LECTURE PRESENTATIONS

For CAMPBELL BIOLOGY, NINTH EDITION

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Chapter 20

Biotechnology

Lectures by
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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

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- 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

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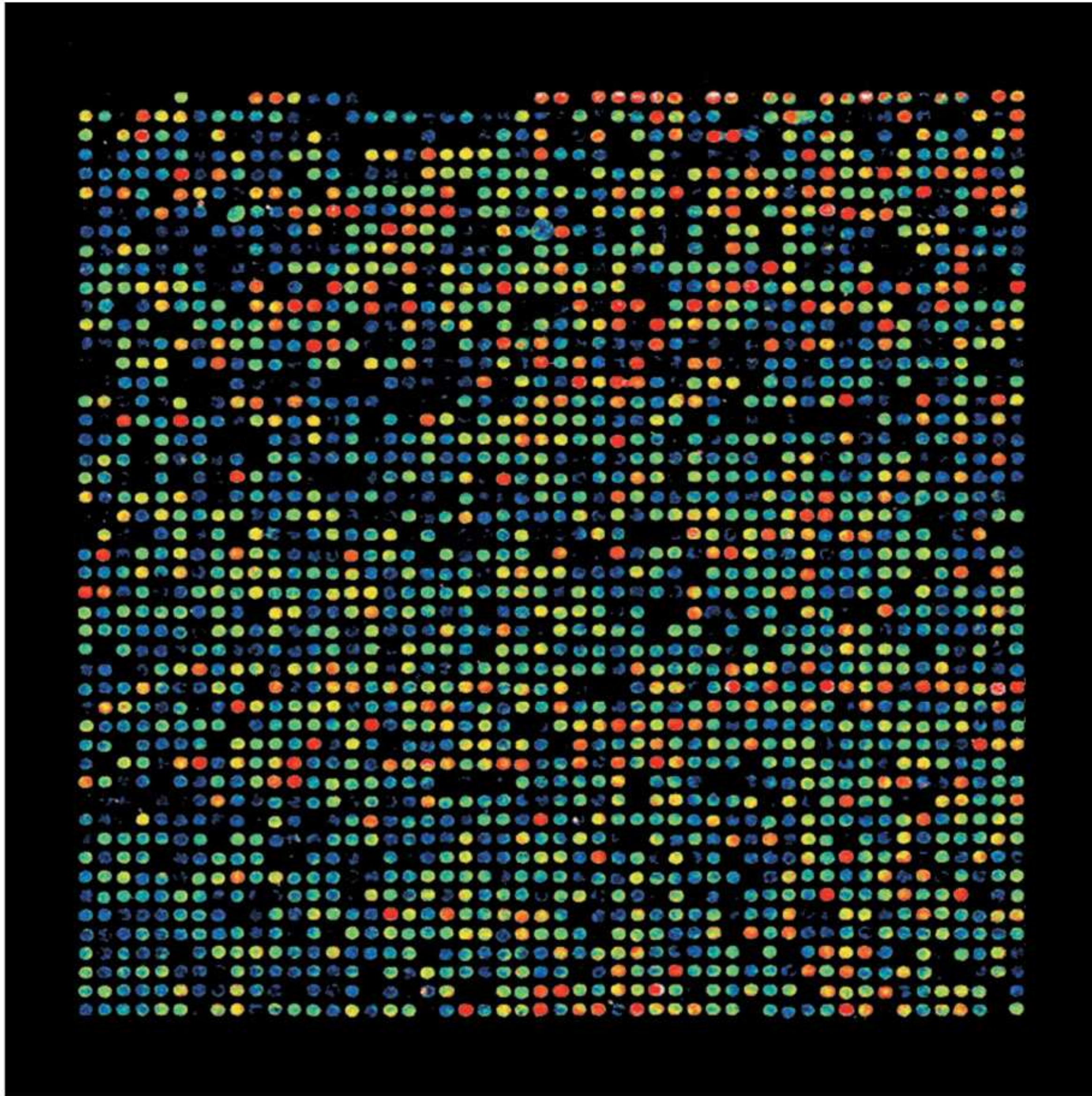
Figure 20.1



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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*

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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

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- **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

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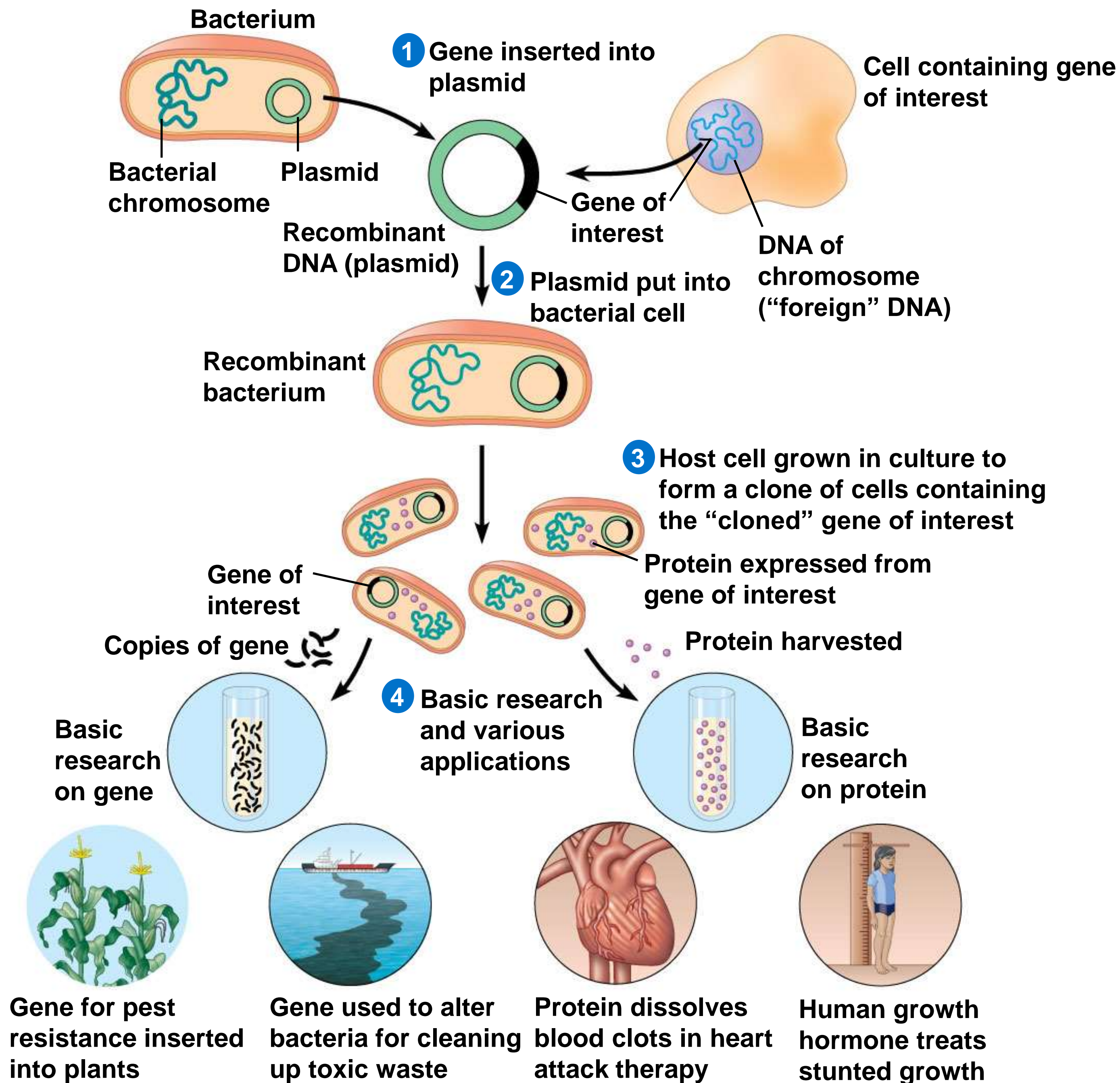
Figure 20.2



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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**."

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

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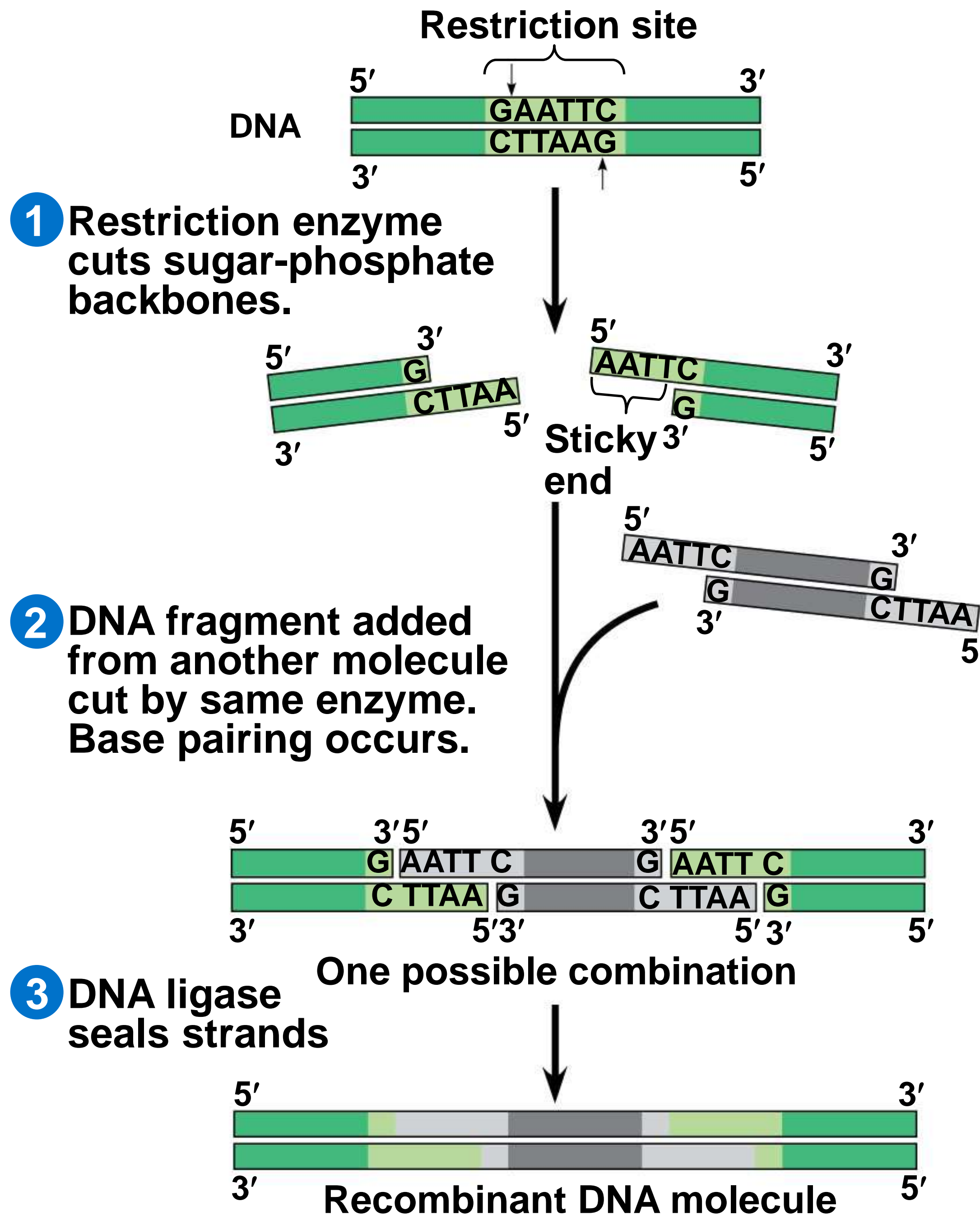
Figure 20.3-3



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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

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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

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- 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

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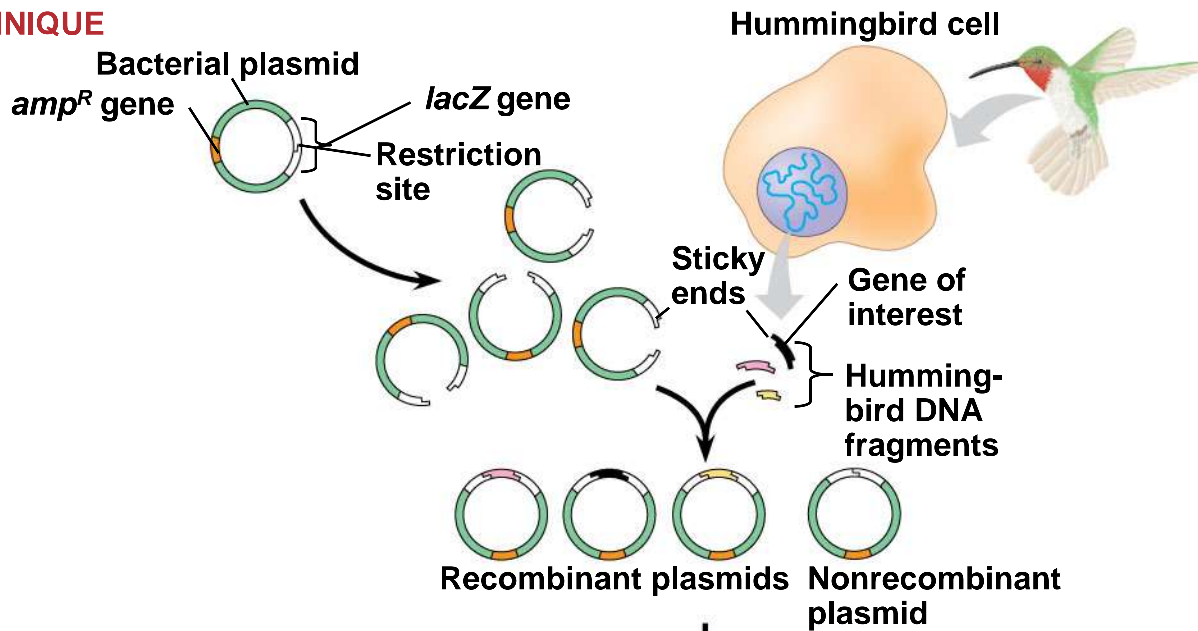
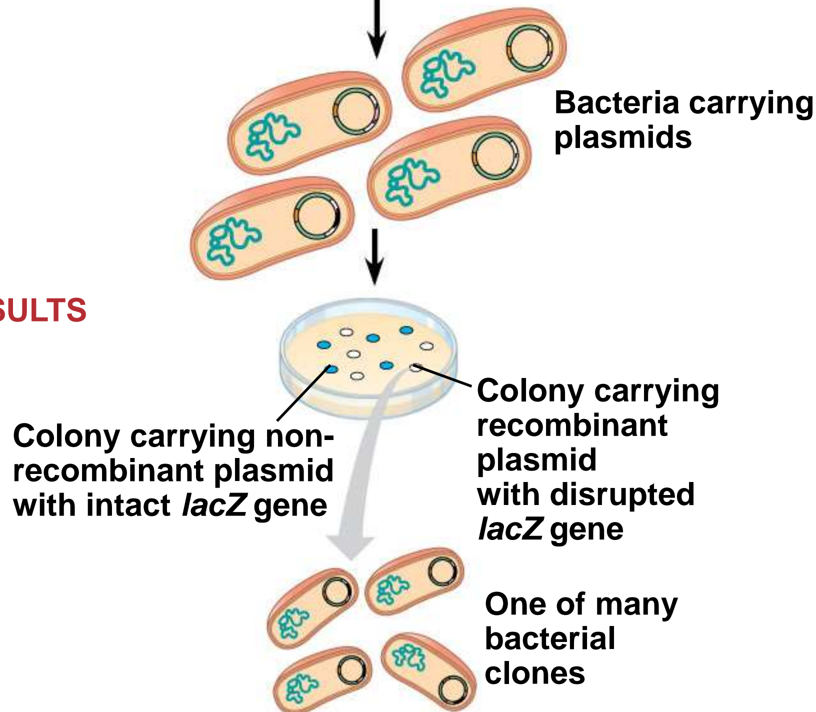
Figure 20.4





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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

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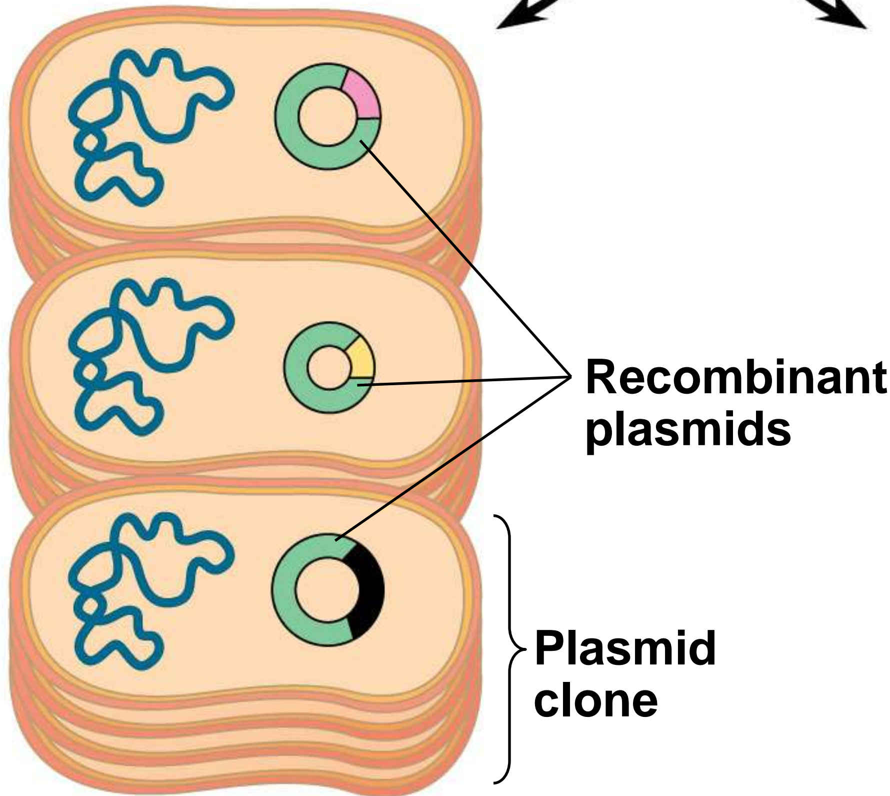
Figure 20.5

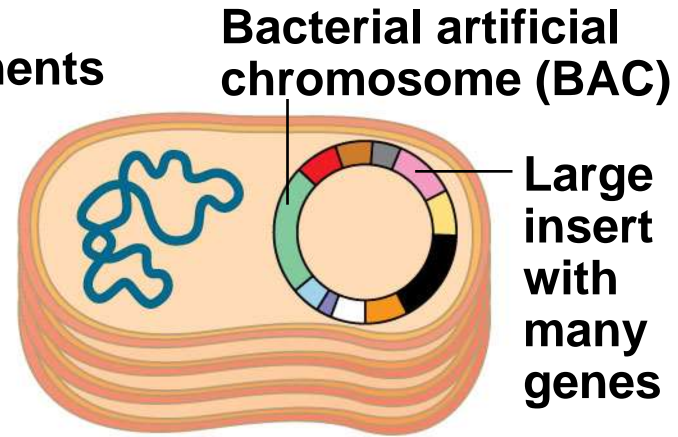
Foreign genome

Cut with restriction enzymes into either

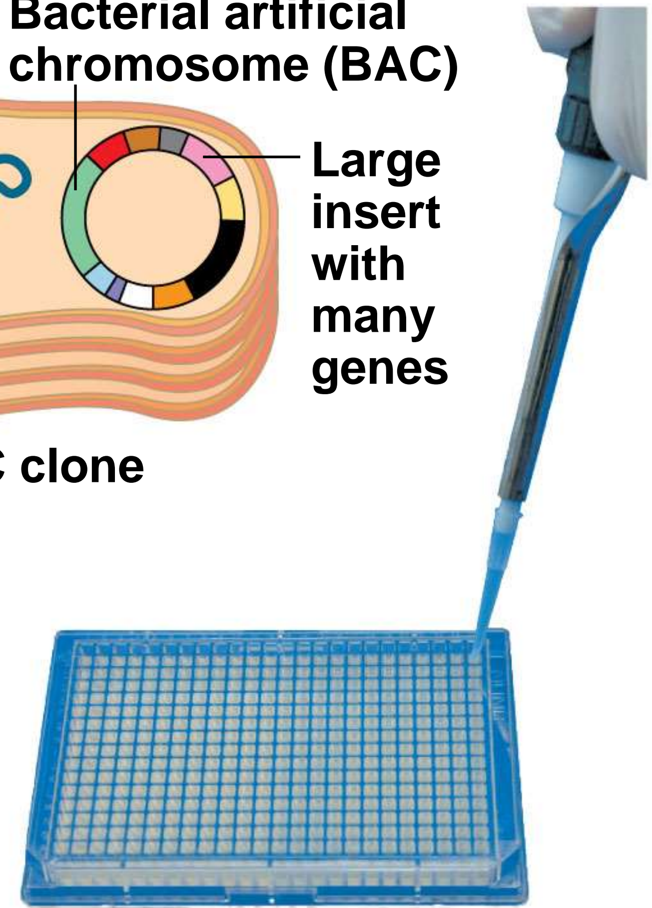
small fragments or large fragments



(a) Plasmid library



(b) BAC clone



(c) Storing genome libraries

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- 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

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- 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

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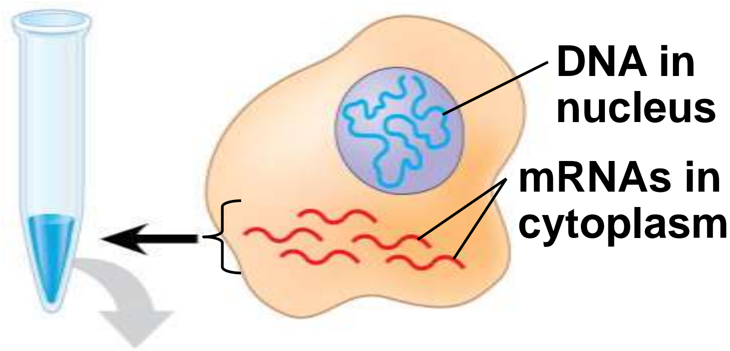
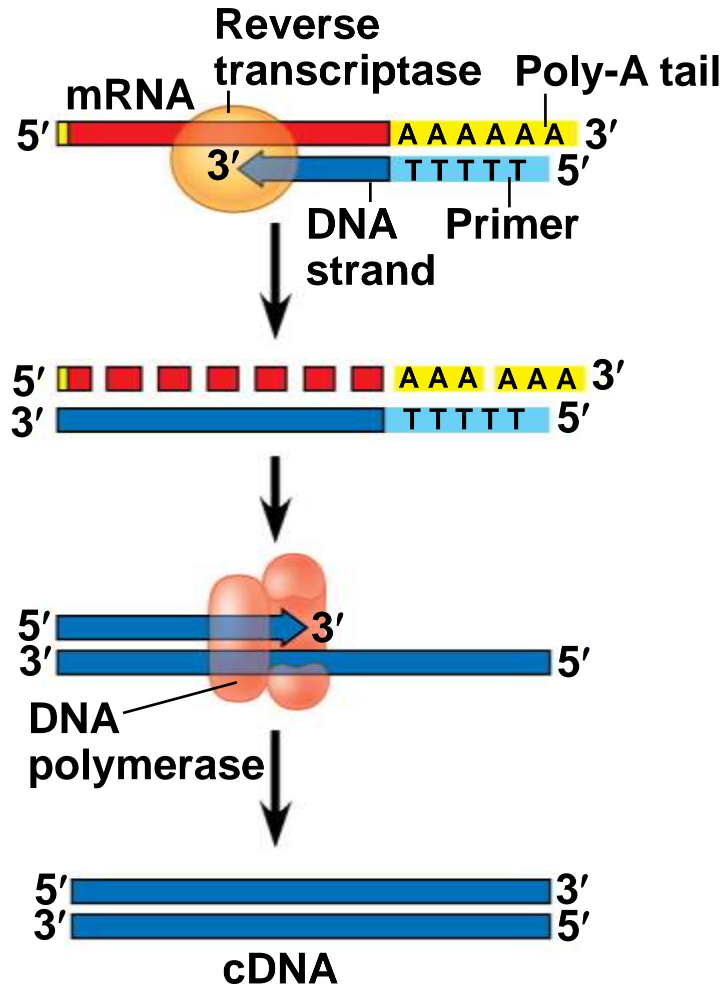
Figure 20.6-5





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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**

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

5′ ··· CTCATCACCGGC··· 3′

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  – Then we would synthesize this probe

3′ GAGTAGTGGCCG 5′

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- 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

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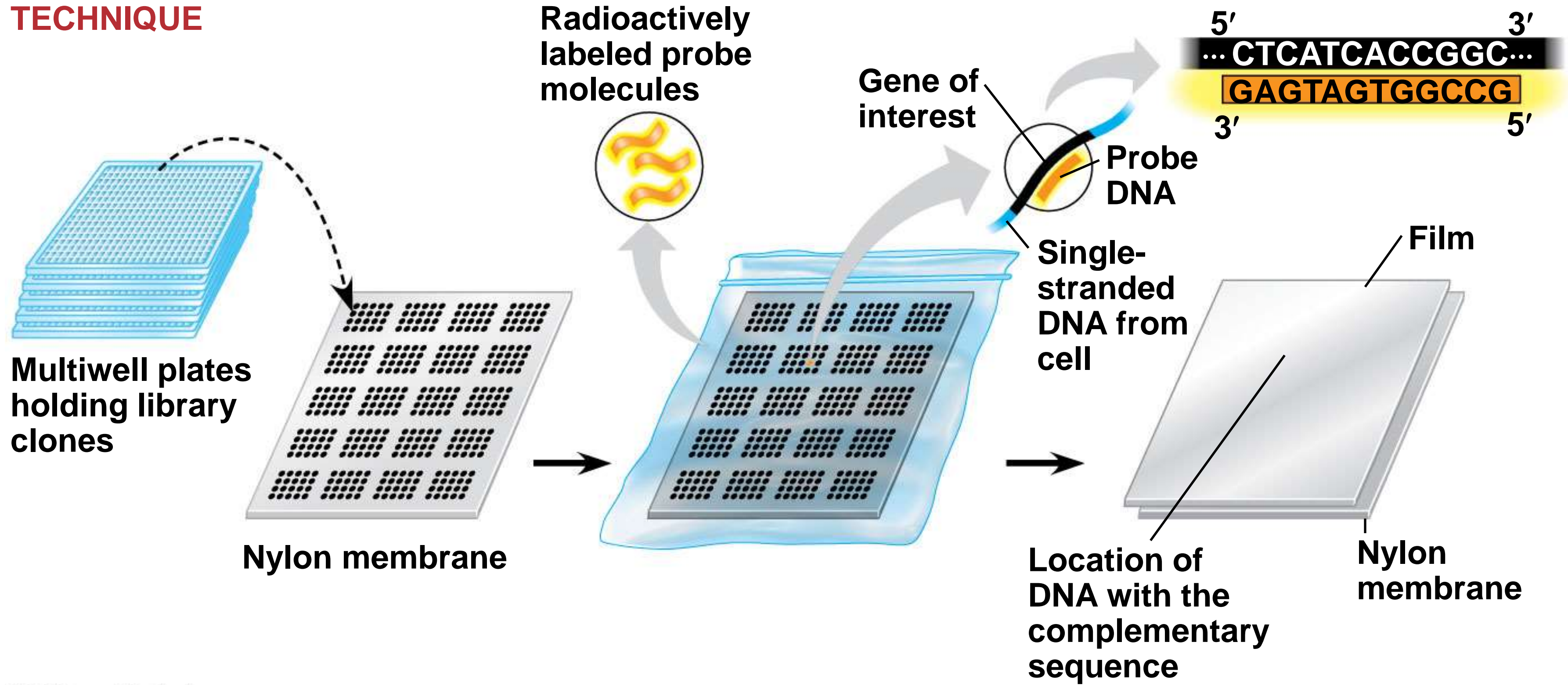
Figure 20.7



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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

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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

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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

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- 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

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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

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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.

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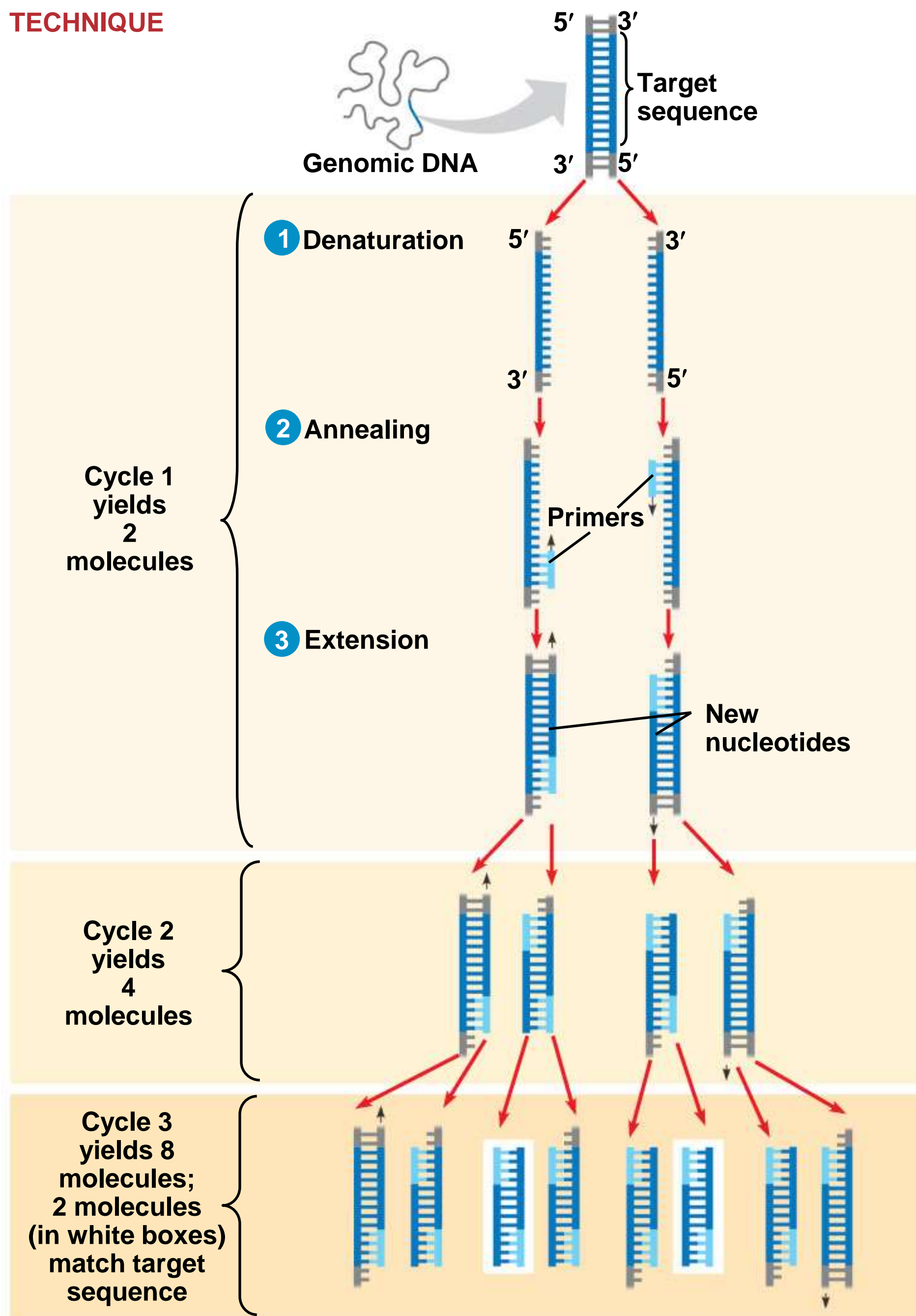
Figure 20.8



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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

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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

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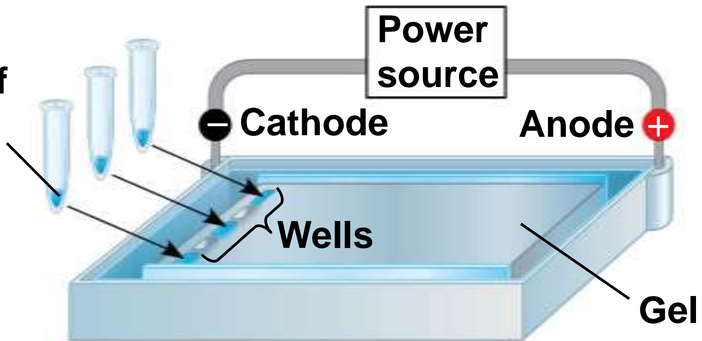
Figure 20.9

TECHNIQUE

1 Mixture of DNA molecules of different sizes



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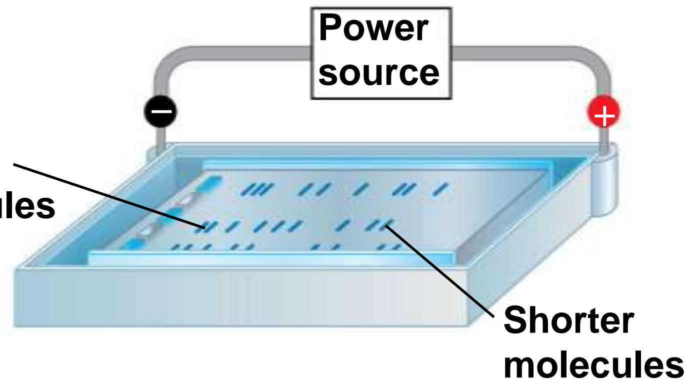

RESULTS

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- 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

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

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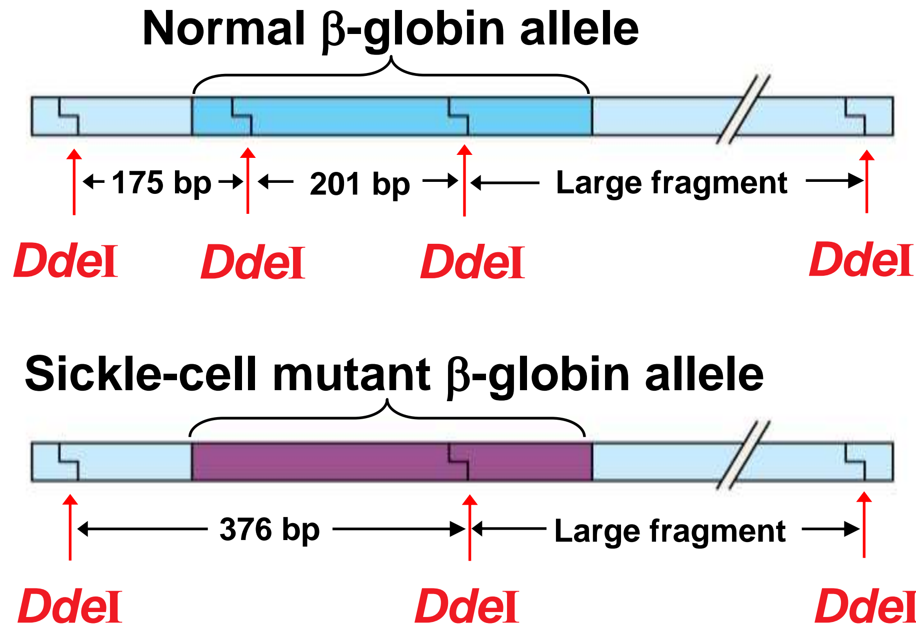
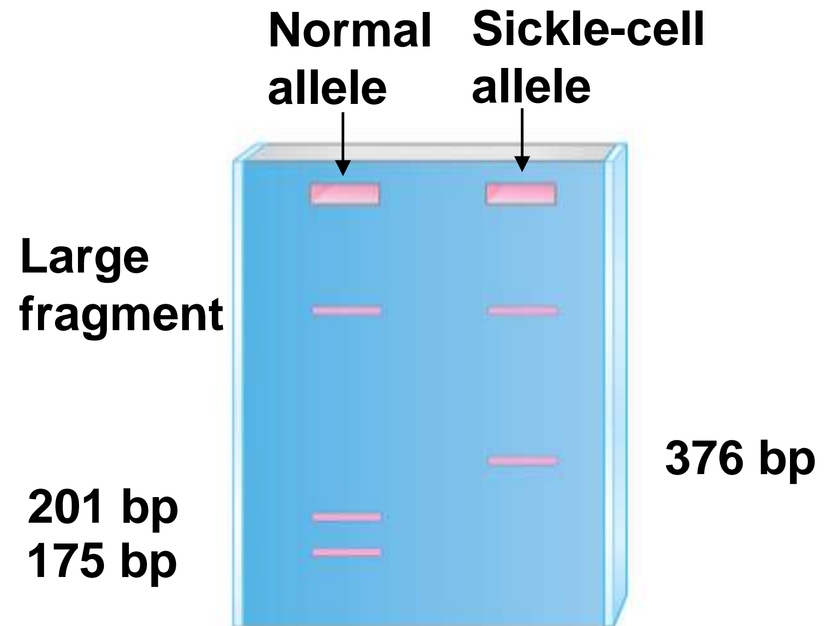
Figure 20.10



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



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

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- 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

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Figure 20.11

TECHNIQUE

DNA + restriction enzyme

Restriction fragments

I Normal β-globin allele

II Sickle-cell allele

III Heterozygote

I II III

Nitrocellulose membrane (blot)

Heavy weight

Gel

Sponge

Alkaline solution

Paper towels

1 Preparation of restriction fragments

2 Gel electrophoresis

3 DNA transfer (blotting)

Radioactively labeled probe for β-globin gene

I II III

Nitrocellulose blot

Probe base-pairs with fragments

Fragment from sickle-cell β-globin allele

Fragment from normal β- globin allele

Film over blot

I II III

4 Hybridization with labeled probe

5 Probe detection

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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

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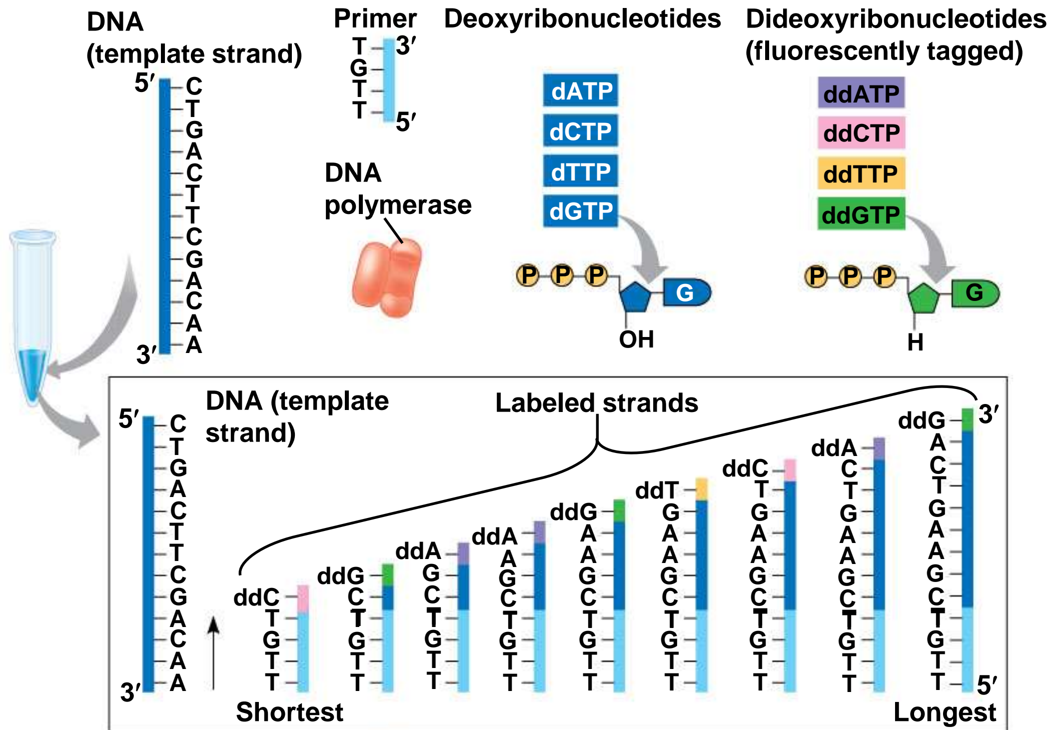
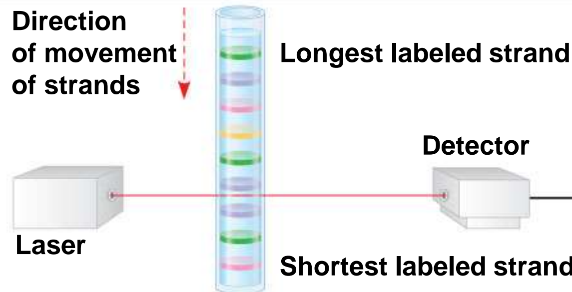
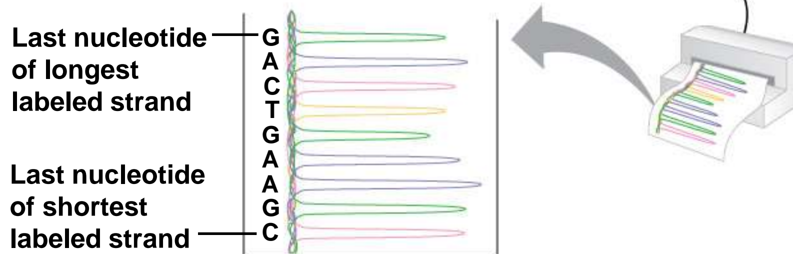
Figure 20.12





RESULTS



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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

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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

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- **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

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- **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

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Figure 20.13

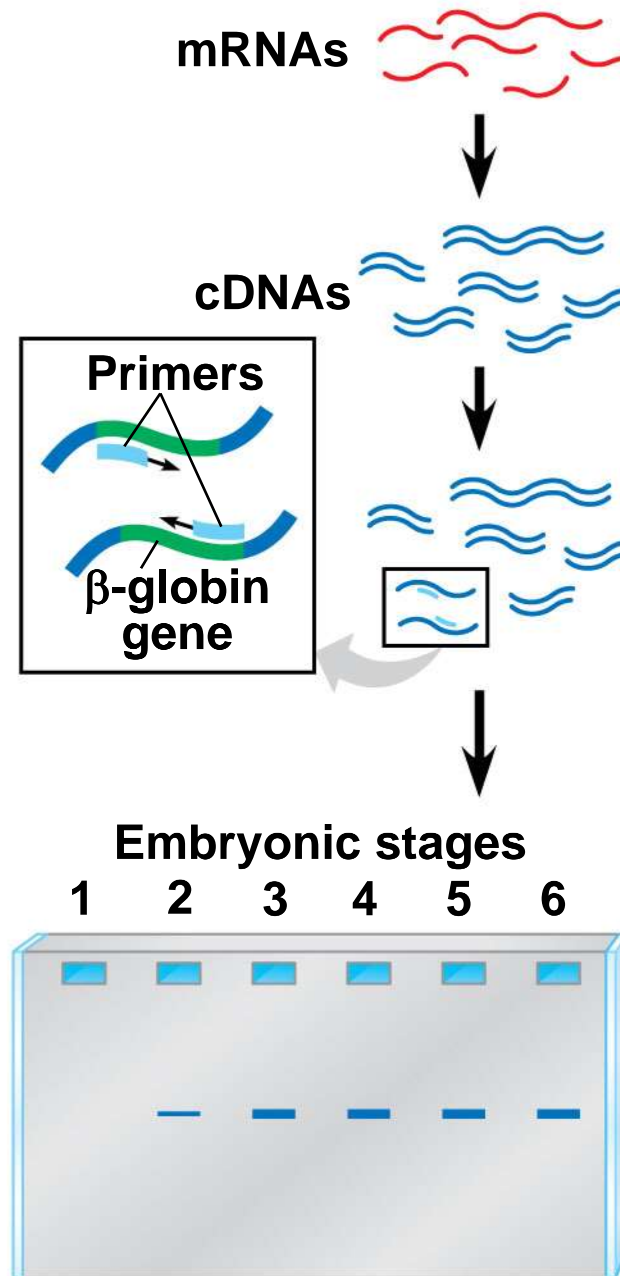
TECHNIQUE

1 cDNA synthesis

2 PCR amplification

3 Gel electrophoresis

RESULTS



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

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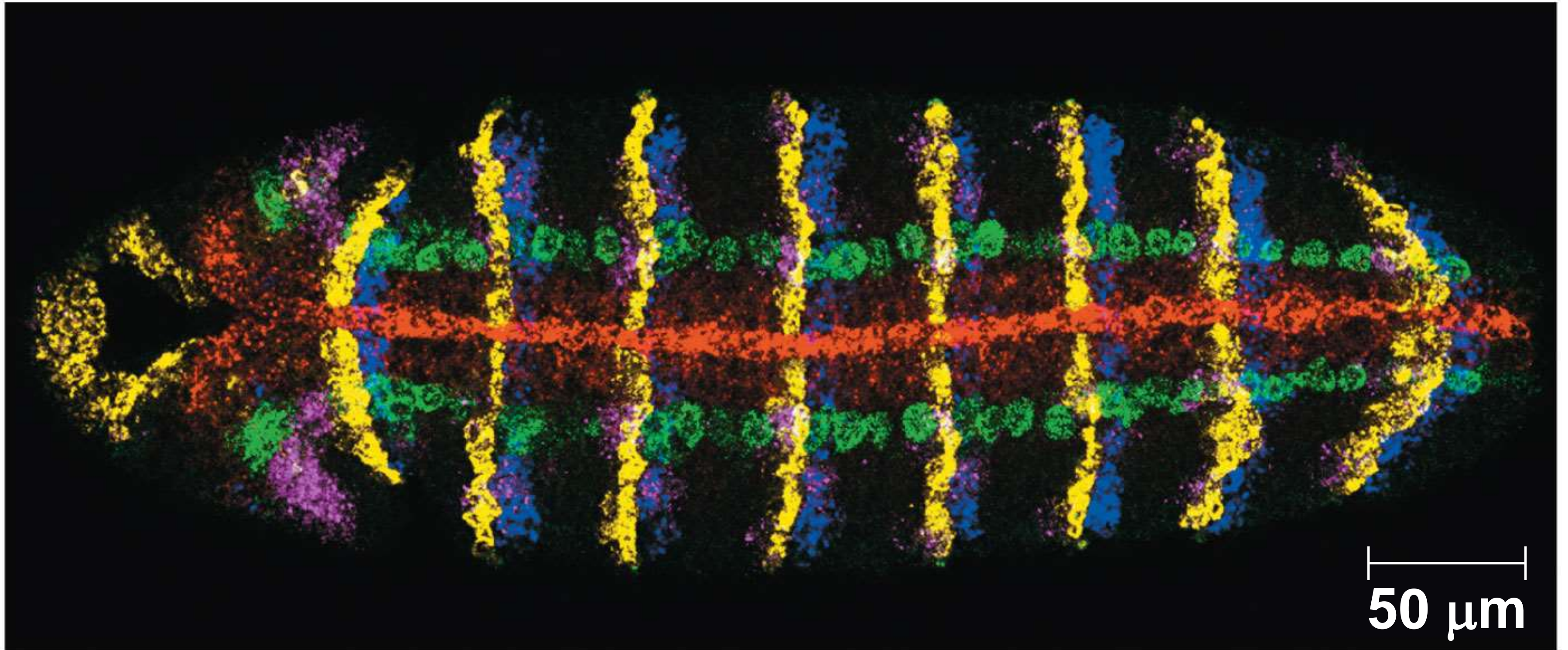
Figure 20.14



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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

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Figure 20.15

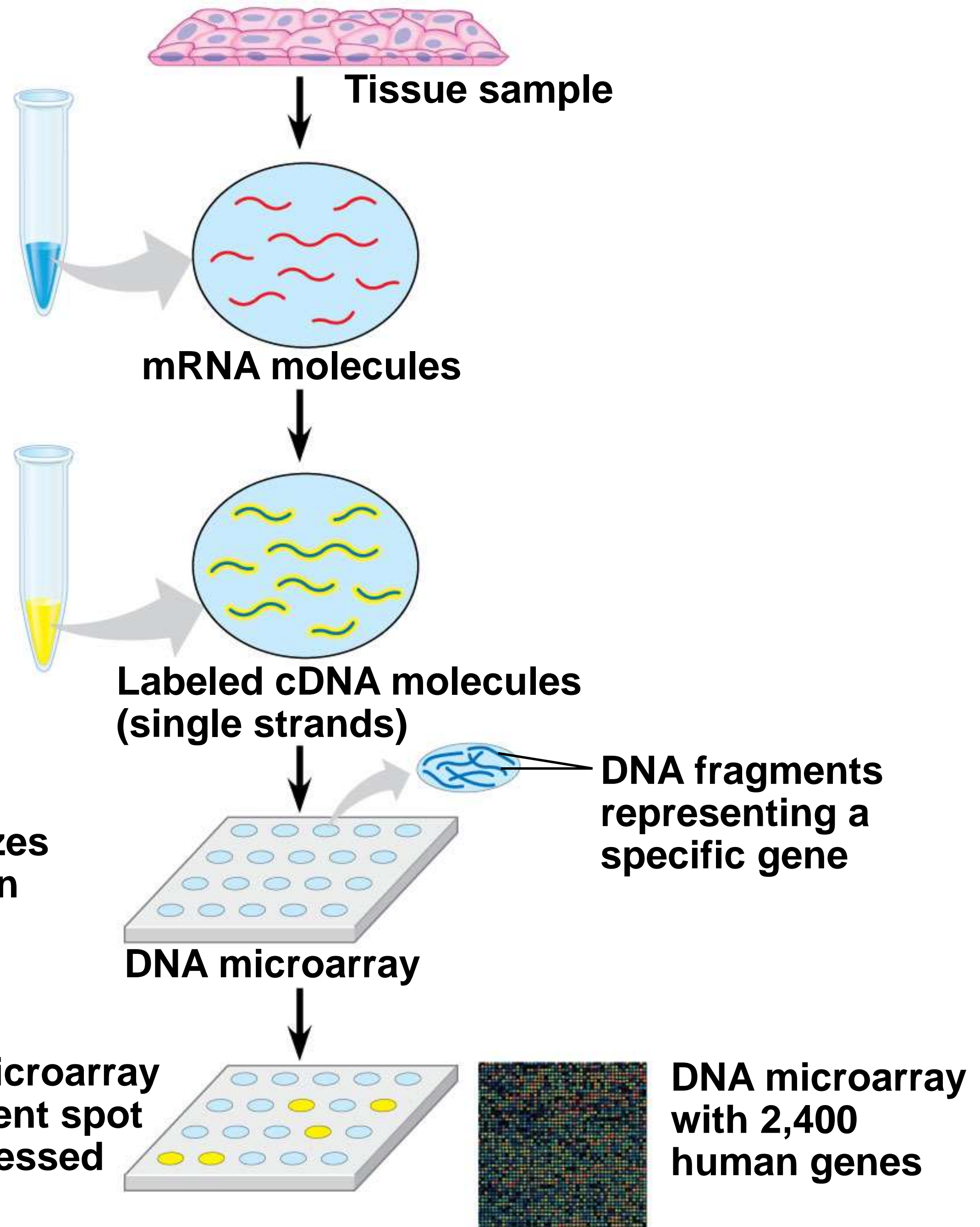
TECHNIQUE

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.



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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

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- 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

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- 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

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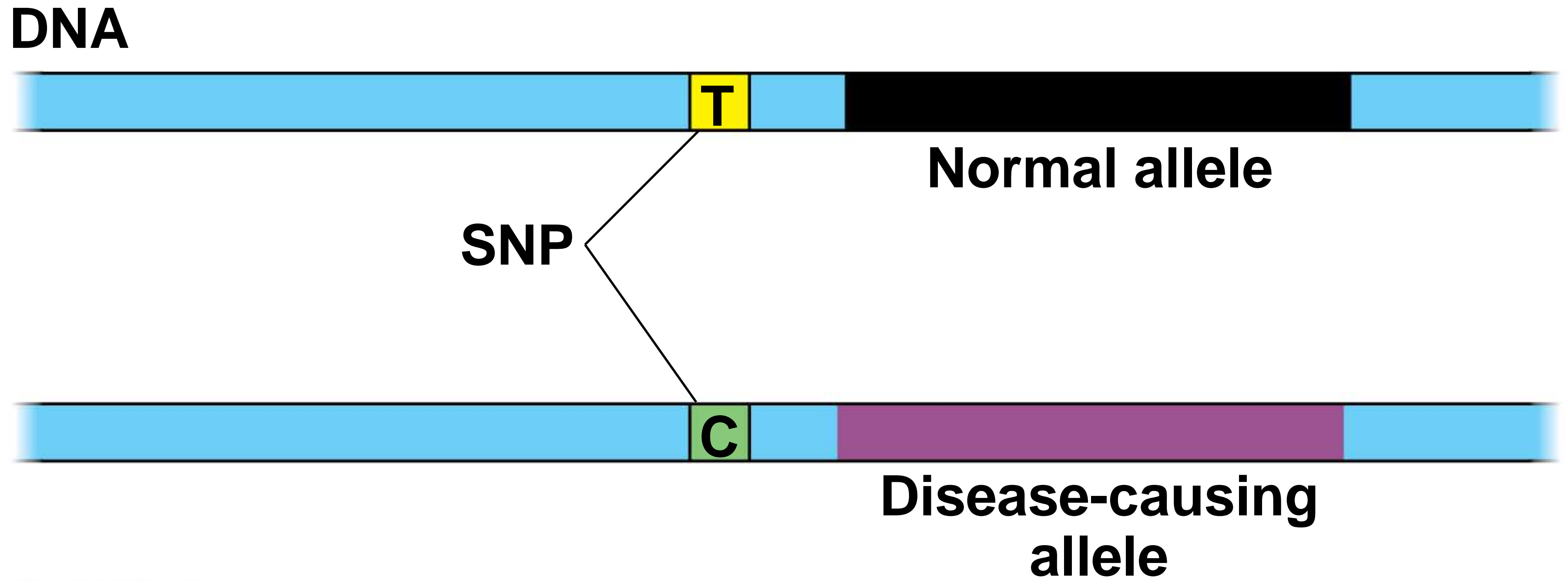
Figure 20.16



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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

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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

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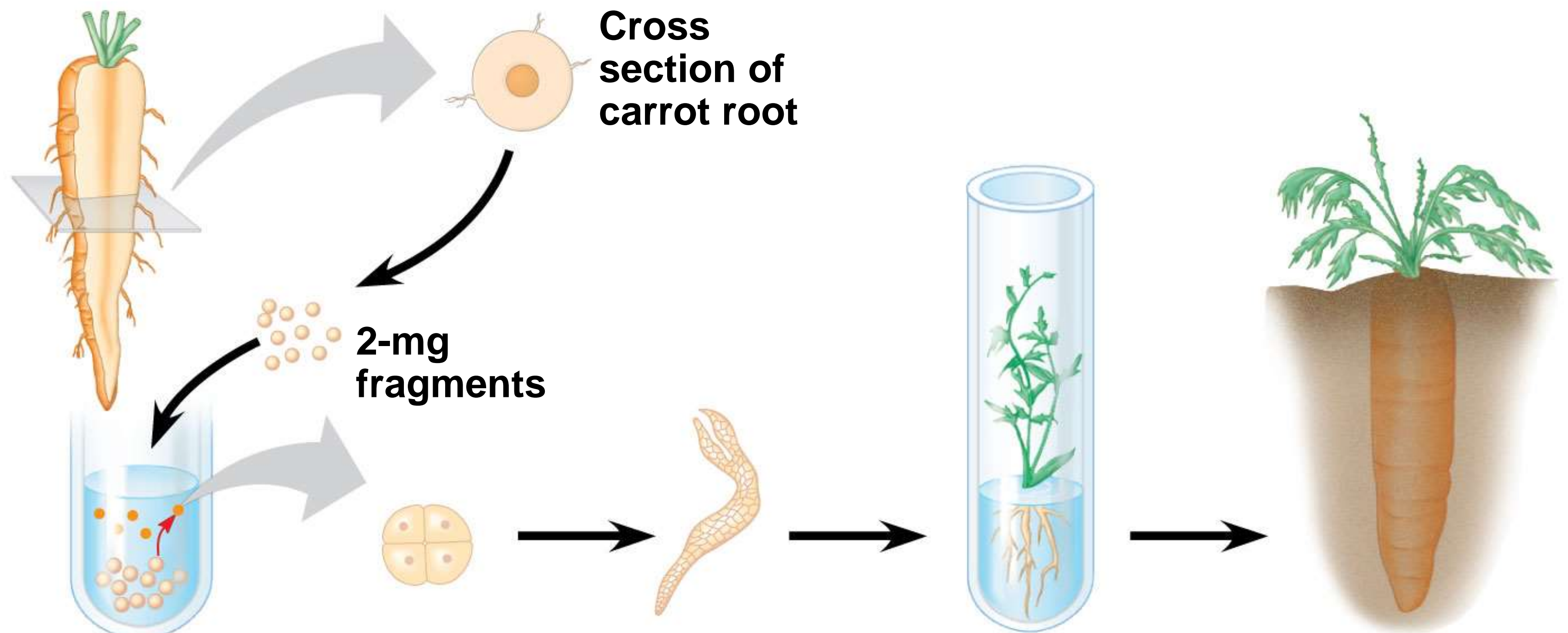
Figure 20.17



Fragments were cultured in nu-trient medium; stirring caused single cells to shear off into the liquid.

Single cells free in suspension began to divide.

Embryonic plant developed from a cultured single cell.

Plantlet was cultured on agar medium. Later it was planted in soil.

Adult plant

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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

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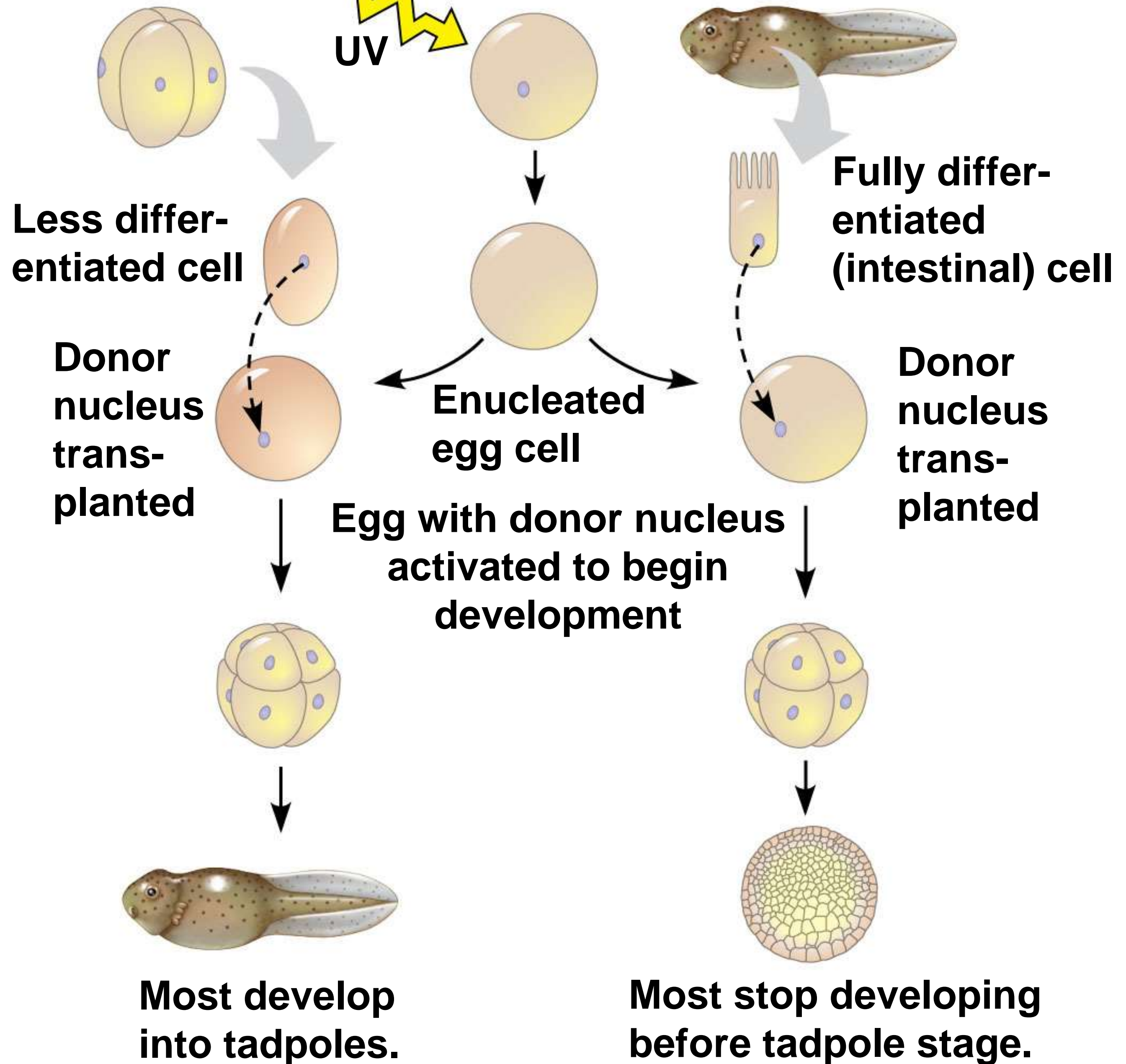
Figure 20.18

EXPERIMENT

RESULTS



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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

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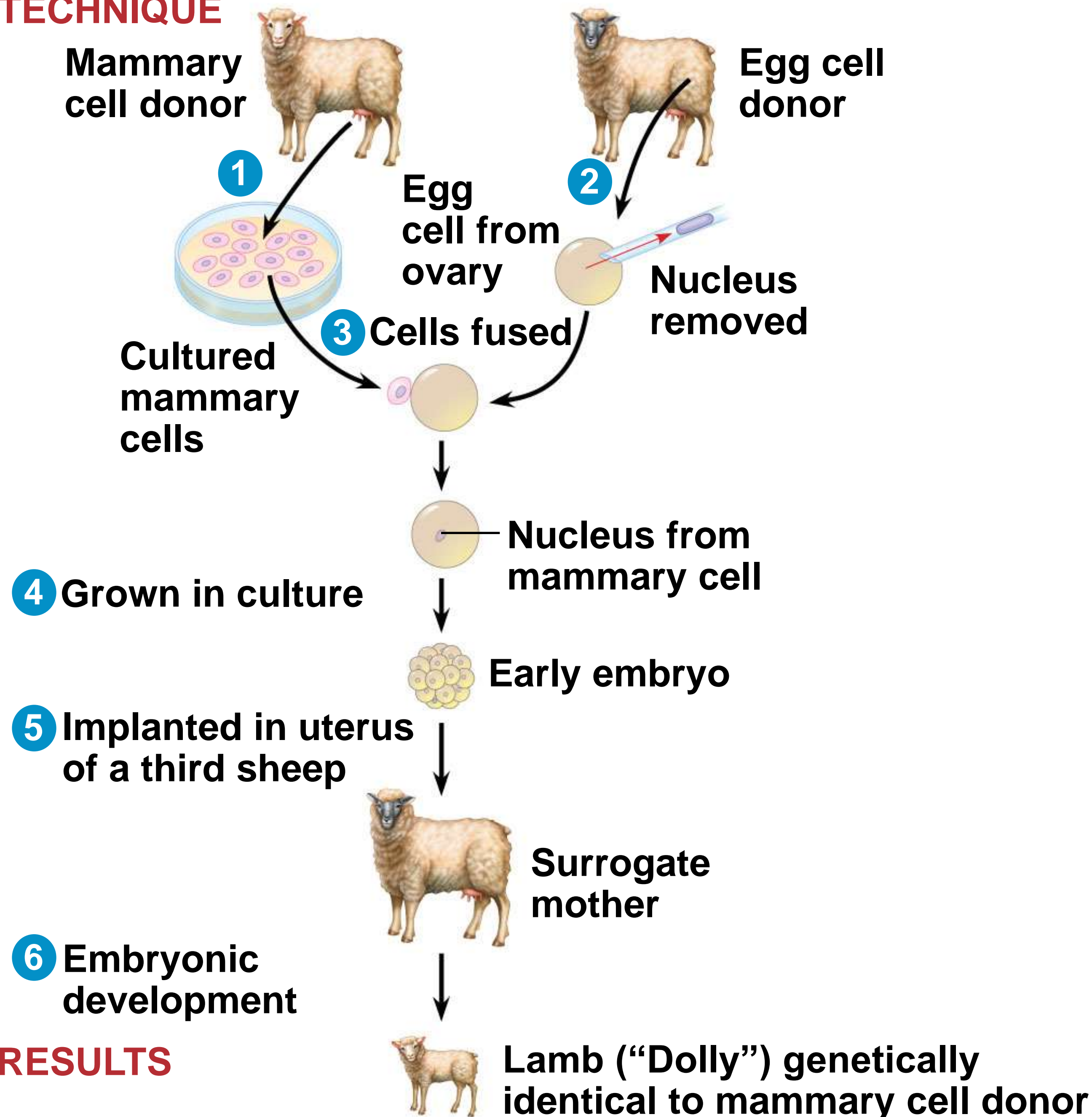
Figure 20.19



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- 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

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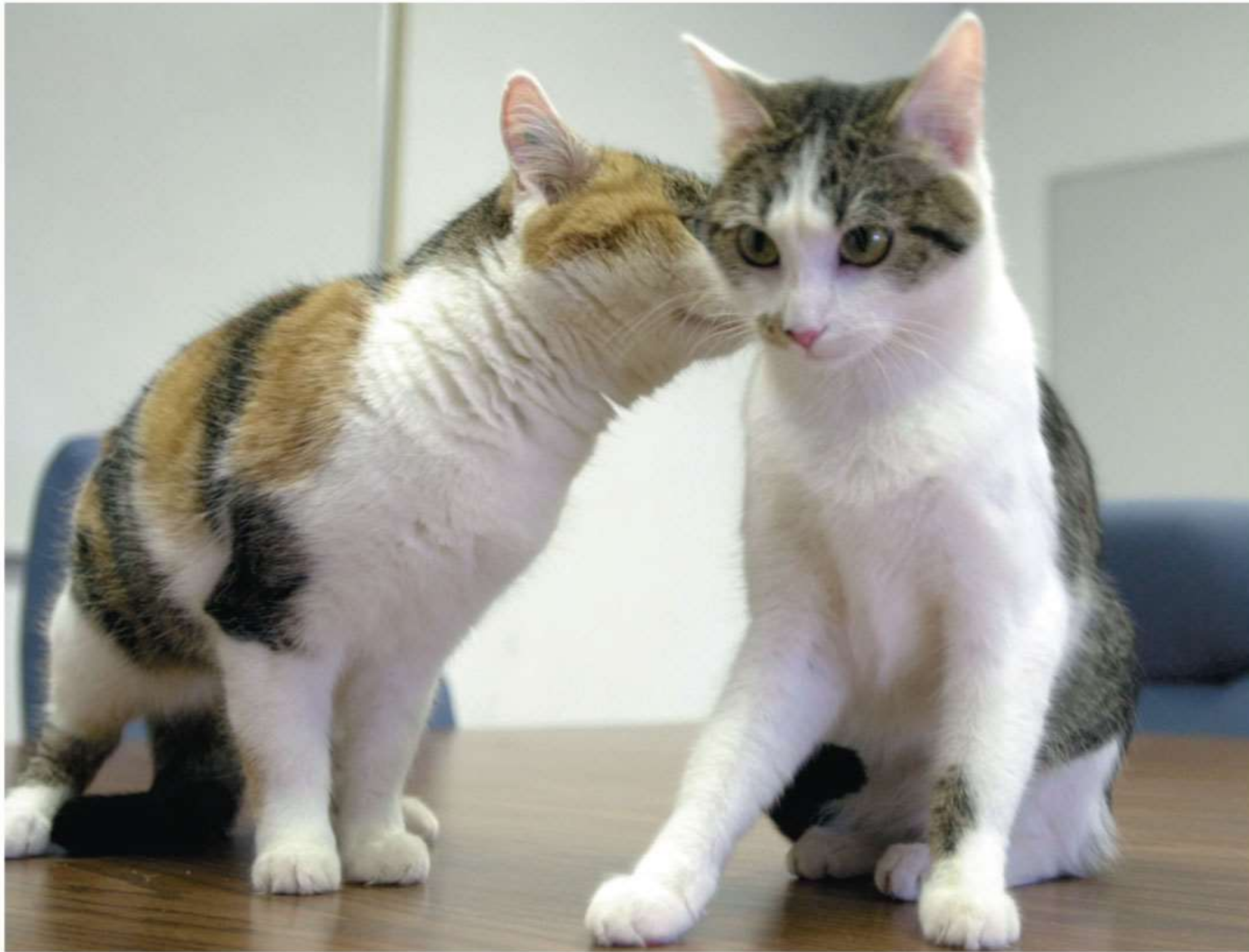
Figure 20.20



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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

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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

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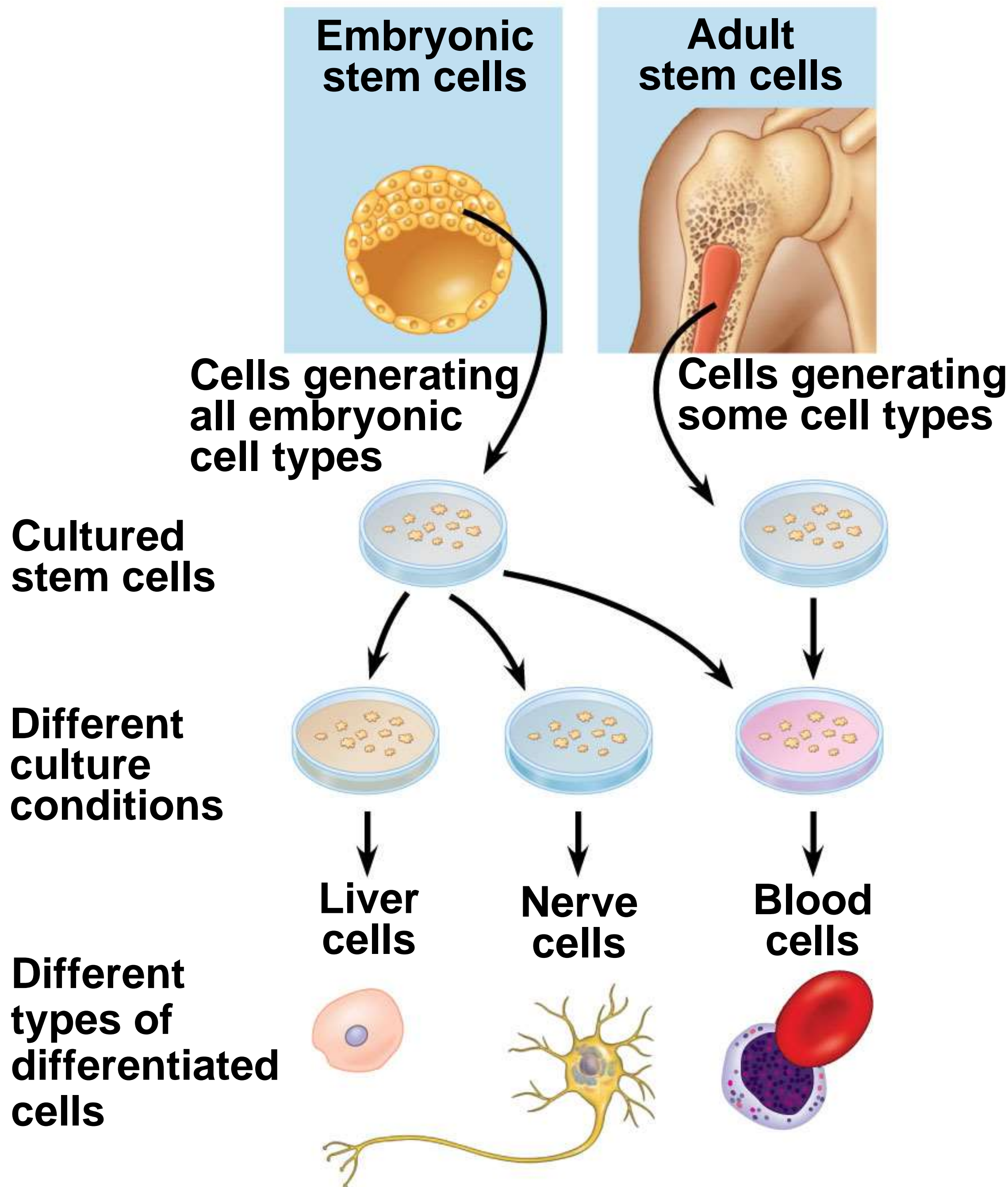
Figure 20.21



- 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

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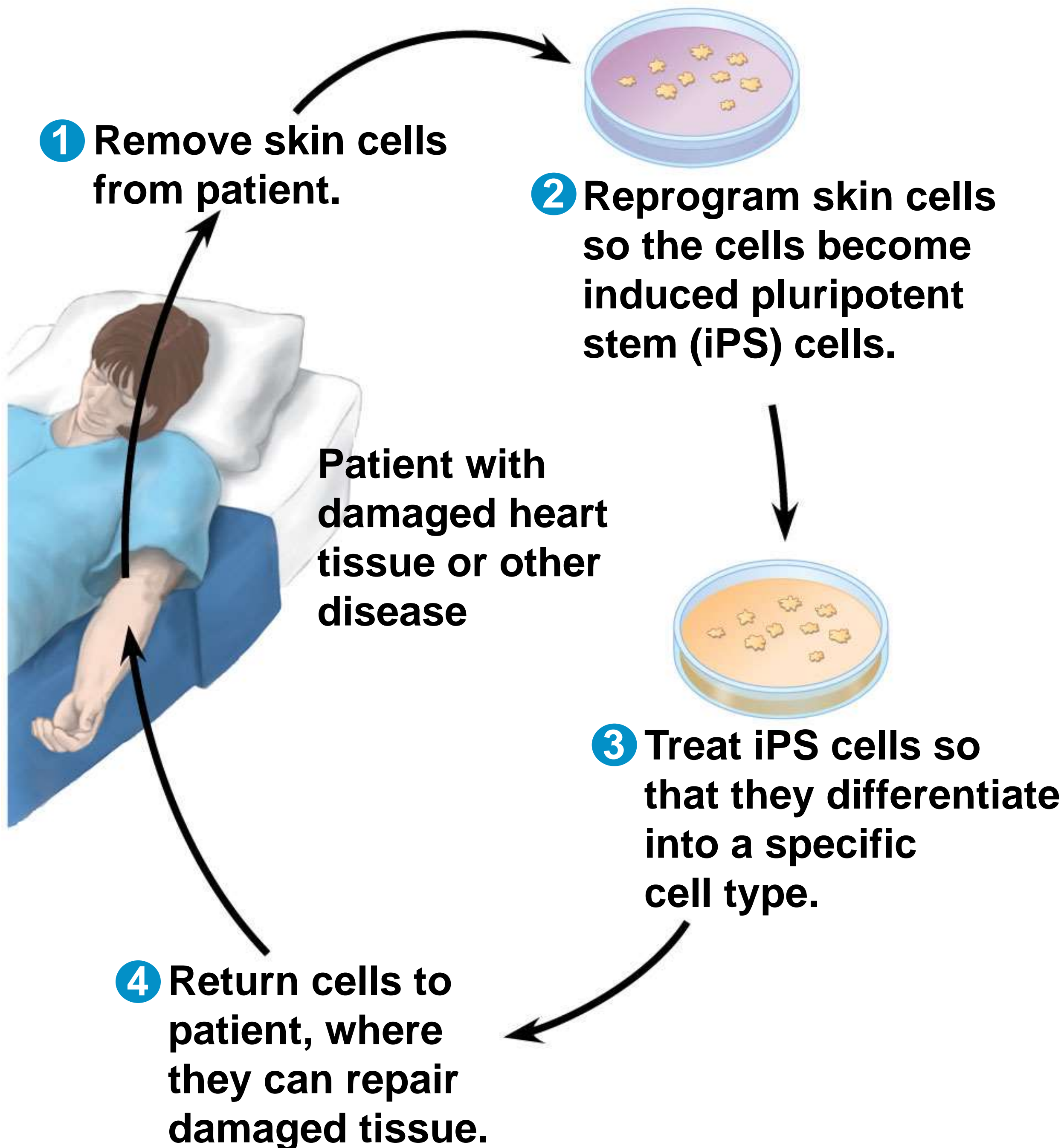
Figure 20.22



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

- Many fields benefit from DNA technology and genetic engineering

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Medical Applications

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

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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

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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

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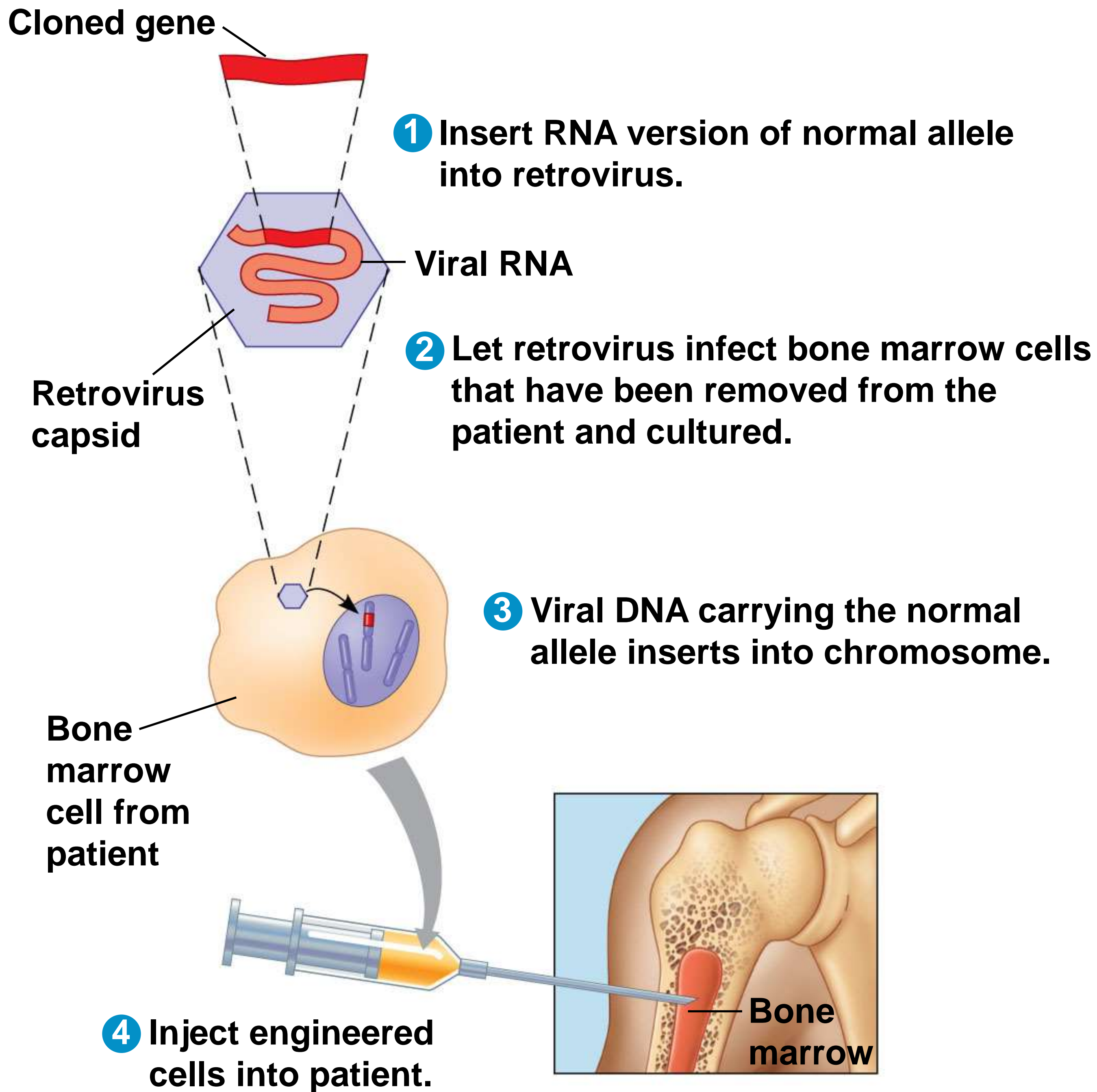
Figure 20.23



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Pharmaceutical Products

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

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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

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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

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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

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Figure 20.24

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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

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- 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

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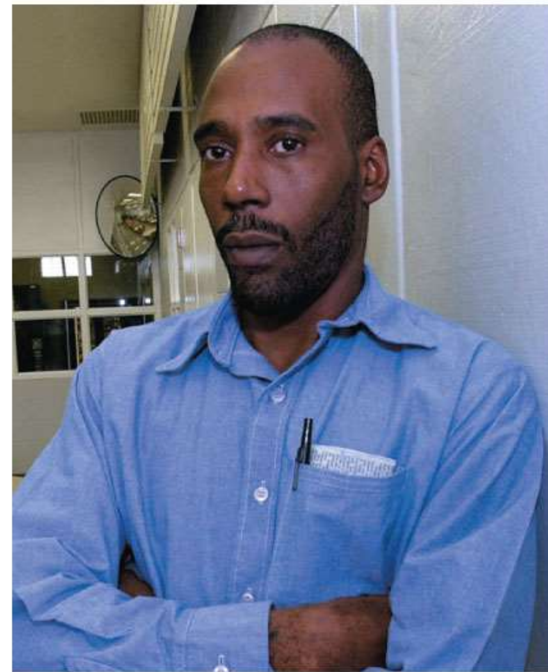
Figure 20.25

(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.

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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

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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

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- 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

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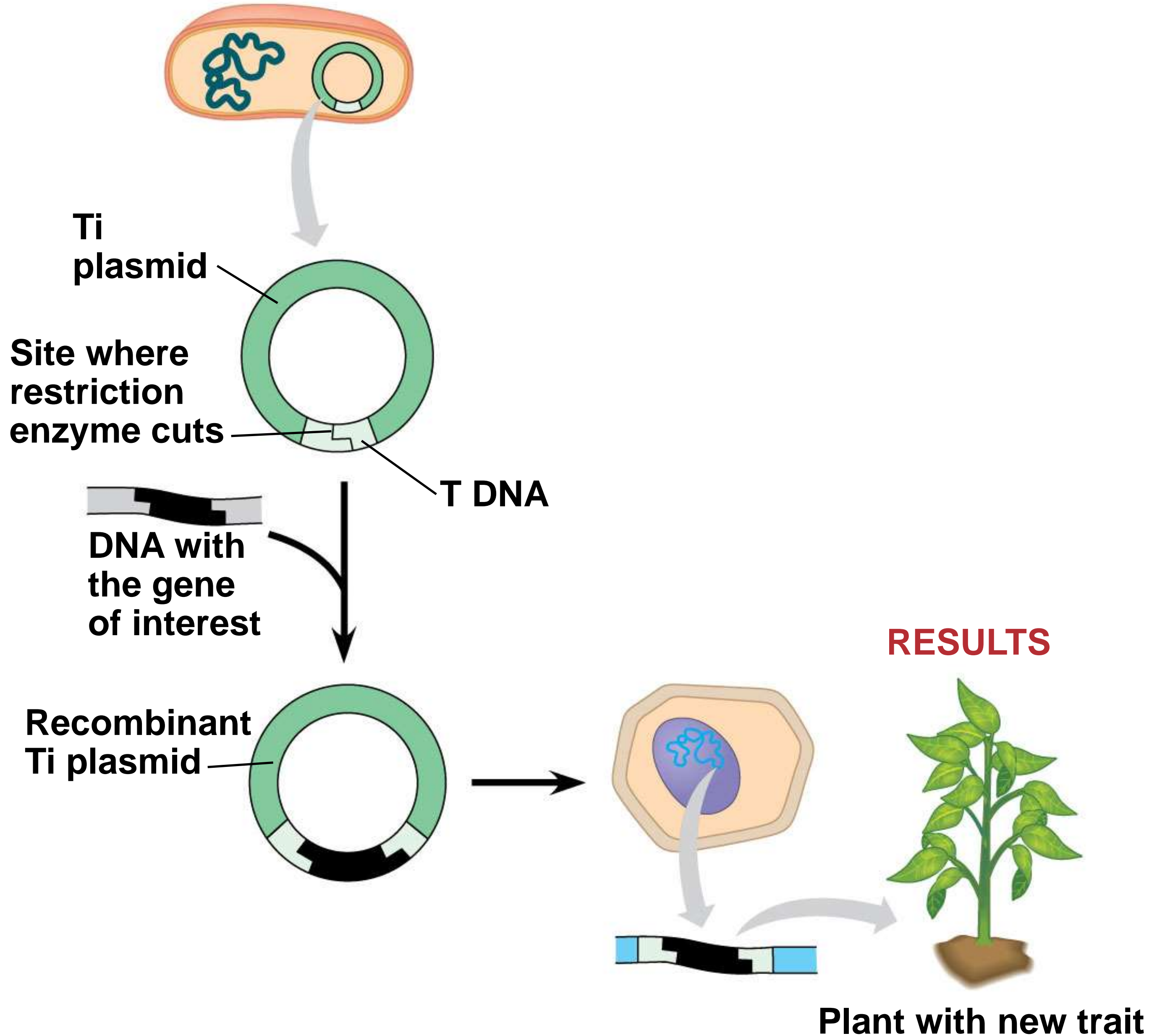
Figure 20.26



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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

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- 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

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- 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

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Figure 20.UN03

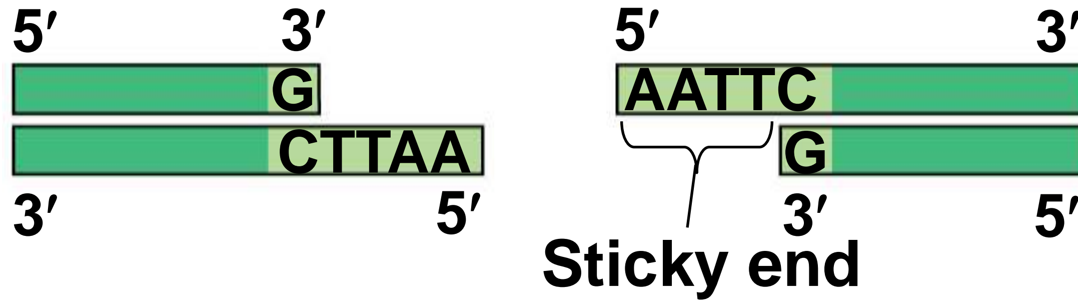

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Figure 20.UN04

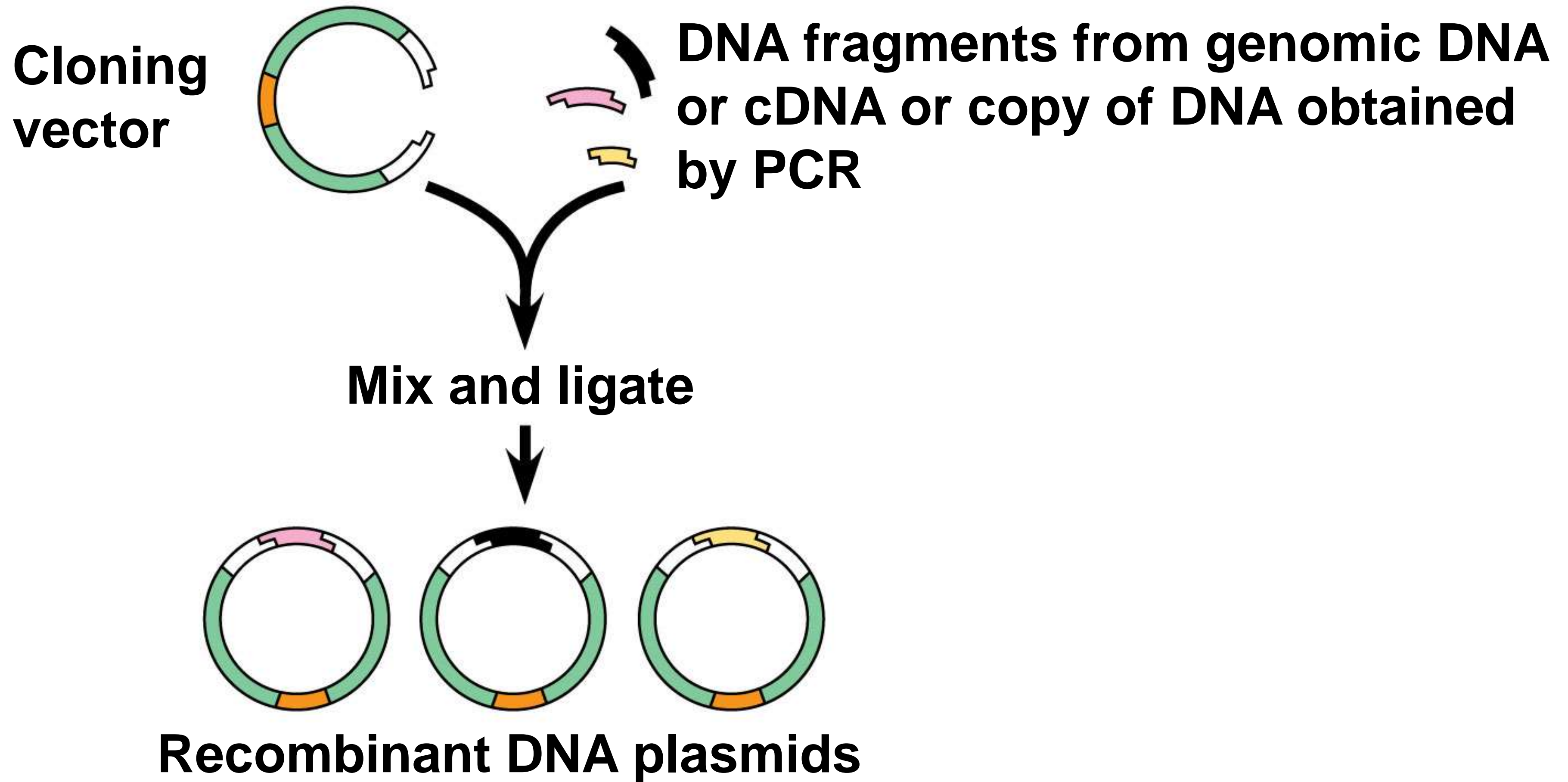

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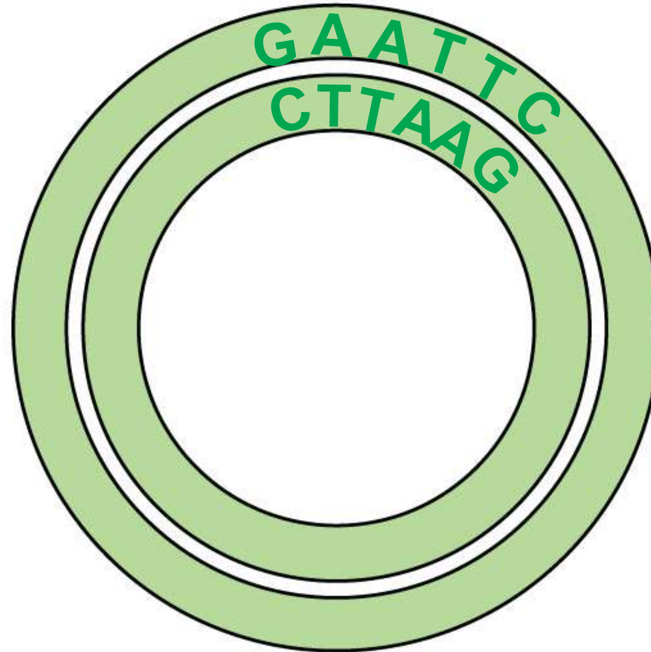
Figure 20.UN05

5′ TCCATGAATTCTAAAGCGCTTATGAATTCACGGC 3′

3′ AGGTACTTAAGATTTCGCGAATACTTAAGTGCCG 5′

Aardvark DNA



Plasmid

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Figure 20.UN06

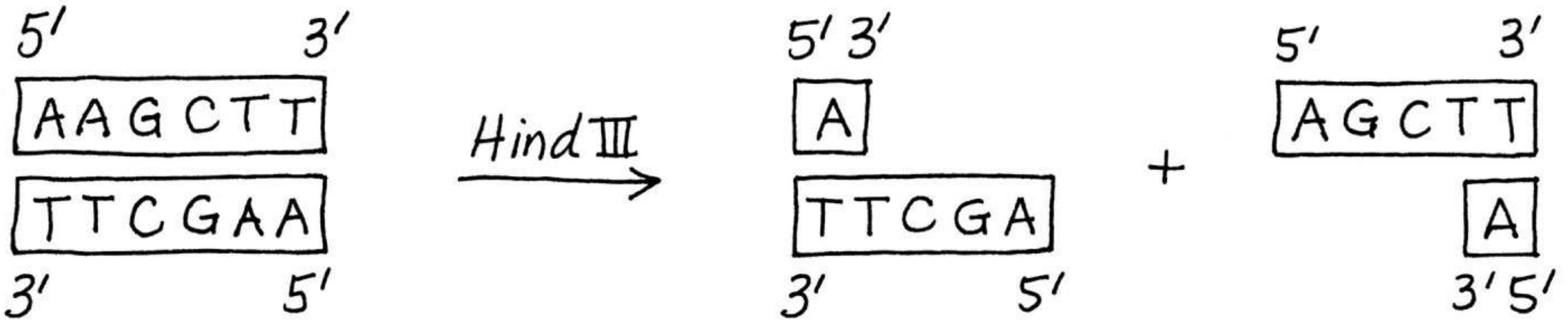

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Figure 20.UN07

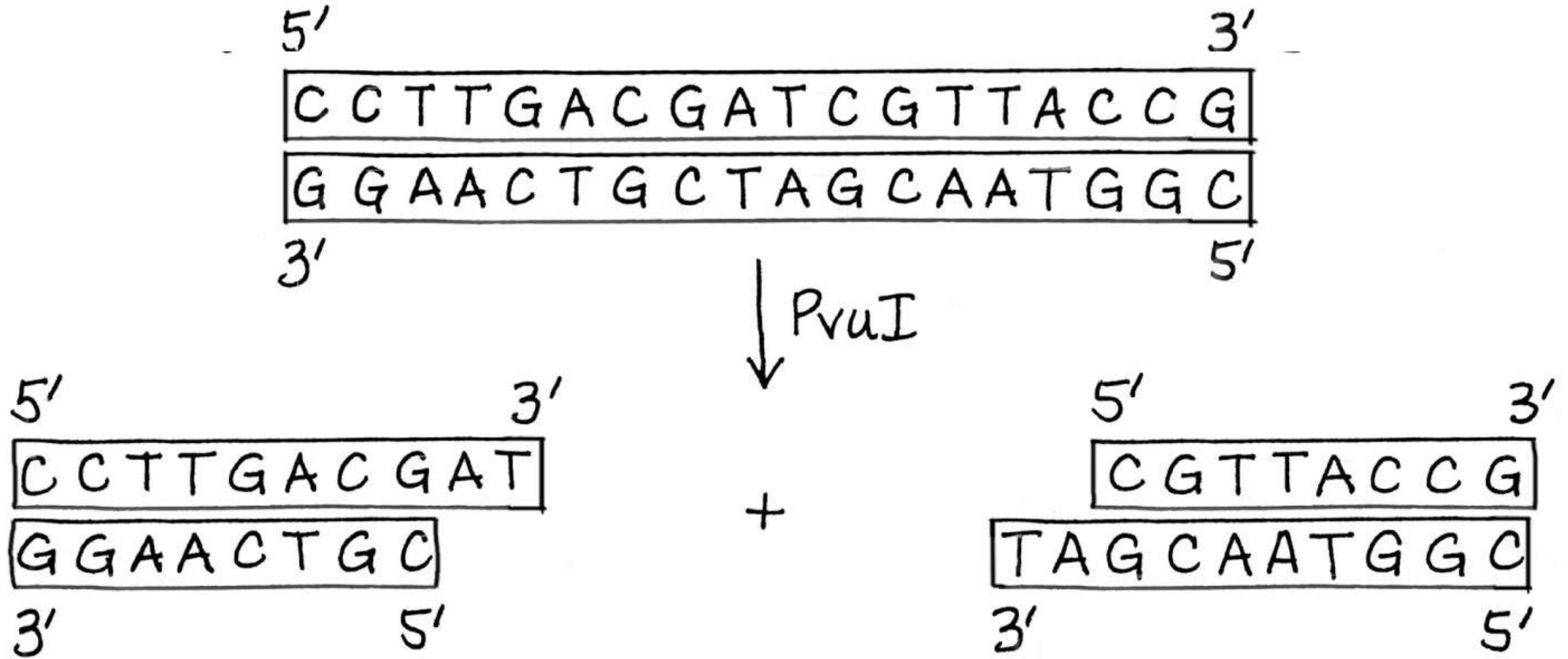

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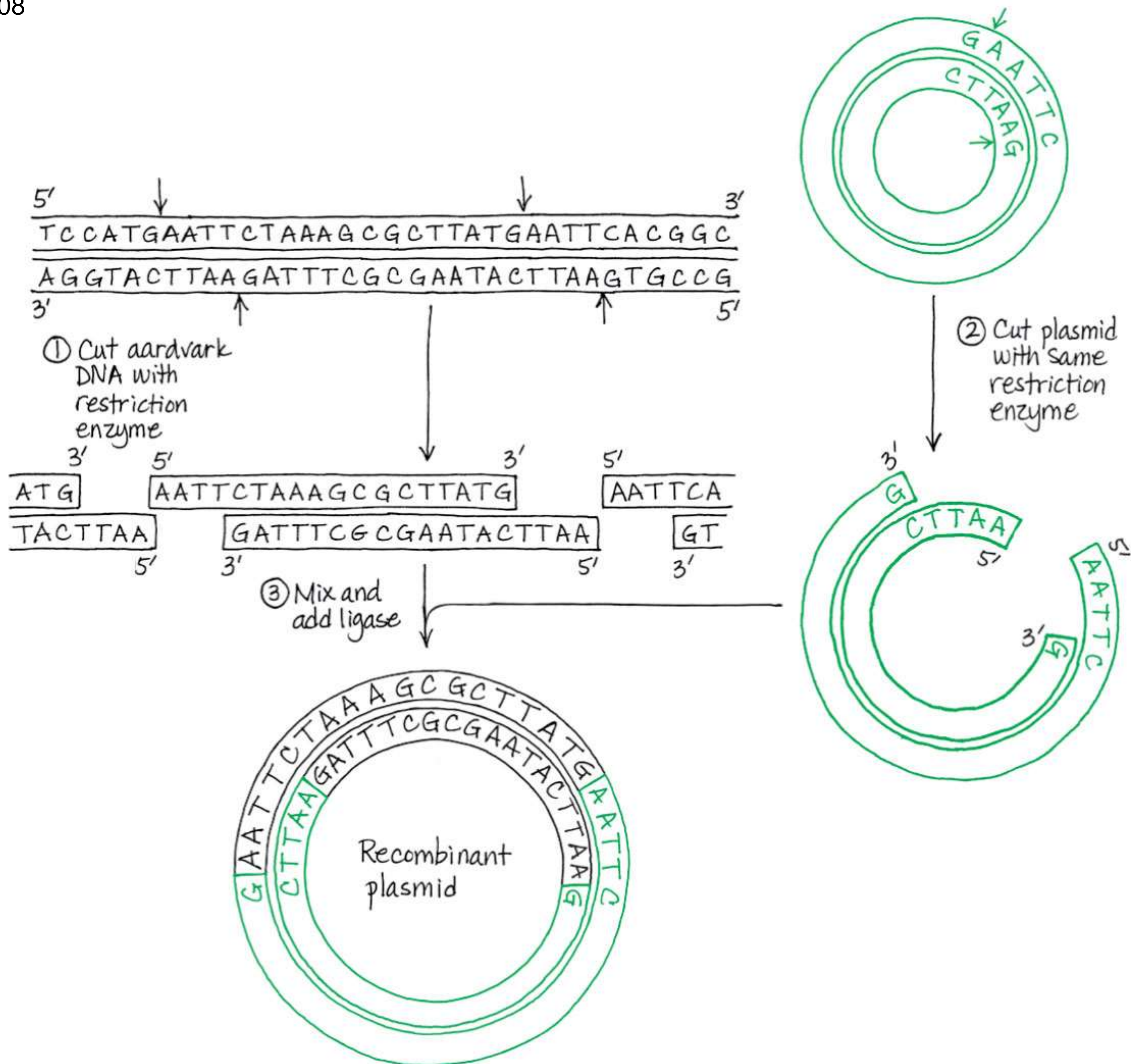
Figure 20.UN08



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CH 21 – Genome and Evolution

Overview: Reading the Leaves from the Tree of Life

- Complete genome sequences exist for a human, chimpanzee, *E. coli*, brewer's yeast, corn, fruit fly, house mouse, rhesus macaque, and other organisms
- Comparisons of genomes among organisms provide information about the evolutionary history of genes and taxonomic groups

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- **Genomics** is the study of whole sets of genes and their interactions
- **Bioinformatics** is the application of computational methods to the storage and analysis of biological data

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Figure 21.1

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Concept 21.1: New approaches have accelerated the pace of genome sequencing

- The most ambitious mapping project to date has been the sequencing of the human genome
- Officially begun as the **Human Genome Project** in 1990, the sequencing was largely completed by 2003
- The project had three stages
  - Genetic (or linkage) mapping
  - Physical mapping
  - DNA sequencing

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Three-Stage Approach to Genome Sequencing

- A **linkage map** (genetic map) maps the location of several thousand genetic markers on each chromosome
- A genetic marker is a gene or other identifiable DNA sequence
- Recombination frequencies are used to determine the order and relative distances between genetic markers

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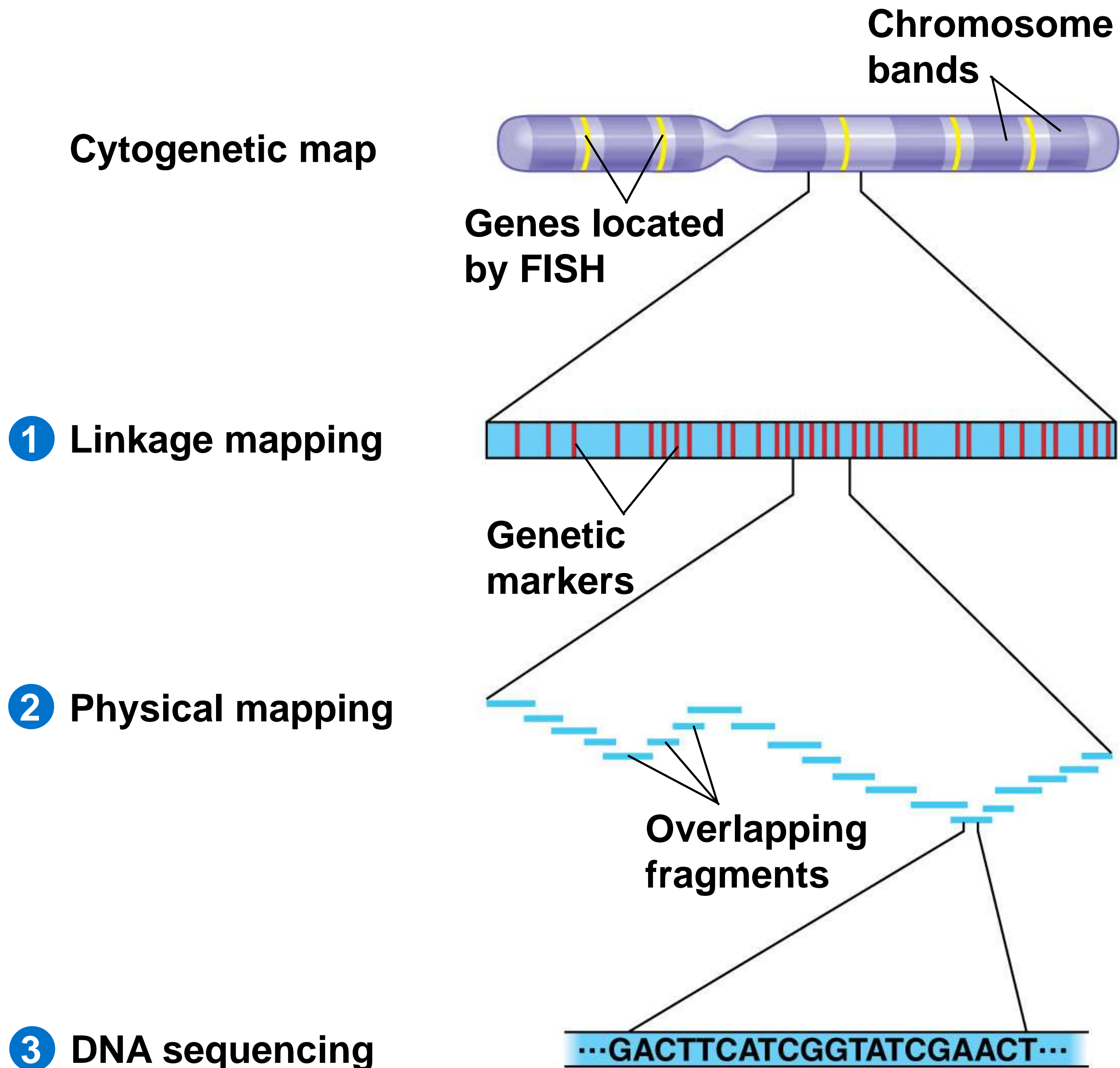
Figure 21.2-4



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- A **physical map** expresses the distance between genetic markers, usually as the number of base pairs along the DNA
- It is constructed by cutting a DNA molecule into many short fragments and arranging them in order by identifying overlaps

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- Sequencing machines are used to determine the complete nucleotide sequence of each chromosome
- A complete haploid set of human chromosomes consists of 3.2 billion base pairs

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Whole-Genome Shotgun Approach to Genome Sequencing

- The whole-genome shotgun approach was developed by J. Craig Venter in 1992
- This approach skips genetic and physical mapping and sequences random DNA fragments directly
- Powerful computer programs are used to order fragments into a continuous sequence

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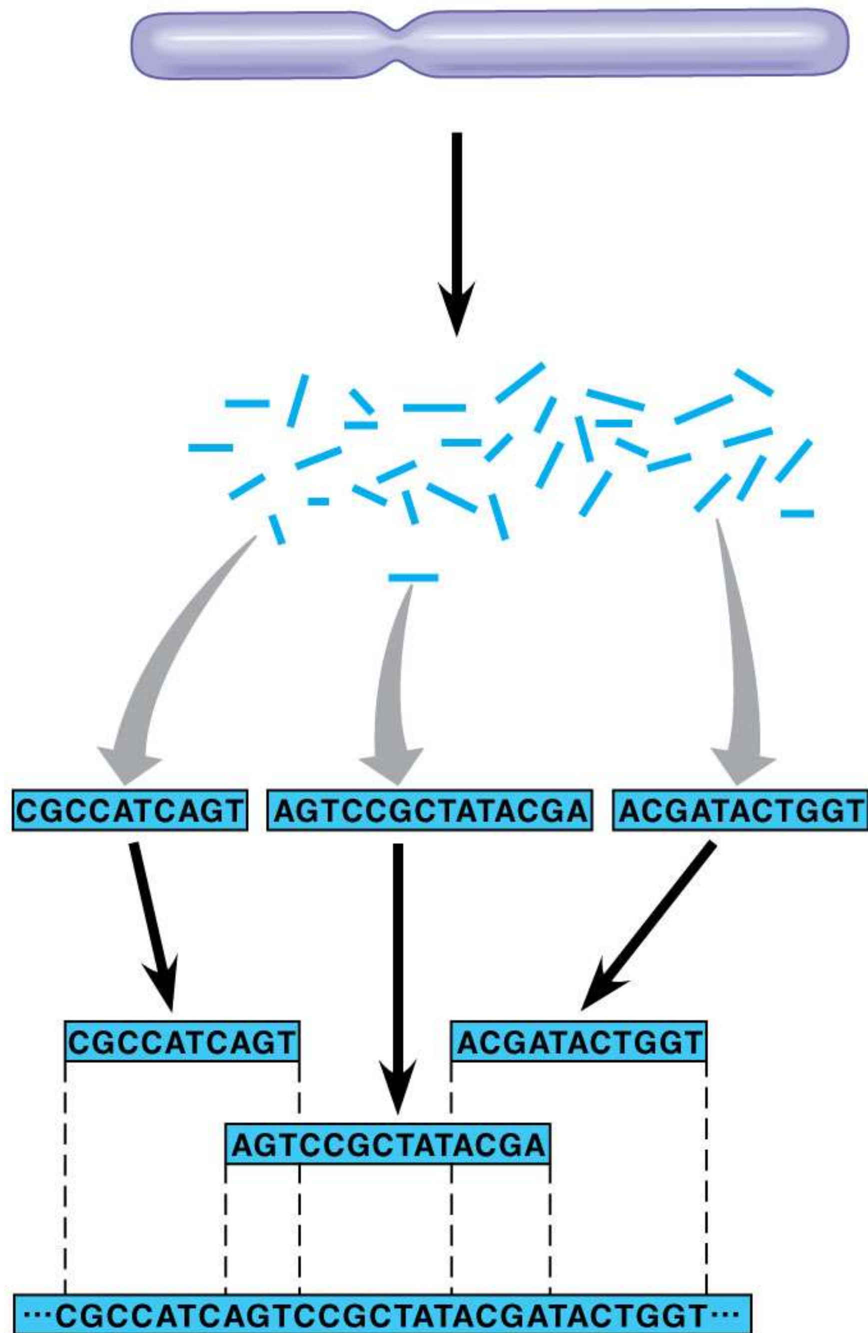
Figure 21.3-3

1 Cut the DNA into overlapping fragments short enough for sequencing.

2 Clone the fragments in plasmid or phage vectors.

3 Sequence each fragment.

4 Order the sequences into one overall sequence with computer software.



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- Both the three-stage process and the whole-genome shotgun approach were used for the Human Genome Project and for genome sequencing of other organisms
- At first many scientists were skeptical about the whole-genome shotgun approach, but it is now widely used as the sequencing method of choice
- The development of newer sequencing techniques has resulted in massive increases in speed and decreases in cost

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- Technological advances have also facilitated **metagenomics**, in which DNA from a group of species (a metagenome) is collected from an environmental sample and sequenced
- This technique has been used on microbial communities, allowing the sequencing of DNA of mixed populations, and eliminating the need to culture species in the lab

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Concept 21.2 Scientists use bioinformatics to analyze genomes and their functions

- The Human Genome Project established databases and refined analytical software to make data available on the Internet
- This has accelerated progress in DNA sequence analysis

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Concept 21.3 Genomes vary in size, number of genes, and gene density

- By early 2010, over 1,200 genomes were completely sequenced, including 1,000 bacteria, 80 archaea, and 124 eukaryotes
- Sequencing of over 5,500 genomes and over 200 metagenomes is currently in progress

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Genome Size

- Genomes of most bacteria and archaea range from 1 to 6 million base pairs (Mb); genomes of eukaryotes are usually larger
- Most plants and animals have genomes greater than 100 Mb; humans have 3,000 Mb
- Within each domain there is no systematic relationship between genome size and phenotype

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Table 21.1

Table 21.1 Genome Sizes and Estimated Numbers of Genes*

Organism	Haploid Genome Size (Mb)	Number of Genes	Genes per Mb
Bacteria			
Haemophilus influenzae	1.8	1,700	940
Escherichia coli	4.6	4,400	950
Archaea			
Archaeoglobus fulgidus	2.2	2,500	1,130
Methanosarcina barkeri	4.8	3,600	750
Eukaryotes			
Saccharomyces cerevisiae (yeast, a fungus)	12	6,300	525
Caenorhabditis elegans (nematode)	100	20,100	200
Arabidopsis thaliana (mustard family plant)	120	27,000	225
Drosophila melanogaster (fruit fly)	165	13,700	83
Oryza sativa (rice)	430	42,000	98
Zea mays (corn)	2,300	32,000	14
Mus musculus (house mouse)	2,600	22,000	11
Ailuropoda melanoleuca (giant panda)	2,400	21,000	9
Homo sapiens (human)	3,000	<21,000	7
Fritillaria assyriaca (lily family plant)	124,000	ND	ND

*Some values given here are likely to be revised as genome analysis continues. Mb = million base pairs. ND = not determined.

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Number of Genes

- Free-living bacteria and archaea have 1,500 to 7,500 genes
- Unicellular fungi have from about 5,000 genes and multicellular eukaryotes up to at least 40,000 genes

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- Number of genes is not correlated to genome size
- For example, it is estimated that the nematode *C. elegans* has 100 Mb and 20,000 genes, while *Drosophila* has 165 Mb and 13,700 genes
- Vertebrate genomes can produce more than one polypeptide per gene because of alternative splicing of RNA transcripts

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Gene Density and Noncoding DNA

- Humans and other mammals have the lowest gene density, or number of genes, in a given length of DNA
- Multicellular eukaryotes have many introns within genes and noncoding DNA between genes

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Concept 21.4: Multicellular eukaryotes have much noncoding DNA and many multigene families

- The bulk of most eukaryotic genomes neither encodes proteins nor functional RNAs
- Much evidence indicates that noncoding DNA (previously called “junk DNA”) plays important roles in the cell
- For example, genomes of humans, rats, and mice show high sequence conservation for about 500 noncoding regions

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- Sequencing of the human genome reveals that 98.5% does not code for proteins, rRNAs, or tRNAs
- About a quarter of the human genome codes for introns and gene-related regulatory sequences

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- Intergenic DNA is noncoding DNA found between genes
  - **Pseudogenes** are former genes that have accumulated mutations and are nonfunctional
  - **Repetitive DNA** is present in multiple copies in the genome
- About three-fourths of repetitive DNA is made up of transposable elements and sequences related to them

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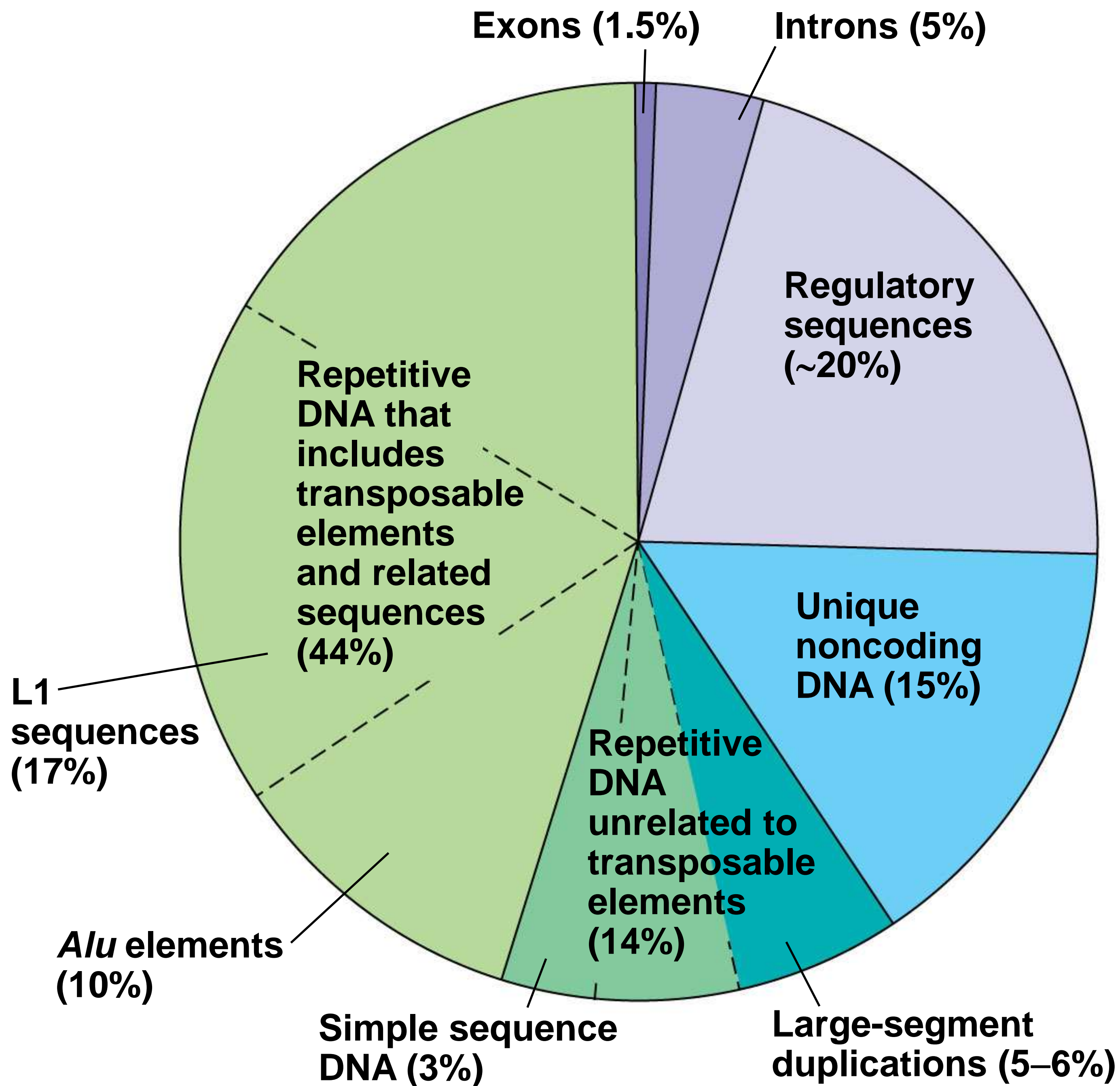
Figure 21.7



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Transposable Elements and Related Sequences

- The first evidence for mobile DNA segments came from geneticist Barbara McClintock's breeding experiments with Indian corn
- McClintock identified changes in the color of corn kernels that made sense only by postulating that some genetic elements move from other genome locations into the genes for kernel color
- These **transposable elements** move from one site to another in a cell's DNA; they are present in both prokaryotes and eukaryotes

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Figure 21.8

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Movement of Transposons and Retrotransposons

- Eukaryotic transposable elements are of two types
  - **Transposons**, which move by means of a DNA intermediate
  - **Retrotransposons**, which move by means of an RNA intermediate

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Figure 21.9

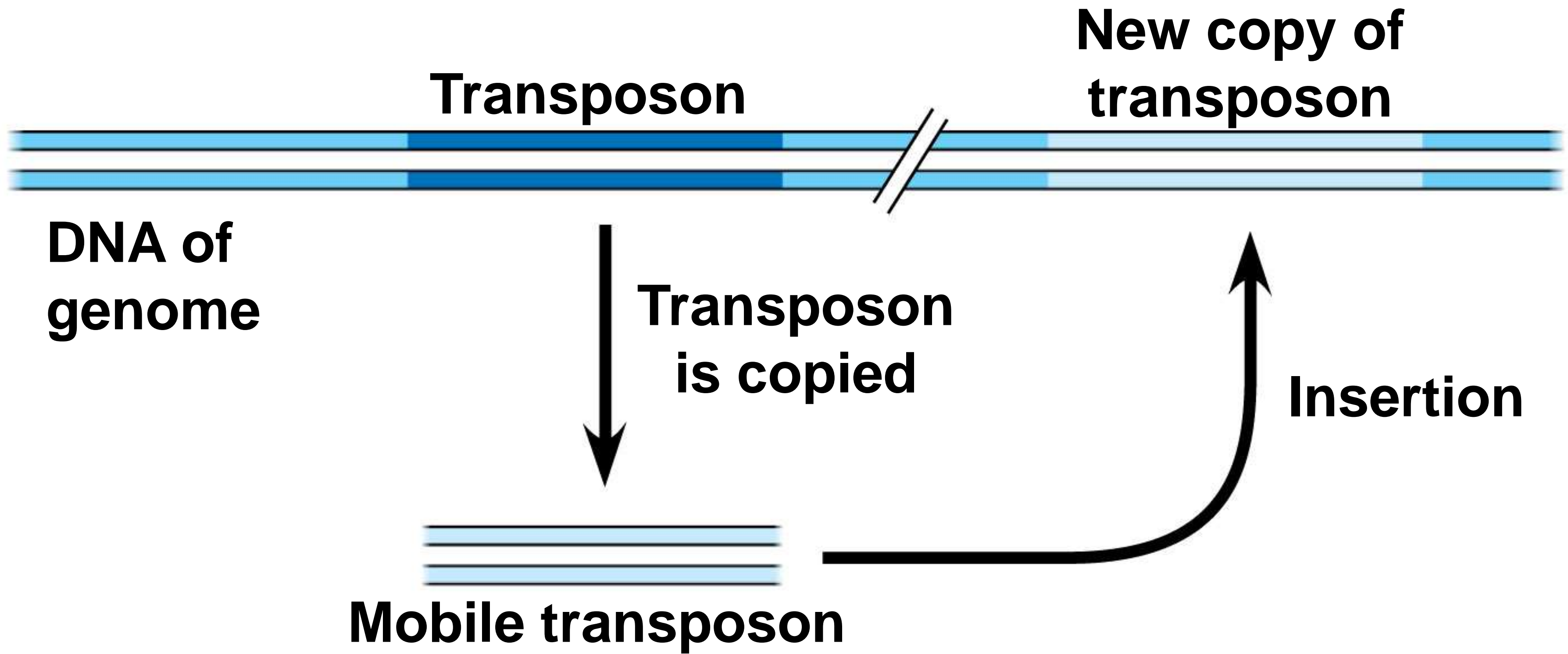

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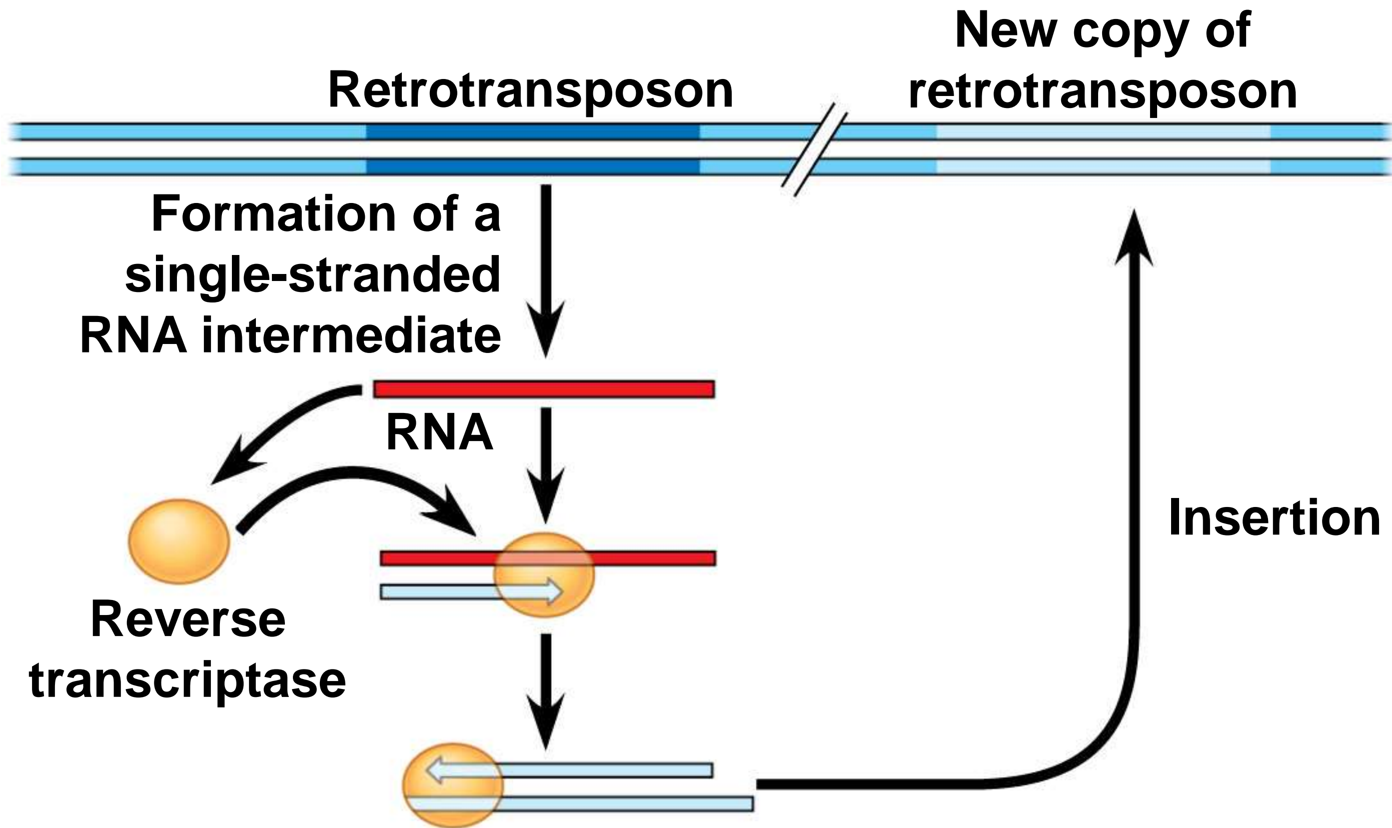
Figure 21.10



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Sequences Related to Transposable Elements

- Multiple copies of transposable elements and related sequences are scattered throughout the eukaryotic genome
- In primates, a large portion of transposable element–related DNA consists of a family of similar sequences called *Alu elements*
- Many *Alu* elements are transcribed into RNA molecules; however their function, if any, is unknown

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- The human genome also contains many sequences of a type of retrotransposon called *LINE-1* (*L1*)
- L1 sequences have a low rate of transposition and may help regulate gene expression

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Other Repetitive DNA, Including Simple Sequence DNA

- About 15% of the human genome consists of duplication of long sequences of DNA from one location to another
- In contrast, **simple sequence DNA** contains many copies of tandemly repeated short sequences

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- A series of repeating units of 2 to 5 nucleotides is called a **short tandem repeat** (**STR**)
- The repeat number for STRs can vary among sites (within a genome) or individuals
- Simple sequence DNA is common in centromeres and telomeres, where it probably plays structural roles in the chromosome

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CH 18 – Gene Regulation

Overview: Conducting the Genetic Orchestra

- Prokaryotes and eukaryotes alter gene expression in response to their changing environment
- In multicellular eukaryotes, gene expression regulates development and is responsible for differences in cell types
- RNA molecules play many roles in regulating gene expression in eukaryotes

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Concept 18.1: Bacteria often respond to environmental change by regulating transcription

- Natural selection has favored bacteria that produce only the products needed by that cell
- A cell can regulate the production of enzymes by feedback inhibition or by gene regulation
- Gene expression in bacteria is controlled by the operon model

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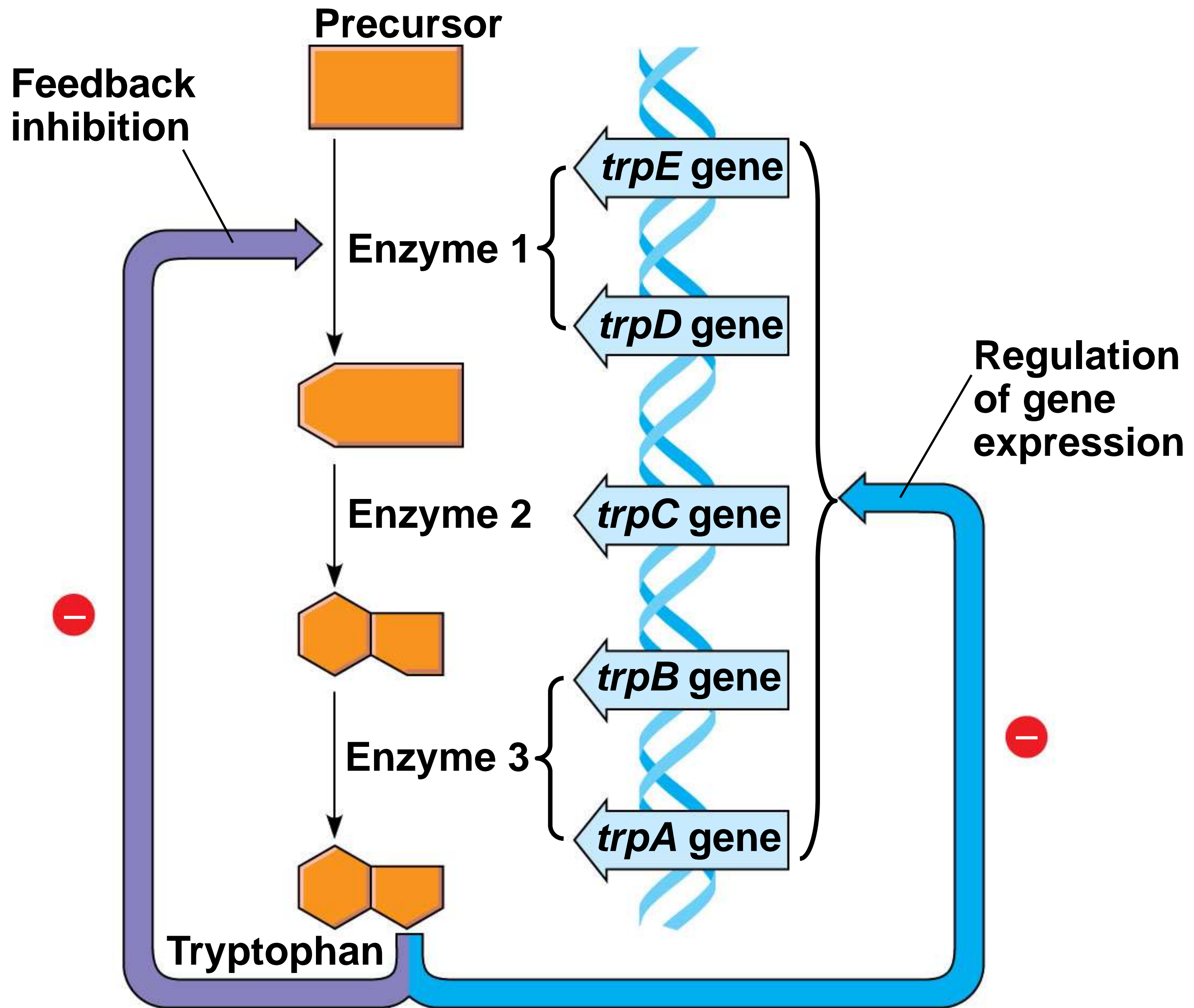
Figure 18.2



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Operons: The Basic Concept

- A cluster of functionally related genes can be under coordinated control by a single "on-off switch"
- The regulatory "switch" is a segment of DNA called an **operator** usually positioned within the promoter
- An **operon** is the entire stretch of DNA that includes the operator, the promoter, and the genes that they control

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- The operon can be switched off by a protein **repressor**
- The repressor prevents gene transcription by binding to the operator and blocking RNA polymerase
- The repressor is the product of a separate **regulatory gene**

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- The repressor can be in an active or inactive form, depending on the presence of other molecules
- A **corepressor** is a molecule that cooperates with a repressor protein to switch an operon off
- For example, *E. coli* can synthesize the amino acid tryptophan

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- By default the *trp* operon is on and the genes for tryptophan synthesis are transcribed
- When tryptophan is present, it binds to the *trp* repressor protein, which turns the operon off
- The repressor is active only in the presence of its corepressor tryptophan; thus the *trp* operon is turned off (repressed) if tryptophan levels are high

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Figure 18.3

trp operon

Promoter

Promoter

Genes of operon

DNA

trpR

trpE *trpD* *trpC* *trpB* *trpA*

Operator

Regulatory gene

RNA polymerase

Start codon

Stop codon

3′

mRNA

5′

mRNA 5′

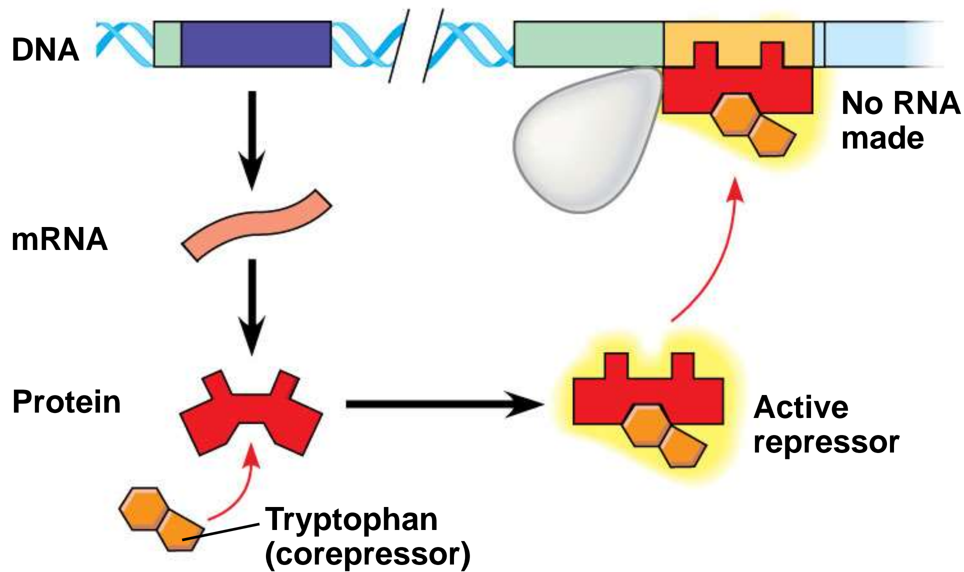
E D C B A

Protein

Inactive repressor

Polypeptide subunits that make up enzymes for tryptophan synthesis

(a) Tryptophan absent, repressor inactive, operon on



(b) Tryptophan present, repressor active, operon off

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Repressible and Inducible Operons: Two Types of Negative Gene Regulation

- A repressible operon is one that is usually on; binding of a repressor to the operator shuts off transcription
- The *trp* operon is a repressible operon
- An inducible operon is one that is usually off; a molecule called an inducer inactivates the repressor and turns on transcription

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- The *lac* operon is an inducible operon and contains genes that code for enzymes used in the hydrolysis and metabolism of lactose
- By itself, the *lac* repressor is active and switches the *lac* operon off
- A molecule called an **inducer** inactivates the repressor to turn the *lac* operon on

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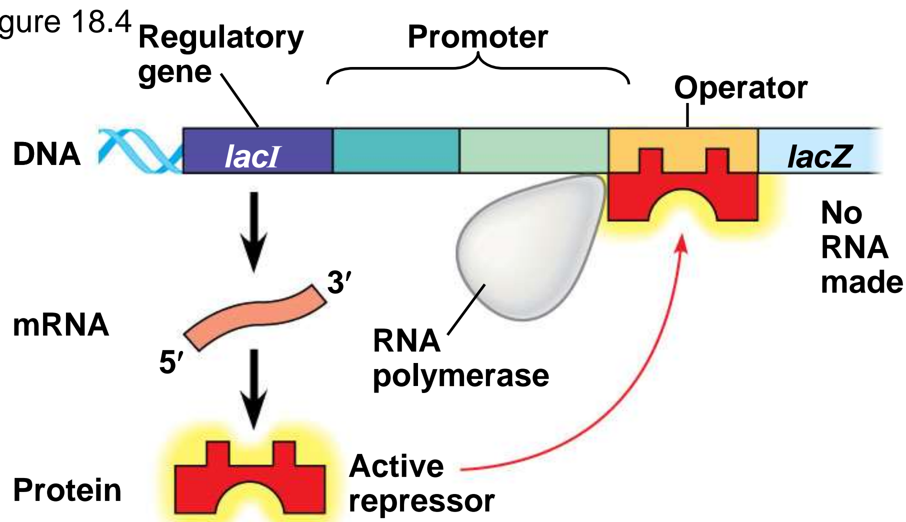
Figure 18.4



(a) Lactose absent, repressor active, operon off

lac operon
DNA
lacI
lacZ
lacY
lacA
RNA polymerase
mRNA
3′
5′
mRNA 5′
Protein
Allolactose (inducer)
Inactive repressor
β-Galactosidase
Permease
Transacetylase

(b) Lactose present, repressor inactive, operon on

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- Inducible enzymes usually function in catabolic pathways; their synthesis is induced by a chemical signal
- Repressible enzymes usually function in anabolic pathways; their synthesis is repressed by high levels of the end product
- Regulation of the *trp* and *lac* operons involves negative control of genes because operons are switched off by the active form of the repressor

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Positive Gene Regulation

- Some operons are also subject to positive control through a stimulatory protein, such as catabolite activator protein (CAP), an **activator** of transcription
- When glucose (a preferred food source of *E. coli*) is scarce, CAP is activated by binding with **cyclic AMP (cAMP)**
- Activated CAP attaches to the promoter of the *lac* operon and increases the affinity of RNA polymerase, thus accelerating transcription

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- When glucose levels increase, CAP detaches from the *lac* operon, and transcription returns to a normal rate
- CAP helps regulate other operons that encode enzymes used in catabolic pathways

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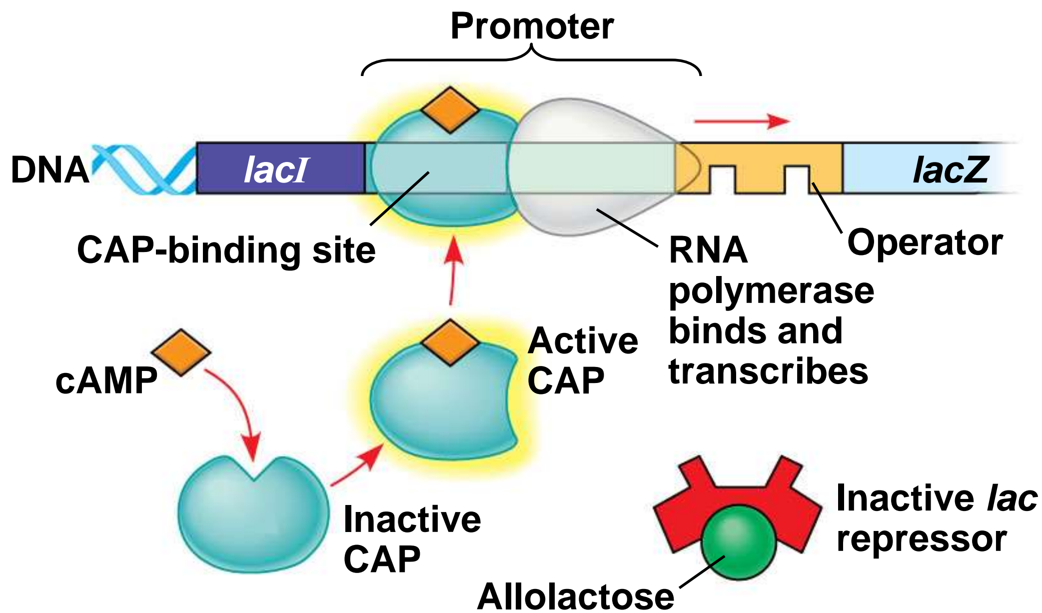
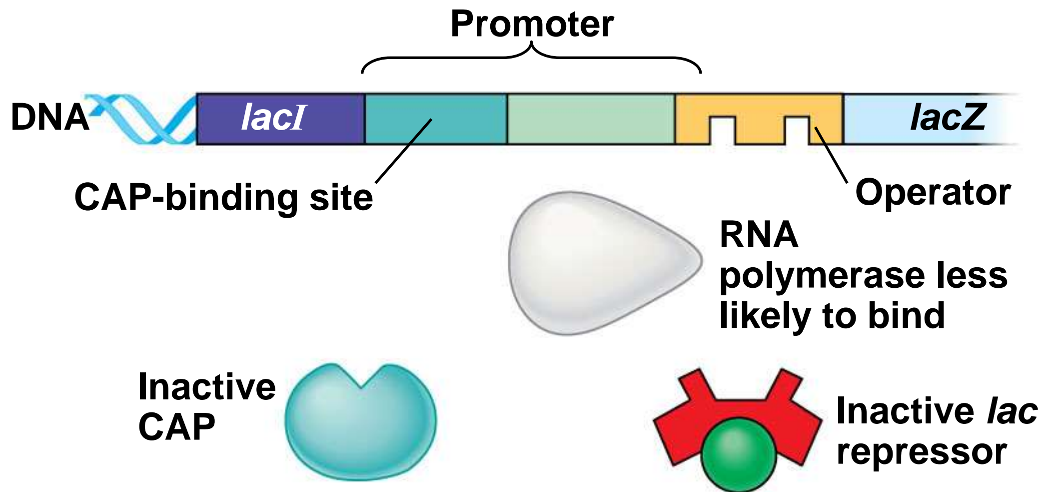
Figure 18.5



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized



(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

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