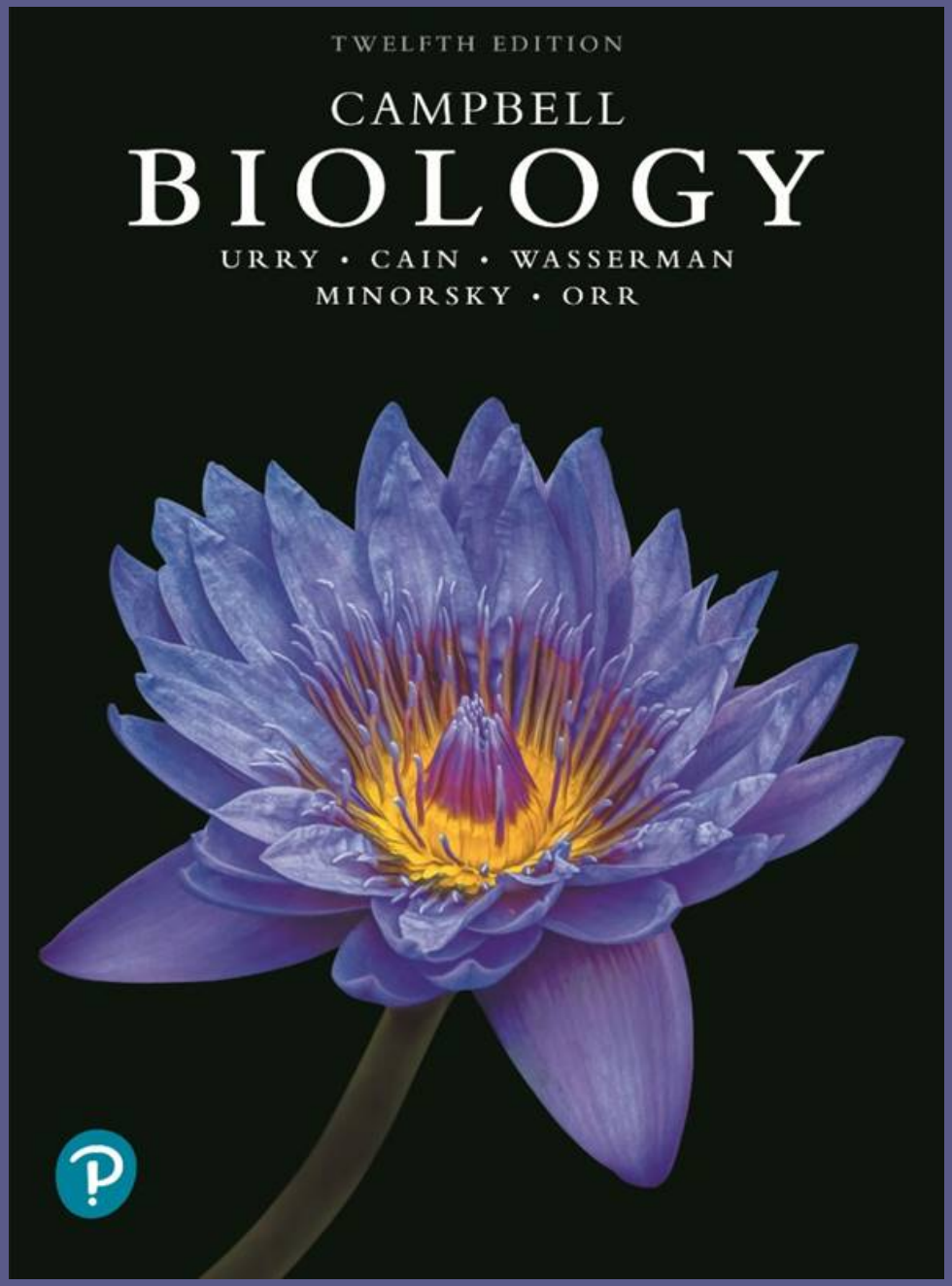


Chapter 20

DNA Tools and Biotechnology

Lecture Presentations by
Nicole Tunbridge and
Kathleen Fitzpatrick

Figure 20.1a

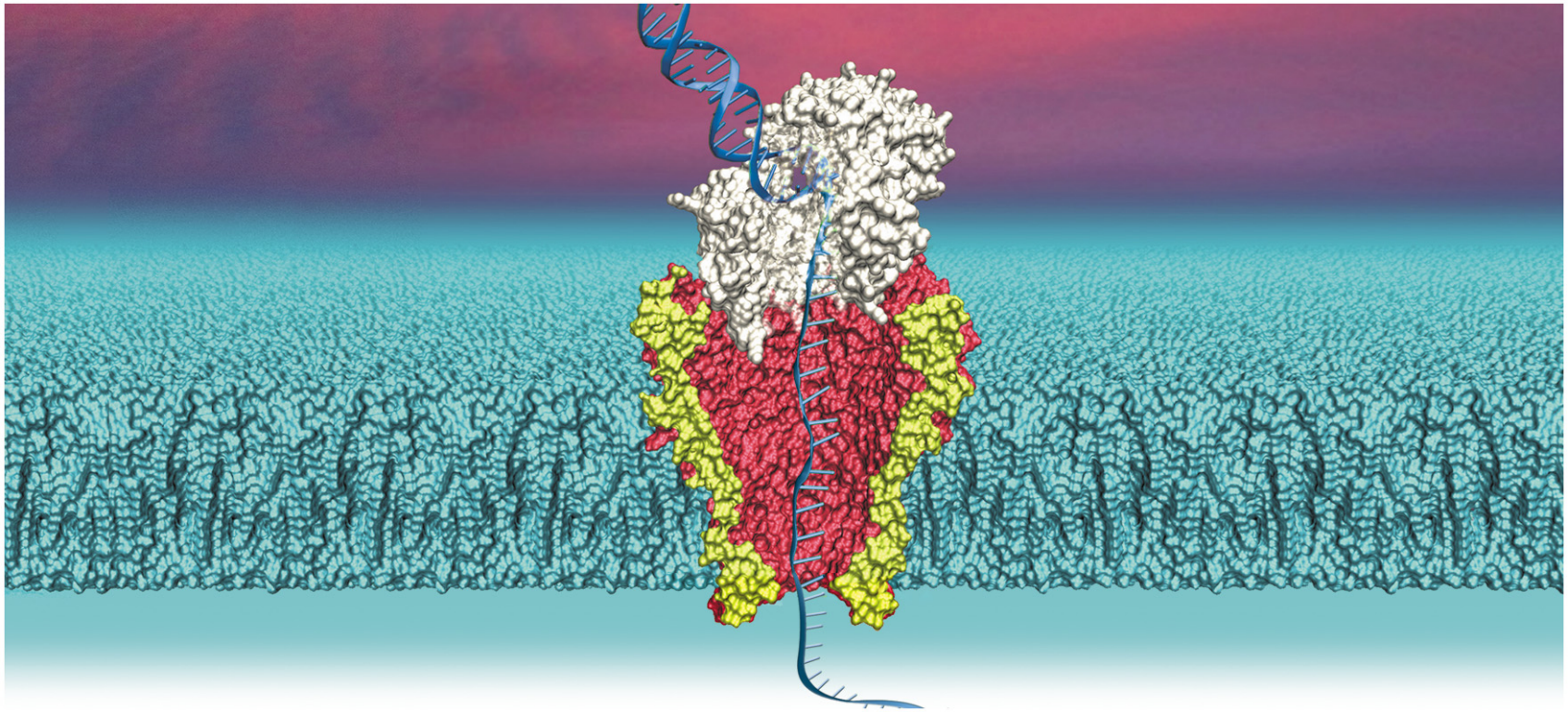
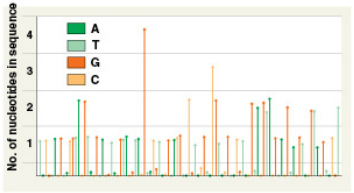


Figure 20.1b

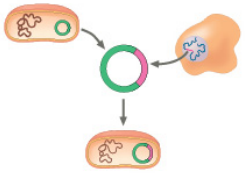
What are the main techniques and applications of biotechnology?

TECHNIQUES

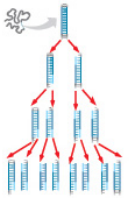
DNA sequencing



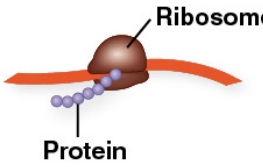
Gene cloning



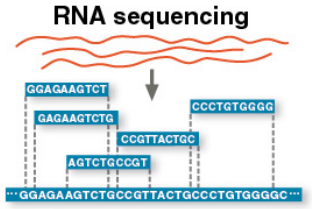
PCR amplification



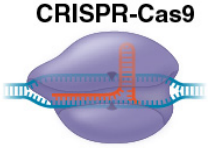
Expressing genes



Analyzing gene expression



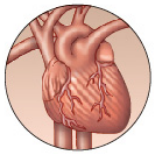
Gene editing



APPLICATIONS

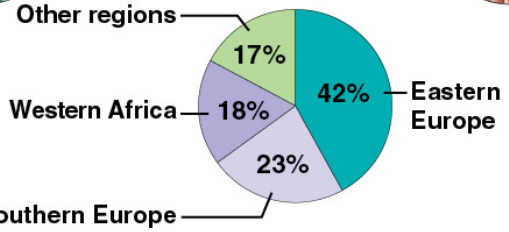
Agriculture

Medicine



Environmental cleanup

Ancestry



Forensics

CONCEPT 20.1: DNA sequencing and DNA cloning are valuable tools for genetic engineering and biological inquiry

- The main technologies for sequencing and manipulating DNA are called **DNA technology**
- The complementarity of the two DNA strands is the basis for **nucleic acid hybridization**, the base pairing of one strand of nucleic acid to the complementary sequence on another strand
- **Genetic engineering** is the direct manipulation of genes for practical purposes

DNA Sequencing

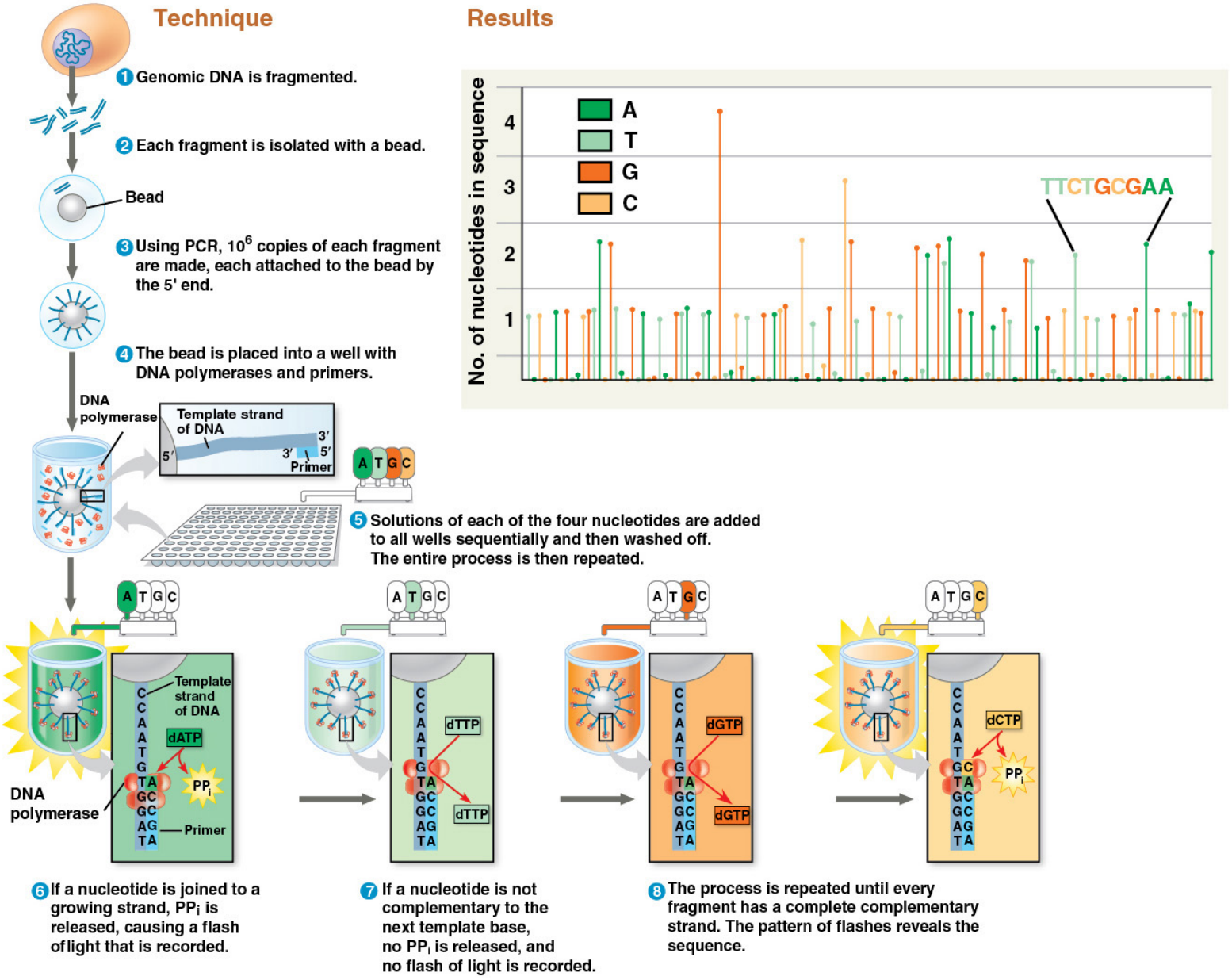
- A gene's complete nucleotide sequence can be determined using a process called **DNA sequencing**
- The first automated procedure was based on a technique called dideoxy or chain termination sequencing, developed by Frederick Sanger
- In the first decade of this century, "next-generation sequencing" techniques have been developed that are rapid and inexpensive

Figure 20.2

Video: Sanger Method of DNA Sequencing

- In sequencing by synthesis, many DNA fragments are copied to produce an enormous number of identical fragments
- A single strand of each fragment is immobilized and the complementary strand synthesized one nucleotide at a time
- Thousands or hundreds of thousands of fragments about 300 nucleotides long can be sequenced in parallel
- This is an example of “high-throughput” technology

Figure 20.3

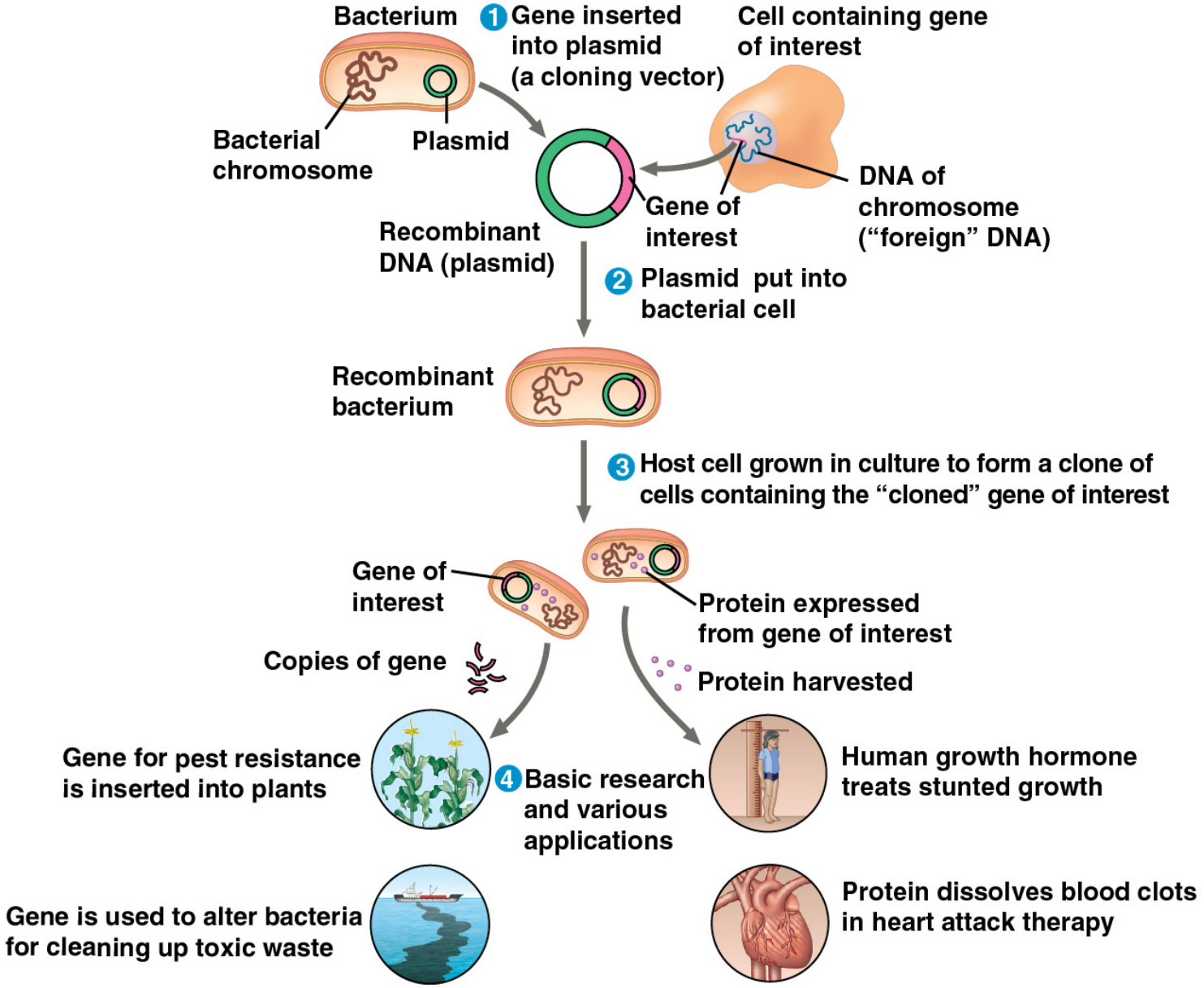

Technique
Results
1 Genomic DNA is fragmented.
2 Each fragment is isolated with a bead.
Bead
3 Using PCR, 10^6 copies of each fragment are made, each attached to the bead by the 5' end.
4 The bead is placed into a well with DNA polymerases and primers.
DNA polymerase
Template strand of DNA
5′
3′
3′
5′
Primer
A T G C
5 Solutions of each of the four nucleotides are added to all wells sequentially and then washed off. The entire process is then repeated.
Template strand of DNA
dATP
PPi
DNA polymerase
Primer
dTTP
dTTP
dGTP
dGTP
dCTP
PPi
6 If a nucleotide is joined to a growing strand, PPi is released, causing a flash of light that is recorded.
7 If a nucleotide is not complementary to the next template base, no PPi is released, and no flash of light is recorded.
8 The process is repeated until every fragment has a complete complementary strand. The pattern of flashes reveals the sequence.
No. of nucleotides in sequence
4
3
2
1
A
T
G
C
TTCTGCGAA

- “Third-generation sequencing” techniques are even faster and less expensive
- In these methods, a single long DNA molecule is sequenced as it moves through a very small pore (*nanopore*) in a membrane
- In one approach, each base is identified by the way it interrupts an electric current as it passes through the pore
- Associated software allows identification and analysis of the DNA sequence

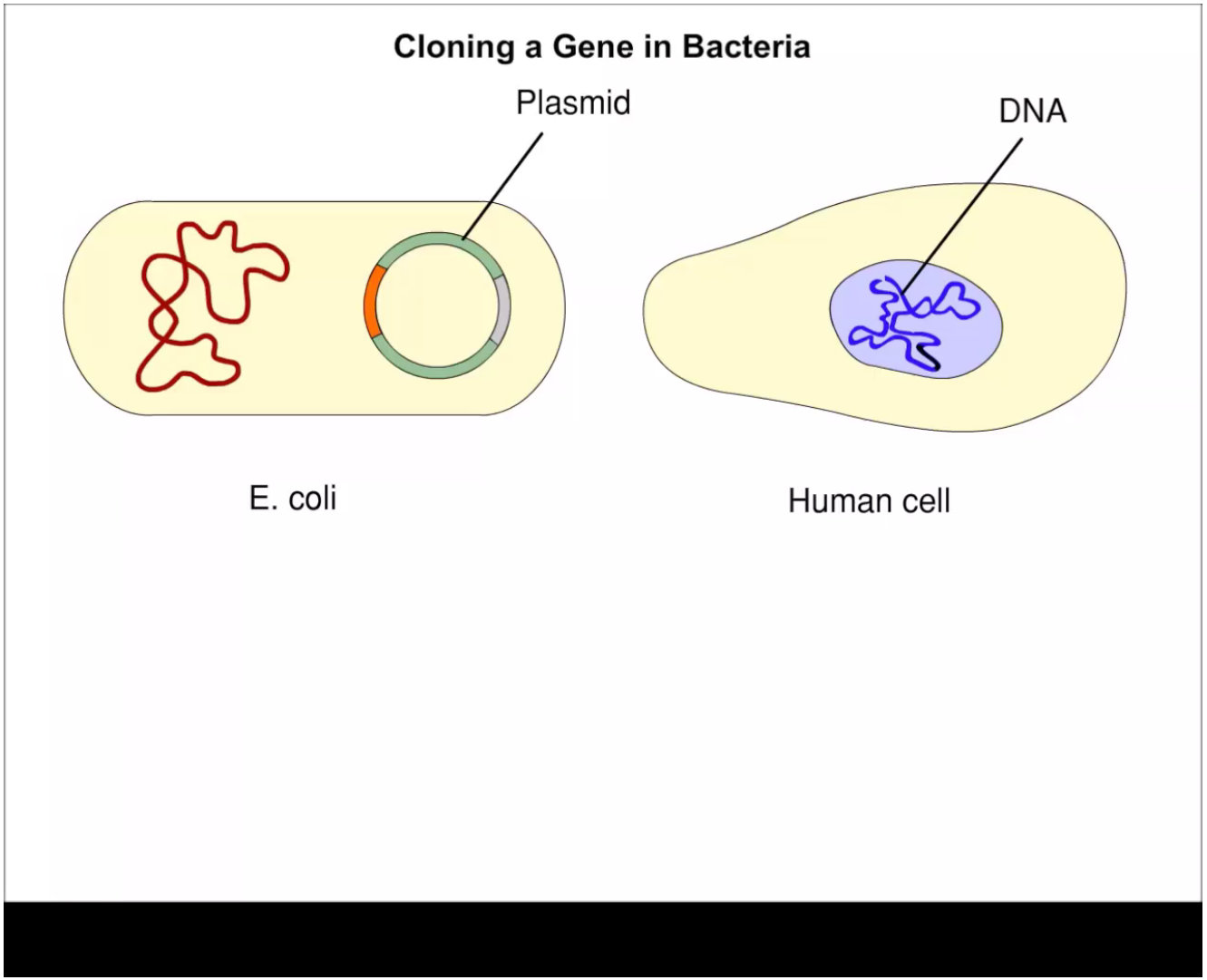
Making Multiple Copies of a Gene or Other DNA Segment

- To work directly with specific genes, scientists prepare well-defined DNA segments in multiple identical copies by a process called **DNA cloning**
- **Plasmids** are small, circular DNA molecules that replicate separately from the bacterial chromosome
- Researchers can insert DNA into a plasmid to produce a **recombinant DNA molecule**, which contains DNA from two different sources

Figure 20.4


Bacterium
1 Gene inserted into plasmid (a cloning vector)
Cell containing gene of interest
Bacterial chromosome
Plasmid
DNA of chromosome ("foreign" DNA)
Gene of interest
Recombinant DNA (plasmid)
2 Plasmid put into bacterial cell
Recombinant bacterium
3 Host cell grown in culture to form a clone of cells containing the "cloned" gene of interest
Gene of interest
Protein expressed from gene of interest
Copies of gene
Protein harvested
Gene for pest resistance is inserted into plants
4 Basic research and various applications
Human growth hormone treats stunted growth
Gene is used to alter bacteria for cleaning up toxic waste
Protein dissolves blood clots in heart attack therapy

Animation: Recombinant DNA



- Reproduction of a recombinant plasmid in a bacterial cell results in cloning of the plasmid including the foreign DNA
- This production of multiple copies of a single gene is a type of DNA cloning called **gene cloning**
- A plasmid used to clone a foreign gene is called a **cloning vector**

- Bacterial plasmids are widely used as cloning vectors because they are:
  - readily obtained
  - easily manipulated
  - easily introduced into bacterial cells
  - rapidly multiplied once in the bacteria
- Gene cloning is useful for amplifying genes to produce a protein product for research, medical, or other purposes

Using Restriction Enzymes to Make a Recombinant DNA Plasmid

- Bacterial **restriction enzymes** cut DNA molecules at specific DNA sequences called **restriction sites**
- A restriction enzyme usually makes many cuts in a long DNA molecule, yielding **restriction fragments**
- The most useful restriction enzymes cut DNA in a staggered way, producing fragments with at least one single-stranded end called a **sticky end**

- Sticky ends can bond with complementary sticky ends of other fragments
- DNA ligase is an enzyme that seals the bonds between restriction fragments
- This allows researchers to join two DNA fragments from different sources

Animation: Restriction Enzymes

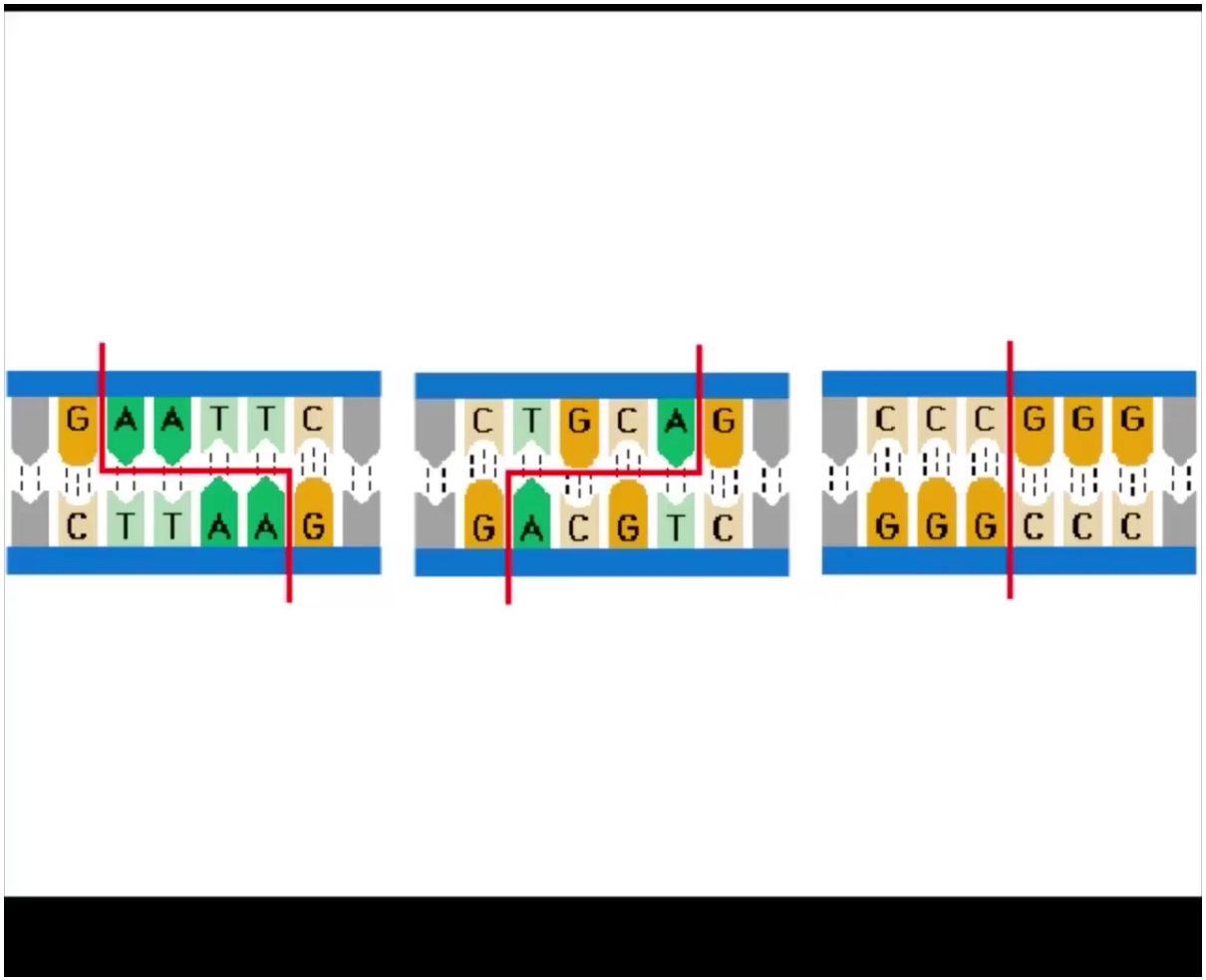
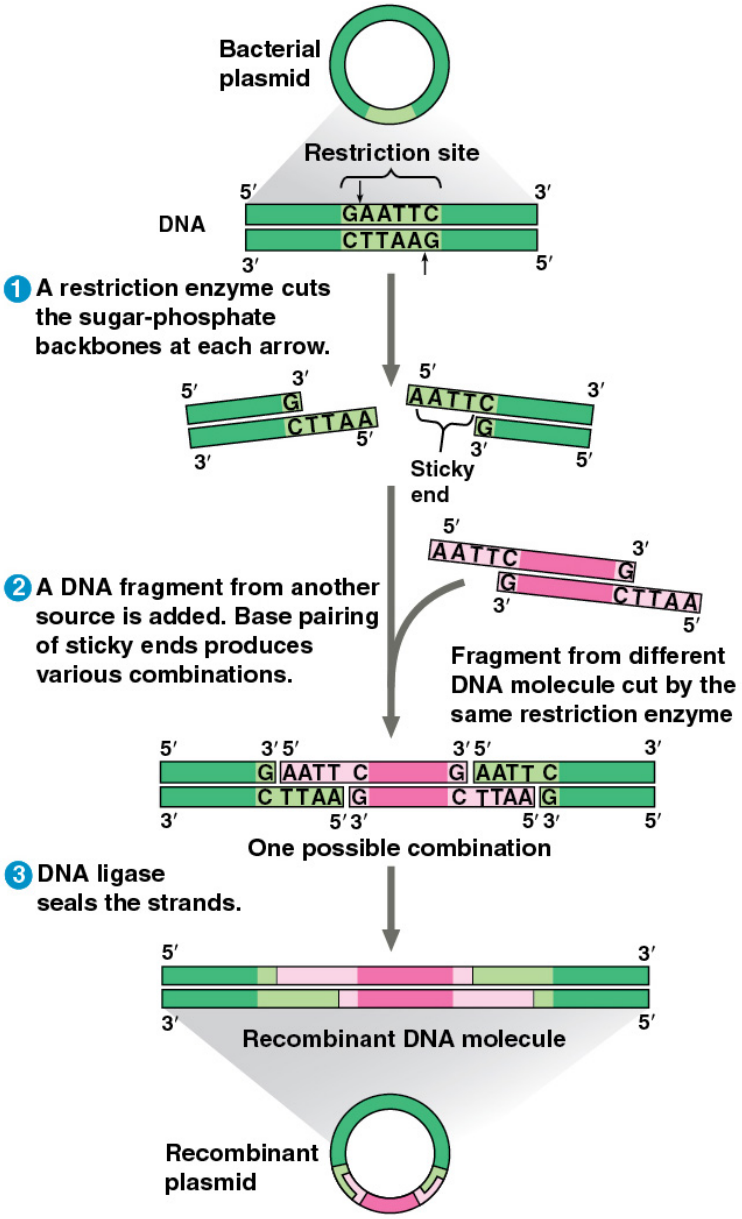
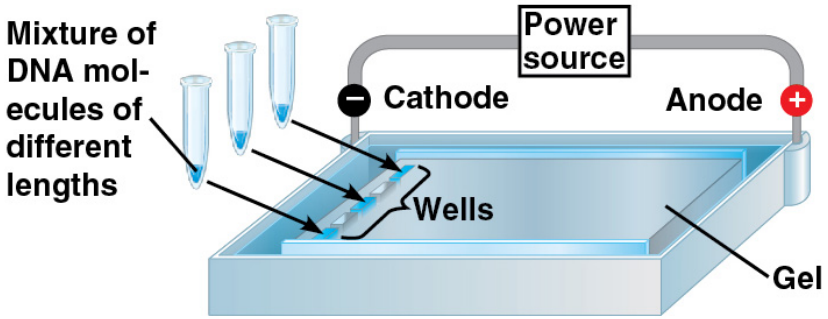


Figure 20.5

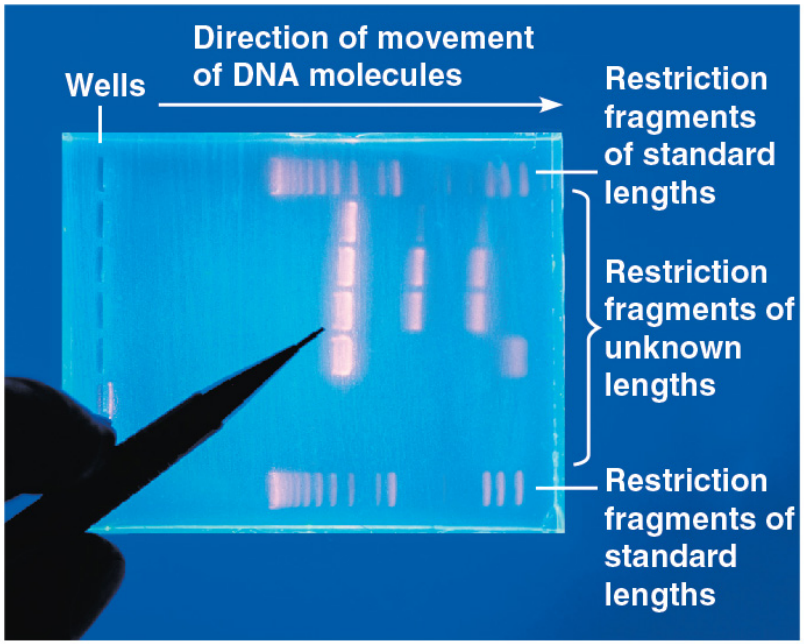

Bacterial plasmid
Restriction site
DNA
5′
3′
GAATTC
CTTAAG
3′
5′
1 A restriction enzyme cuts the sugar-phosphate backbones at each arrow.
G
CTTAA
AATTC
G
Sticky end
2 A DNA fragment from another source is added. Base pairing of sticky ends produces various combinations.
Fragment from different DNA molecule cut by the same restriction enzyme
G AATT C
C TTAA G
One possible combination
3 DNA ligase seals the strands.
Recombinant DNA molecule
Recombinant plasmid

- To check the recombinant plasmid, researchers might cut the products again using the same restriction enzyme
- To separate and visualize the fragments produced, **gel electrophoresis** is carried out
- This technique uses a gel made of a polymer that has microscopic holes of different sizes, through which shorter fragments can travel faster

Figure 20.6



(a) Negatively charged DNA molecules will move toward the positive electrode.



(b) Shorter molecules are slowed down less than longer molecules, so shorter molecules move faster through the gel.

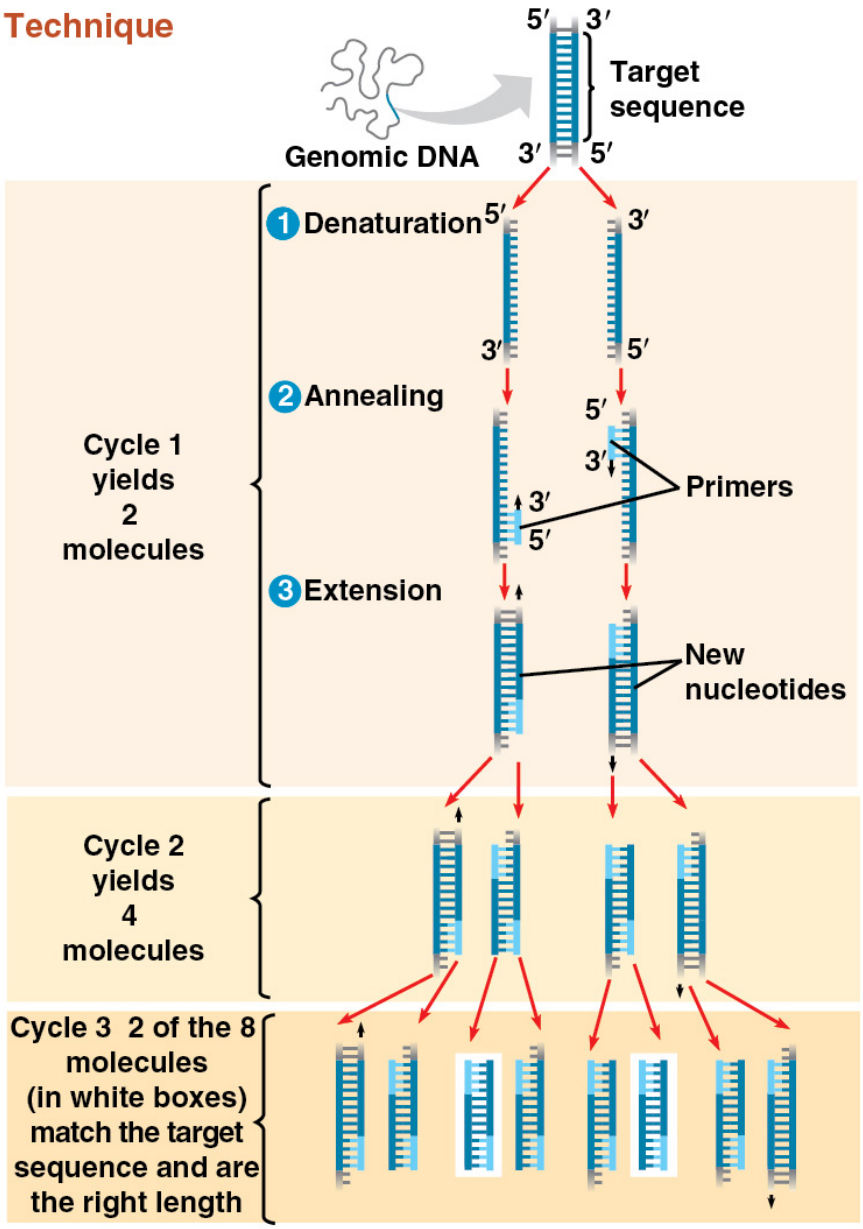
Animation: Gel Electrophoresis of DNA

Amplifying DNA: The Polymerase Chain Reaction (PCR) and Its Use in DNA Cloning

- The **polymerase chain reaction**, **PCR**, can produce many copies of a specific target segment of DNA
- A three-step cycle—heating (denaturing), cooling (annealing), and extension—brings about a chain reaction that produces an exponentially growing population of identical DNA molecules
- The process uses primers, short single-stranded DNA molecules complementary to sequences to either side of the target sequence

- The key to PCR is an unusual, heat-stable DNA polymerase called *Taq* polymerase
- Other polymerases may be used as well; some are more accurate and stable than *Taq*, such as *Pfu* polymerase
- The primers used are specific for the sequence to be amplified
- PCR amplification occasionally incorporates errors into the amplified strands and so cannot substitute for gene cloning in cells

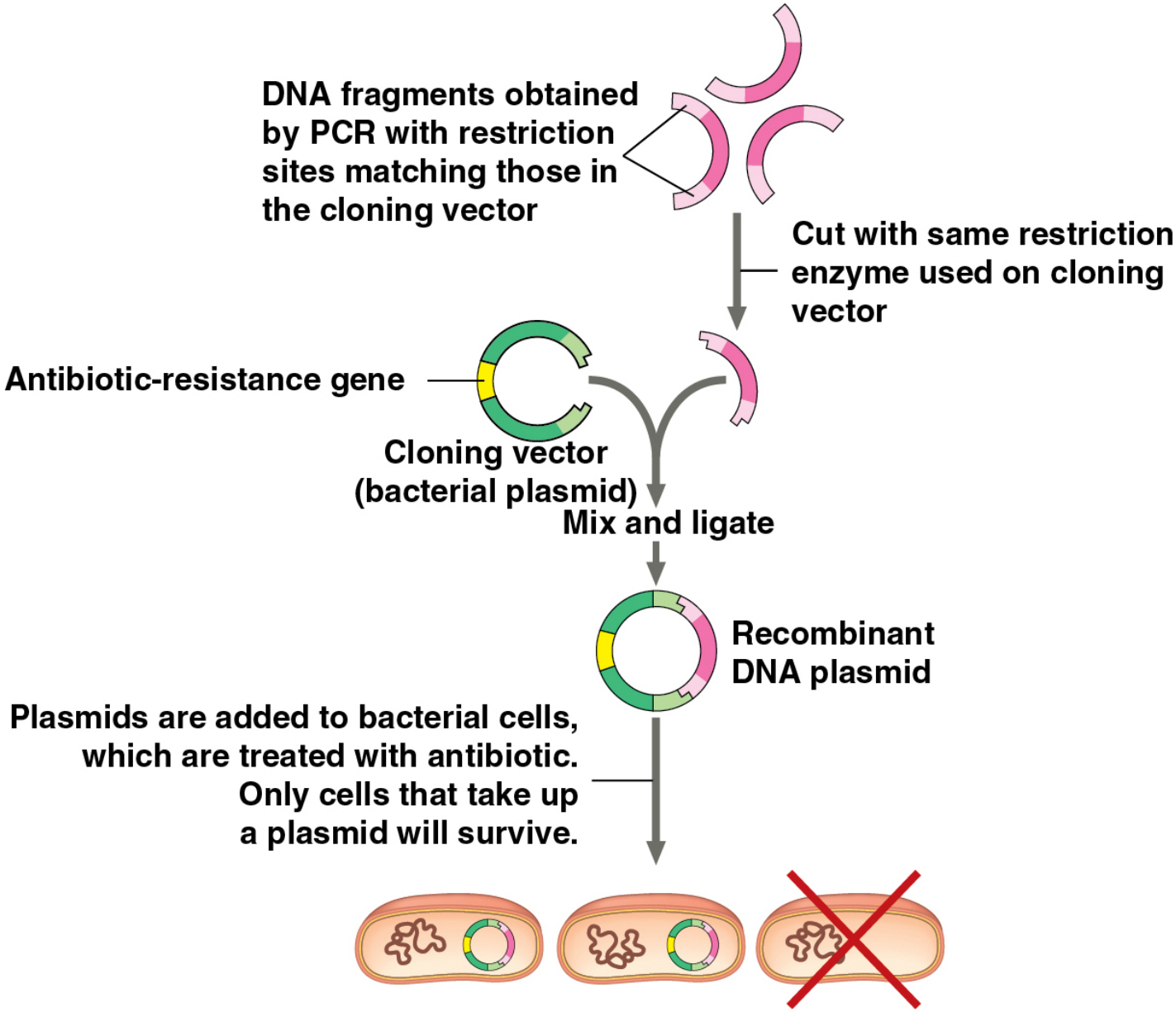
Figure 20.7


Technique
5′
3′
Target sequence
Genomic DNA
3′
5′
1 Denaturation
5′
3′
3′
5′
2 Annealing
5′
3′
Primers
3′
5′
Cycle 1 yields 2 molecules
3 Extension
New nucleotides
Cycle 2 yields 4 molecules
Cycle 3 2 of the 8 molecules (in white boxes) match the target sequence and are the right length

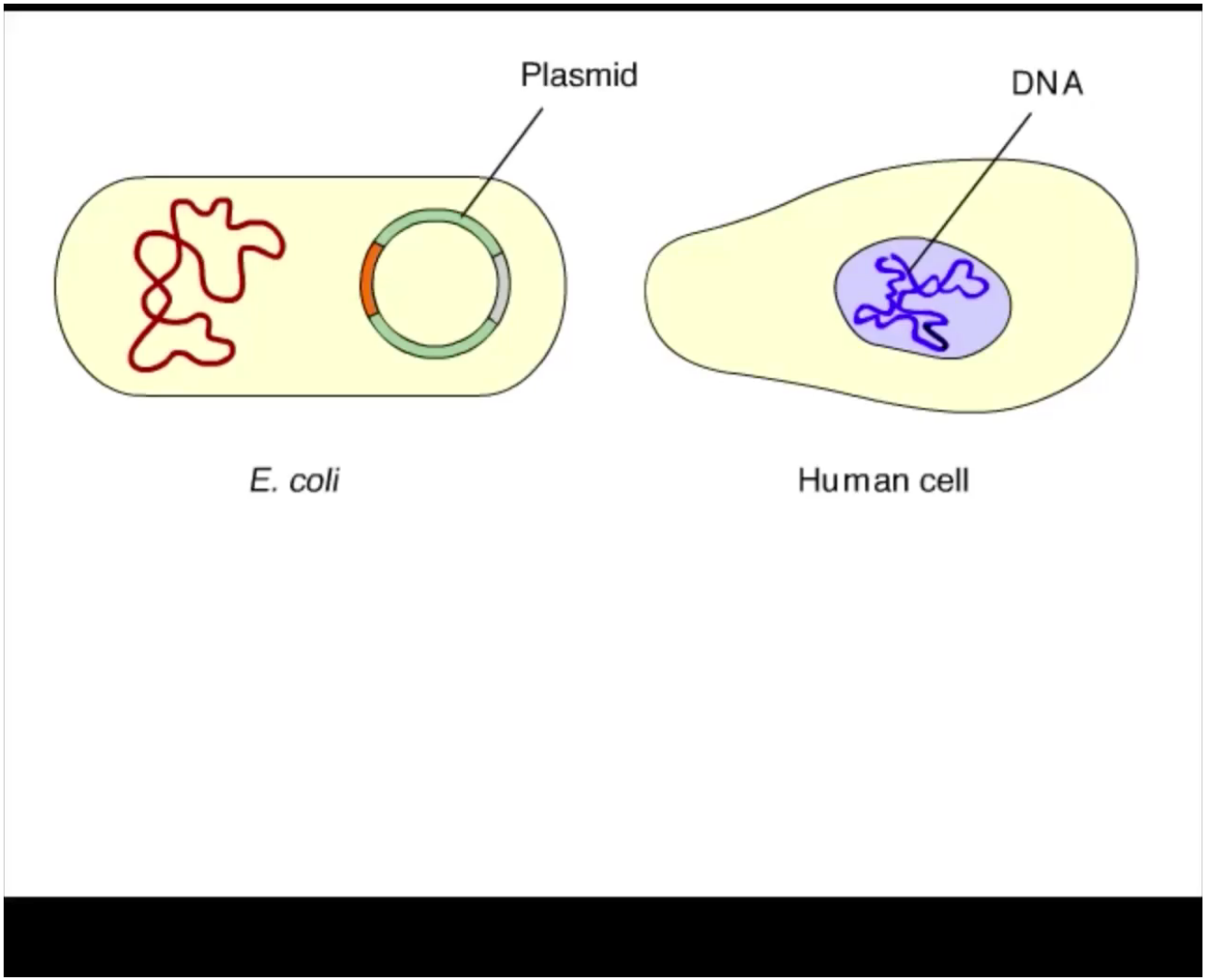
- PCR is used to produce the specific DNA fragment for cloning
- PCR primers can be designed to include restriction sites that allow the product to be cloned into plasmid vectors
- The resulting clones are sequenced and error-free inserts selected

Video: Polymerase Chain Reaction (PCR)

Figure 20.8


DNA fragments obtained by PCR with restriction sites matching those in the cloning vector
Cut with same restriction enzyme used on cloning vector
Antibiotic-resistance gene
Cloning vector (bacterial plasmid)
Mix and ligate
Recombinant DNA plasmid
Plasmids are added to bacterial cells, which are treated with antibiotic. Only cells that take up a plasmid will survive.

Animation: Copying DNA Through PCR



- PCR has had a major impact on biological research and genetic engineering
- DNA can be amplified from many sources such as:
  - A 40,000 year-old frozen woolly mammoth
  - A fingerprint
  - Tiny amounts of blood, tissue or semen found at a crime scene
  - Single embryonic cells
  - Cells infected with viruses that are difficult to detect

Expressing Cloned Eukaryotic Genes

- Once a gene has been cloned, its protein product can be produced in larger amounts for research or practical applications
- Cloned genes can be expressed 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

- Another problem with eukaryotic gene expression in bacteria is the presence of introns in most eukaryotic genes
- Researchers can avoid this problem by using cDNA, complementary to the mRNA, which contains only exons

Eukaryotic DNA 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 be able to modify expressed mammalian proteins correctly
- 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

CONCEPT 20.2: Biologists use DNA technology to study gene expression and function

- Analysis of when and where a gene or group of genes is expressed can provide important clues about gene function

Analyzing Gene Expression

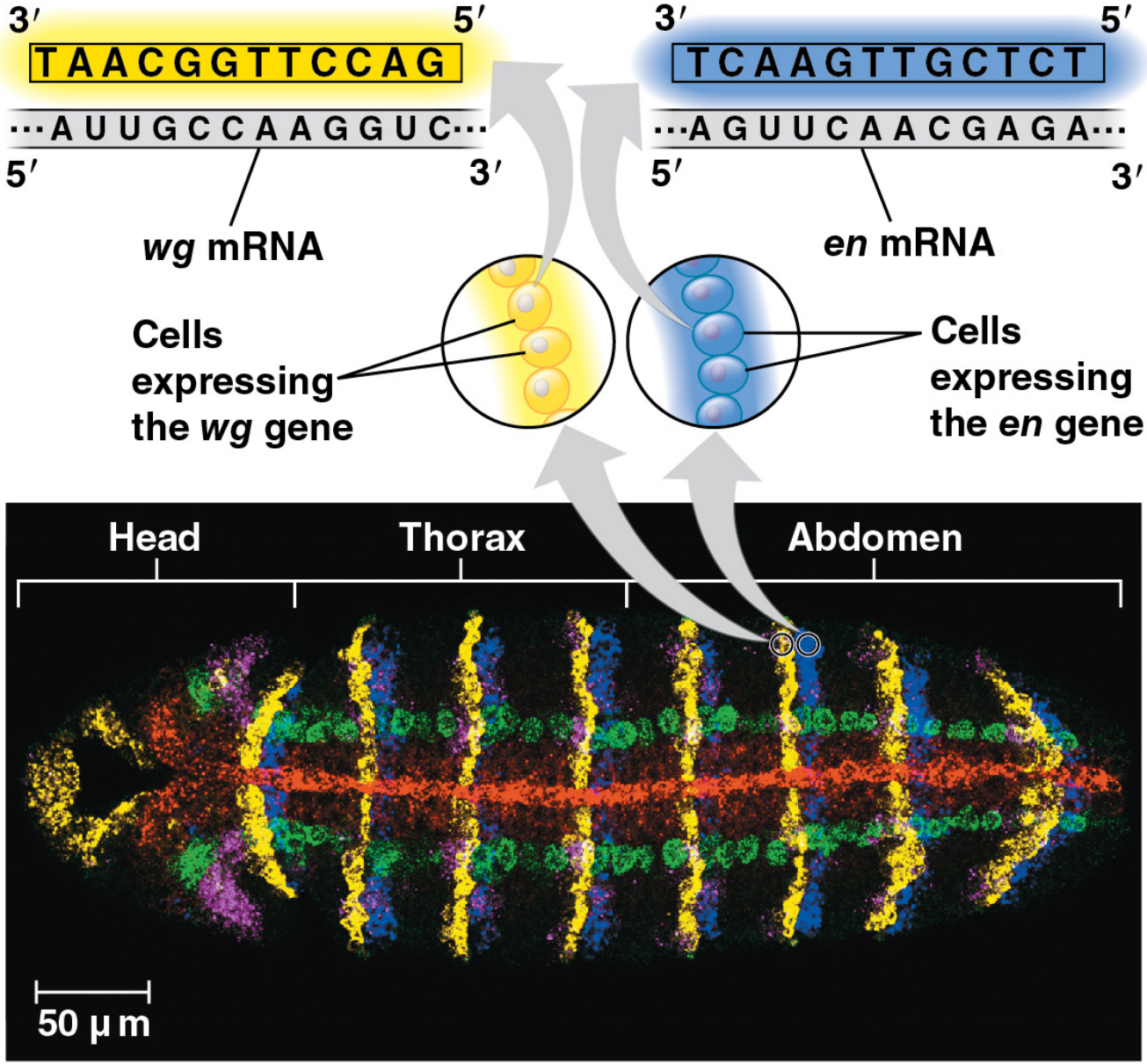
- The most straightforward way to discover which genes are expressed in certain cells is to identify the mRNAs being made

Studying the Expression of Single Genes

- mRNA can be detected by nucleic acid hybridization with complementary molecules
- These complementary molecules, of either DNA or RNA, are **nucleic acid probes**

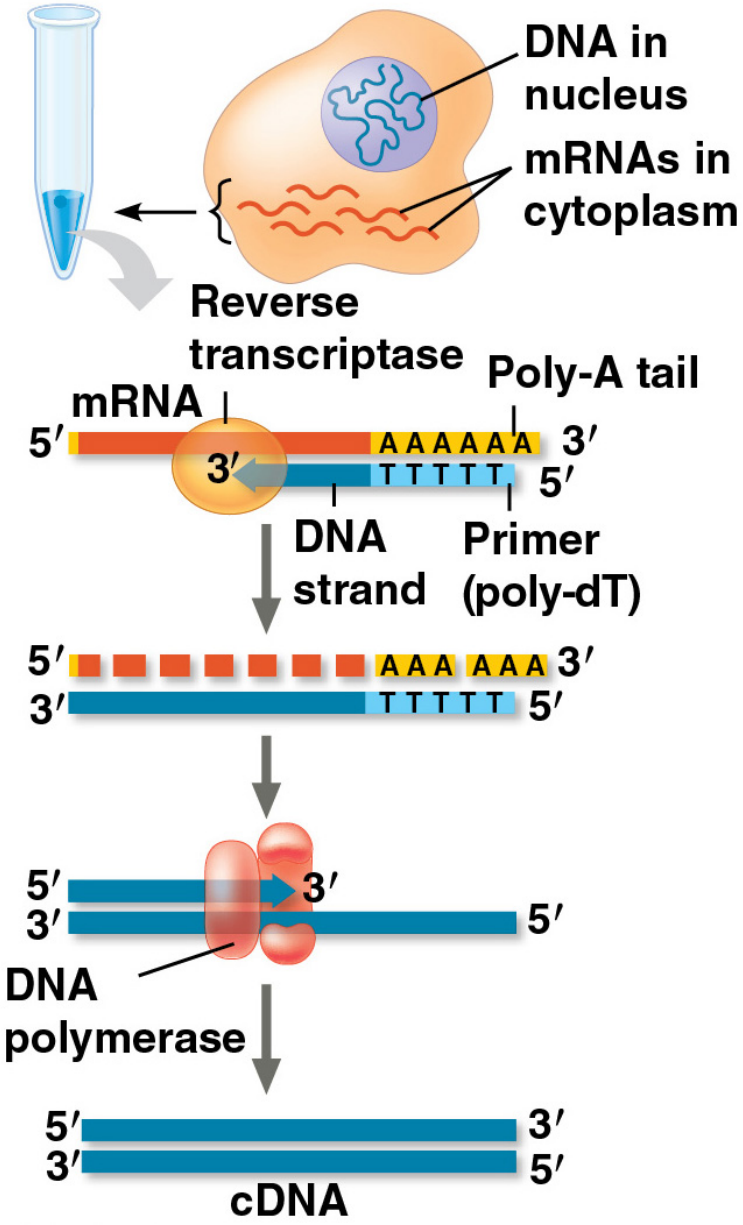
- ***In situ* hybridization** uses fluorescent dyes attached to probes to identify the location of specific mRNAs in place in the intact organism
- Different probes can be labeled with different fluorescent dyes, sometimes with strikingly beautiful results

Figure 20.9


3′
5′
TAACGGTTCCAG
···AUUGCCAAGGUC···
5′
3′
wg mRNA
3′
5′
TCAAGTTGCTCT
···AGUUCAACGAGA···
5′
3′
en mRNA
Cells expressing the wg gene
Cells expressing the en gene
Head
Thorax
Abdomen
50 µm

- **Reverse transcriptase-polymerase chain reaction (RT-PCR)** is useful for comparing amounts of specific mRNAs in several samples at the same time
- Reverse transcriptase is used to synthesize a **complementary DNA (cDNA)** copy of each mRNA in the sample
- A second DNA strand, complementary to the first is synthesized by DNA polymerase

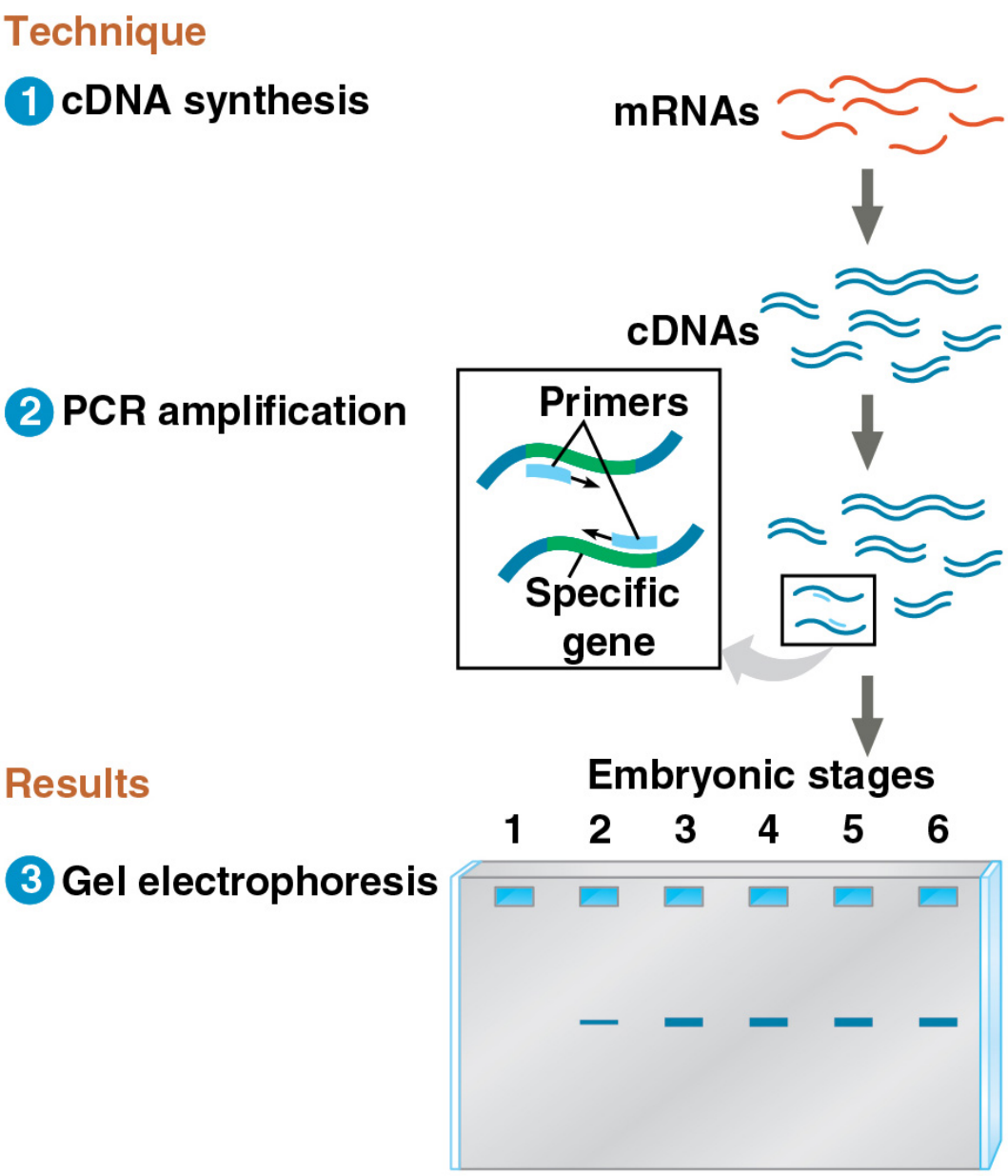
Figure 20.10


DNA in nucleus
mRNAs in cytoplasm
Reverse transcriptase
Poly-A tail
mRNA
5′
3′
A A A A A A
T T T T T
DNA strand
Primer (poly-dT)
A A A
A A A
5′
3′
DNA polymerase
cDNA

- Next, PCR is used to amplify DNA segments of interest from the cDNAs
- The products are run on a gel to determine which samples expressed the gene of interest
- This method only shows the presence or absence of the mRNA in a sample

- An enhancement called *quantitative RT-PCR (qRT-PCR)* uses a dye that fluoresces only when bound to a double-stranded PCR product
- Newer methods of this type do not require gel electrophoresis
- It can be used to discover which tissue is producing a specific mRNA and gives quantitative data

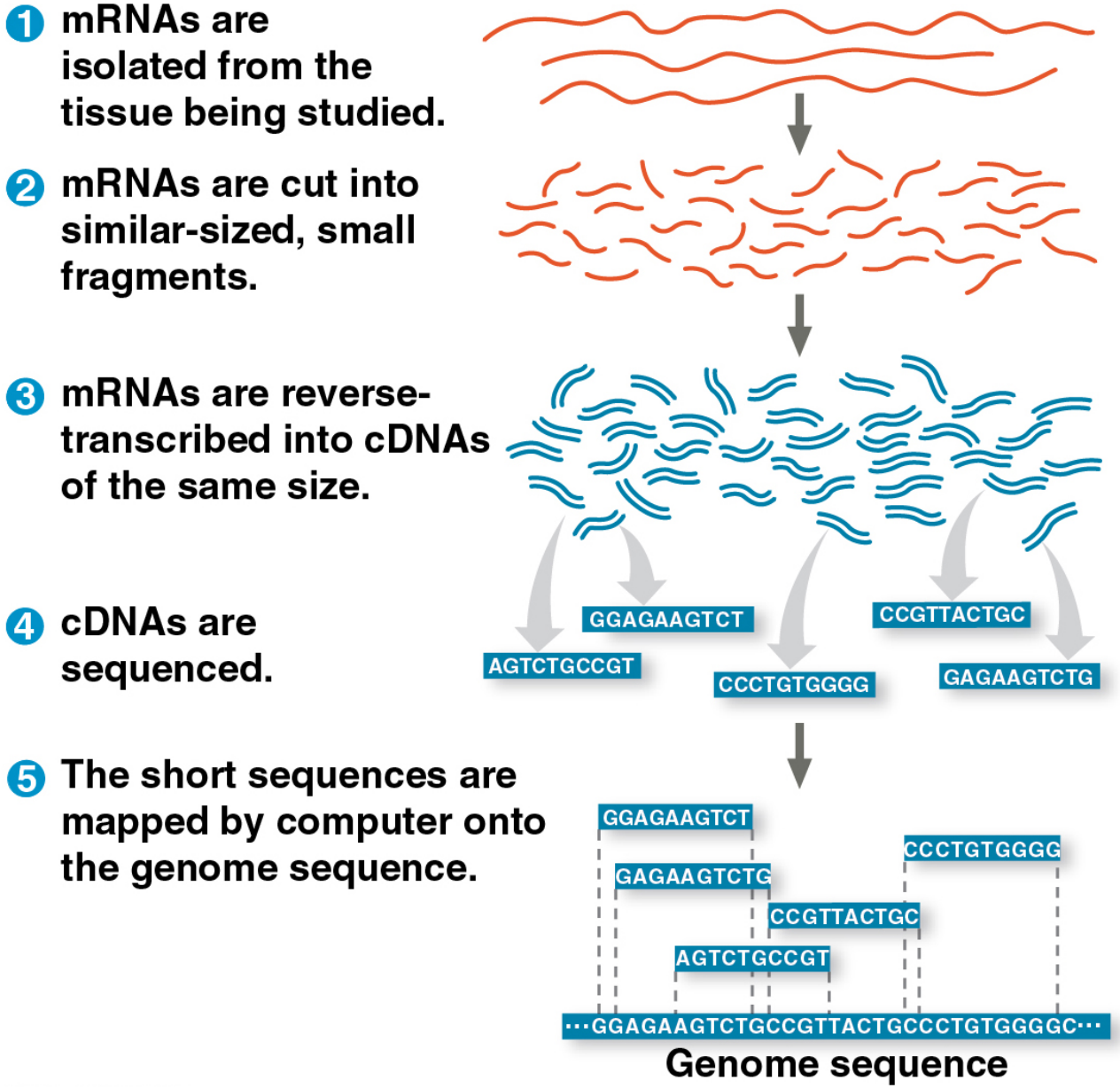
Figure 20.11


Technique
1 cDNA synthesis
mRNAs
cDNAs
2 PCR amplification
Primers
Specific gene
Results
Embryonic stages
1 2 3 4 5 6
3 Gel electrophoresis

Studying the Expression of Interacting Groups of Genes

- The study of expression of thousands of genes at one time constitutes a *systems approach*
- With rapid and inexpensive sequencing methods, researchers can sequence cDNA samples from different tissues or embryonic stages to determine the gene expression differences between them
- This approach is called **RNA sequencing** or **RNA-seq**
- RNAs are isolated, cut into short, similar-sized fragments, converted into cDNAs, and sequenced

Figure 20.12

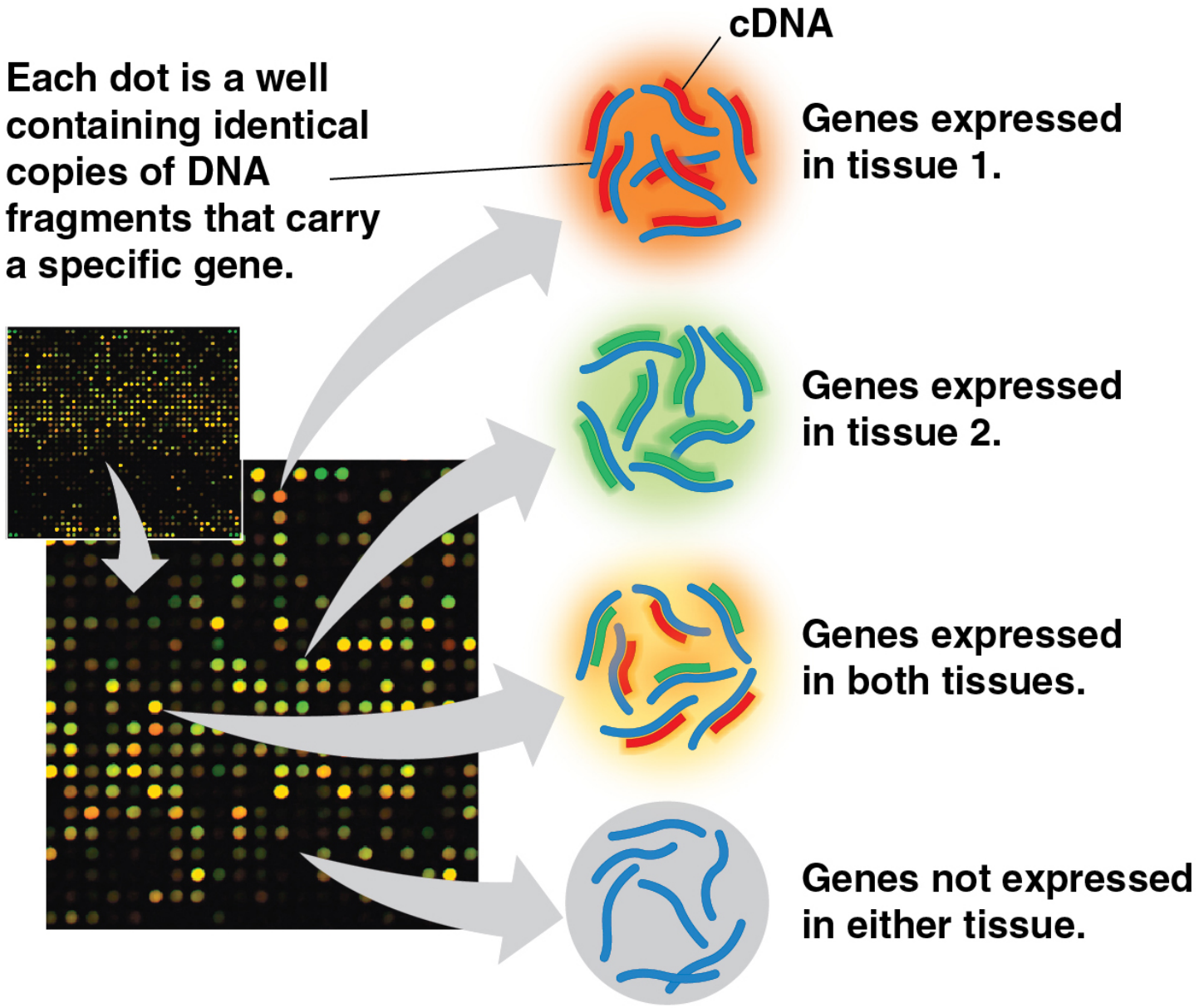

1 mRNAs are isolated from the tissue being studied.
2 mRNAs are cut into similar-sized, small fragments.
3 mRNAs are reverse-transcribed into cDNAs of the same size.
4 cDNAs are sequenced.
5 The short sequences are mapped by computer onto the genome sequence.
GGAGAAGTCT
AGTCTGCCGT
CCCTGTGGGG
CCGTTACTGC
GAGAAGTCTG
GGAGAAGTCT
GAGAAGTCTG
CCGTTACTGC
AGTCTGCCGT
CCCTGTGGGG
···GGAGAAGTCTGCCGTTACTGCCCTGTGGGGC···
Genome sequence

- RNA-seq is a powerful technique for several reasons:
  - It does not depend on knowing genomic sequences
  - It can measure levels of expression over a wide range
  - Careful analysis provides a wealth of information about expression of a particular gene
- However, expression of an individual gene still needs to be confirmed by RT-PCR

- An older method of genome-wide expression studies is less powerful than RNA-seq but is still used for applications such as fetal testing
- **DNA microarray assays** compare patterns of gene expression in different tissues at different times or under different conditions

- mRNAs from the cells to be studied are reverse-transcribed into cDNAs and marked with a fluorescent label
- Different labels are used for different cell types so that multiple samples can be tested in the same experiment

Figure 20.13


cDNA
Each dot is a well containing identical copies of DNA fragments that carry a specific gene.
Genes expressed in tissue 1.
Genes expressed in tissue 2.
Genes expressed in both tissues.
Genes not expressed in either tissue.

- Scientists can now measure expression of thousands of genes at one time
- Information from these methods should provide a grander view of how ensembles of genes interact to form an organism and maintain its vital systems

Determining Gene Function

- One way to determine function is to disable the gene and observe the consequences

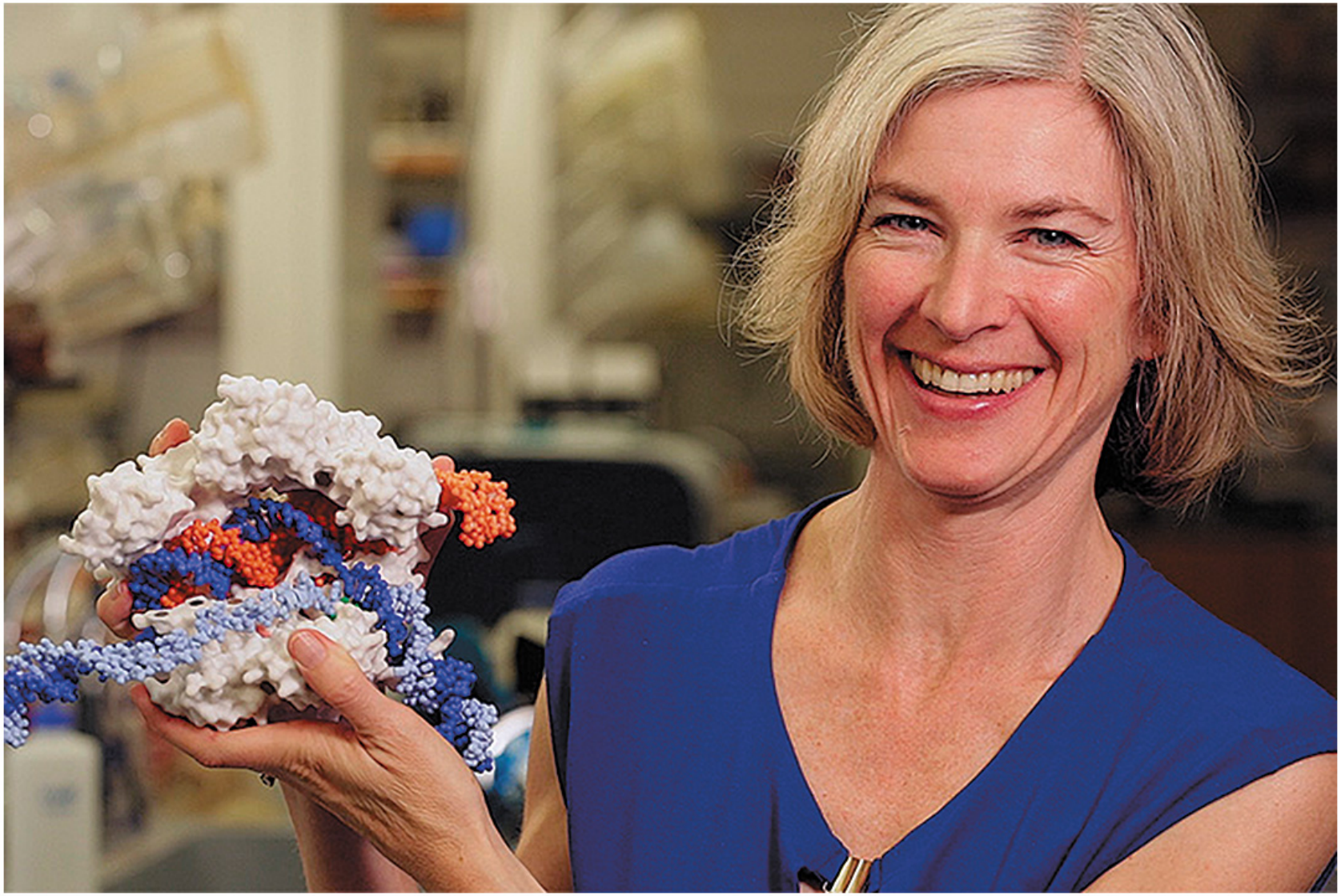
Editing Genes and Genomes

- Using ***in vitro* mutagenesis**, specific 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

- The CRISPR-Cas9 system is a powerful new technique for gene editing in living cells and organisms
- It is an effective way for researchers to knock out a given gene in order to study what the gene does
- Modifications of the technique allow researchers to repair a gene that has a mutation

- In another application, scientists are trying to use CRISPR-Cas9 to address the global problem of insect-borne diseases
- They are trying to alter genes in the insects so they cannot transmit the disease
- An extra twist is to engineer the new allele so that it is favored for inheritance over the wild type allele
- This technology is called **gene drive**

Figure 20.14



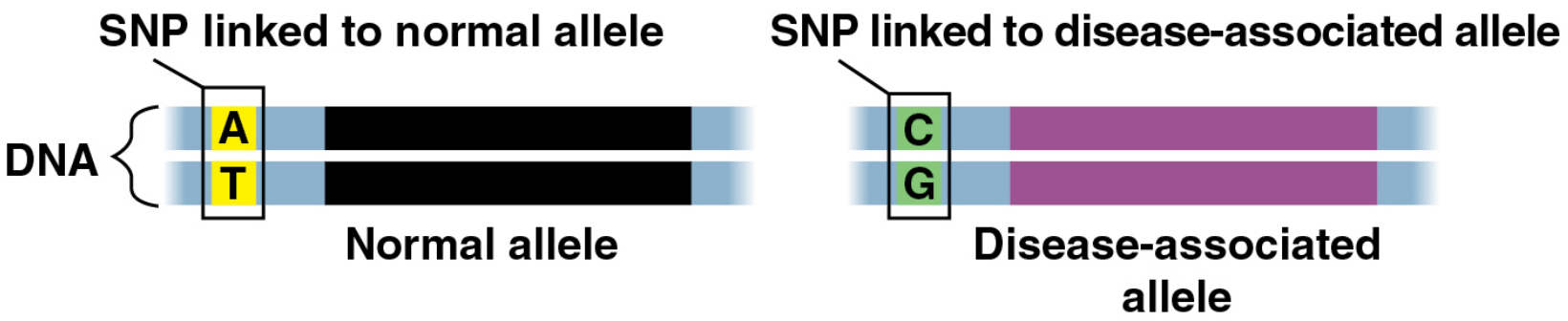
Other Methods for Studying Gene Function

- 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
- These **genome-wide association studies** test for genetic markers, sequences that vary among individuals
- **SNPs (single nucleotide polymorphisms)**, single nucleotide variants, are among the most useful genetic markers

- SNPs that are found frequently associated with a particular inherited disorder alert researchers to the most likely location for the disease-causing gene
- SNPs are rarely directly involved in the disease; they are most often in noncoding regions of the genome
- However, if a SNP is close to a mutation in a gene that causes a condition, it will rarely be passed on separately from the mutation
- Therefore it acts as a marker for the disease-causing allele

Figure 20.15


SNP linked to normal allele
SNP linked to disease-associated allele
DNA
A
T
C
G
Normal allele
Disease-associated allele

CONCEPT 20.3: Cloned organisms and stem cells are useful for basic research and other applications

- Organismal cloning produces one or more organisms genetically identical to the “parent” that donated the single cell
- A **stem cell** is a relatively unspecialized cell that can reproduce itself indefinitely, or under certain conditions can differentiate into one or more types of specialized cells

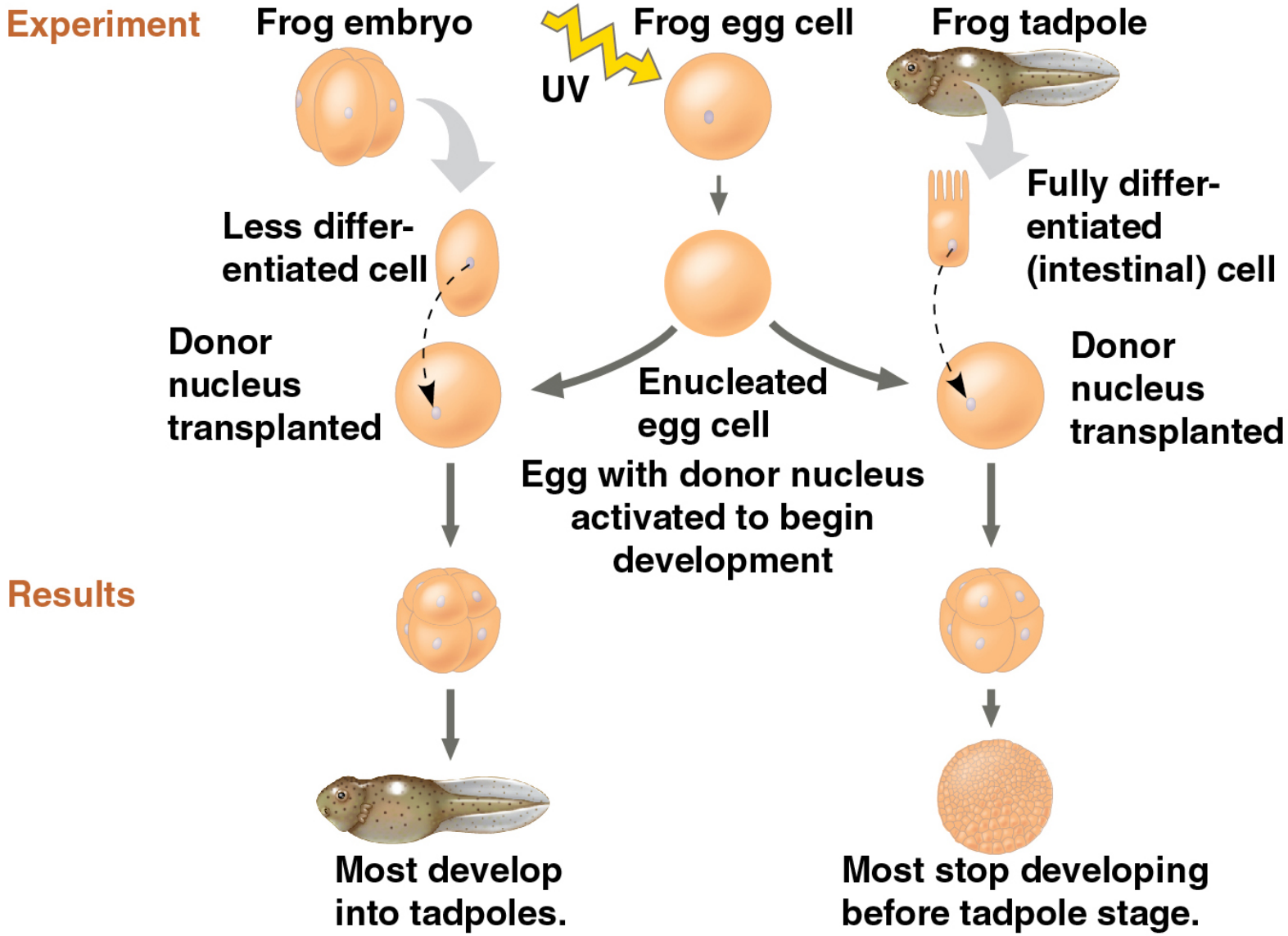
Cloning Plants: Single-Cell Cultures

- In plants, mature cells can “dedifferentiate” and then give rise to all the specialized cell types of the organism
- A **totipotent** cell, such as this, is one that can generate a complete new organism
- Plant cloning is used extensively in agriculture

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
- This suggested the something in the nucleus changes as animal cells differentiate

Figure 20.16



Data from J. B. Gurdon et al., The developmental capacity of nuclei transplanted from keratinized cells of adult frogs, *Journal of Embryology and Experimental Morphology* 34:93–112 (1975).

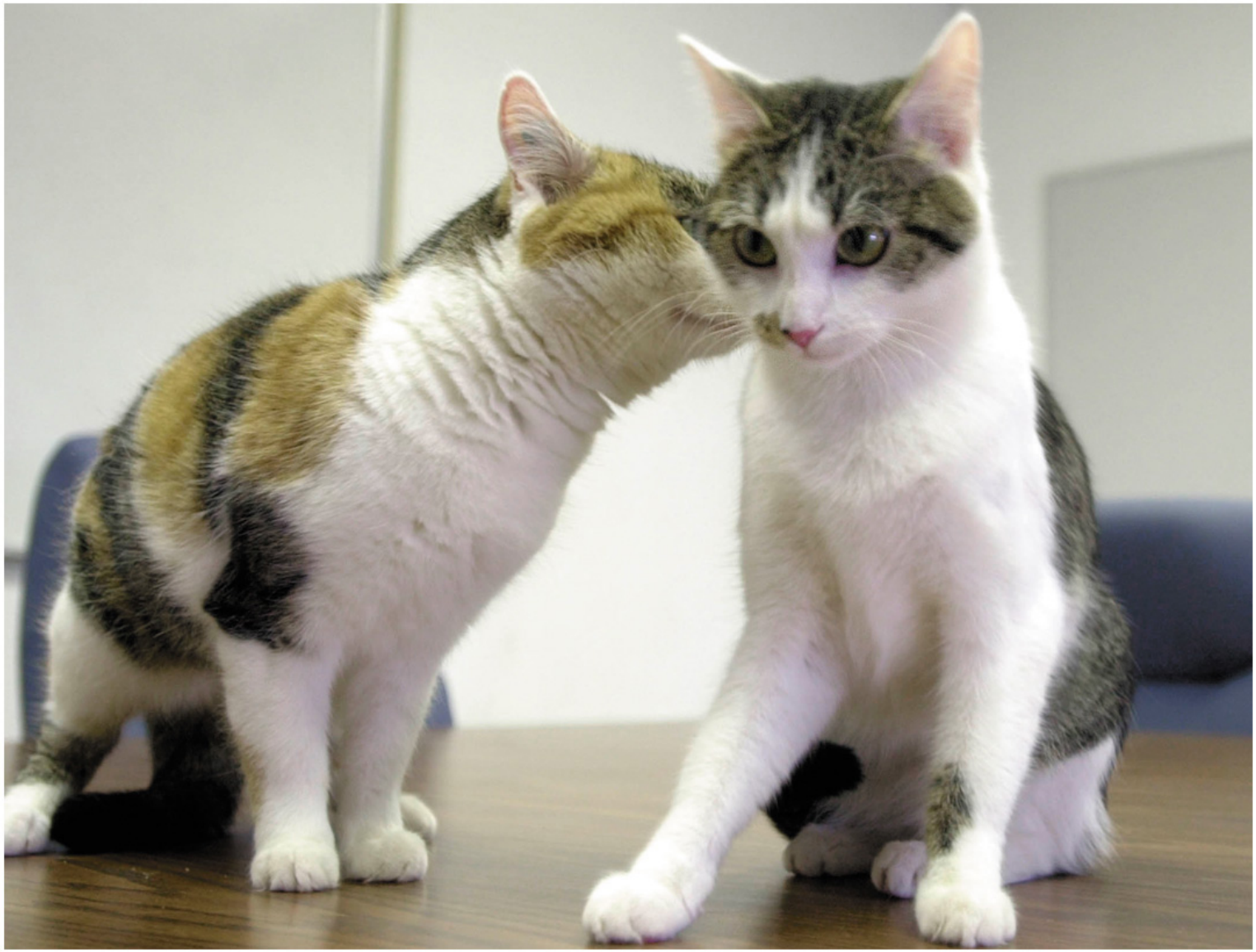
Reproductive Cloning of Mammals

- 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 that of another cloned sheep from another experiment, led to speculation that her cells were not as healthy as those of a normal sheep
- This possibly reflects incomplete reprogramming of the original transplanted nucleus

Figure 20.17

- Since 1997, cloning has been demonstrated in many mammals, including mice, cats, cows, horses, mules, pigs, and dogs
- CC (“Carbon Copy”) was the first cat cloned; however, CC differed somewhat from her single female parent
- Cloned animals do not always look or behave exactly the same
- Even identical human twins, natural “clones,” are always slightly different

Figure 20.18



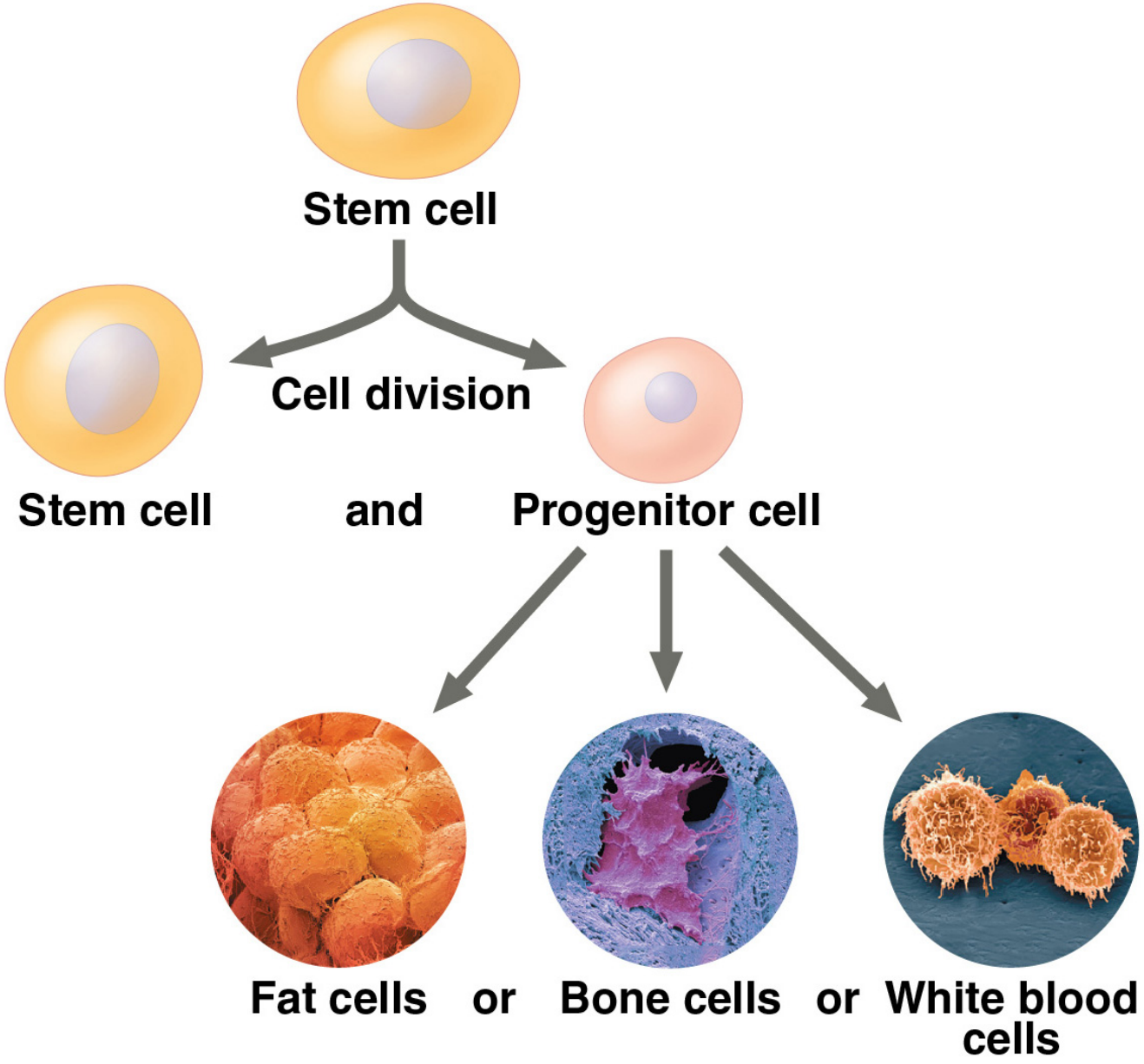
Epigenetic Differences in Cloned Animals

- In most nuclear transplantation studies, only a small percentage of cloned embryos have developed normally to birth
- 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

- Stem cells are relatively unspecialized cells that can both reproduce indefinitely and, under certain conditions, differentiate into one or more specialized cell types

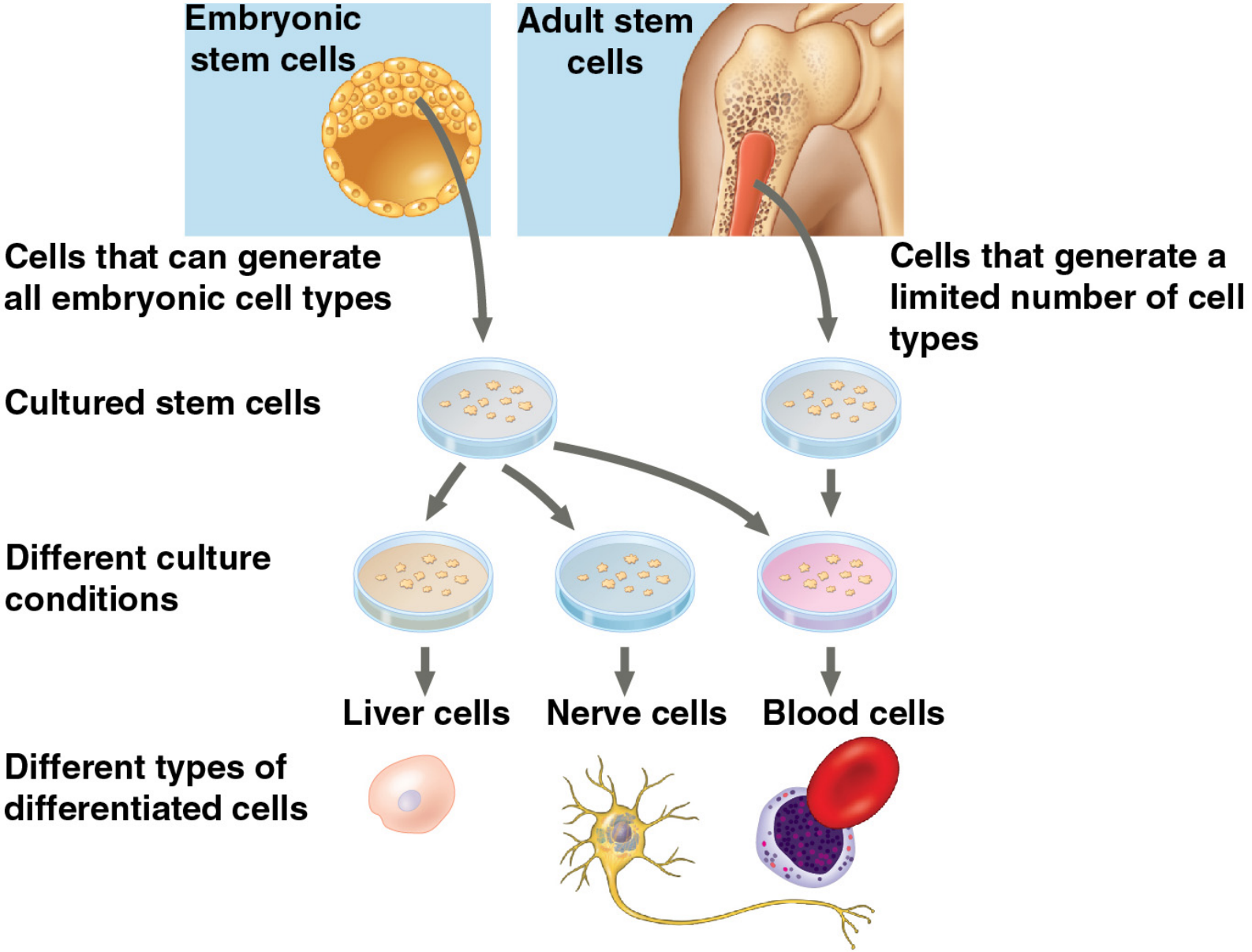
Figure 20.19


Stem cell
Cell division
Stem cell
and
Progenitor cell
Fat cells
or
Bone cells
or
White blood cells

Embryonic and Adult Stem Cells

- Many early embryos contain stem cells capable of giving rise to differentiated embryonic cells of any type
- In culture, these *embryonic stem (ES)* cells reproduce indefinitely, and depending on culture conditions, can be made to differentiate into a variety of specialized cells
- *Adult stem cells* can generate multiple (but not all) cell types and are used in the body to replace nonreproducing cells as needed

Figure 20.20


Embryonic stem cells
Adult stem cells
Cells that can generate all embryonic cell types
Cells that generate a limited number of cell types
Cultured stem cells
Different culture conditions
Liver cells
Nerve cells
Blood cells
Different types of differentiated cells

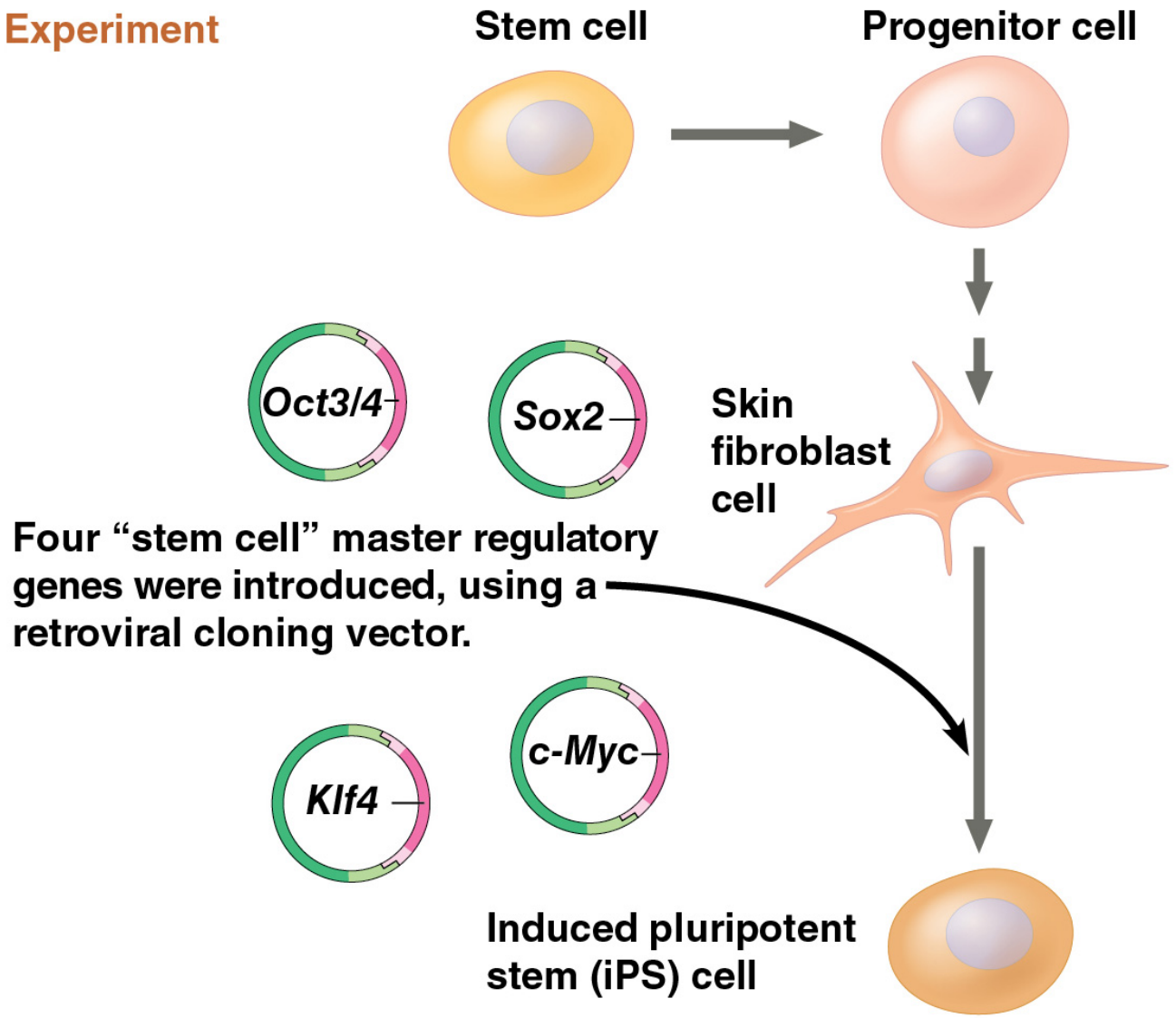
- Embryonic stem cells are **pluripotent**, capable of differentiating into many different cell types
- Originally, ES cells were obtained only from embryos donated by patients undergoing infertility treatments
- The techniques for cloning human embryos are still being optimized, but they represent a potential new (less controversial) source of ES cells

- The main aim of cloning ES cells is to produce cells for treating disease
- The process is thus called *therapeutic cloning*
- Opinions vary about the morality of therapeutic cloning

Induced Pluripotent Stem (iPS) Cells

- Researchers can treat differentiated cells and reprogram them to act like ES cells
- Researchers use retroviruses to induce extra copies of four stem cell master regulatory genes to produce *induced pluripotent stem (iPS)* cells
- Shinya Yamanaka received the 2012 Nobel Prize in Medicine for this work, shared with John Gurdon, for his work on nuclear transplantation in frogs

Figure 20.21



Data from K. Takahashi et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, *Cell* 131:861–872 (2007).

- iPS cells can perform most of the functions of ES cells
- However, there are some differences in gene expression and cell division
- It is likely that ES cells with continue to make important contributions to the development of stem cell therapies

- iPS cells can be used as models for study of certain diseases and potentially as replacement cells for patients
- Human iPS lines have been developed from individuals with type 1 diabetes, Parkinson's disease, Huntington's disease, Down syndrome, and other diseases

- iPS cells can also be used in regenerative medicine
- Currently reprogramming cells is too expensive to use as a standard treatment
- Development of techniques to direct iPS cells to become specific cell types for regenerative medicine is an area of intensive research
- Cells created this way could be used without raising most ethical objections

CONCEPT 20.4: The practical applications of DNA-based biotechnology affect our lives in many ways

- **Biotechnology** is the manipulation of organisms or their components to make useful products
- Applications of DNA technology and genetic engineering include the following fields:
  - Medicine
  - Forensic evidence
  - Genetic profiles
  - Environmental cleanup
  - Agriculture

Medical Applications

- One benefit of DNA technology is identification of human genes in which mutation plays a role in genetic diseases
- Researchers use RNA-seq and microarray assays or other techniques to identify genes turned on or off in particular diseases
- The genes and their products are then potential targets for prevention or therapy

Diagnosis and Treatment of Diseases

- Scientists can diagnose many human genetic disorders by using PCR and sequence-specific primers, then sequencing the amplified product to look for the disease-causing mutation
- PCR can also be used to identify symptomless carriers of potentially harmful recessive alleles

Personal Genome Analysis

- SNPs may be linked to disease-causing mutations
- Individuals can be tested by PCR and sequencing for a SNP associated with the abnormal allele
- SNPs may also be correlated with increased risks for conditions such as heart disease, Alzheimer's disease, and some types of cancer

- Direct-to-consumer genome analysis companies offer kits allowing individuals to send in a sample that the company will analyze genetically
- It may be helpful for individuals to learn about their health risks
- Such tests reflect correlations and do not make predictions
- As the size of the database used increases, the results become more refined and accurate

Personalized Medicine

- DNA techniques help understand—for example, which cancer-related genes have been mutated in a patient's tumor
- Knowing the expression levels of particular genes in a given individual can help physicians determine the likelihood that a particular cancer will recur
- This knowledge can help in the design of an appropriate treatment

- Many envision a future of **personalized medicine** where each person's genetic profile can inform them about diseases and conditions for which they may be at risk
- Eventually this genetic profile will likely mean sequencing the complete genome of each individual
- An individual's genomic information can be used to predict the benefits of particular medications, an approach called *pharmacogenetics*

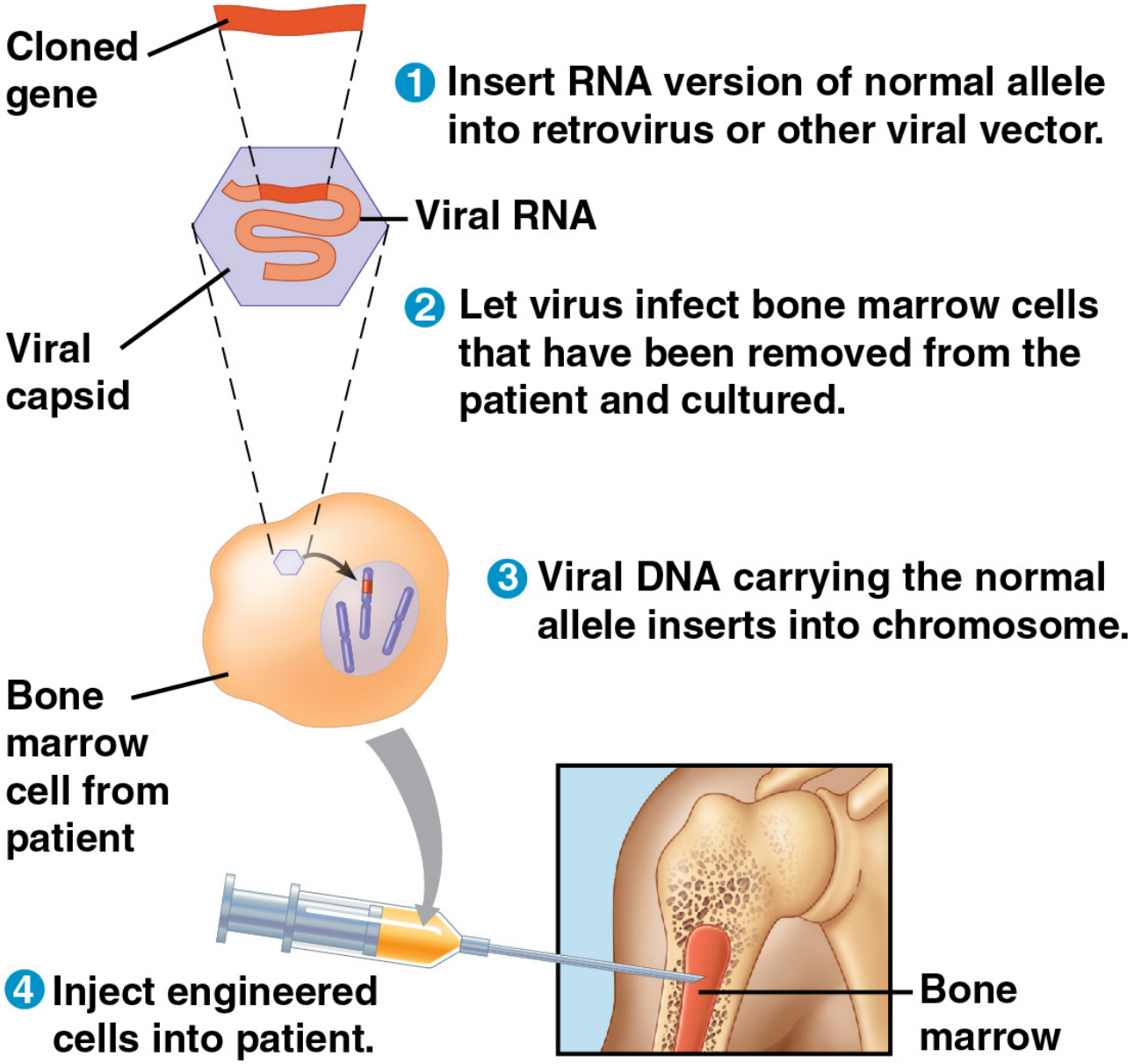
Human Gene Therapy and Gene Editing

- **Gene therapy** is the introduction of genes into an afflicted individual for therapeutic purposes
- It holds great potential for treating disorders traceable to a single defective gene
- Bone marrow cells are prime candidates for gene therapy because they multiply throughout the patient's life

- In France, in 2000, ten young children with severe combined immunodeficiency (SCID) were treated by a gene therapy procedure involving bone marrow cells
- SCID is caused by a single defective gene
- Nine of the patients showed improvement after two years, but three subsequently developed leukemia

- Researchers concluded that the use of a retrovirus as a vector for the therapy was responsible for the leukemias
- It is likely that the retroviral vector inserted near a gene that triggers the proliferation of blood cells
- Approaches that do not use retroviral vectors have been used somewhat successfully

Figure 20.22


Cloned gene
1 Insert RNA version of normal allele into retrovirus or other viral vector.
Viral RNA
Viral capsid
2 Let virus infect bone marrow cells that have been removed from the patient and cultured.
3 Viral DNA carrying the normal allele inserts into chromosome.
Bone marrow cell from patient
4 Inject engineered cells into patient.
Bone marrow

- Gene therapy raises many technical issues:
  - How can the activity of the transferred gene be controlled to make appropriate amounts of the gene product at the right time and place?
  - How can we be sure that the insertion of a therapeutic gene does not harm some other necessary cell function?
- As we learn more about DNA control elements and gene interactions, we may be able to answer such questions

- The CRISPR-Cas9 system avoids the complications of using viral vectors to deliver gene therapy
- In 2018, researchers reported using CRISPR-Cas9 in an attempt to correct the genetic defect that causes sickle-cell disease
- They edited cells from patients with the disease and injected them into bone marrow in mice
- After 19 weeks, the gene remained corrected in 20–40% of the cells

- Gene therapy and gene editing provoke ethical questions
- Some critics believe than any tampering with human genes is unethical
- Others see not difference between transplanting genes into human cells and transplanting organs between people
- Jennifer Doudna, a co-discoverer of CRISPR-Cas9, recognized not only its potential but also the danger of its misapplication

- Under what circumstances, if any, should genomes of human germ lines be altered?
- Would alterations lead to deliberate efforts to control the genetic makeup of human populations (eugenics)?
- It is imperative to consider these questions

Pharmaceutical Products

- Advances in DNA technology and genetic research are important to the development of useful 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 tyrosine kinase
- It is used to treat patients with chronic myelogenous leukemia (CML), caused by overexpression of this kinase
- Using drugs that target particular molecules is feasible only for treatment of cancers in which the molecular basis is well understood

Protein Production in Cell Cultures

- Pharmaceutical products are commonly synthesized on a large scale using cell cultures
- The host cells can be engineered to secrete a protein as it is made, simplifying the task of purifying it
- Human insulin and human growth hormone (HGH) are among the first such products made in this way

Protein Production by "Pharm" Animals

- A **transgene** is a gene that is transferred from one organism into another
- **Transgenic** animals can then express the introduced gene
- Transgenic animals are pharmaceutical "factories," producers of large amounts of otherwise rare substances for medical use

Figure 20.23

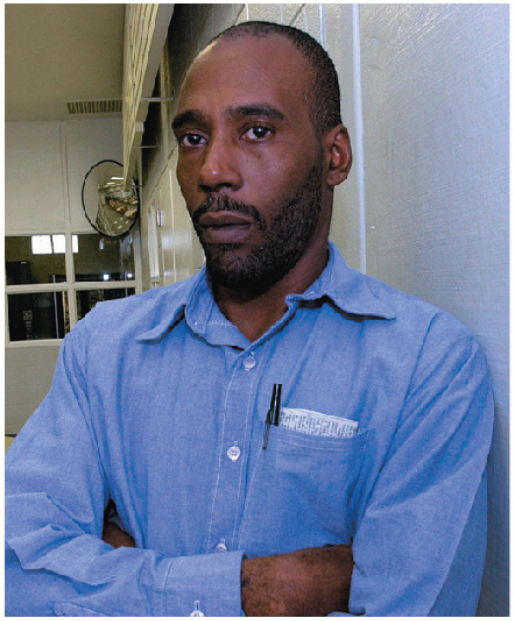
Forensic Evidence and Genetic Profiles

- DNA testing can identify individuals with a high degree of certainty
- An individual's unique set of genetic markers, or **genetic profile**, can be obtained by analysis of tissue or body fluids
- Genetic profiles are currently analyzed using genetic markers **called short tandem repeats (STRs)**
- STRs are variations in the number of repeats of specific DNA sequences; they are analyzed by PCR and gel electrophoresis

- The probability that two people who are not identical twins have the same STR markers is exceptionally small
- The Innocence Project is a nonprofit organization that uses STR analysis of archived samples to revisit old legal cases
- As of 2019, 362 innocent people have been released from prison as a result of DNA-based forensic and legal work

Figure 20.24

(a) Earl 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 (not shown) exonerated Washington and led Tinsley to plead guilty to the murder.

- Genetic profiles can also be used to determine paternity of a child
- They can also be used to identify victims of mass casualties, such as the 2001 attack on the World Trade Center
- Despite some problems that can arise from insufficient data, human error, or flawed evidence, genetic profiles are accepted as compelling evidence by legal experts and scientists

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
- Biotechnologists are trying to engineer microorganisms that can degrade chlorinated hydrocarbons or other harmful compounds
- These could be used in wastewater treatment

Agricultural Applications

- DNA technology is being used to improve agricultural productivity
- The ability of scientists to produce transgenic animals speeds up the selective breeding process, as beneficial genes can be transferred between varieties or species
- Health problems often occur among farm animals carrying genes from other species
- The CRISPR-Cas9 system may emerge as a more useful approach

- Agricultural scientists have endowed a number of crop plants with genes for desirable traits
- Genetic engineering in plants has been used to transfer many useful genes, including those for herbicide or pest resistance, increased resistance to salinity, and improved nutritional value of crops

- The Food and Agriculture Organization of the United Nations predicts that we will need 70% more food by the year 2050 than we are currently producing
- The C_4 Rice Project aims to alter a strain of rice to perform C_4 photosynthesis instead of C_3 photosynthesis
- This would increase crop yield at the anticipated higher temperatures of the future

Safety and Ethical Questions Raised by DNA Technology

- Early concerns about potential dangers of recombinant DNA technology focused on the possibility of producing hazardous new pathogens
- Guidelines are in place in the United States and other countries to ensure safe practices for recombinant DNA technology
- Certain obviously dangerous experiments have been banned

- Most public concern about possible hazards centers on **genetically modified organisms (GMOs)** used as food
- Some are concerned about the safety of GM food and possible environmental consequences
- There are concerns that GM crops might transfer genes to wild plants, producing “super weeds”
- Others fear that protein products of transgenes might lead to allergic reactions

- To address the concerns of many Europeans regarding the safety of GM crops, the European Union established a comprehensive legal framework regarding GMOs in 2015
- Individual member states may ban either the growing or importing of GM crops
- GM crops that are grown or imported must be clearly labeled

- Governments and regulators throughout the world are grappling with how to facilitate the use of biotechnology in agriculture, industry and medicine while ensuring that procedures are safe
- Great benefits could result from biotechnology approaches, but unforeseen problems could arise
- We must proceed with humility and caution

Figure 20.UN01

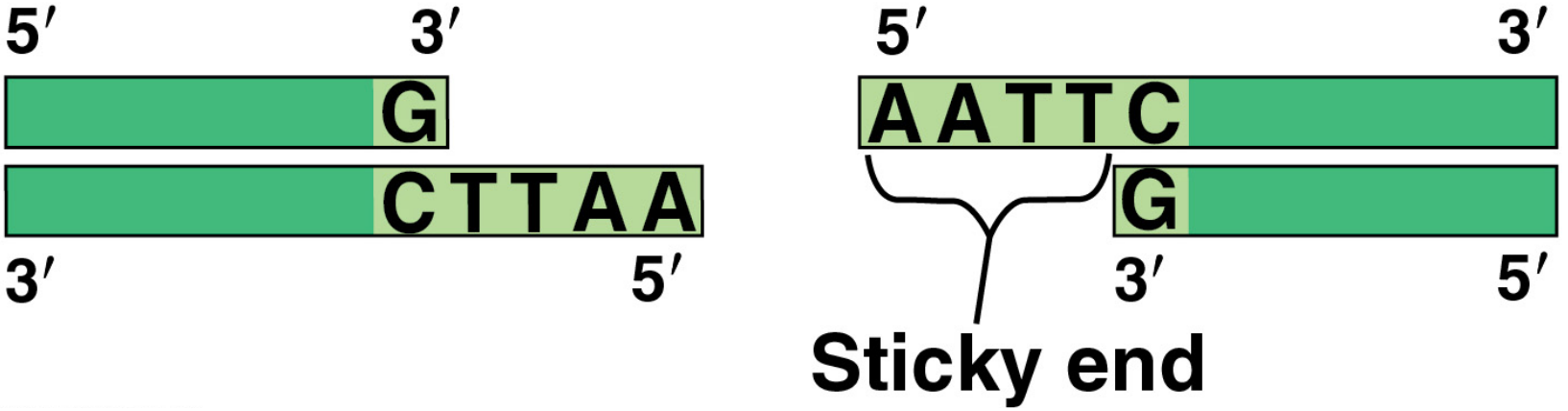


Figure 20.UN02

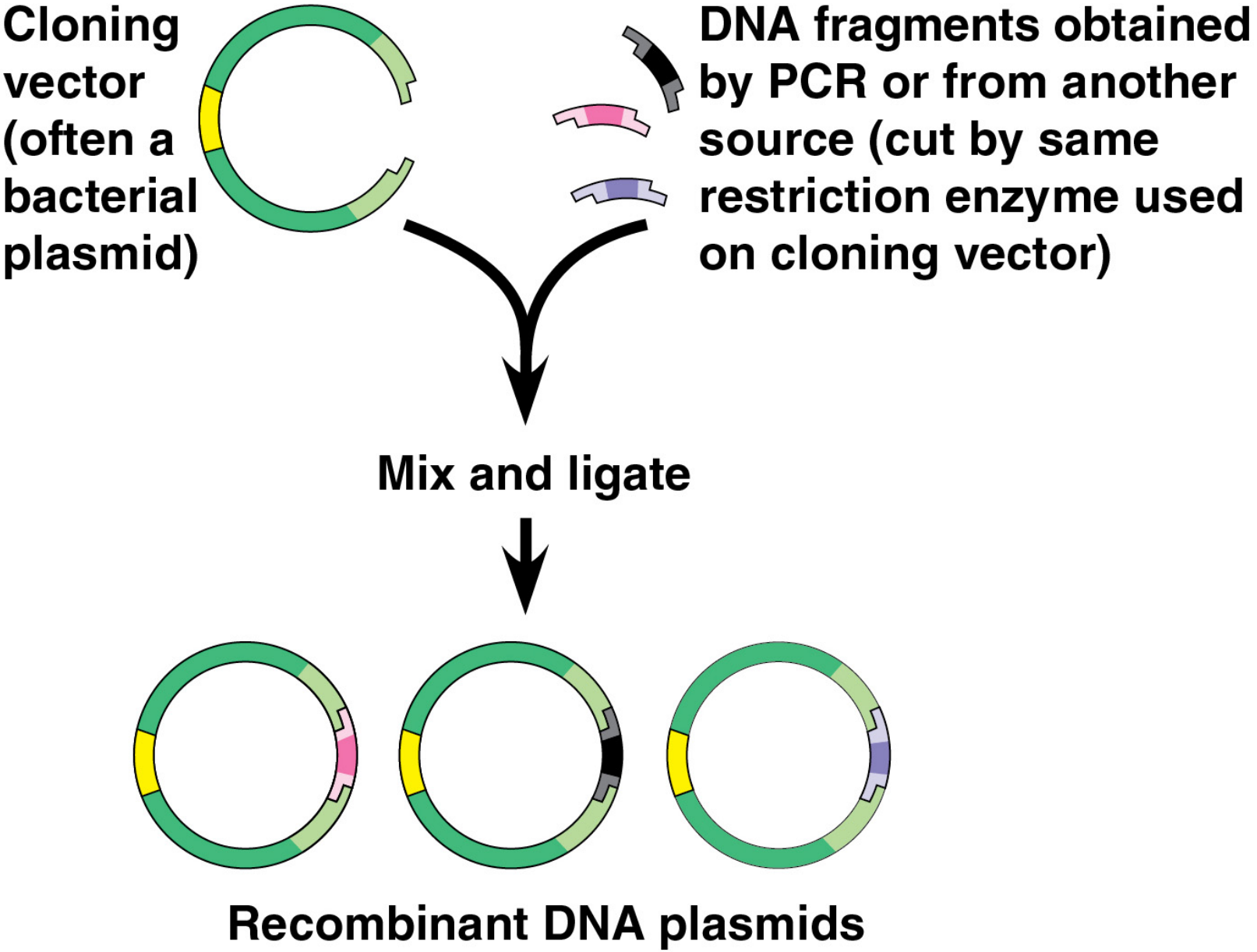


Figure 20.UN03

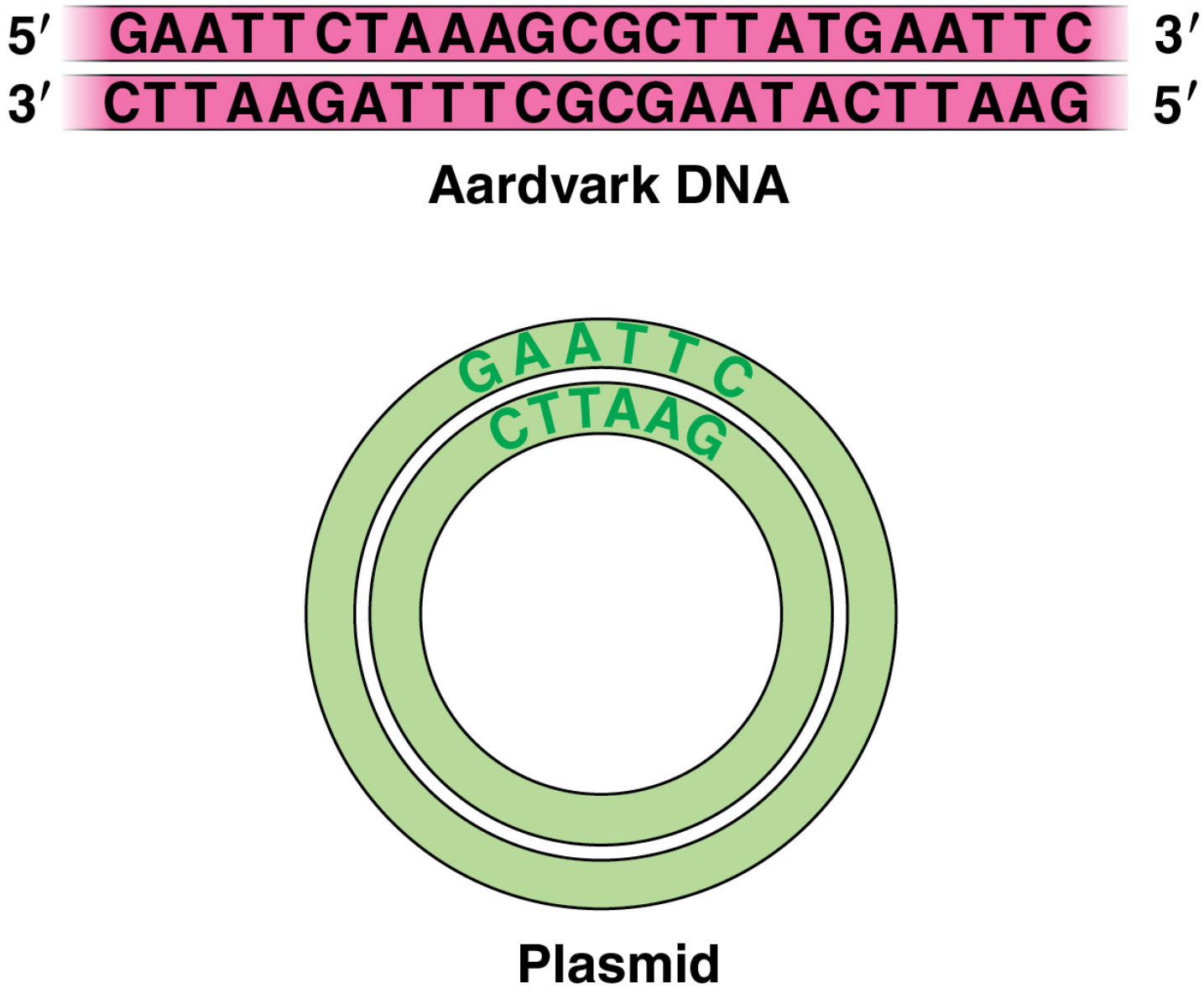


Figure 20.UN04