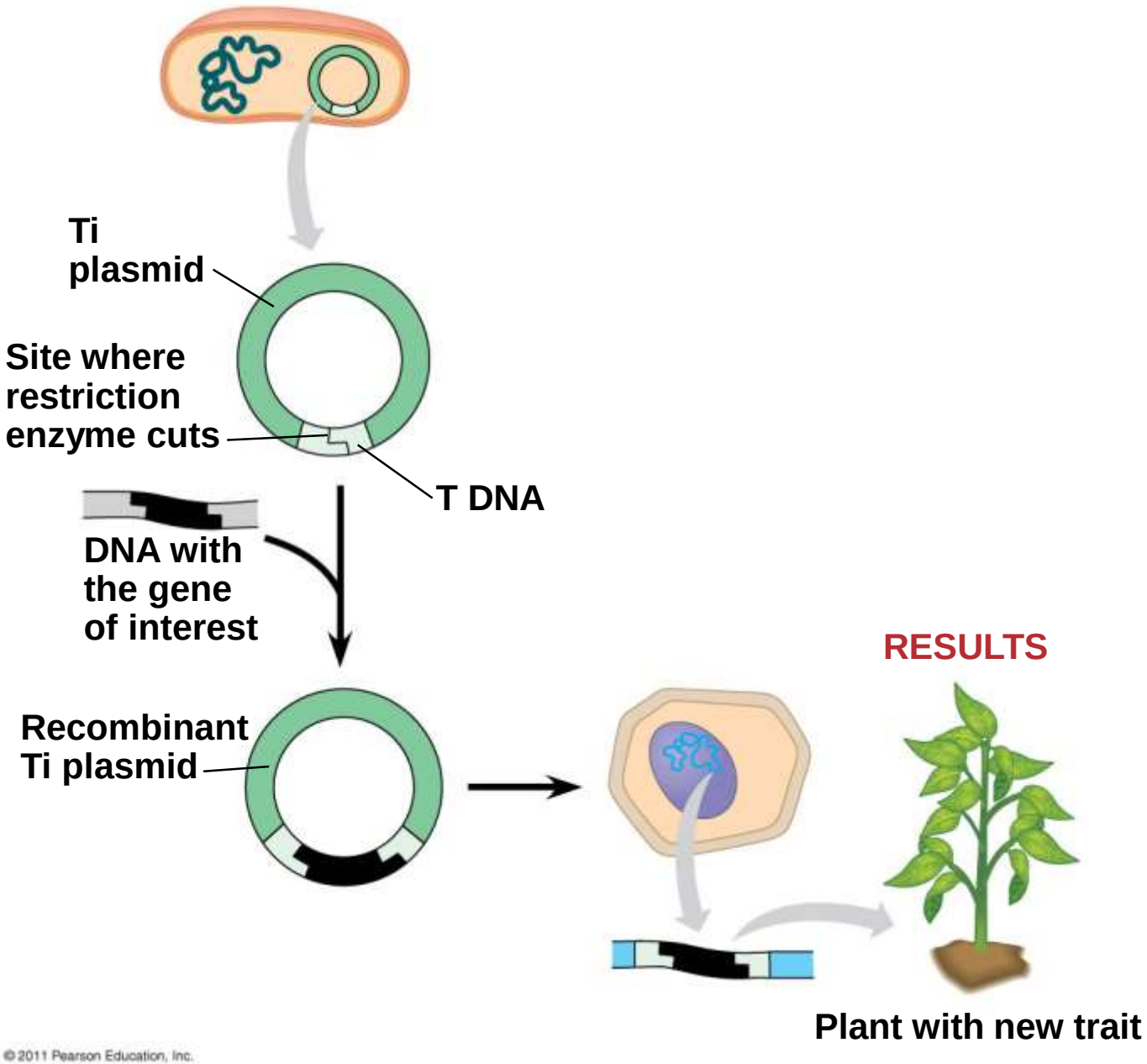
- Genetic engineering can be used to modify the metabolism of microorganisms
- Some modified microorganisms can be used to extract minerals from the environment or degrade potentially toxic waste materials

Agricultural Applications

- DNA technology is being used to improve agricultural productivity and food quality
- Genetic engineering of transgenic animals speeds up the selective breeding process
- Beneficial genes can be transferred between varieties or species

- Agricultural scientists have endowed a number of crop plants with genes for desirable traits
- The **Ti plasmid** is the most commonly used vector for introducing new genes into plant cells
- Genetic engineering in plants has been used to transfer many useful genes including those for herbicide resistance, increased resistance to pests, increased resistance to salinity, and improved nutritional value of crops

Figure 20.26



Safety and Ethical Questions Raised by DNA Technology

- Potential benefits of genetic engineering must be weighed against potential hazards of creating harmful products or procedures
- Guidelines are in place in the United States and other countries to ensure safe practices for recombinant DNA technology

- Most public concern about possible hazards centers on **genetically modified (GM) organisms** used as food
- Some are concerned about the creation of “super weeds” from the transfer of genes from GM crops to their wild relatives
- Other worries include the possibility that transgenic protein products might cause allergic reactions

- As biotechnology continues to change, so does its use in agriculture, industry, and medicine
- National agencies and international organizations strive to set guidelines for safe and ethical practices in the use of biotechnology

Figure 20.UN03

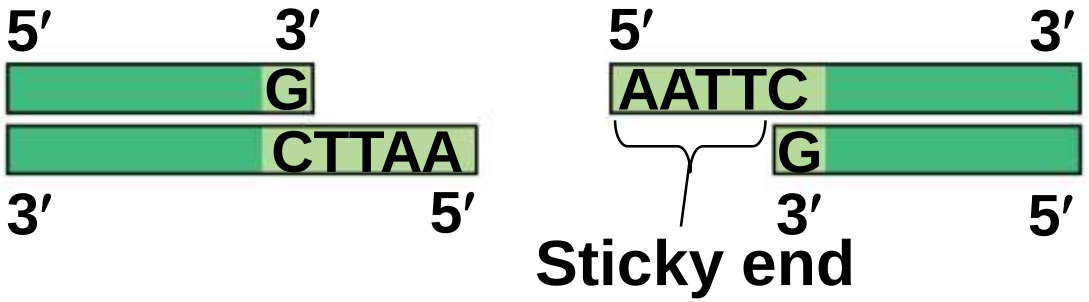


Figure 20.UN04

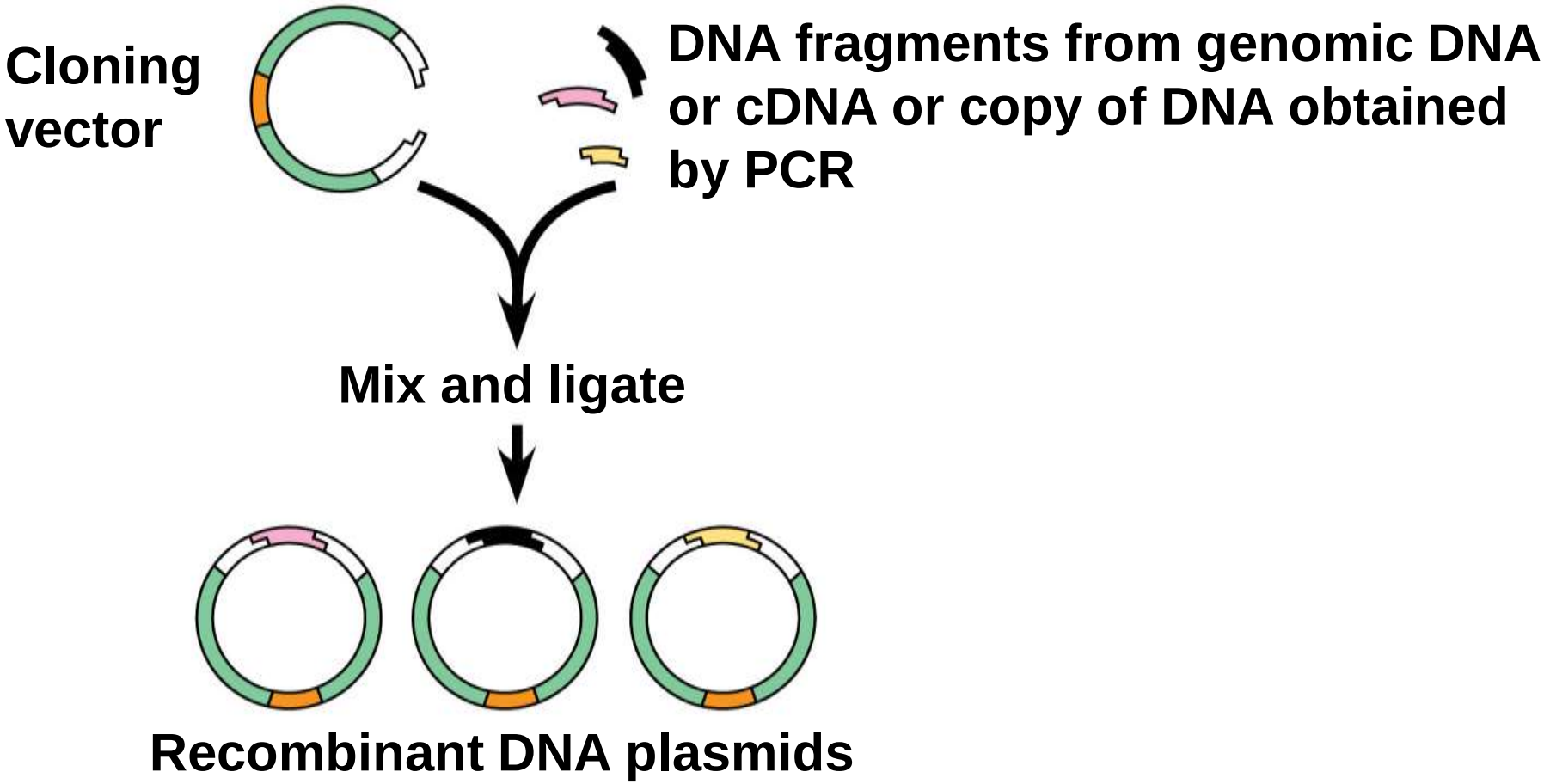


Figure 20.UN05

5′ TCCATGAATTCTAAAGCGCTTATGAATTCACGGC 3′

3′ AGGTACTTAAGATTTCGCGAATACTTAAGTGCCG 5′

Aardvark DNA

Plasmid

Figure 20.UN06

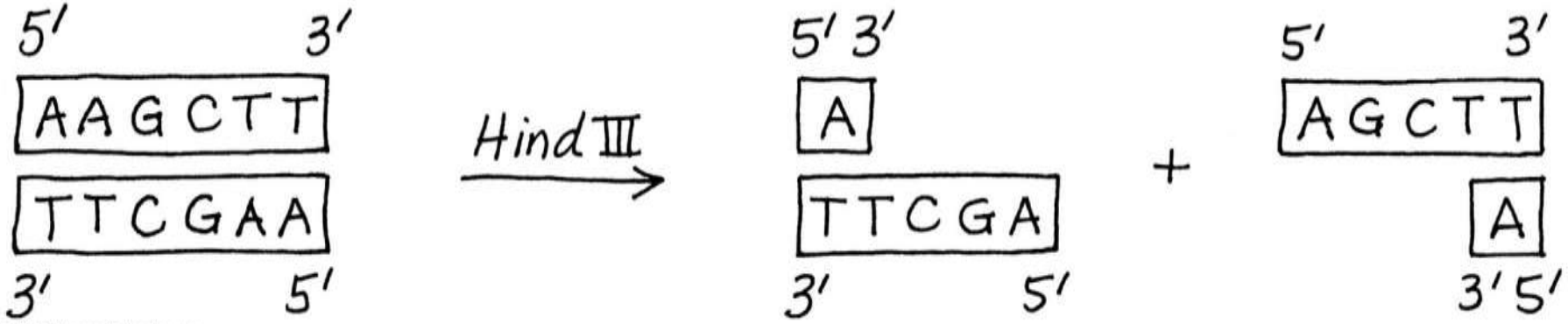


Figure 20.UN07

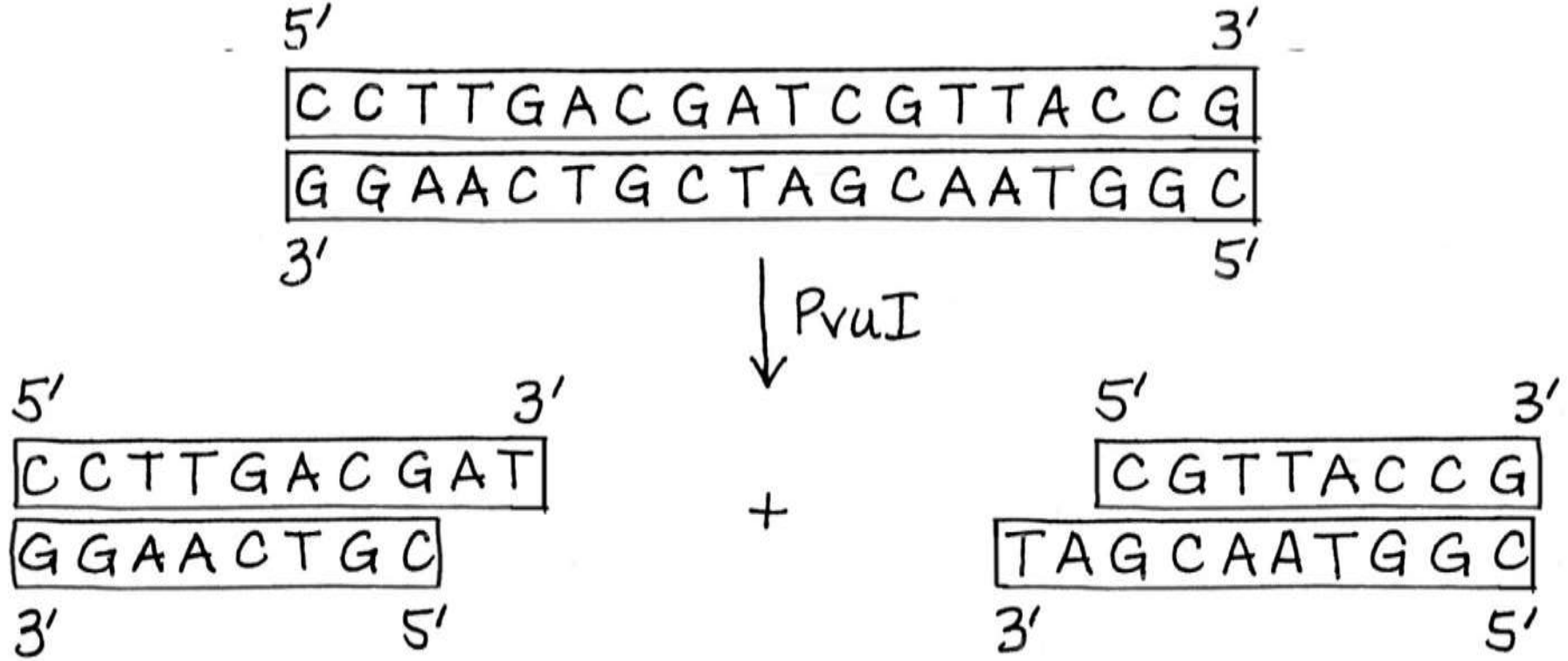


Figure 20.UN08