

Campbell Biology

A Global Approach

10th edition

Chapter 19

DNA Biotechnology

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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

- Recently the genome sequences of two extinct species—Neanderthals and wooly mammoths—have been completed
- Advances in sequencing techniques make genome sequencing increasingly faster and less expensive

Figure 19.1

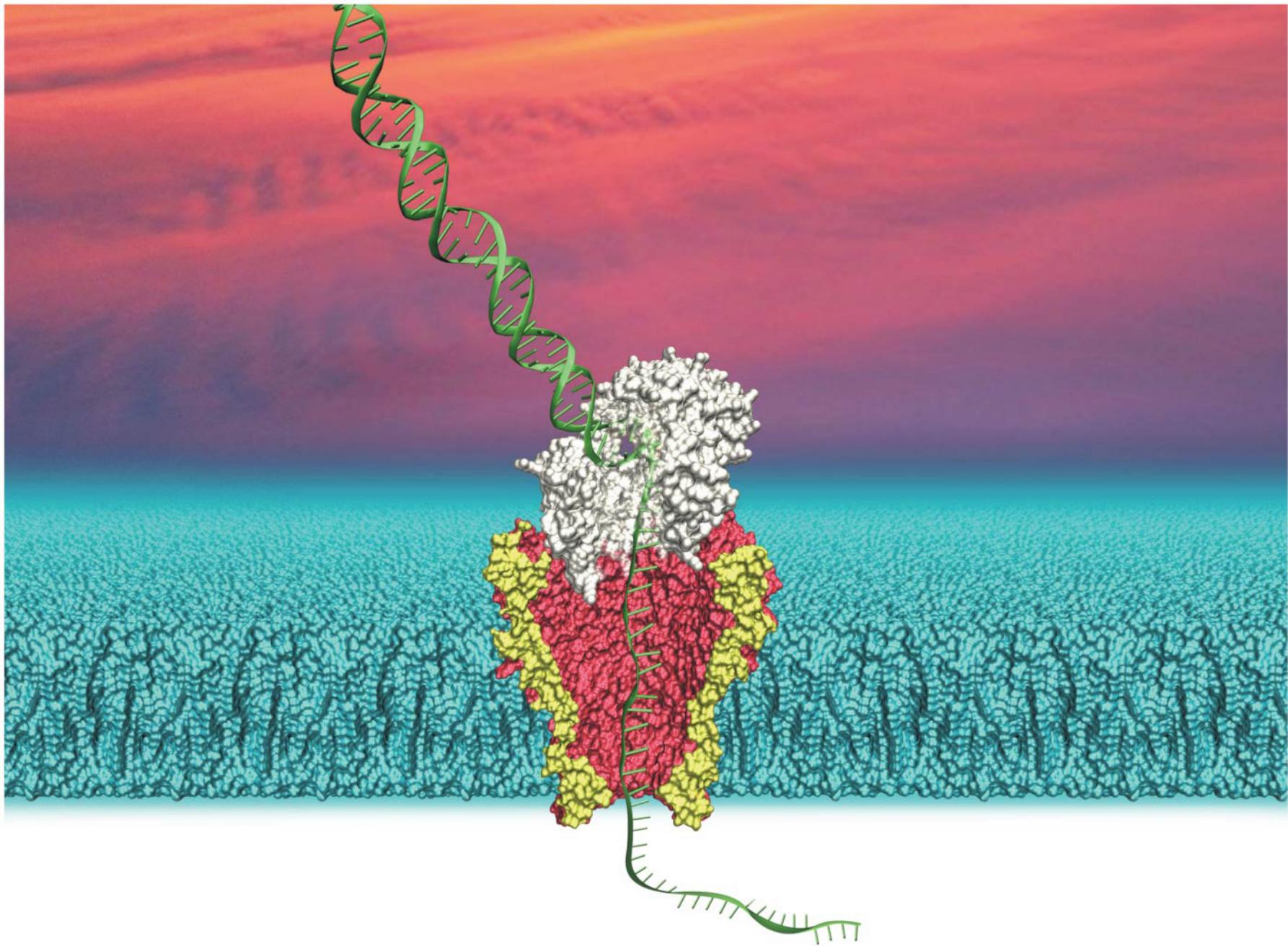


Figure 19.1a



The New Yorker, August 15, 2011
Annals of Evolution
What happened between the Neanderthals and us?



- 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
- The applications of **DNA technology** affect everything from agriculture, to criminal law, to medical research

Concept 19.1

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

- 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

- Researchers can exploit the principle of complementary base pairing to determine a gene's complete nucleotide sequence, called **DNA sequencing**
- The first automated procedure was based on a technique called dideoxy or chain termination sequencing, developed by Sanger

DNA Sequencing

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

Figure 19.2



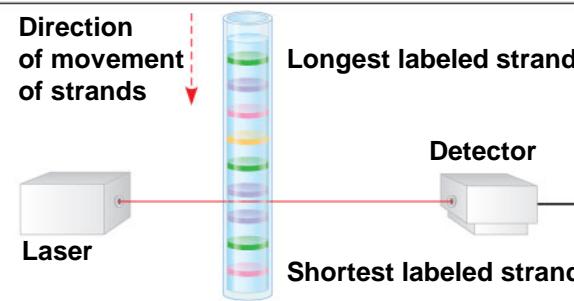
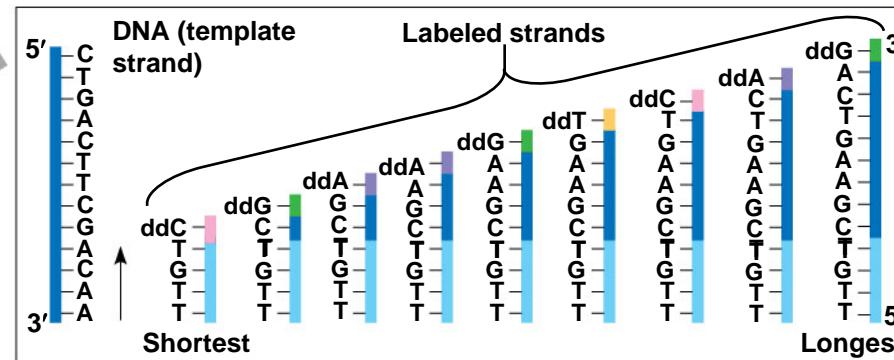
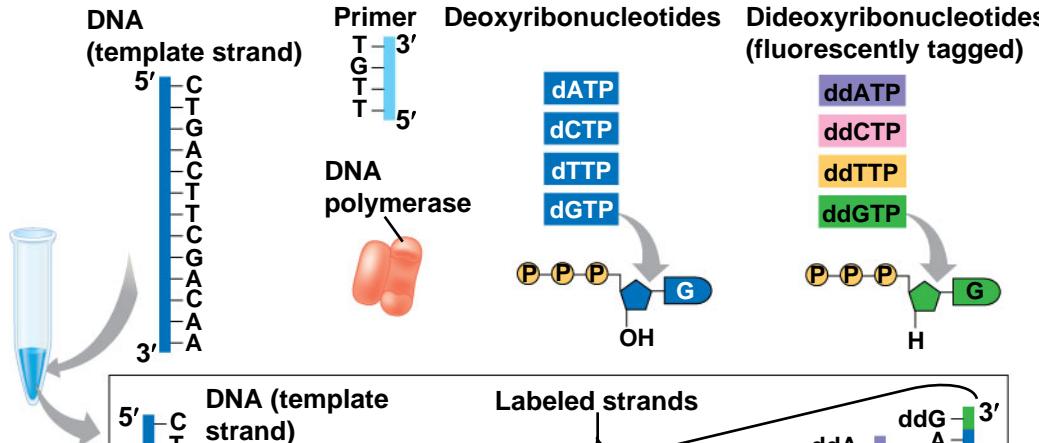
(a) Standard sequencing machine



(b) Next-generation sequencing machines

Figure 19.3

TECHNIQUE



RESULTS

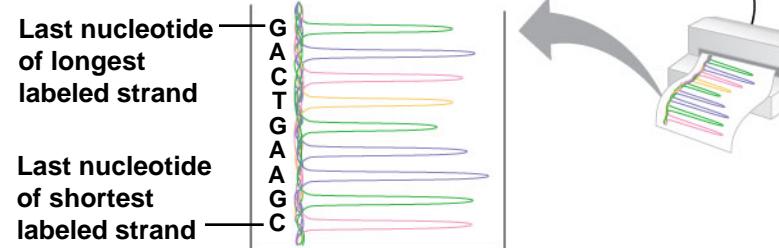


Figure 19.3a

TECHNIQUE

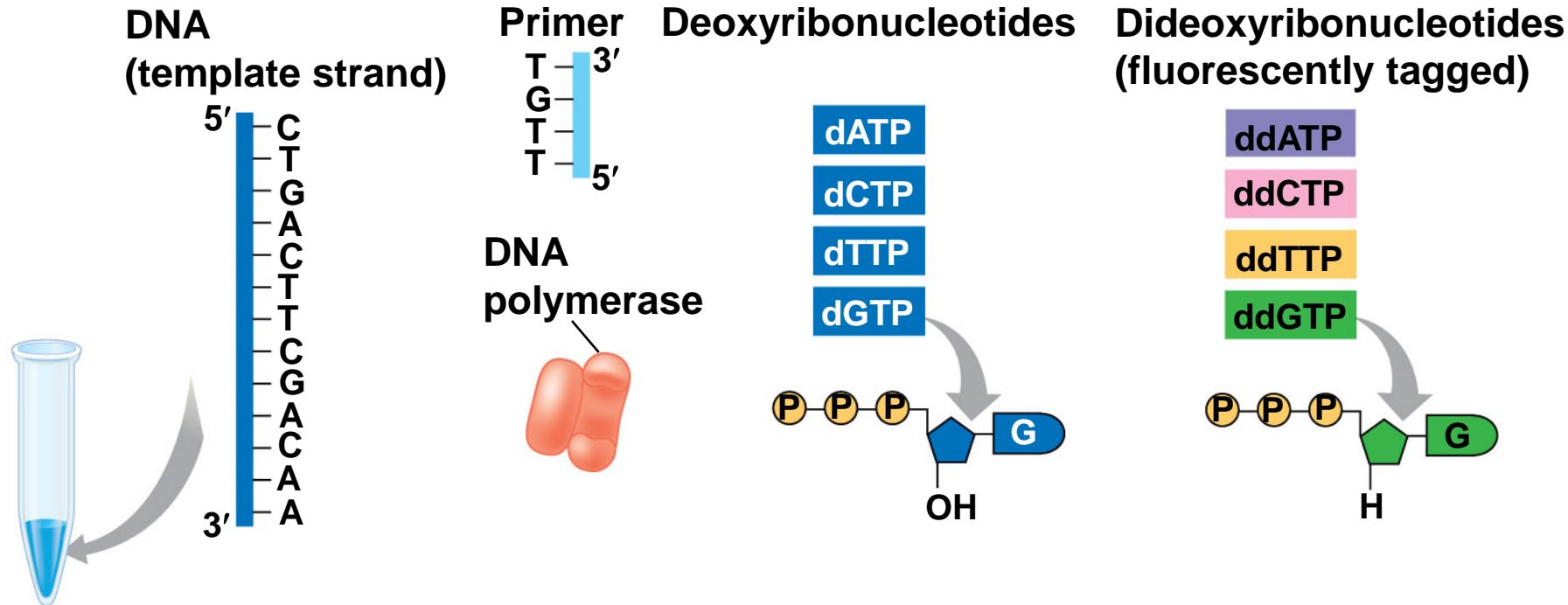


Figure 19.3b

TECHNIQUE (continued)

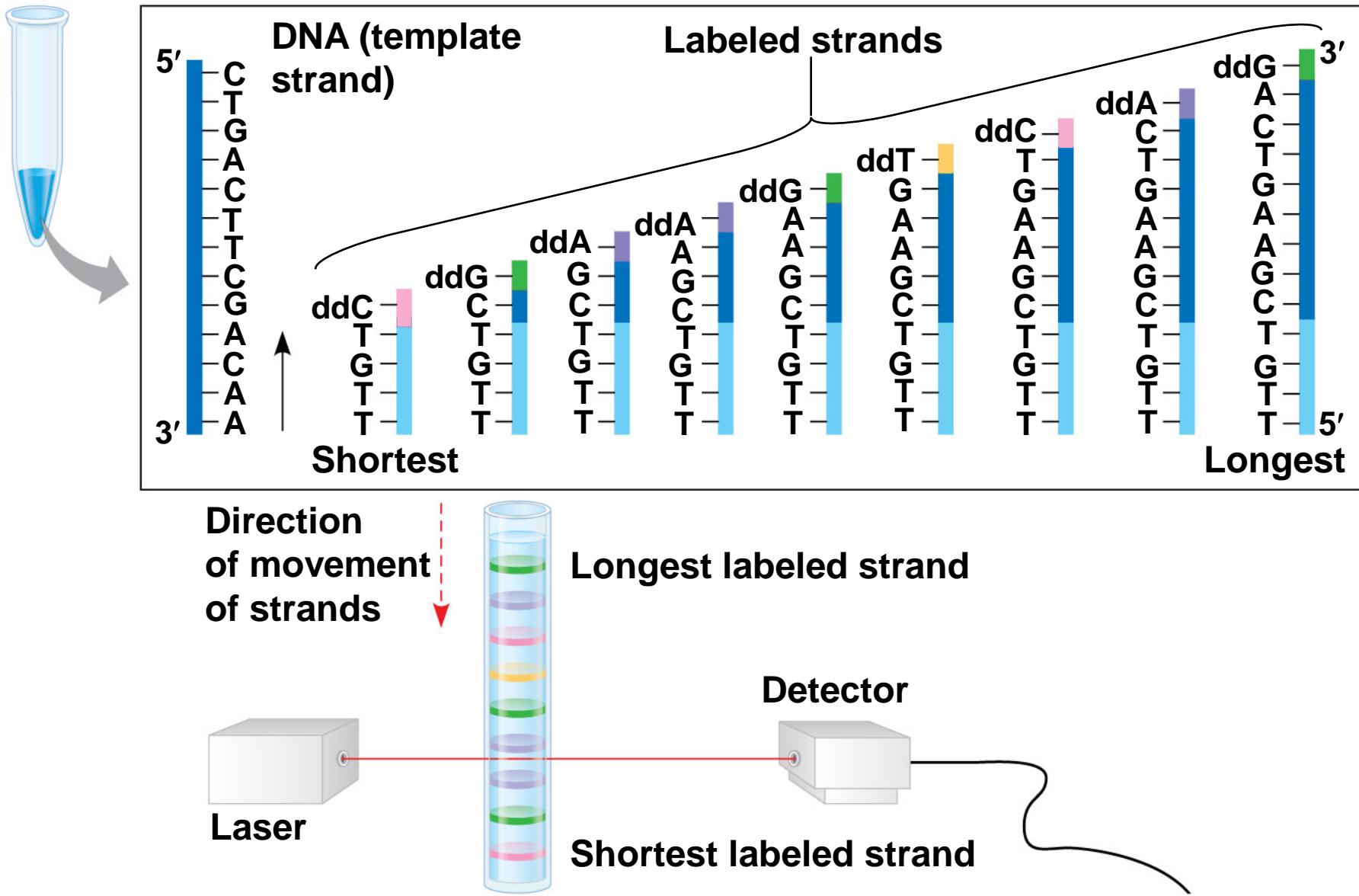
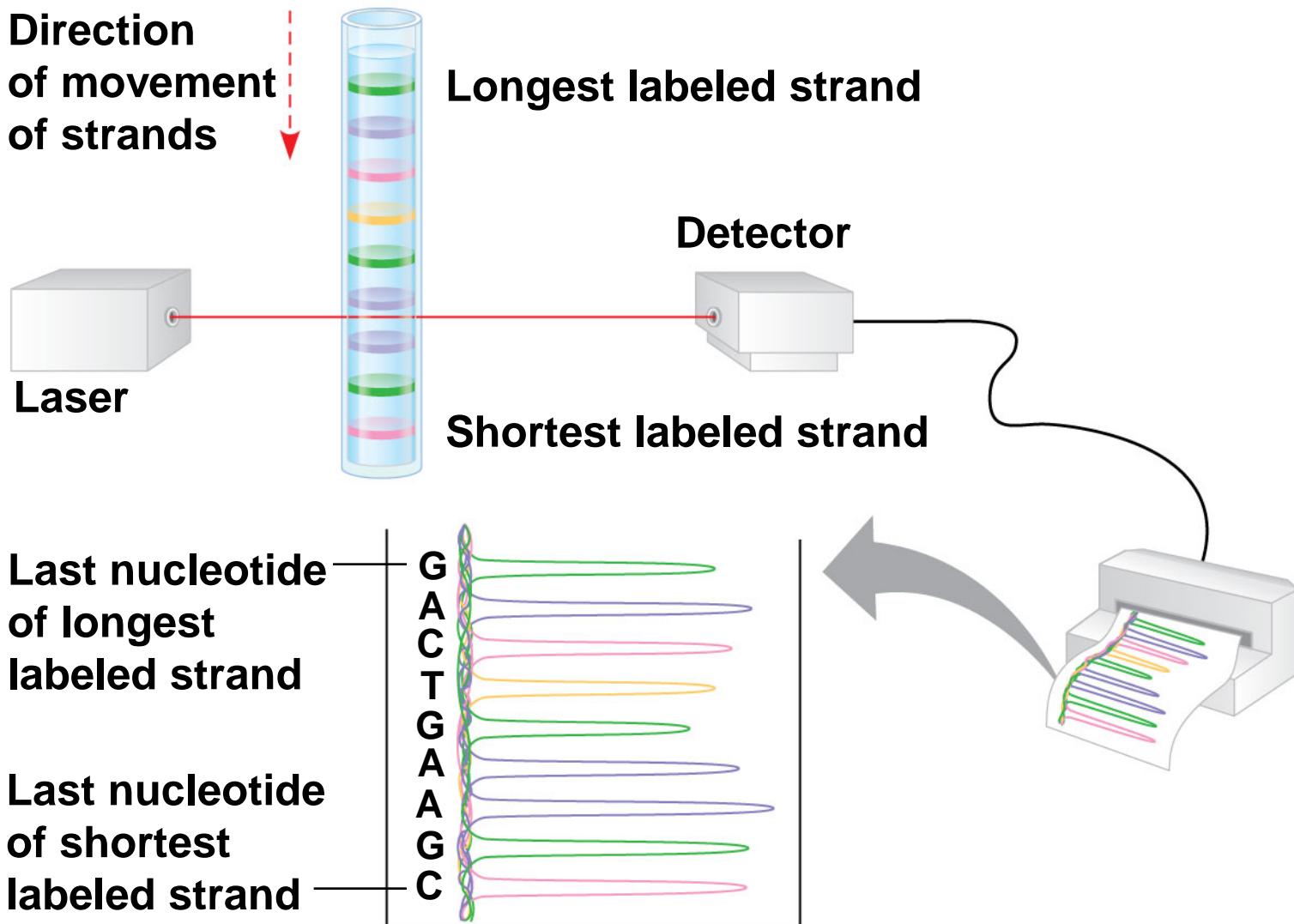


Figure 19.3c



- “**Next-generation sequencing**” techniques use a single template strand that is immobilized and amplified to produce an enormous number of identical fragments
- Thousands or hundreds of thousands of fragments (400–1,000 nucleotides long) are sequenced in parallel
- This is a type of “high-throughput” technology

Figure 19.4

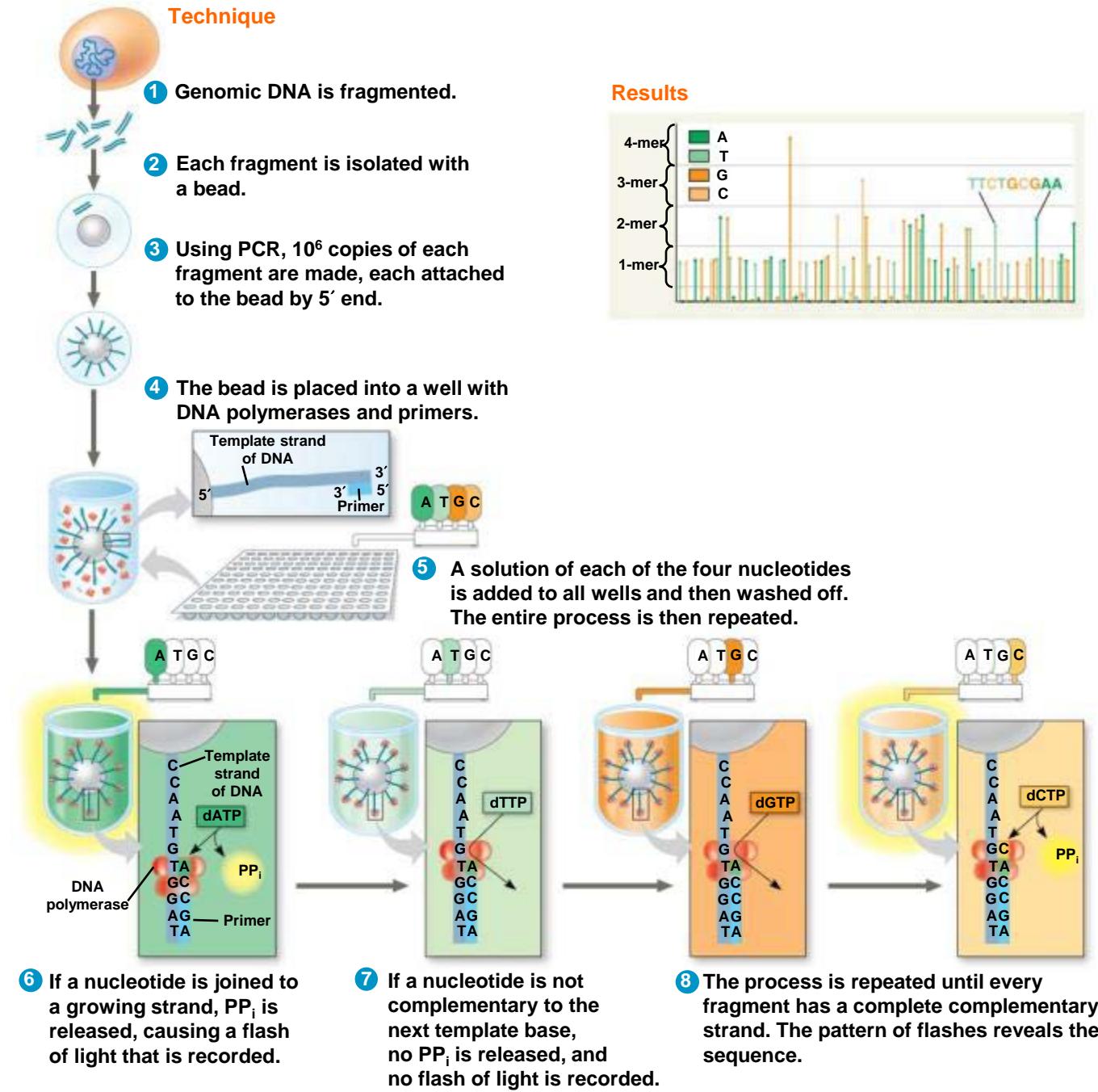
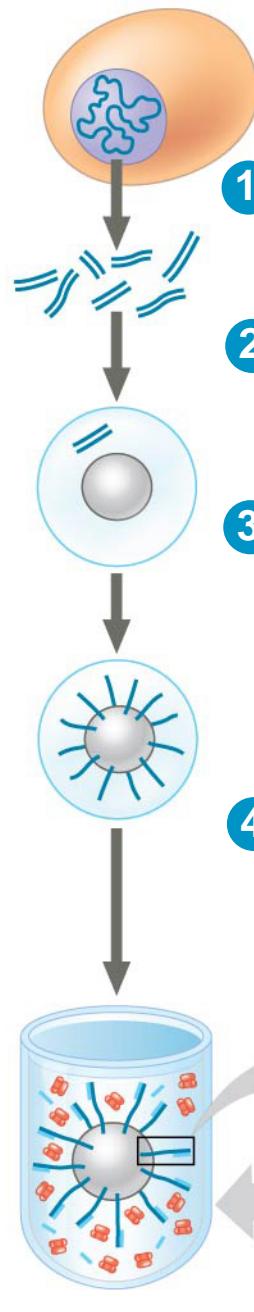


Figure 19.4a

Technique

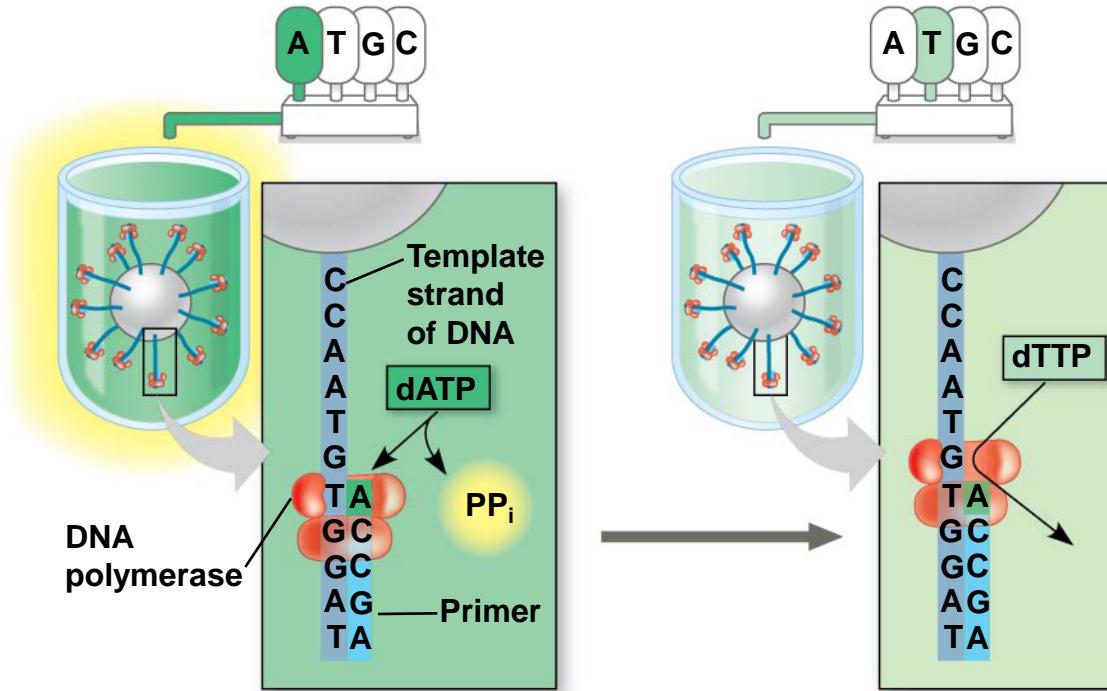


- 1 Genomic DNA is fragmented.
- 2 Each fragment is isolated with a bead.
- 3 Using PCR, 10^6 copies of each fragment are made, each attached to the bead by 5' end.
- 4 The bead is placed into a well with DNA polymerases and primers.

- 5 A solution of each of the four nucleotides is added to all wells and then washed off. The entire process is then repeated.

Figure 19.4b

Technique

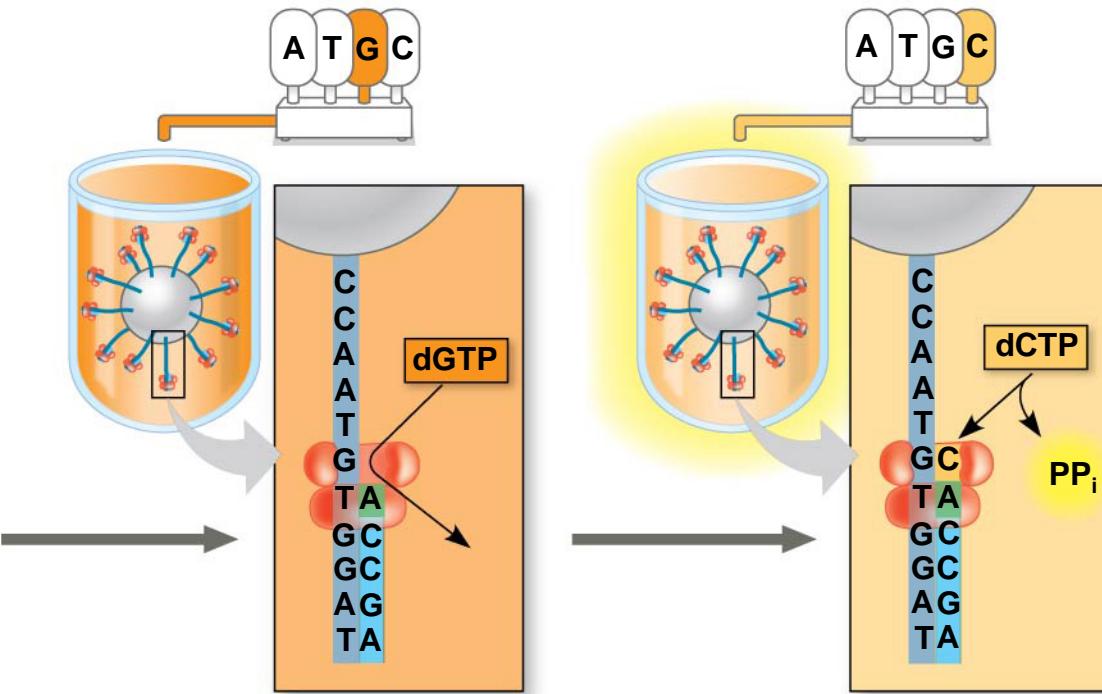


6 If a nucleotide is joined to a growing strand, PP_i is released, causing a flash of light that is recorded.

7 If a nucleotide is not complementary to the next template base, no PP_i is released, and no flash of light is recorded.

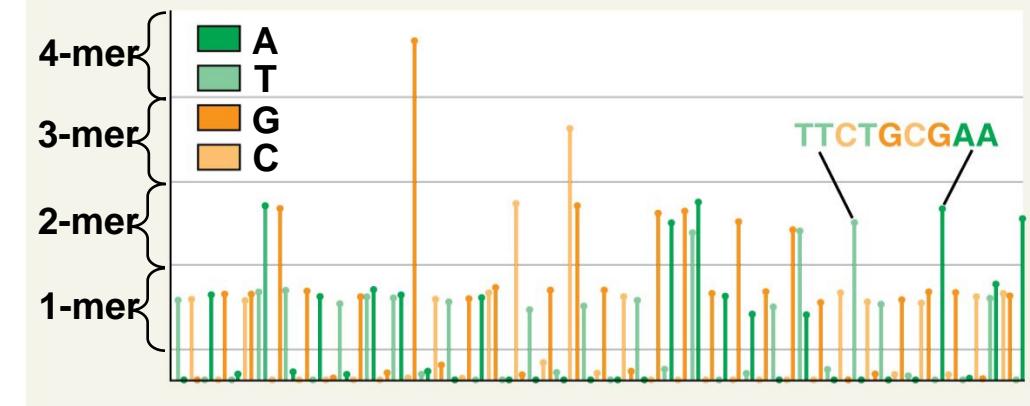
Figure 19.4c

Technique



- 8** The process is repeated until every fragment has a complete complementary strand. The pattern of flashes reveals the sequence.

Results



- In “third-generation sequencing,” the techniques used are even faster and less expensive than the previous

Making 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**
- **Plasmids** are small circular DNA molecules that replicate separately from the bacterial chromosome
- Researchers can insert DNA into plasmids to produce **recombinant DNA**, a molecule with DNA from two different sources

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
- Cloned genes are useful for making copies of a particular gene and producing a protein product

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

Figure 19.5a

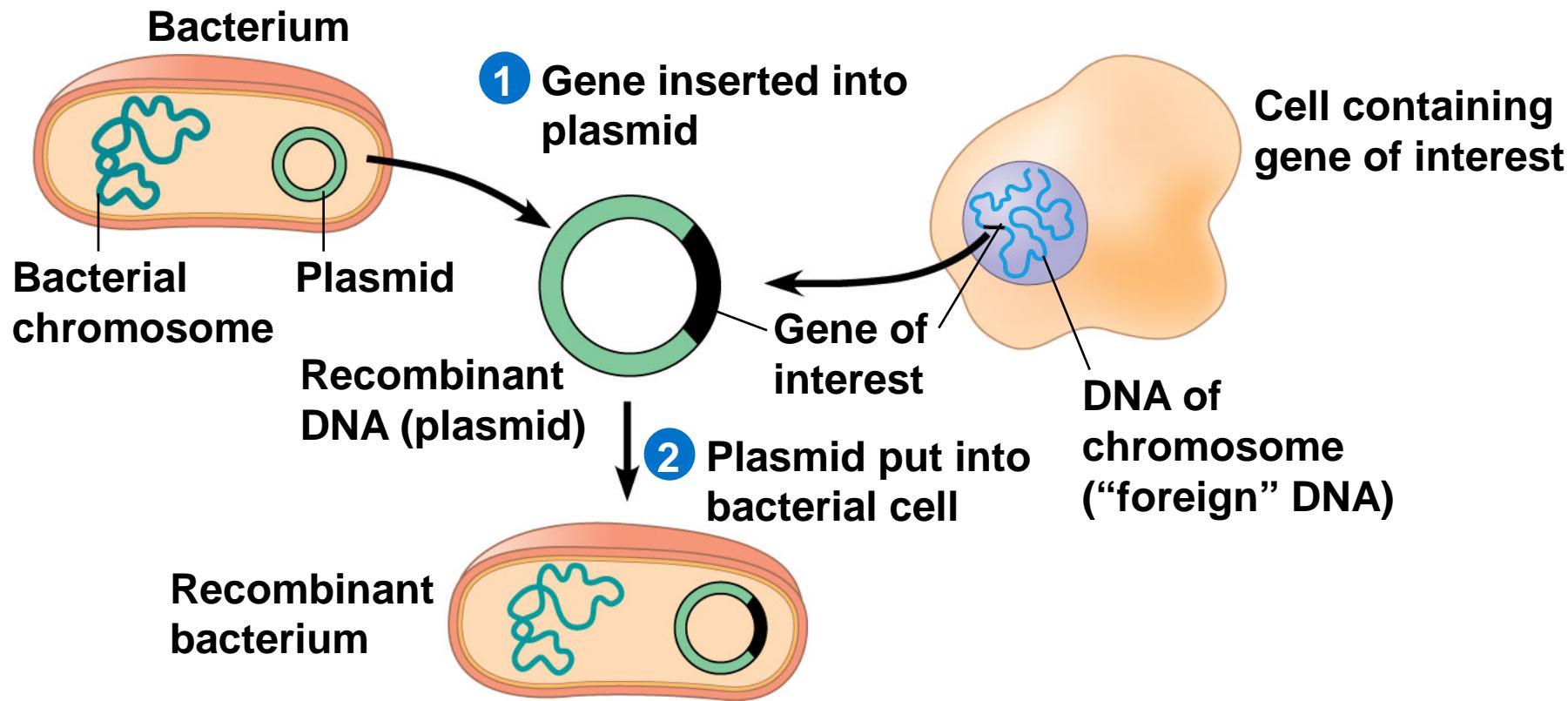
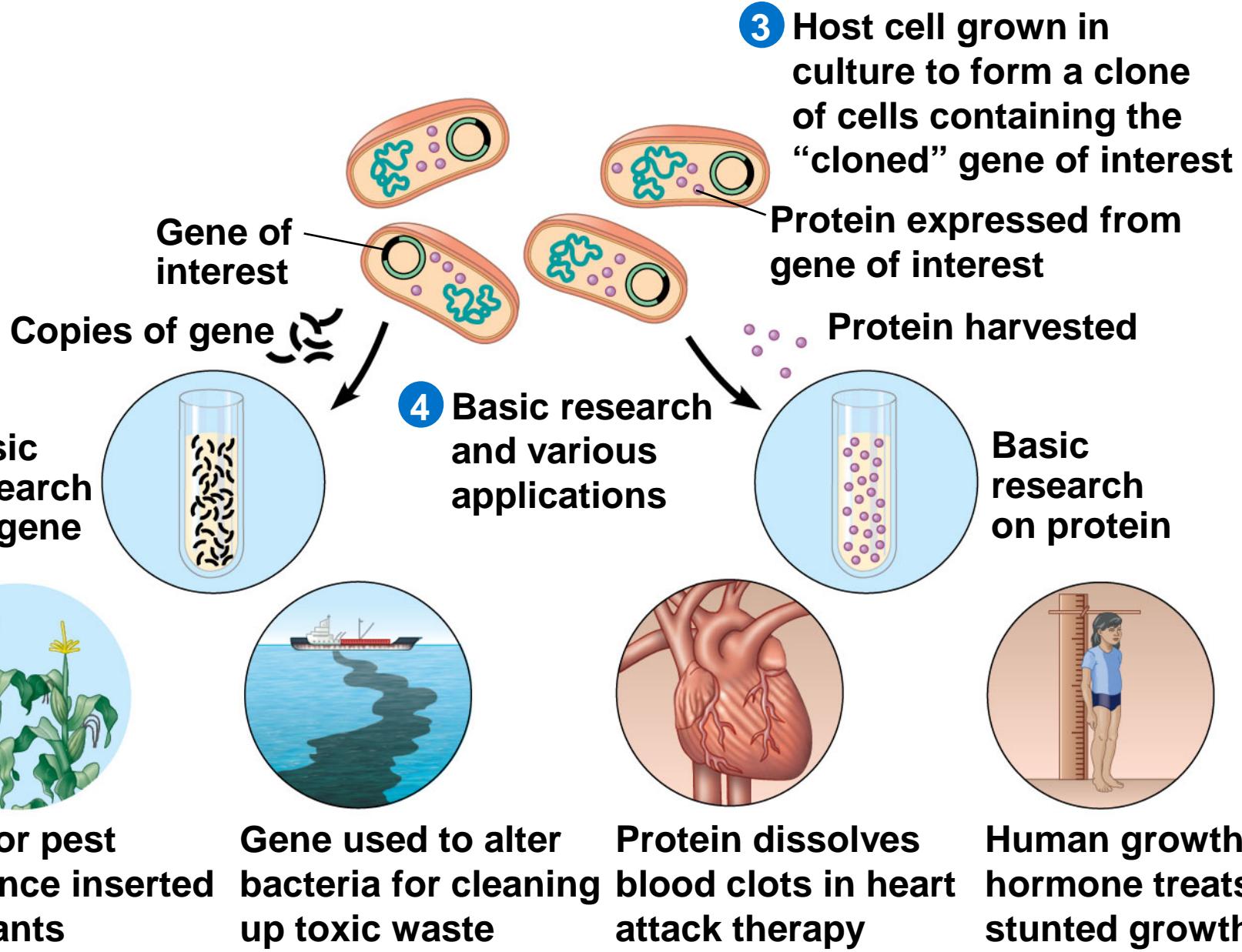


Figure 19.5b



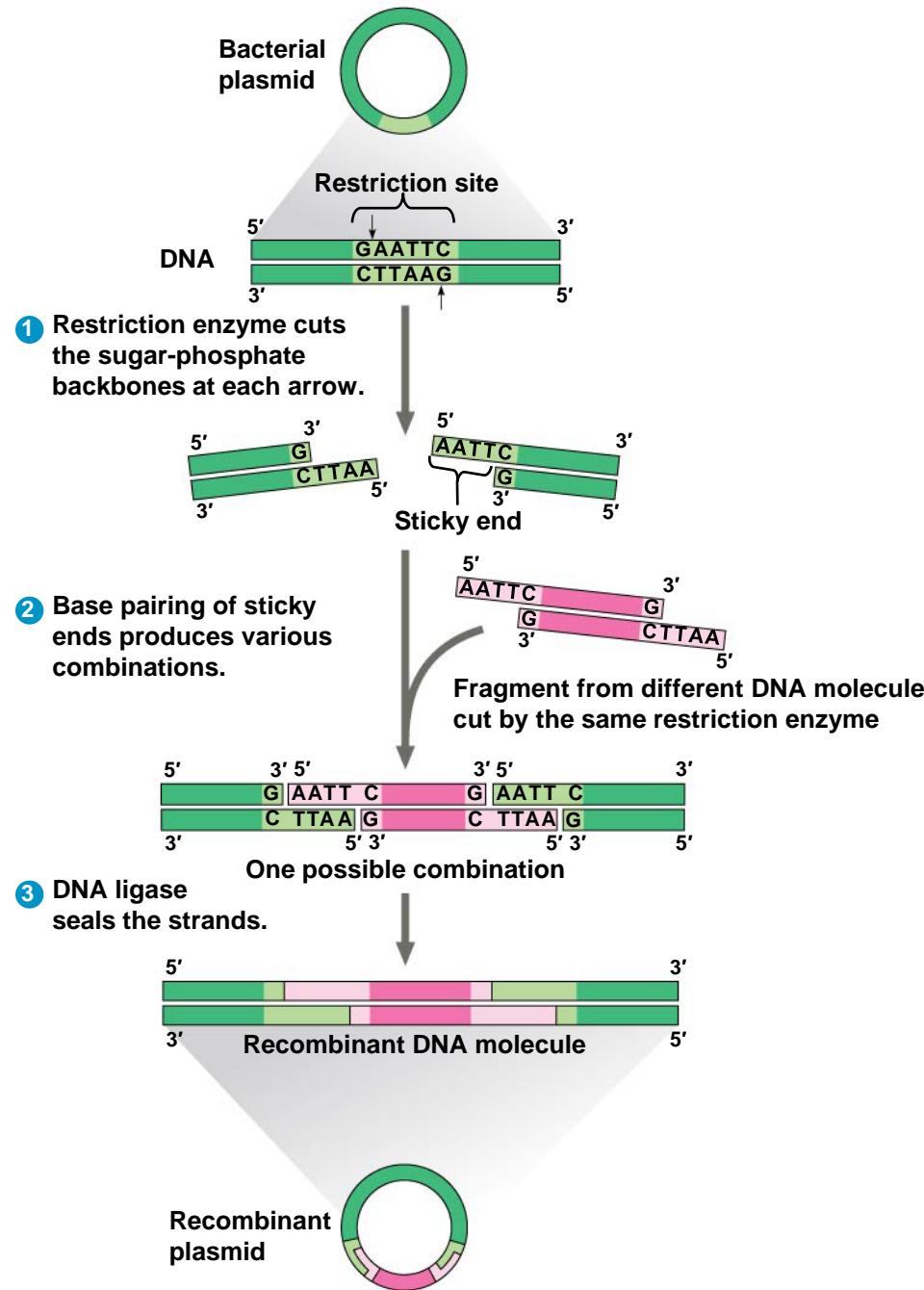
- A plasmid used to clone a foreign gene is called a **cloning vector**
- Bacterial plasmids are widely used as cloning vectors because they are readily obtained, easily manipulated, easily introduced into bacterial cells, and once in the bacteria they multiply rapidly
- Gene cloning is useful for amplifying genes to produce a protein product for research, medical, or other purposes

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**.”

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

Figure 19.6



Cloning a Eukaryotic Gene in a Bacterial Plasmid

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

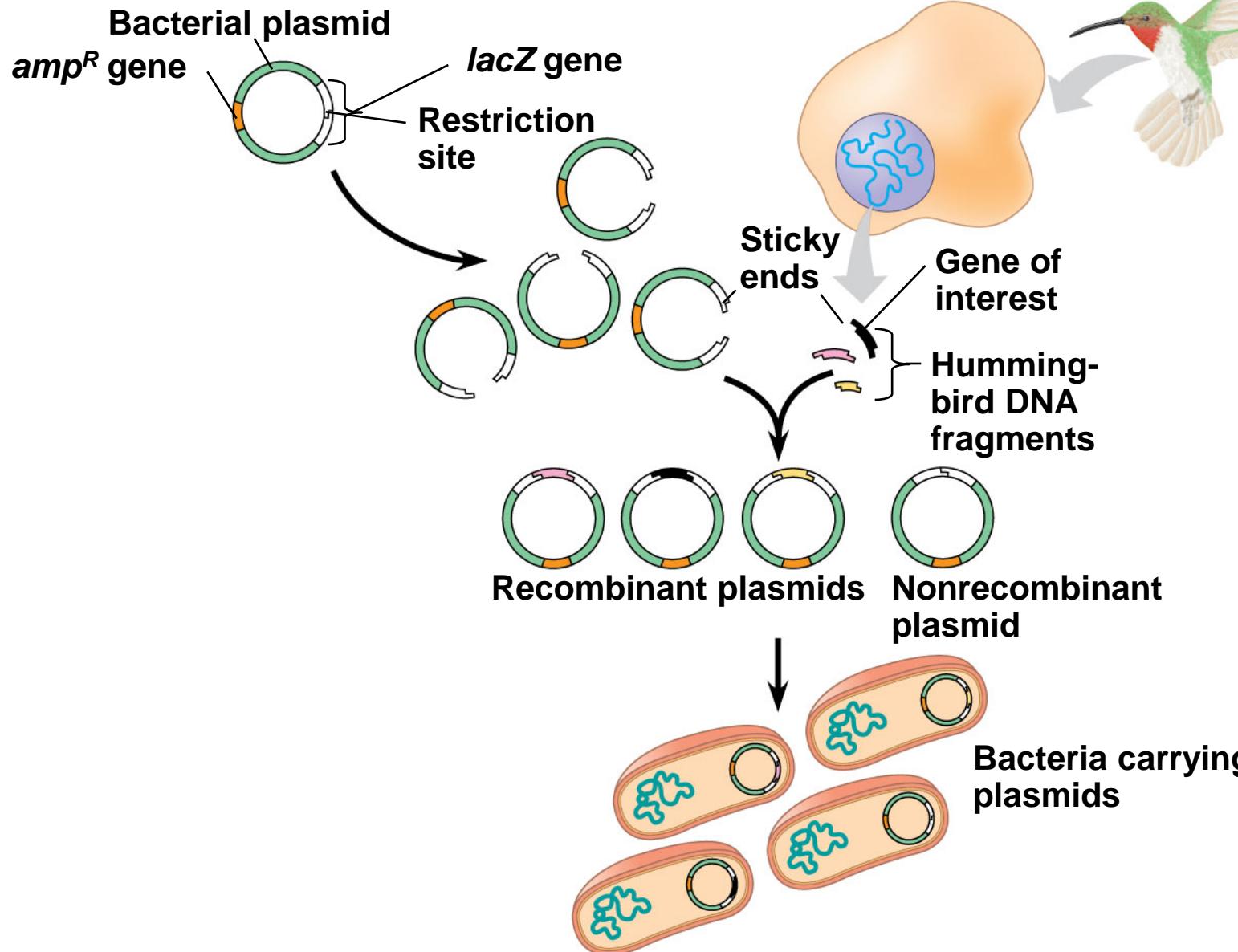
Producing Clones of Cells Carrying Recombinant Plasmids

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

- 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

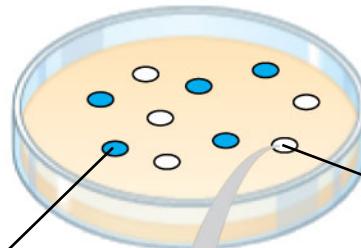
Research Method: Cloning Genes in Bacterial Plasmids

TECHNIQUE

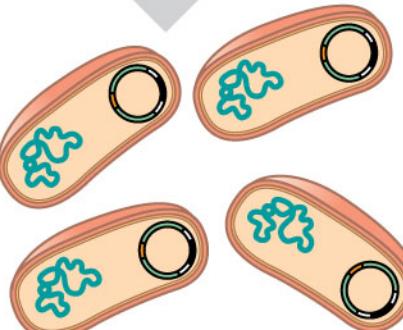


RESULTS

Bacteria carrying plasmids



Colony carrying non-recombinant plasmid with intact *lacZ* gene



Colony carrying recombinant plasmid with disrupted *lacZ* gene

One of many bacterial clones

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

Genomic libraries

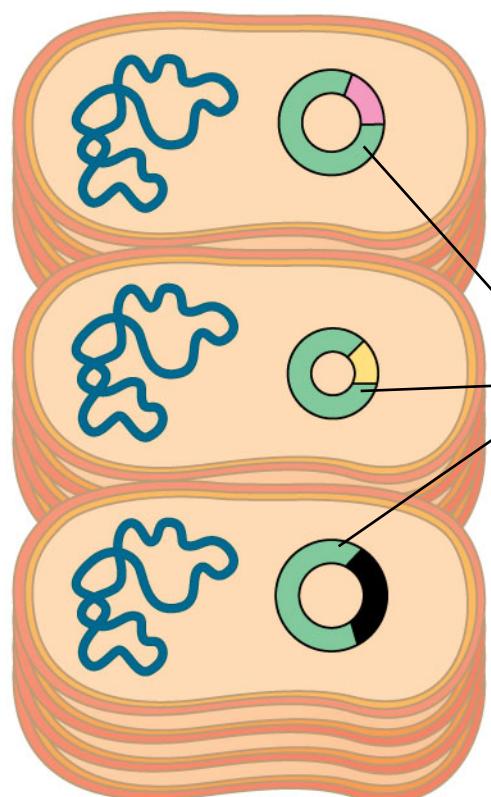
Foreign genome



Cut with restriction enzymes into either

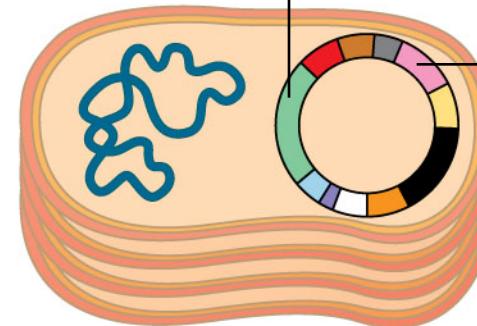
small
fragments

or
large
fragments

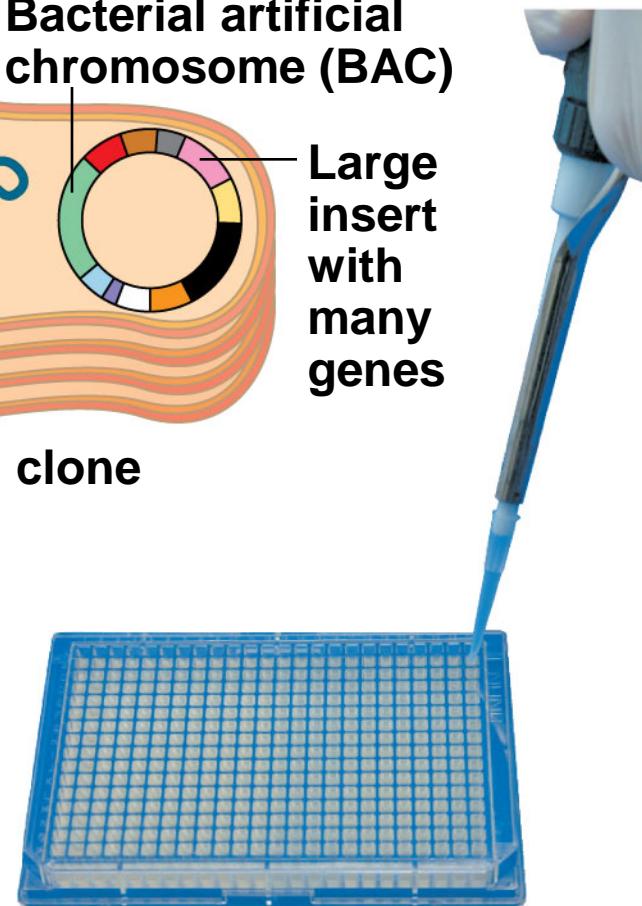


(a) Plasmid library

Bacterial artificial
chromosome (BAC)



(b) BAC clone



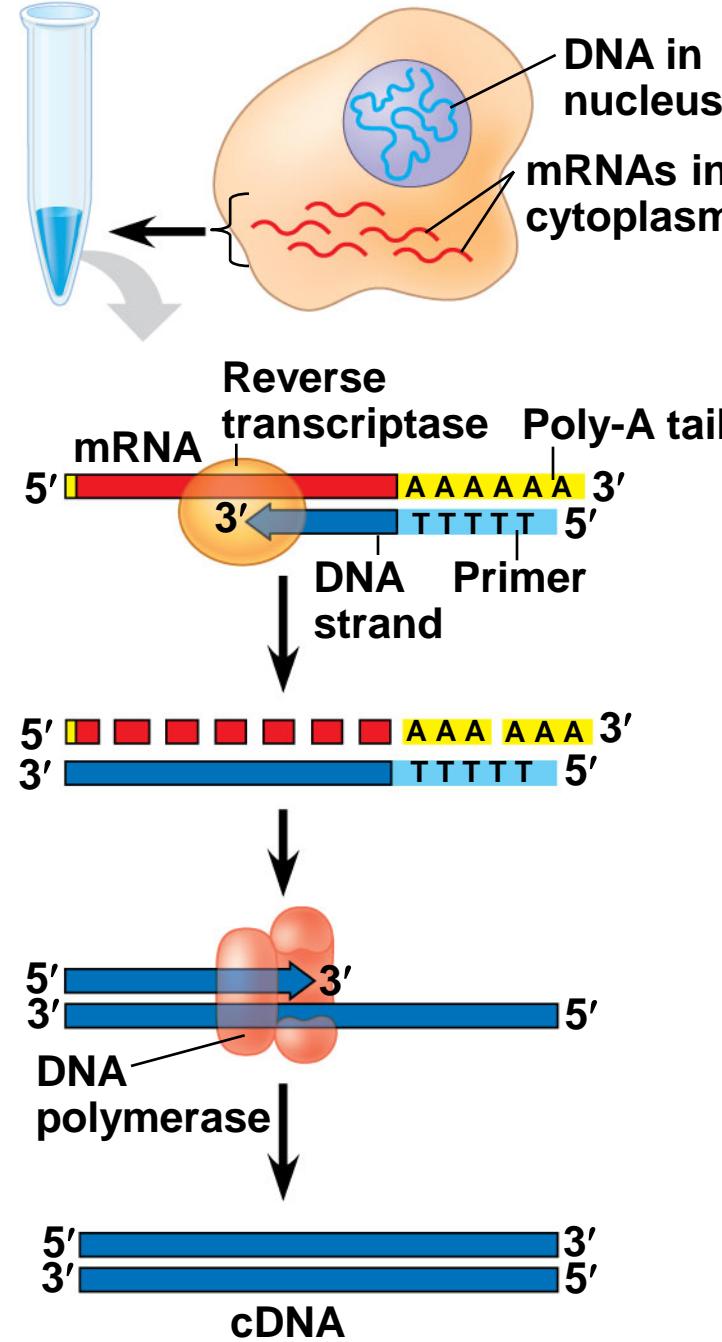
(c) Storing genome libraries

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

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

Figure 19.11

Making cDNA from eukaryotic genes



Screening a Library for Clones Carrying a Gene of Interest

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

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

5' ... CTCATCACCGGC... 3'

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- Then we would synthesize this probe

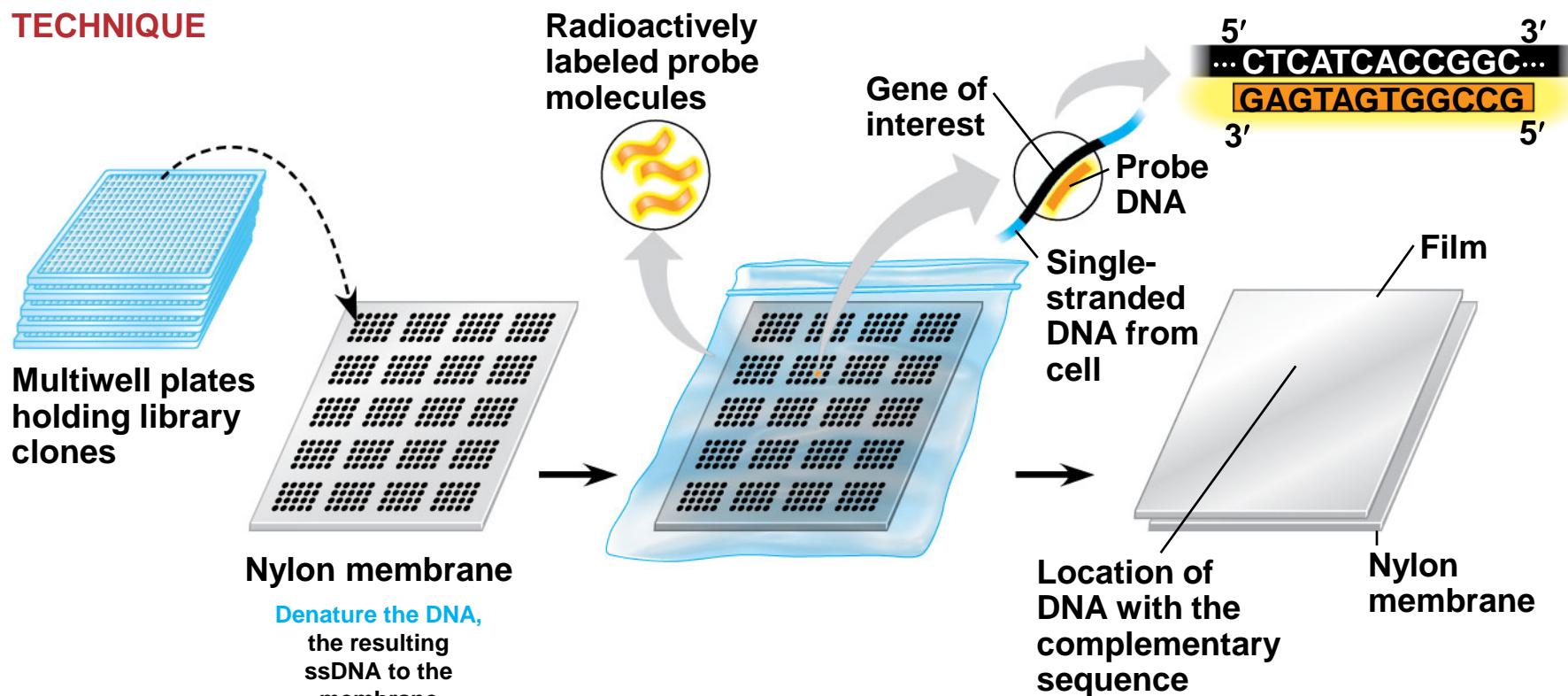
3' GAGTAGTGGCCG 5'

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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

Research Method: Detecting a Specific DNA Sequence by Hybridization with a Nucleic Acid Probe

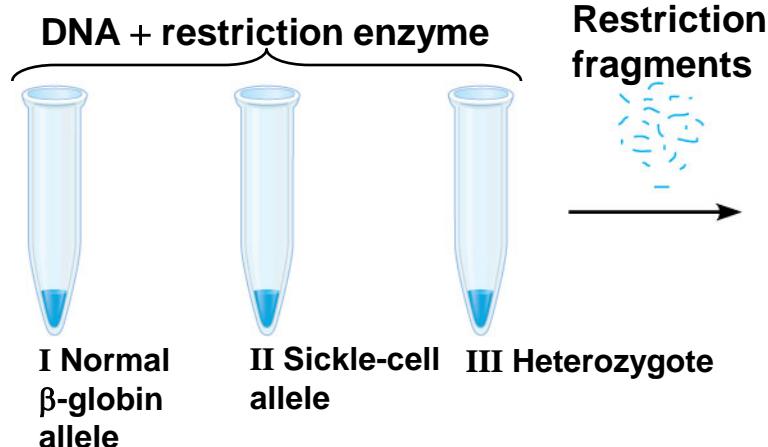
TECHNIQUE



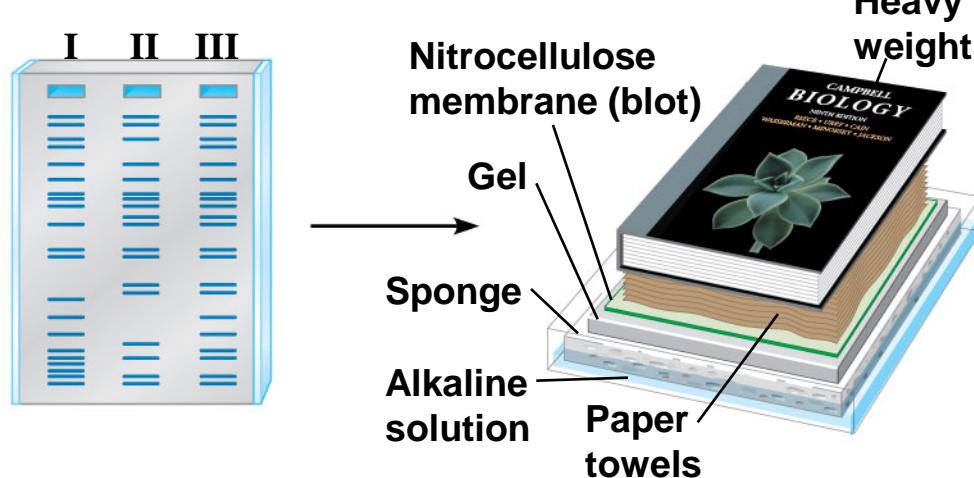
- A technique called **Southern blotting** combines gel electrophoresis of DNA fragments with nucleic acid hybridization
- Specific DNA fragments can be identified by Southern blotting, using labeled probes that hybridize to the DNA immobilized on a “blot” of gel

Research Method: Southern Blotting of DNA Fragments

TECHNIQUE

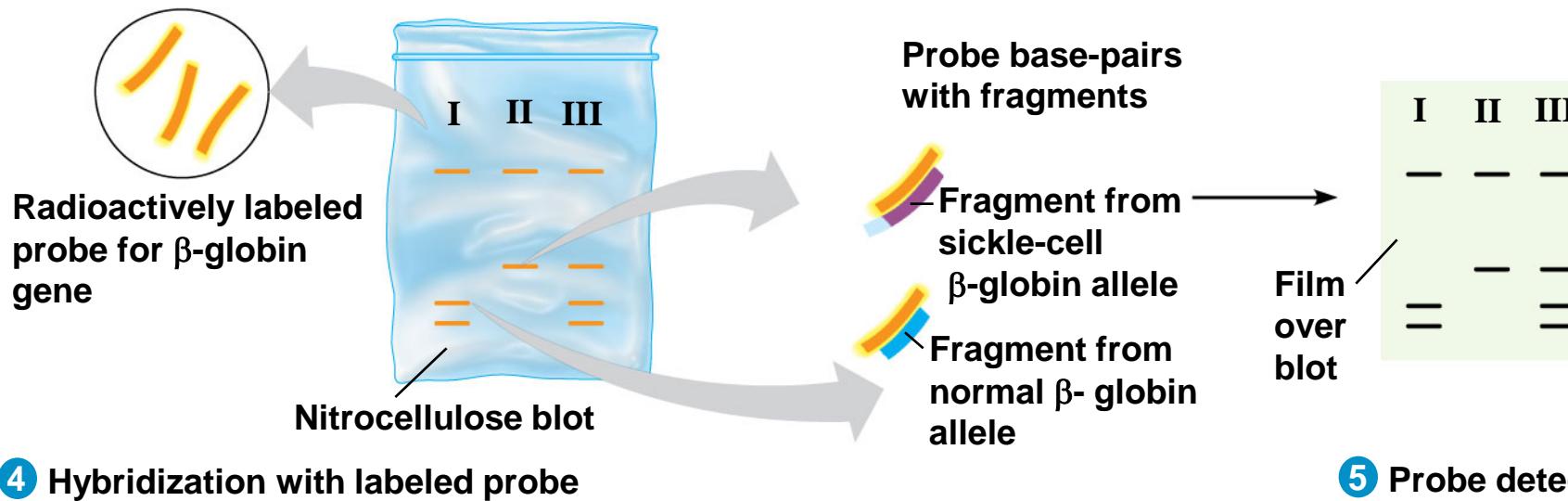


- 1 Preparation of restriction fragments



- 2 Gel electrophoresis

- 3 DNA transfer (blotting)



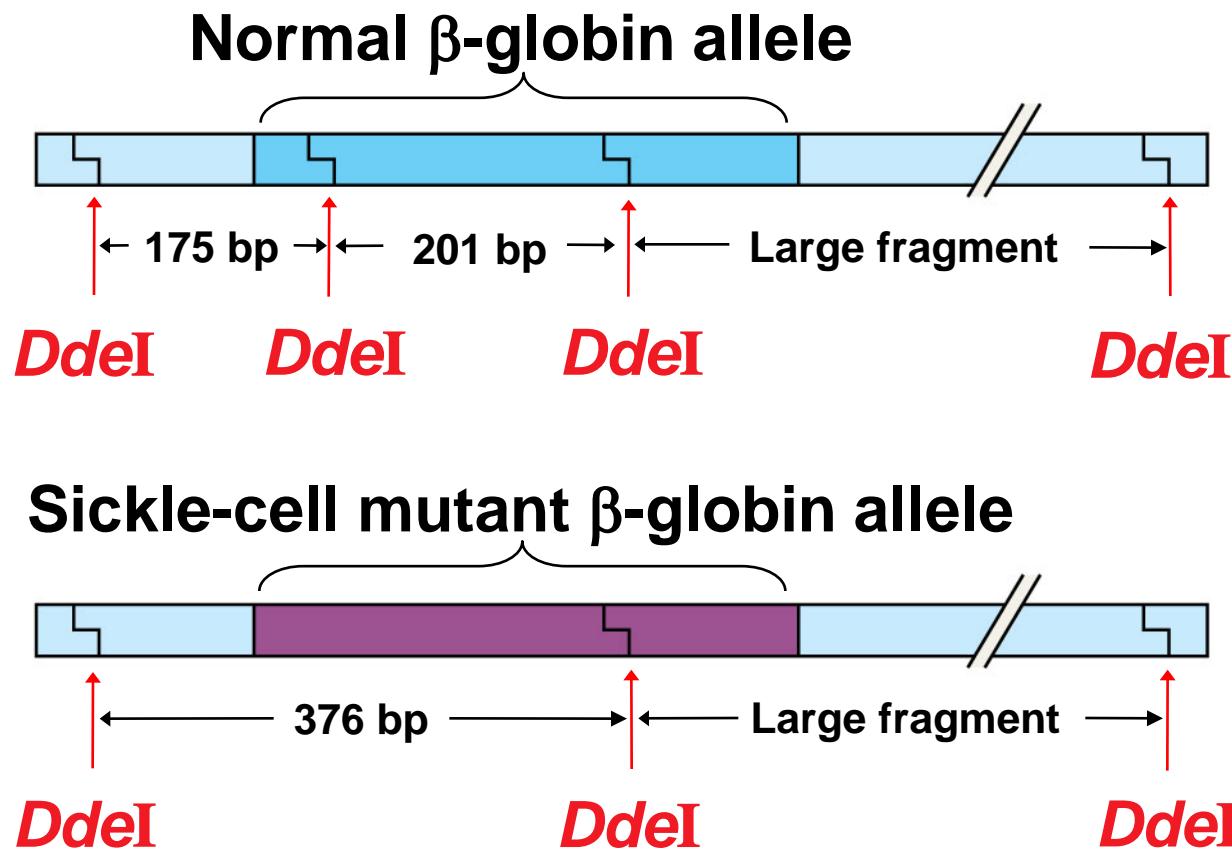
- 4 Hybridization with labeled probe

- 5 Probe detection

Gel Electrophoresis and Southern Blotting

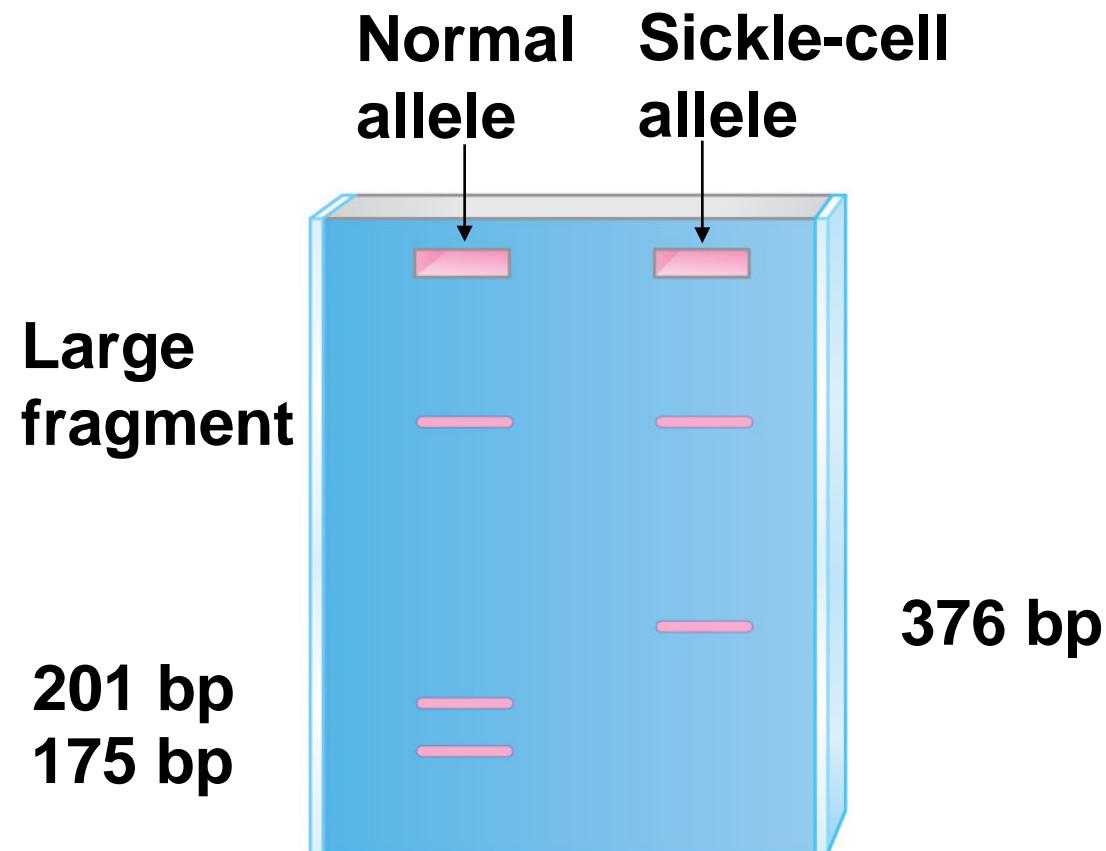
- One indirect method of rapidly analyzing and comparing genomes is **gel electrophoresis**
- This technique uses a gel as a molecular sieve to separate nucleic acids or proteins by size, electrical charge, and other properties
- A current is applied that causes charged molecules to move through the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

Using restriction fragment analysis to distinguish the normal and sickle-cell alleles of the human β -globin gene



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

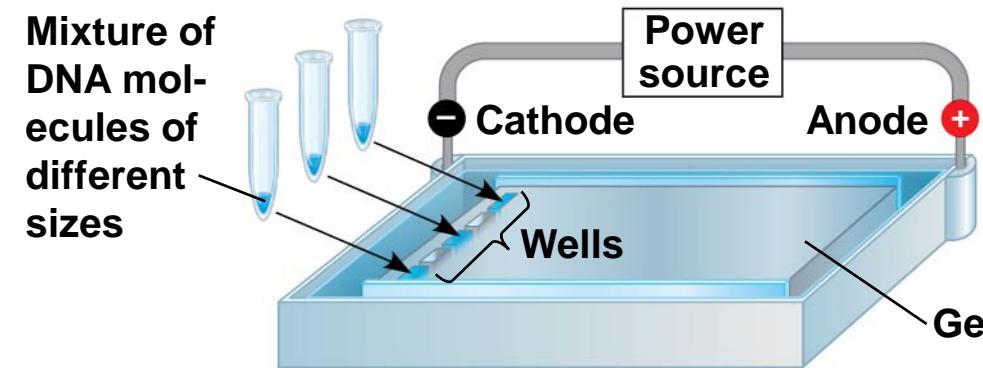
Using restriction fragment analysis to distinguish the normal and sickle-cell alleles of the human β -globin gene



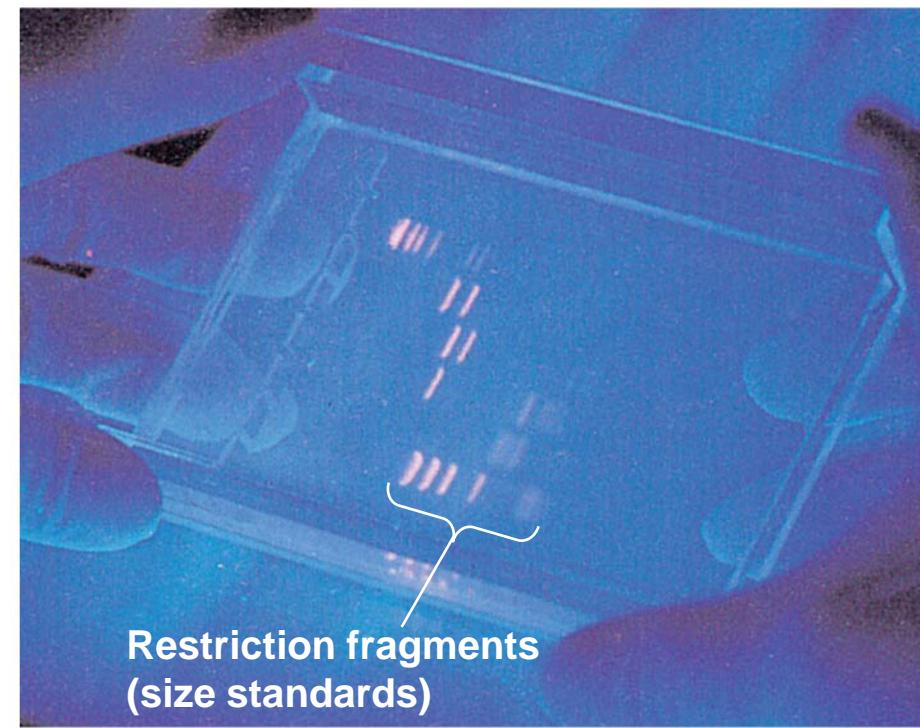
(b) Electrophoresis of restriction fragments from normal and sickle-cell alleles

- 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** would be carried out
- This technique uses a gel made of a polymer to separate a mixture of nucleic acids or proteins based on size, charge, or other physical properties

Figure 19.7



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



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

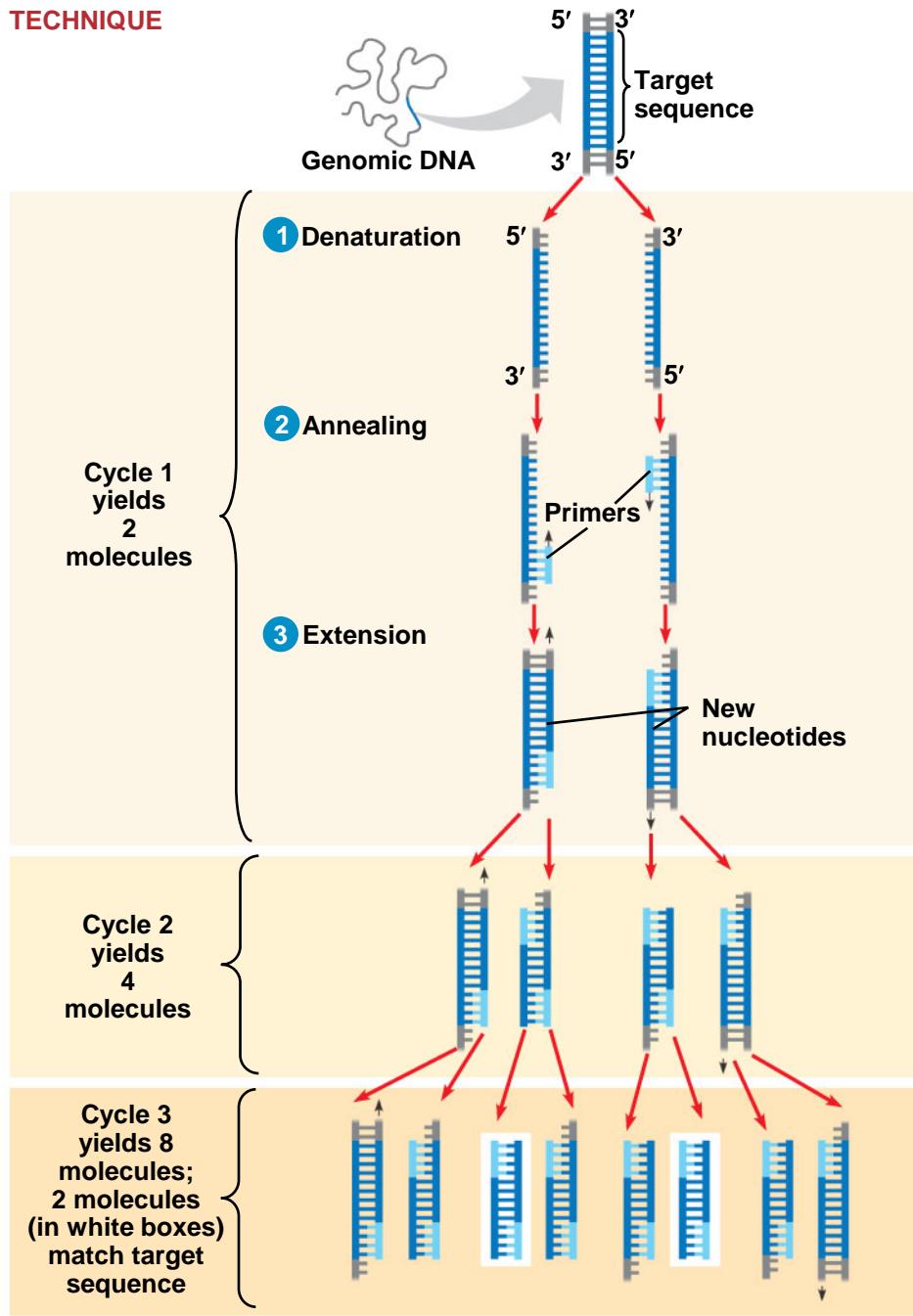
Amplifying DNA *in Vitro*: The Polymerase Chain Reaction (PCR)

- 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*.

- PCR uses a pair of primers 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

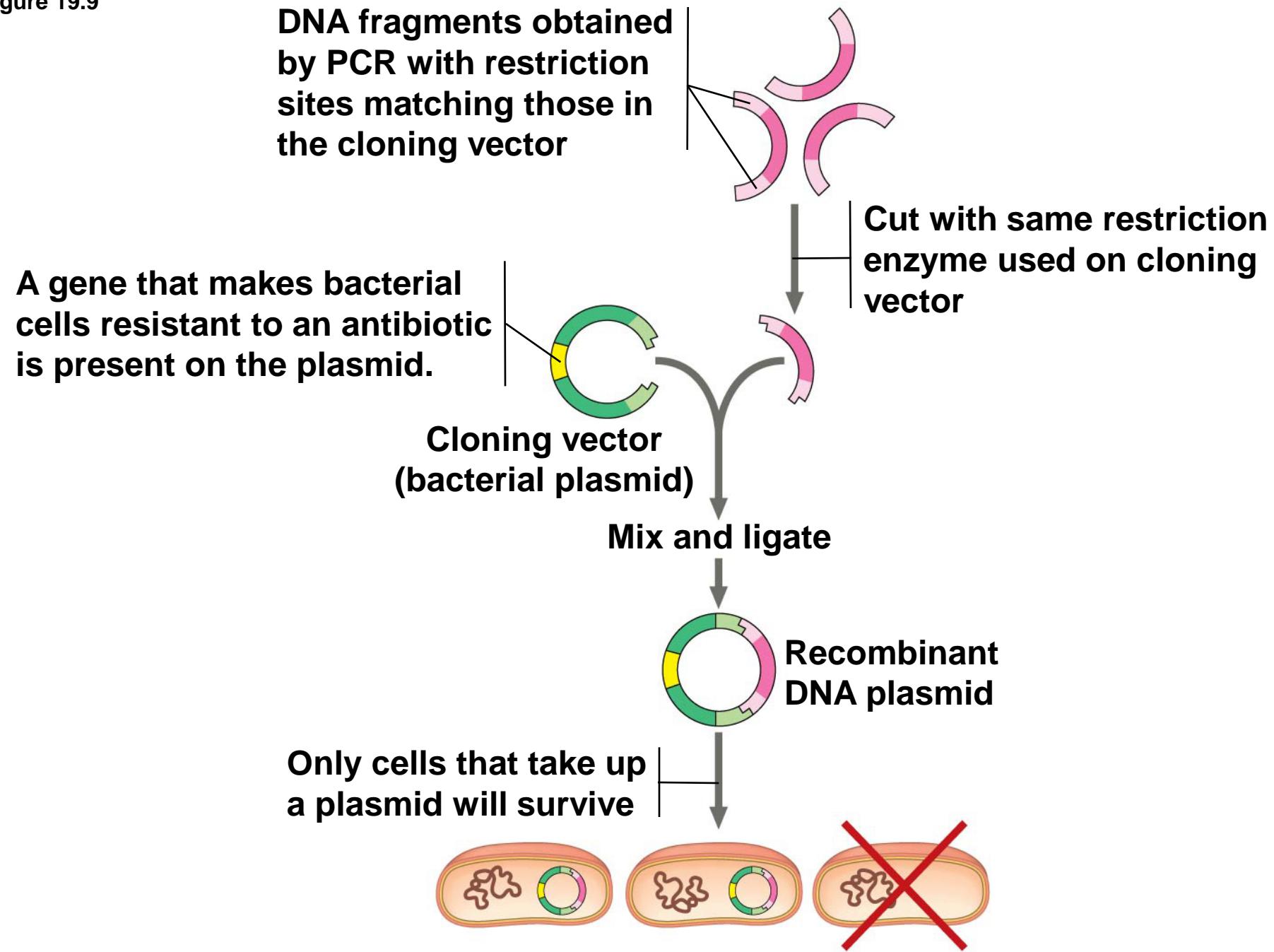
Figure 19.8

TECHNIQUE



- 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

Figure 19.9



Expressing Cloned Eukaryotic Genes

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

Bacterial Expression Systems

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

- Another difficulty 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 Cloning and Expression Systems

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

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

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

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

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 19.2

Biologists use DNA technology to study gene expression and function

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

Studying the Expression of Single Genes

- Changes in the expression of a gene during embryonic development can be tested using
 - Northern blotting
 - Reverse transcriptase-polymerase chain reaction
- Both methods are used to compare mRNA from different developmental stages
- The most straightforward way to discover which genes are expressed in certain cells is to identify the mRNAs being made

- **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
- 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

Figure 19.10

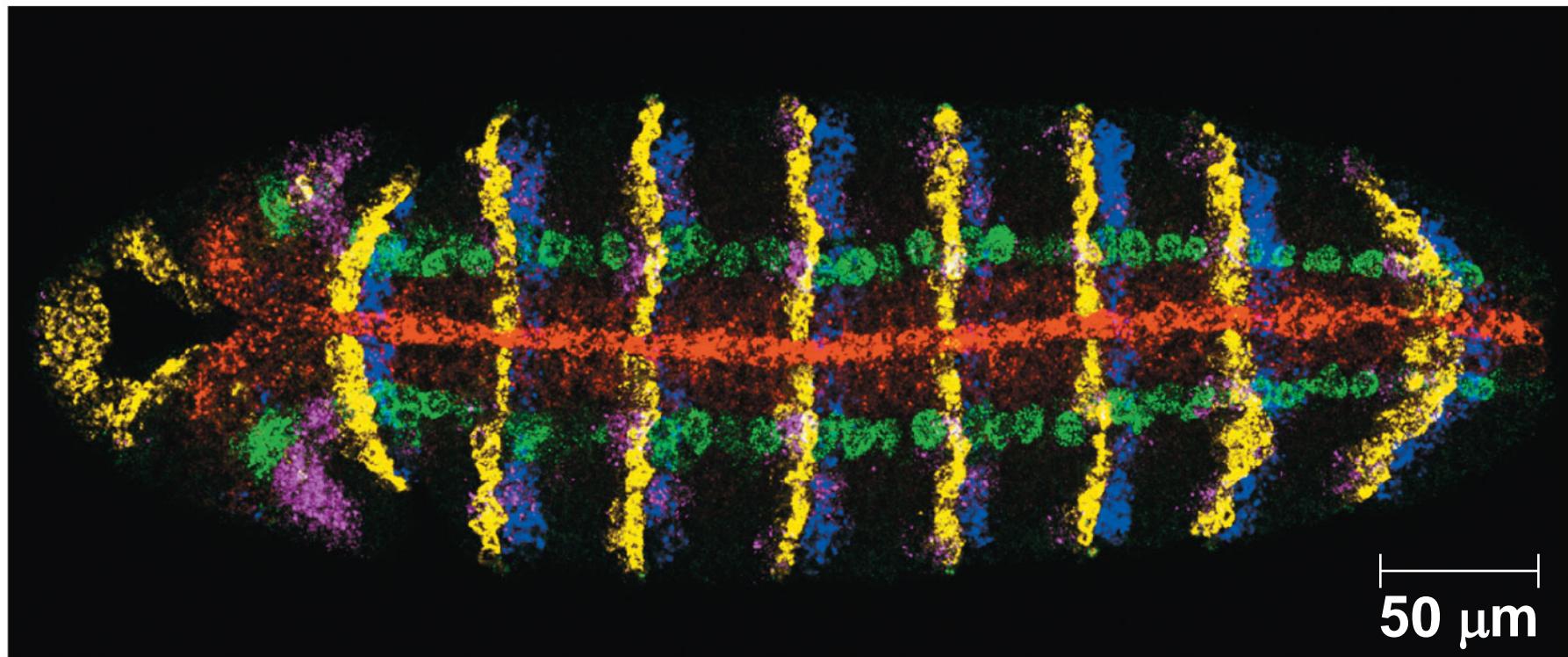
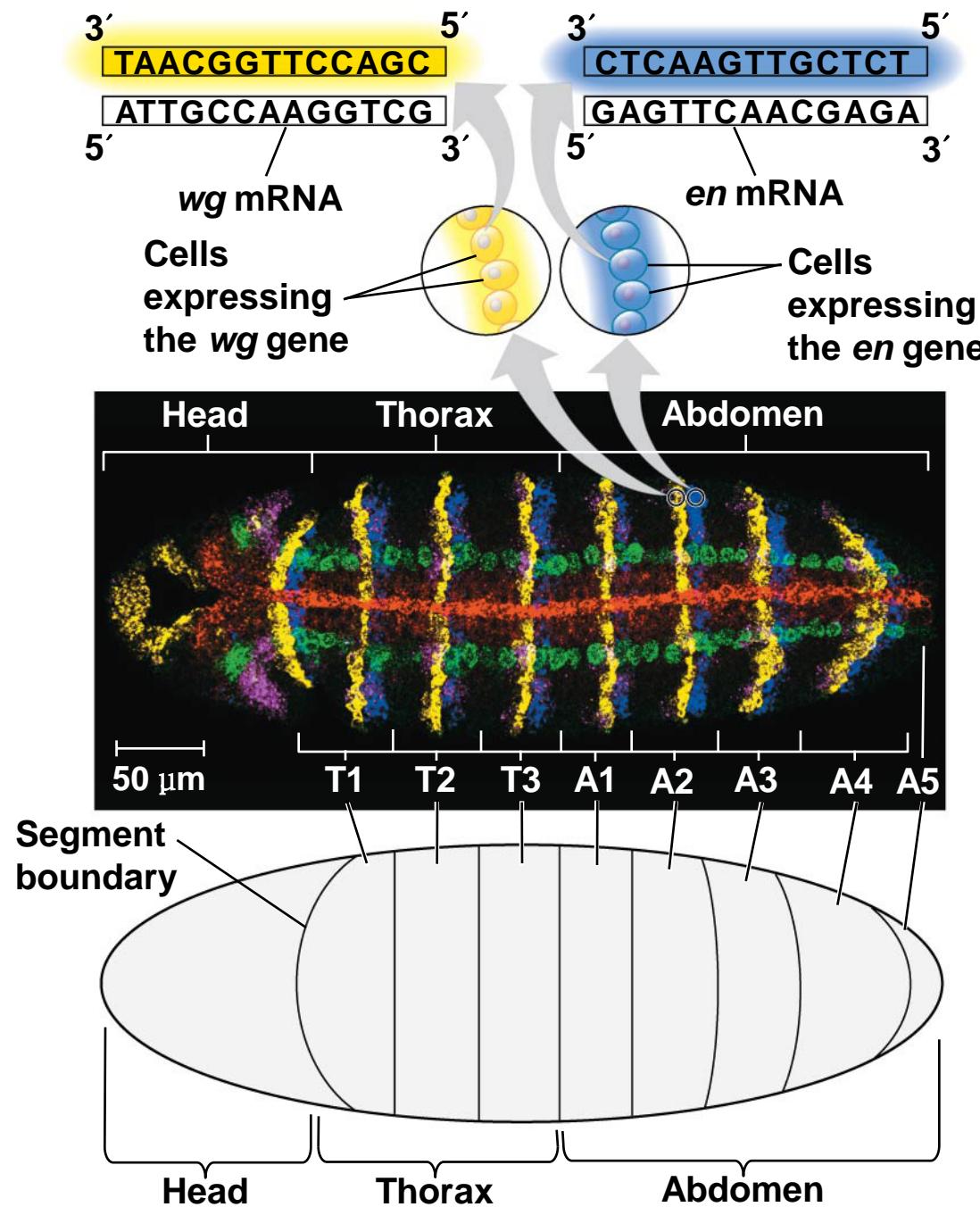


Figure 19.10



- **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 **complementary DNA (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 identified

Figure 19.11

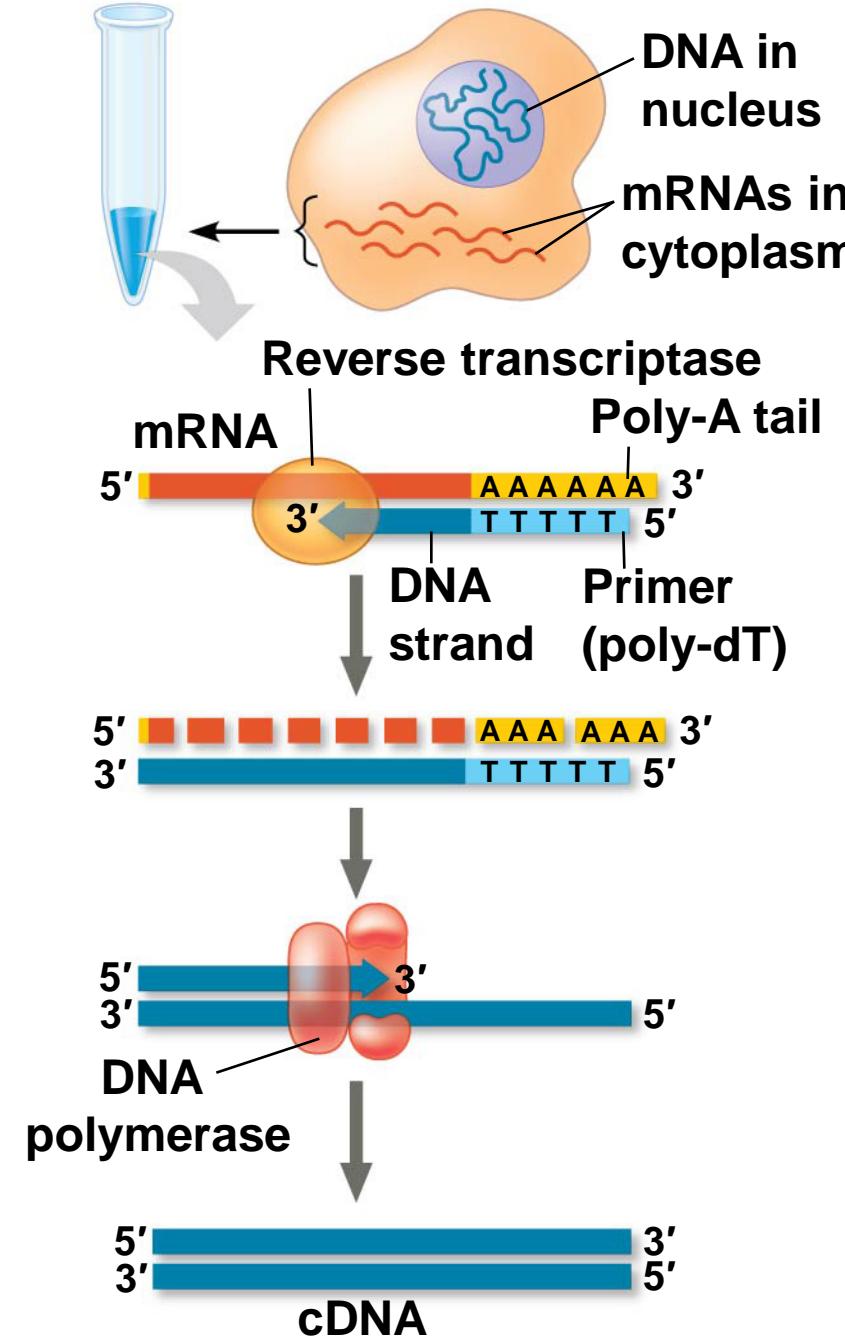


Figure 19.12

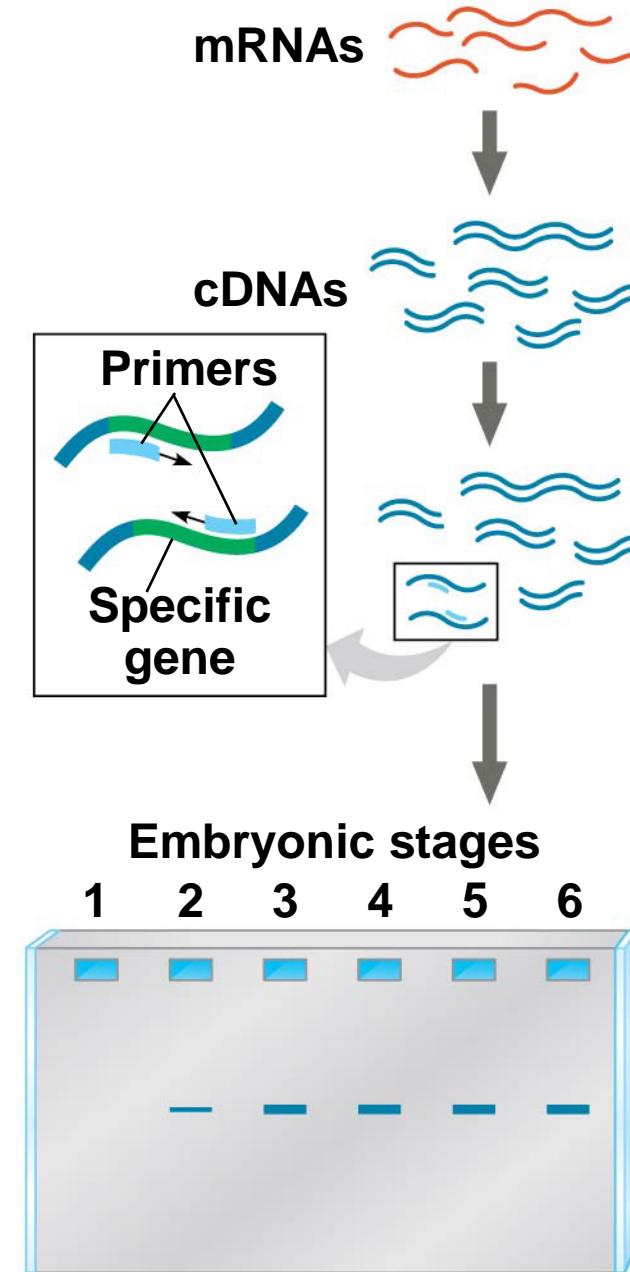
Technique

① cDNA synthesis

② PCR amplification

③ Gel electrophoresis

Results



Studying the Expression of Interacting Groups of Genes

- Automation has allowed scientists to measure 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

Research Method: DNA Microarray Assay of Gene Expression Levels

TECHNIQUE

1 Isolate mRNA.

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

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

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

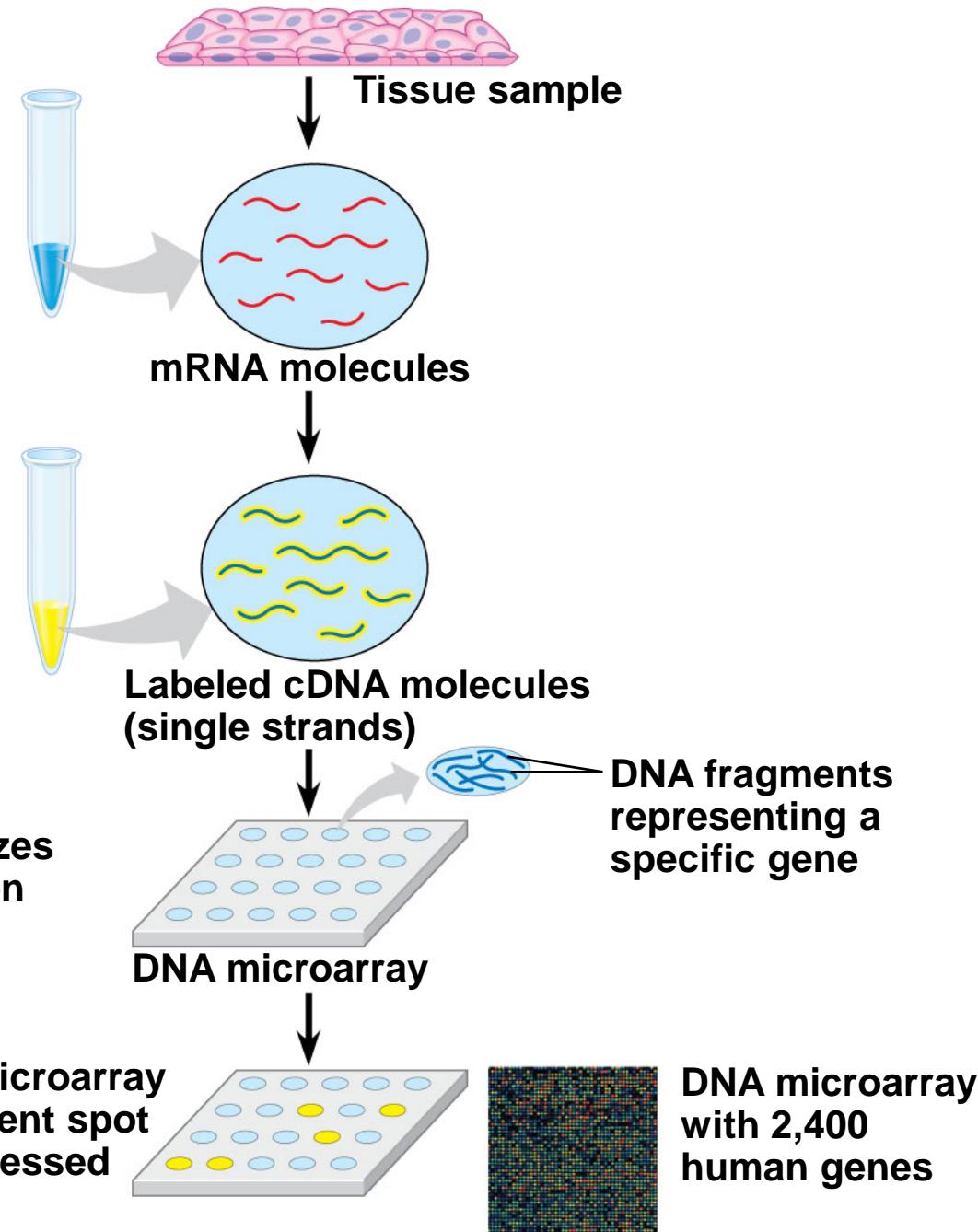
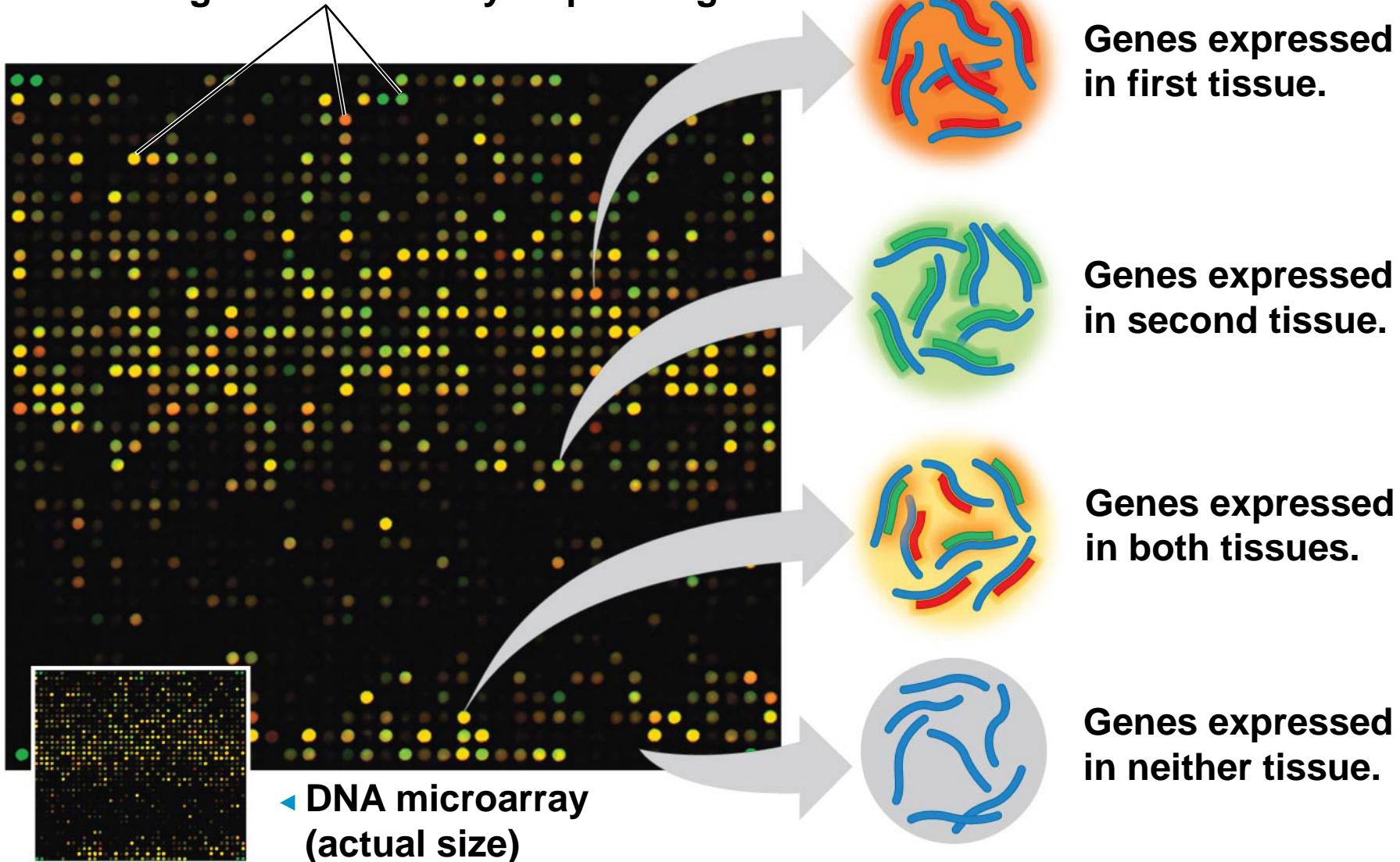


Figure 19.13

Each dot is a well containing identical copies of DNA fragments that carry a specific gene.



- With rapid and inexpensive sequencing methods available, researchers can also just sequence cDNA samples from different tissues or embryonic stages to determine the gene expression differences between them
- By uncovering gene interactions and clues to gene function DNA microarray assays may contribute to understanding of disease and suggest new diagnostic targets

Determining Gene Function

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

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

- In humans, researchers analyze the genomes of many people with a certain genetic condition to try to find nucleotide changes specific to the condition
- These **genome-wide association studies** test for *genetic markers*, sequences that vary among individuals
- 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

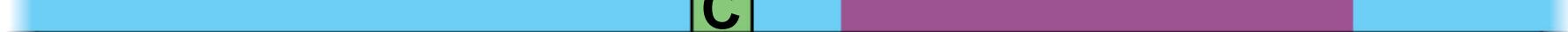
- SNP variants that are found frequently associated with a particular inherited disorder alert researchers to the most likely location for the disease-causing gene
- SNPs are rarely directly involved in the disease; they are most often in noncoding regions of the genome

Figure 19.14

DNA



Normal allele



Disease-causing
allele

Concept 19.3

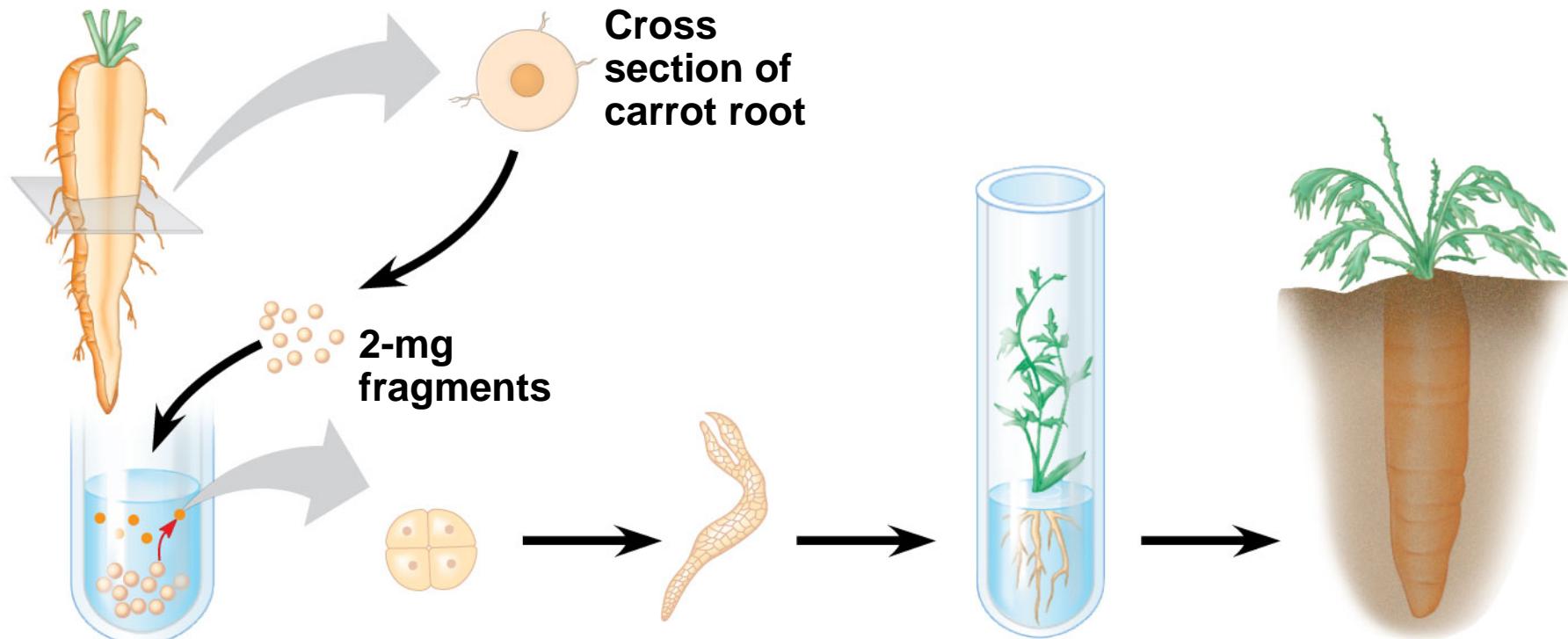
Cloned organisms and stem cells are useful for basic research and other applications

- *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

- One experimental approach for testing genomic equivalence is to see whether a differentiated cell can generate a whole organism
- In plants, cells can differentiate and then give rise to all the specialized cell types of the organism
- A **totipotent** cell is one that can generate a complete new organism
- Plant cloning is used extensively in agriculture

Figure 19.15



Fragments were cultured in nutrient 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

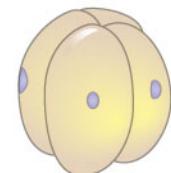
Cloning Animals: Nuclear Transplantation

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

Figure 19.16

EXPERIMENT

Frog embryo

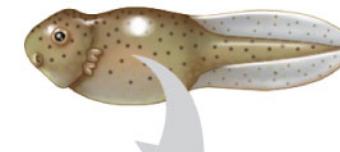


Less differ-
entiated cell

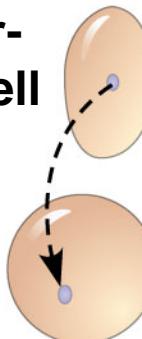
Frog egg cell



Frog tadpole



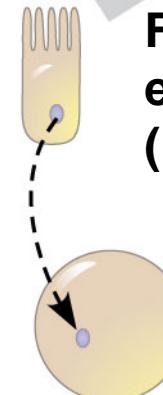
Donor
nucleus
trans-
planted



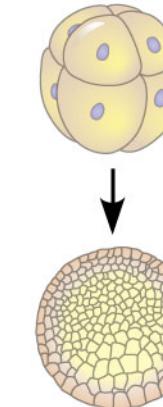
Enucleated
egg cell

Egg with donor nucleus
activated to begin
development

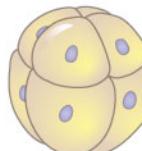
Fully differ-
entiated
(intestinal) cell



Donor
nucleus
trans-
planted



RESULTS



Most develop
into tadpoles.



Most stop developing
before tadpole stage.

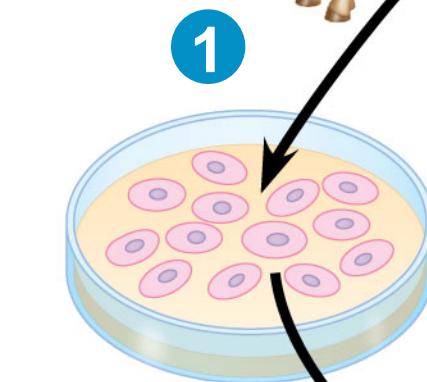
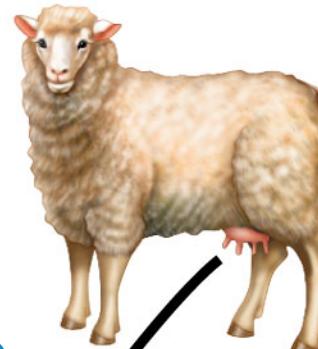
Reproductive Cloning of Mammals

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

Figure 19.17a

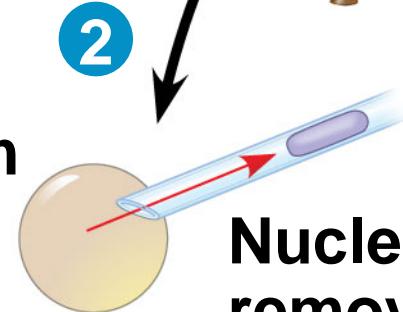
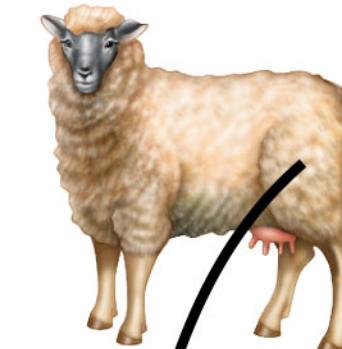
TECHNIQUE

Mammary
cell donor

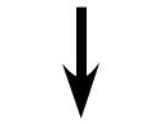


Cultured
mammary
cells

Egg
cell from
ovary



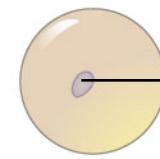
3 Cells fused



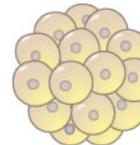
Nucleus from
mammary cell

Figure 19.17b

4 Grown in culture



Nucleus from
mammary cell



Early embryo

5 Implanted in uterus
of a third sheep



Surrogate
mother

6 Embryonic
development



RESULTS

Lamb (“Dolly”) genetically
identical to mammary cell donor

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

Figure 19.18



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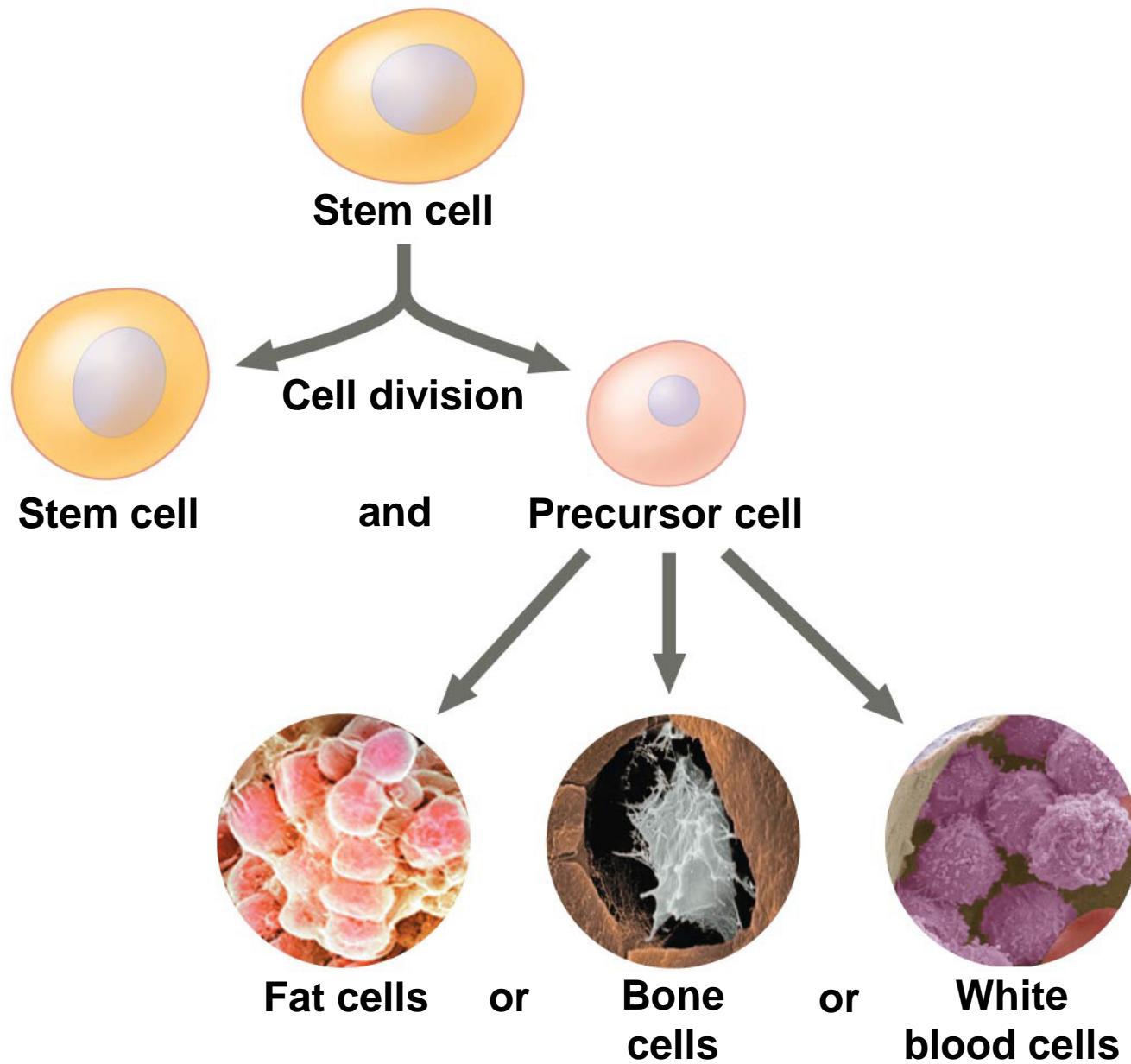
Faulty Gene Regulation in Cloned Animals

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

Stem Cells of Animals

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

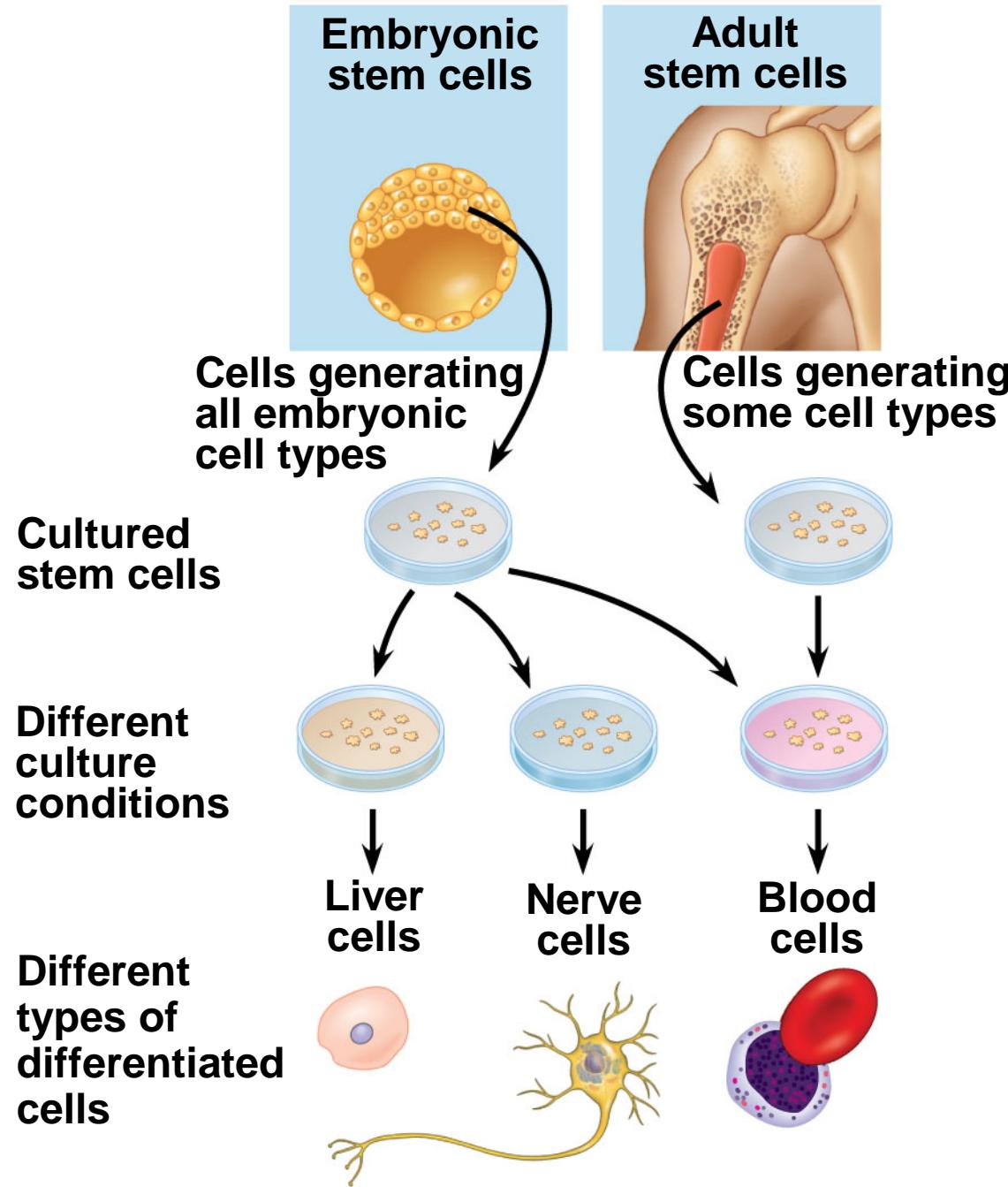
Figure 19.19



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 cells reproduce indefinitely
- Depending on culture conditions, they 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 19.20



- Embryonic stem (ES) cells are **pluripotent**, capable of differentiating into many different cell types
- The ultimate aim of research with stem cells is to supply cells for the repair of damaged or diseased organs
- ES cells present ethical and political issues

Induced Pluripotent Stem (iPS) Cells

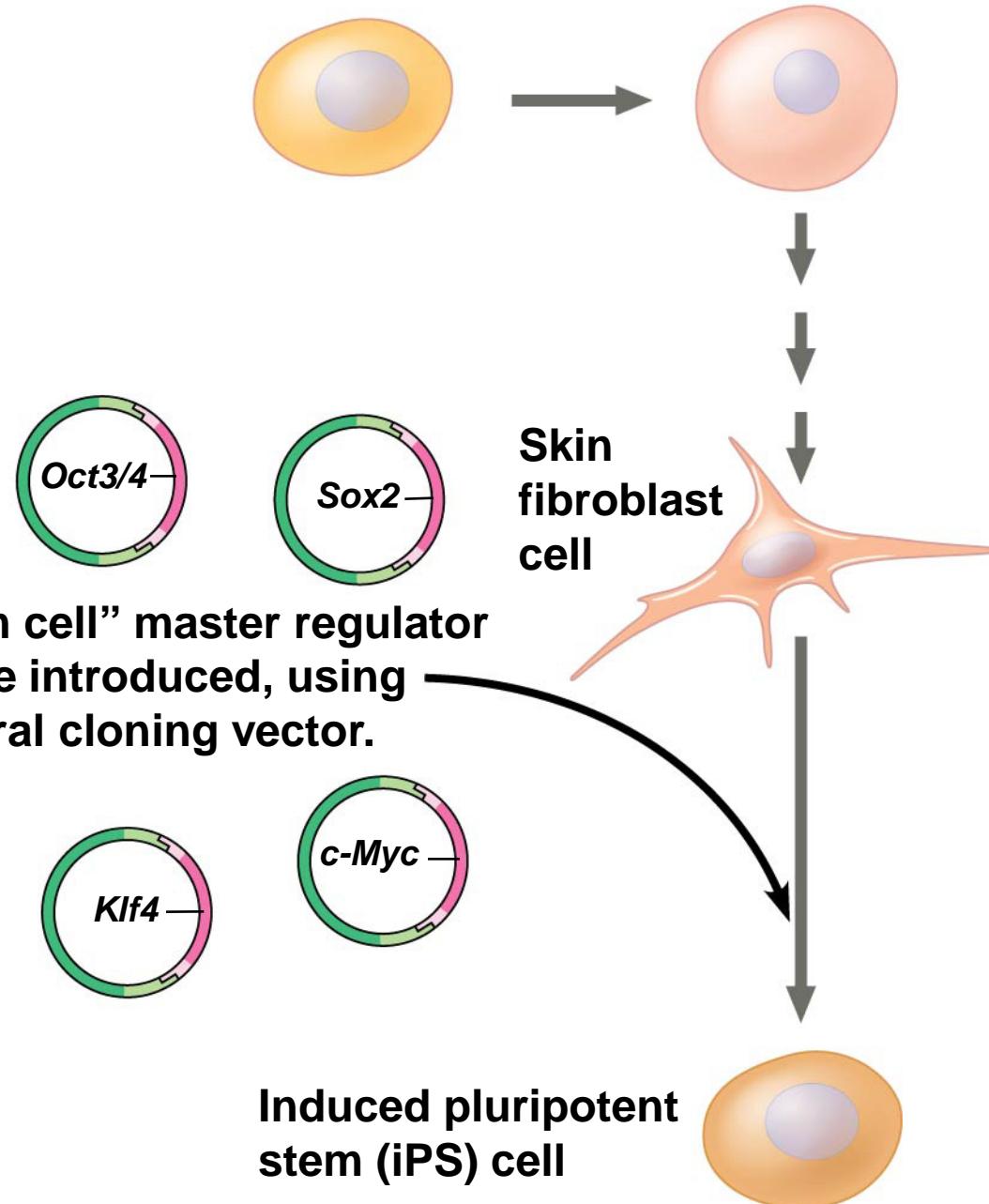
- Researchers can treat differentiated cells, and reprogram them to act like ES cells
- Researchers used retroviruses to induce extra copies of four stem cell master regulatory genes to produce *induced pluripotent stem (iPS) cells*
- iPS cells can perform most of the functions of ES cells
- iPS cells can be used as models for study of certain diseases and potentially as replacement cells for patients

Figure 19.21

Experiment

Stem cell

Precursor cell



Induction of Pluripotency from Adult Human Fibroblasts by Defined Factors

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DOI 10.1016/j.cell.2007.11.019

SUMMARY

Successful reprogramming of differentiated human somatic cells into a pluripotent state allows creation of patient- and disease-specific stem cells. We previously reported the generation of induced pluripotent stem (iPS) cells by germline transmission, from mouse fibroblasts by transduction of four defined transcription factors. Here, we demonstrate the generation of iPS cells from adult human fibroblasts with the same four factors: Oct4, Sox2, Klf4, and c-Myc. Human iPS cells are similar to human embryonic stem (ES) cells in morphology, proliferation, surface gene expression, epigenetic status, telomerase activity, and telomere length. Furthermore, these cells could differentiate into cell types of the three germ layers and form teratomas. These findings demonstrate that iPS cells can be generated from adult human fibroblasts.

INTRODUCTION

Embryonic stem (ES) cells, derived from the inner cell mass of mammalian blastocysts, have the ability to self-renew indefinitely while maintaining pluripotency (Kaufman, 1981; Martin, 1981). These properties are consistent with expectations that human ES cells might be used to understand disease mechanisms, to screen for safe drugs, and to treat patients of various diseases, such as juvenile diabetes and spinal cord injuries (Thomson et al., 1998). Use of human embryonic stem cells faces ethical controversies that hinder the application of human ES cells. In addition, it is difficult to generate patient- or disease-specific ES cells, which are required for their effective application. One way to circum-

Induction of Pluripotency: From Mouse to Human

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DOI 10.1016/j.cell.2007.11.020

In this issue of *Cell*, Takahashi et al. (2007) transfer their seminal work on somatic cell reprogramming from the mouse to human. By overexpressing the transcription factor quartet of Oct4, Sox2, Klf4, and c-Myc in adult human fibroblasts, they successfully isolate human pluripotent stem cells that resemble human embryonic stem cells by all measured criteria. This is a significant turning point in nuclear reprogramming research with broad implications for generating patient-specific pluripotent stem cells for research and therapeutic applications.

This year's three Physiology or Medicine Nobel Laureates—Martin Evans, Mario Capecchi, and Oliver Smithies—will be honored in Stockholm in 10 days' time for their discovery of DNA recombination and the development of mouse embryonic stem (ES) cell technology. It was Martin Evans who discovered how to make mouse ES cells, enabling any genetic alteration to be transferred to the germline and hence to the next generation (Evans and Kaufman, 1981; Martin, 1981). Before this breakthrough, researchers studied mouse embryonal carcinoma cells derived from tumors, which could form every mouse cell lineage except the germline. Combining DNA recombination and mouse ES cell technology revolutionized an entire field of research, forming the basis for studying and understanding the roles

of numerous genes in embryonic development, adult physiology, disease, and aging. To date, more than 500 mouse models of human disorders have been generated. Now, with the study by Takahashi et al. (2007) published in this issue of *Cell*, another important revolution is taking place.

Last summer, Takahashi and Yamanaka (2006) stunned the scientific community with their study showing molecular reprogramming of mouse somatic cells into induced pluripotent stem (iPS) cells using just four factors: Oct4, Sox2, Klf4, and c-Myc. Their elegant but demanding approach of screening for a cocktail of factors that could reprogram mouse fibroblasts starting from 24 candidate genes paid off with their detailed description of iPS cells, which are almost indistinguishable from mouse ES cells. As with all

scientific discoveries, these exciting findings had to be reproduced. Several studies published this year not only reproduced but also extended the Takahashi and Yamanaka findings by demonstrating the pluripotency and differentiation potential of mouse iPS cells in rigorous developmental assays (Maherali et al., 2007; Okita et al., 2007; Wernig et al., 2007).

In their new study, Takahashi, Yamanaka, and their colleagues (Takahashi et al., 2007) now translate their remarkable findings from mouse to human (see Figure 1). They selected adult human dermal fibroblasts and two other human fibroblast populations (from synovial tissue and neonatal foreskin) from different human donors as their reprogramming target cell populations. They then transduced the human fibroblast cultures

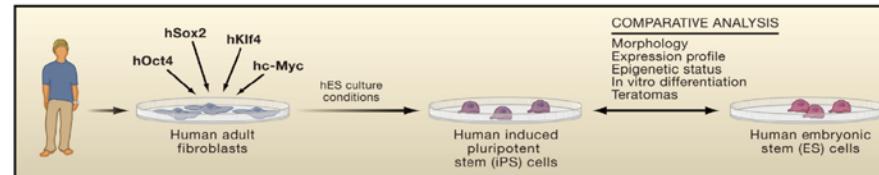


Figure 1. Transcription Factor-Induced Pluripotency

Adult fibroblasts from human donors were exposed to retroviral vectors expressing a cocktail of four transgenes encoding the human factors hOct4, hSox2, hKlf4, and hc-Myc (Takahashi et al., 2007). Thirty days after transduction and further cultivation under human ES cell growth conditions, human induced pluripotent stem (iPS) cell colonies (among others) that could be propagated and expanded further were isolated. Comparative analysis of human iPS cells and human ES cells using assays for morphology, surface-marker expression, gene expression profiling, epigenetic status, and in vitro and in vivo differentiation potential revealed a remarkable degree of similarity between these two pluripotent stem cell types.

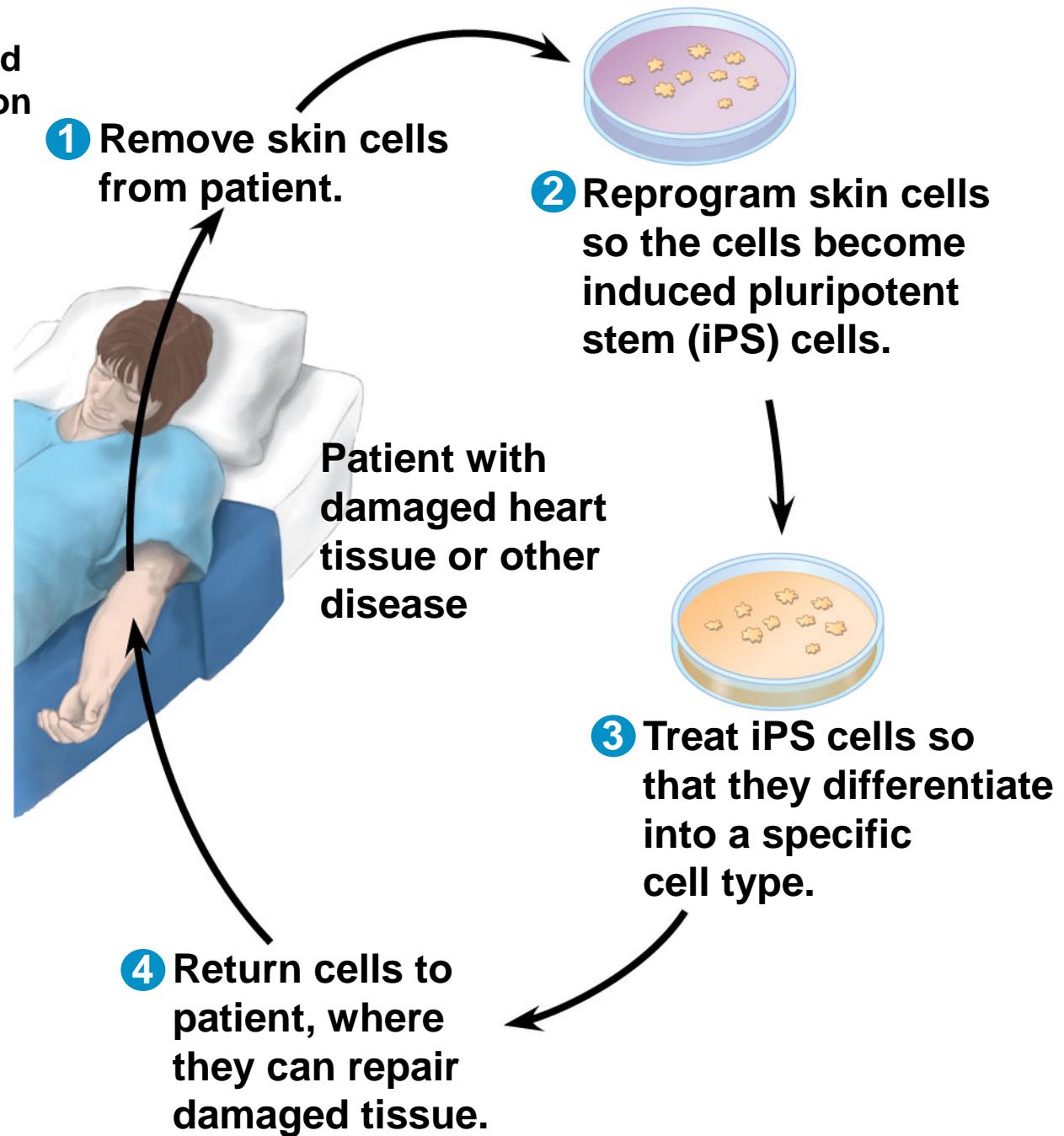
Figure 6. Directed Differentiations of Human iPS Cells

- (A) Phase-contrast image of differentiated iPS cells after 18 days cultivation on PA6.
- (B) Immunocytochemistry of the cells shown in (A) with β -tubulin (red) and tyrosine hydroxylase (green) antibodies. Nuclei were stained with Hoechst 33342 (blue).
- (C) RT-PCR analyses of dopaminergic neuron markers.
- (D) Phase-contrast image of iPS cells differentiated into cardiomyocytes.
- (E) RT-PCR analyses of cardiomyocyte markers. Bars = 200 μ m (A and D) and 100 μ m (B).

Figure 7. Teratoma Derived from Human iPS Cells

Hematoxylin and eosin staining of teratoma derived from iPS cells (clone 201B7). Cells were transplanted subcutaneously into four parts of a SCID mouse. A tumor developed from one injection site.

Impact: The Impact of Induced Pluripotent Stem (iPS) Cells on Regenerative Medicine



Concept 19.4

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

- Many fields benefit from DNA technology and genetic engineering

Medical Applications

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

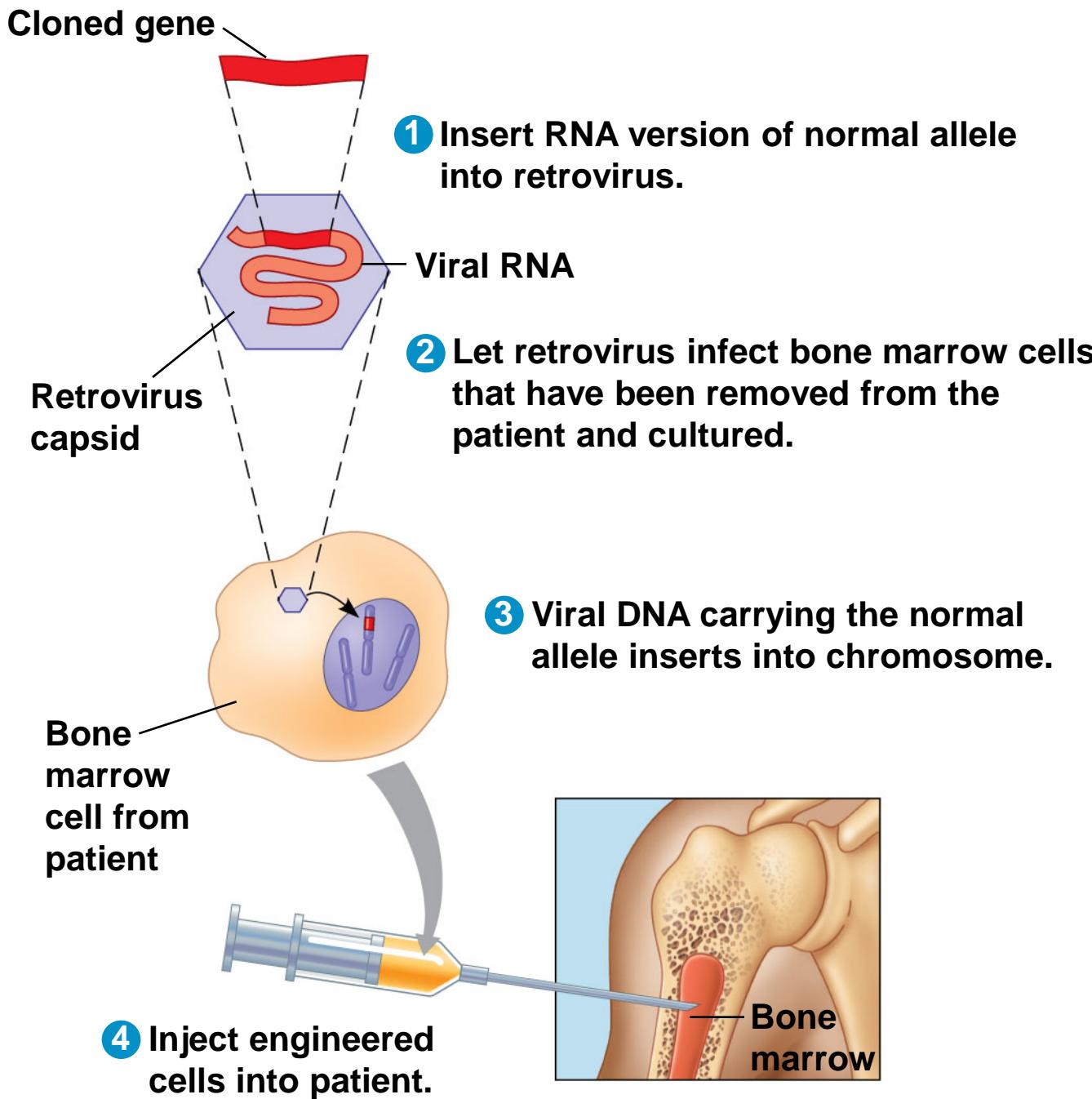
Diagnosis and Treatment of Diseases

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

Human Gene Therapy

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

Figure 19.22



Pharmaceutical Products

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

1. 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

2. 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

3. Protein Production by “Pharm” Animals

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

Figure 19.23



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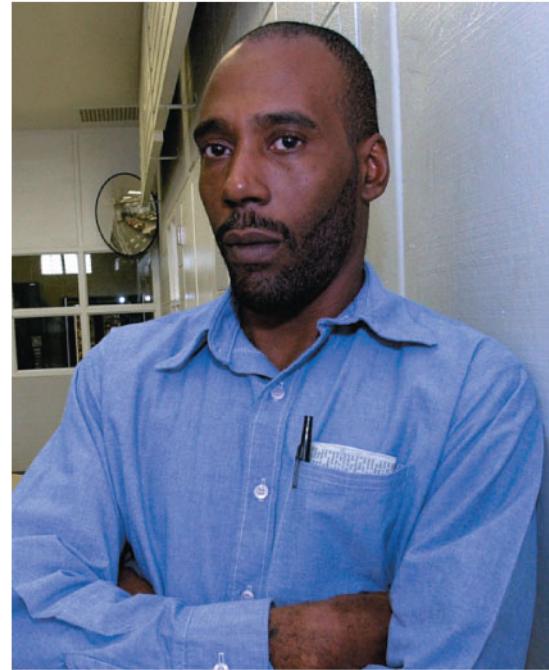
Forensic Evidence and Genetic Profiles

- 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

- 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
- As of 2013 more than 300 innocent people have been released from prison as a result of STR analysis of old DNA evidence

Figure 19.24

(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.

Environmental Cleanup

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

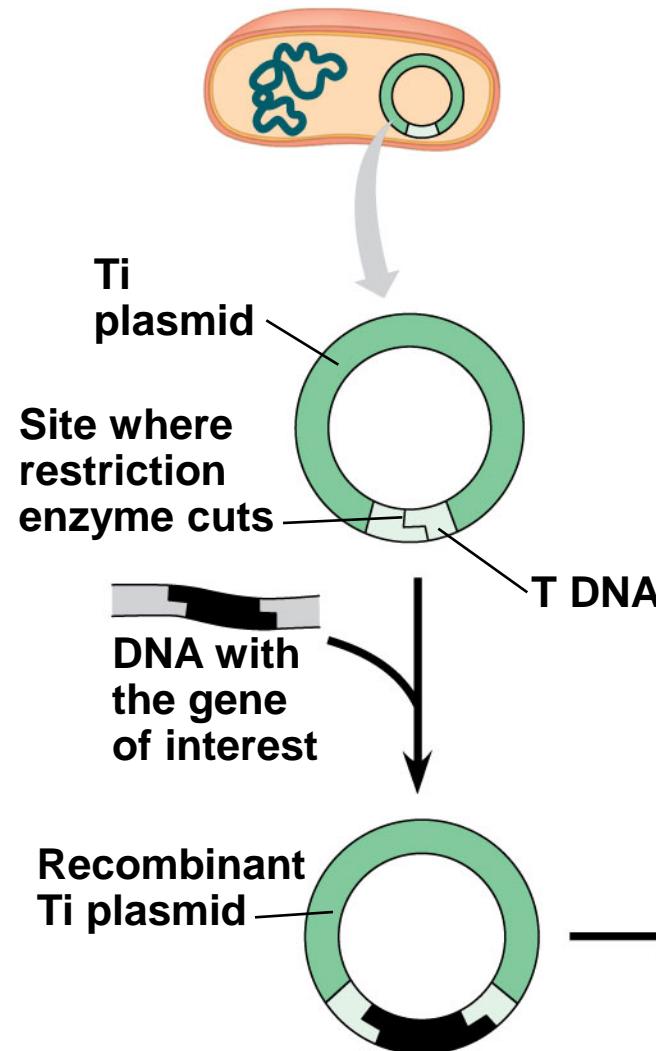
Agricultural Applications

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

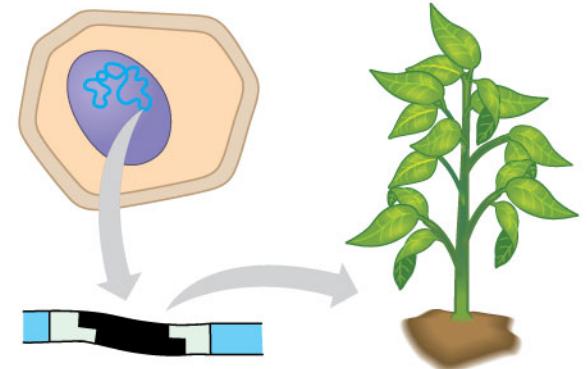
- Agricultural scientists have endowed a number of crop plants with genes for desirable traits
- The **Ti plasmid** is the most commonly used vector for introducing new genes into plant cells
- Genetic engineering in plants has been used to transfer many useful genes including those for herbicide resistance,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

Research Method: Using the Ti Plasmid to Produce Transgenic Plants

TECHNIQUE *Agrobacterium tumefaciens*



RESULTS



Safety and Ethical Questions Raised by DNA Technology

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

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

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