Chapter 20

Biotechnology

PowerPoint® Lecture Presentations for

Biology

Eighth Edition

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Overview: The DNA Toolbox

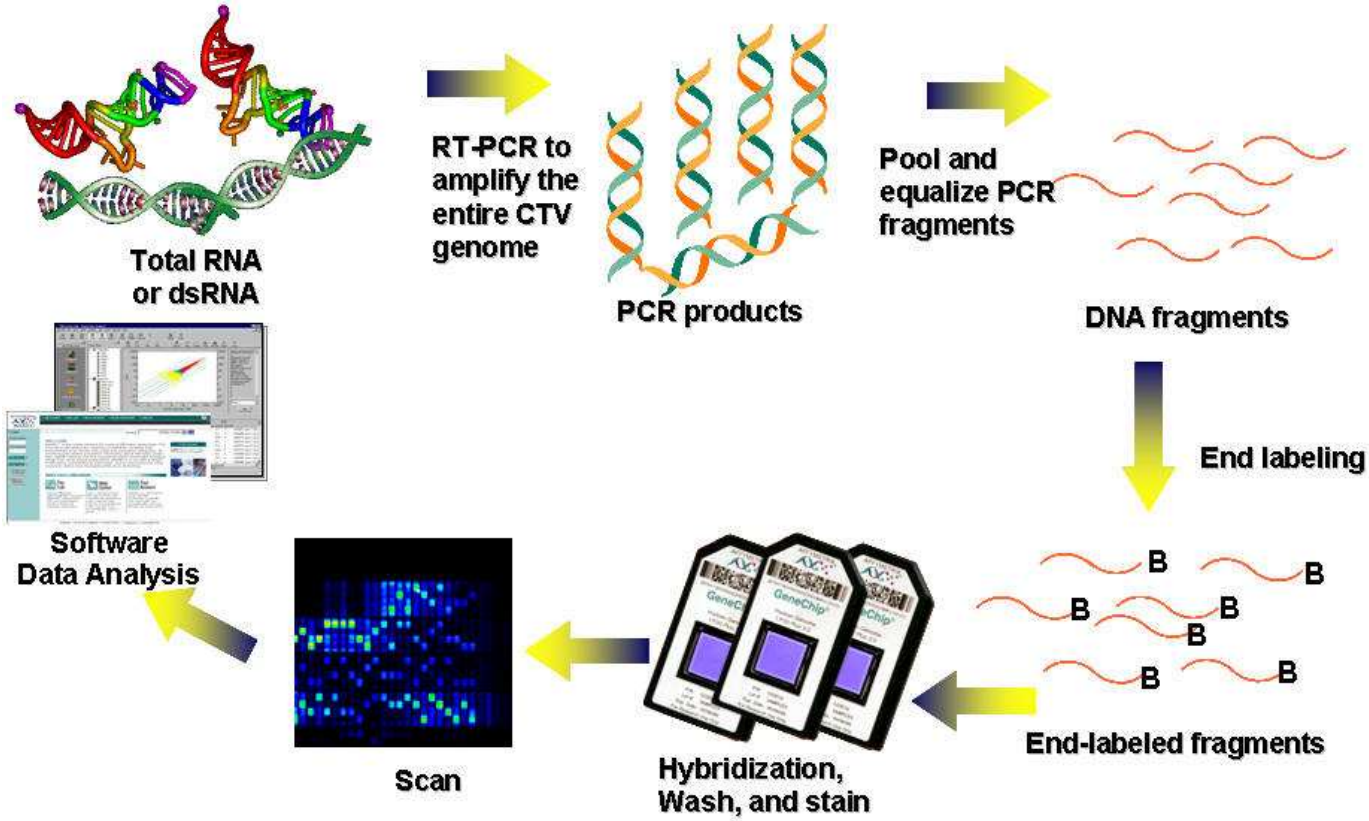
- **One of the greatest achievements of modern science has been the sequencing of the human genome (2003)**--- depended on advances in **recombinant DNA technology**

- In **recombinant DNA**, nucleotide sequences from two different sources, often two species, are combined *in vitro* into the same DNA molecule

- Methods for making recombinant DNA are central to **genetic engineering**, the direct manipulation of genes for practical purposes
- DNA technology has revolutionized **biotechnology**
  - the manipulation of organisms or their genetic components to make useful products

Fig. 20-1

An example of DNA technology is the **microarray**, a measurement of gene expression of tens of thousands of different genes



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

- **The first step in recombinant DNA technology**
  - ***DNA cloning***
- It is the production of multiple identical copies of a specific gene (**gene cloning**) or other DNA segment

DNA Cloning and Its Applications: *A Preview*

- **How to do DNA cloning:**
  - (1) 目標 ：**DNA of interest**
  - (2) 載體： **Plasmids (cloning vector):** **replication origin, multiple cloning sites, selection marker (ampicillin resistance gene, LacZ gene : b-galactosidae** digests **X-gal** into blue product**)**
  - (3) 宿主：**Host**: bacteria (E. Coli), yeast
- Cloned genes are useful for making copies of a particular gene and producing a protein product

Fig. 20-2a

A preview of gene cloning and some uses of cloned genes

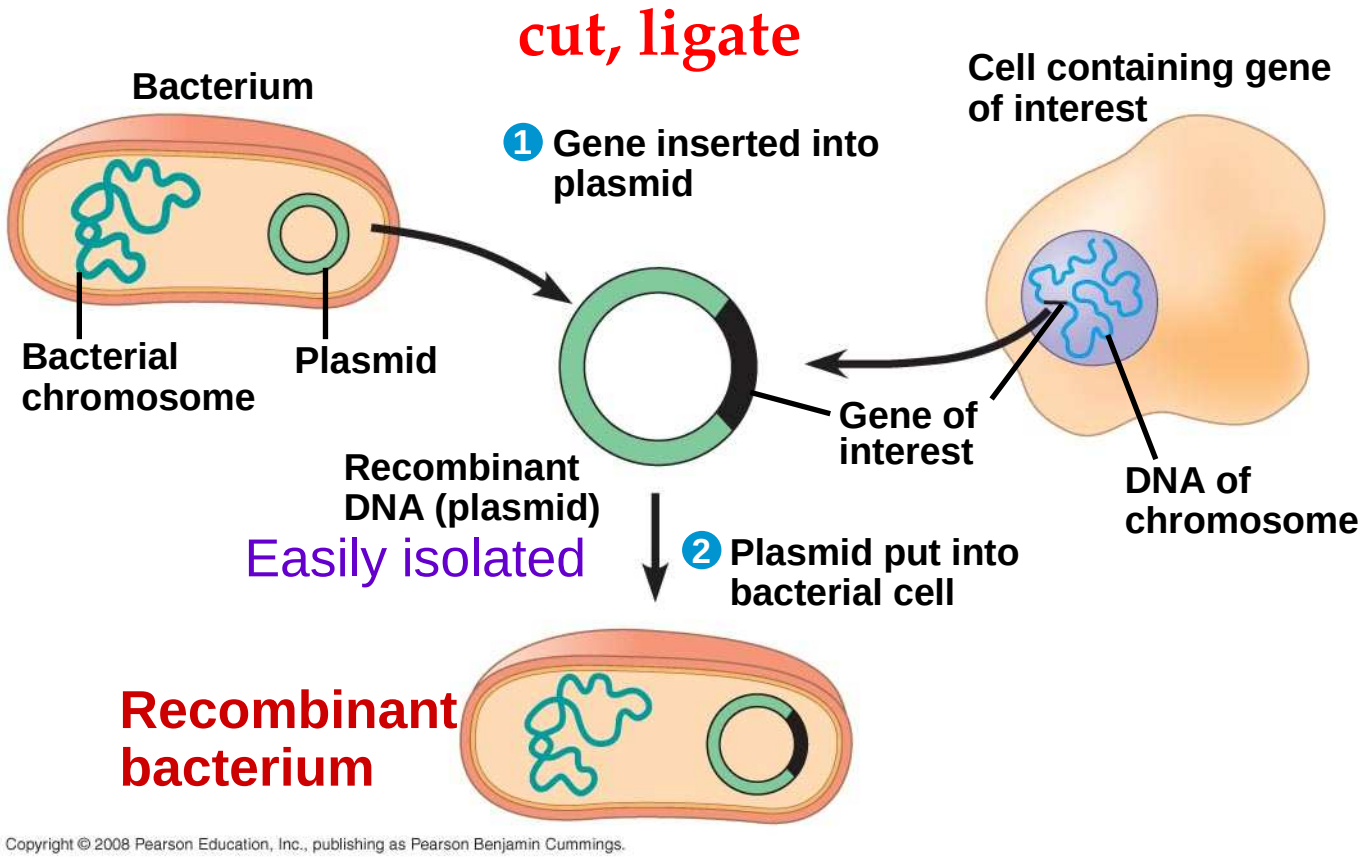
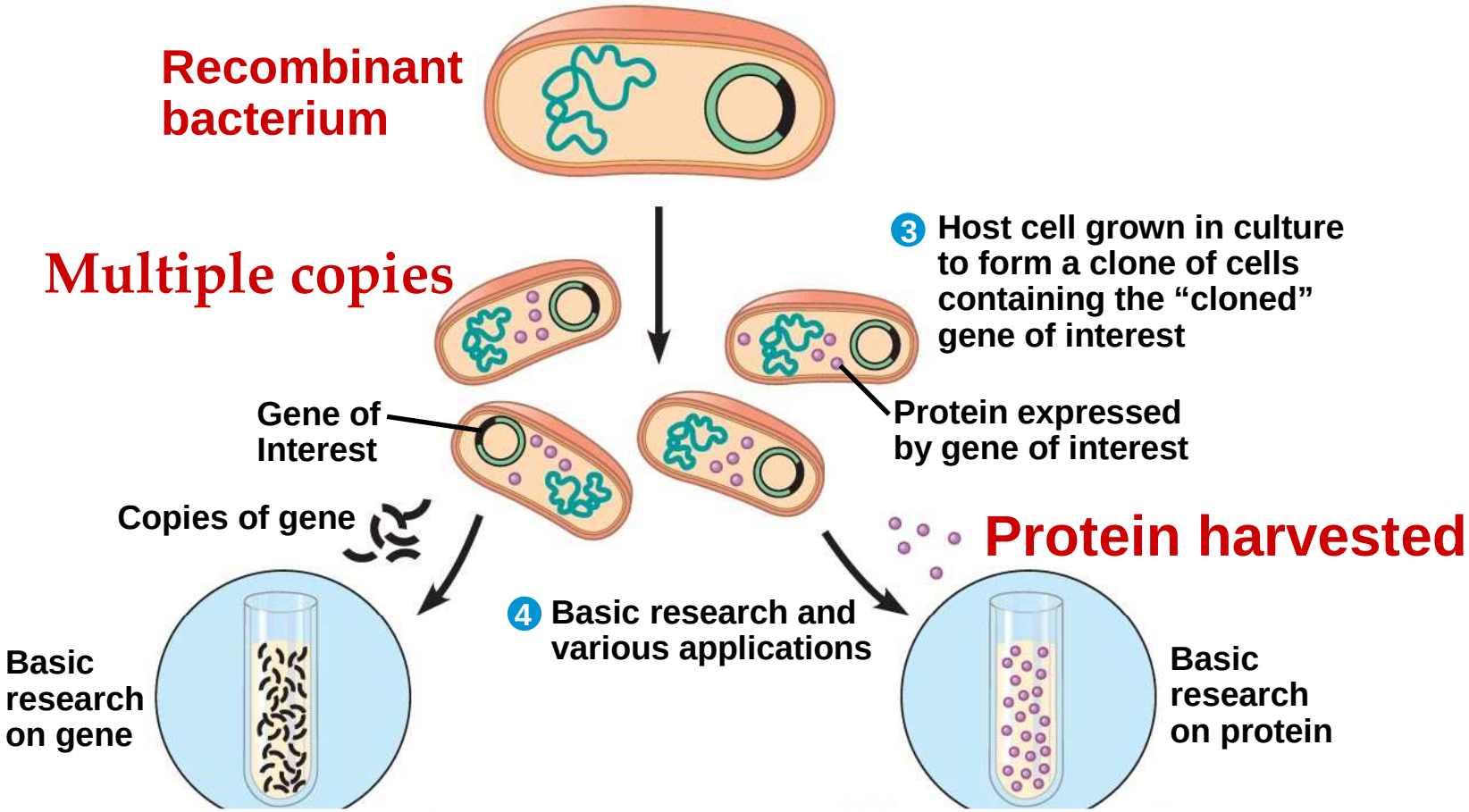
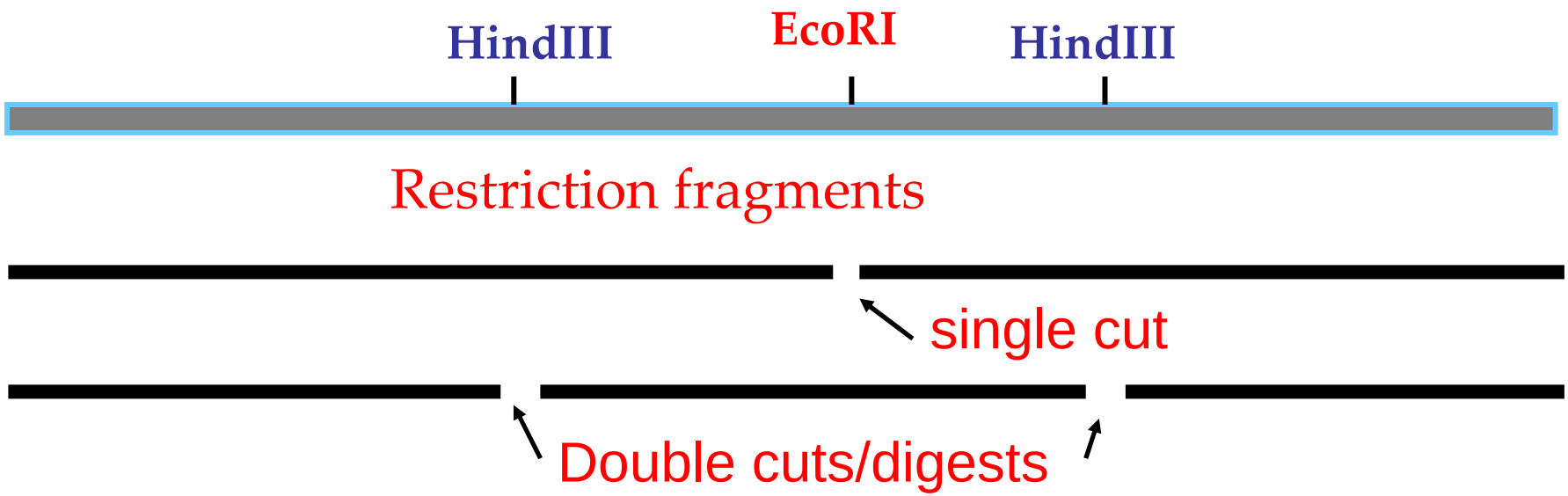


Fig. 20-2b


Recombinant bacterium
Multiple copies
3 Host cell grown in culture to form a clone of cells containing the "cloned" gene of interest
Gene of Interest
Protein expressed by gene of interest
Copies of gene
Protein harvested
4 Basic research and various applications
Basic research on gene
Basic research on protein

How to Make Recombinant DNA: Restriction Enzymes

(1) Bacterial **restriction enzymes** -- cut DNA molecules at a limited number of specific DNA sequences (4-8 nucleotides), called **restriction sites** – GAATTC (EcoRI),



Using Restriction Enzymes to Make Recombinant DNA

- The most useful restriction enzymes cut DNA in a staggered way, producing fragments with "**sticky ends**" that bond with complementary sticky ends of other fragments

PLAY Animation: Restriction Enzymes

- **DNA ligase** is an enzyme that seals the bonds between restriction fragments

Fig. 20-3-1

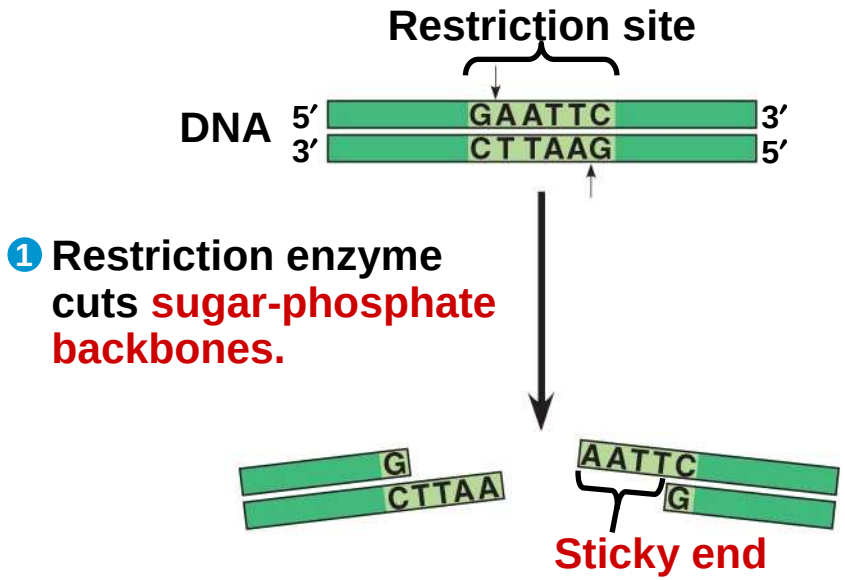


Fig. 20-3-2

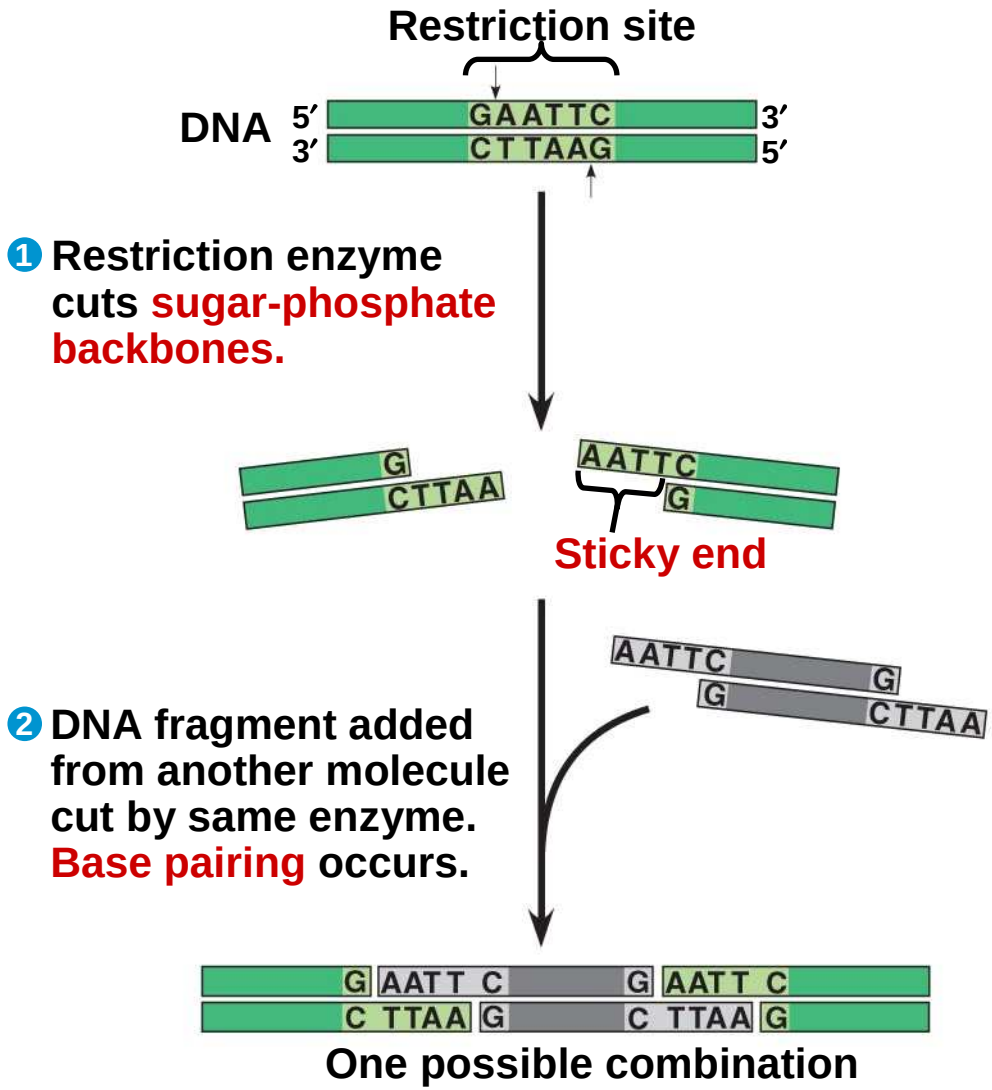
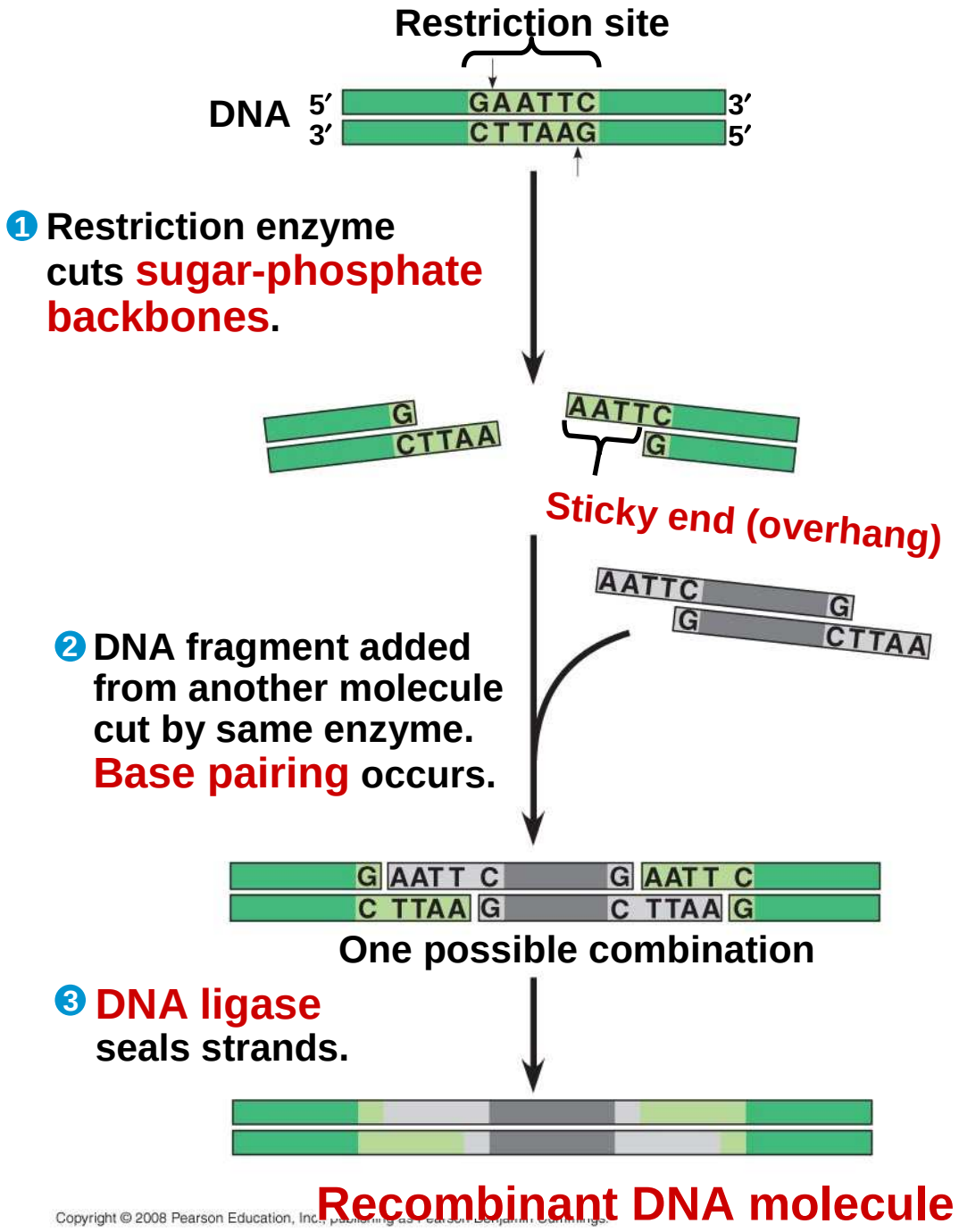


Fig. 20-3-3



Cloning a Eukaryotic Gene in a Bacterial Plasmid

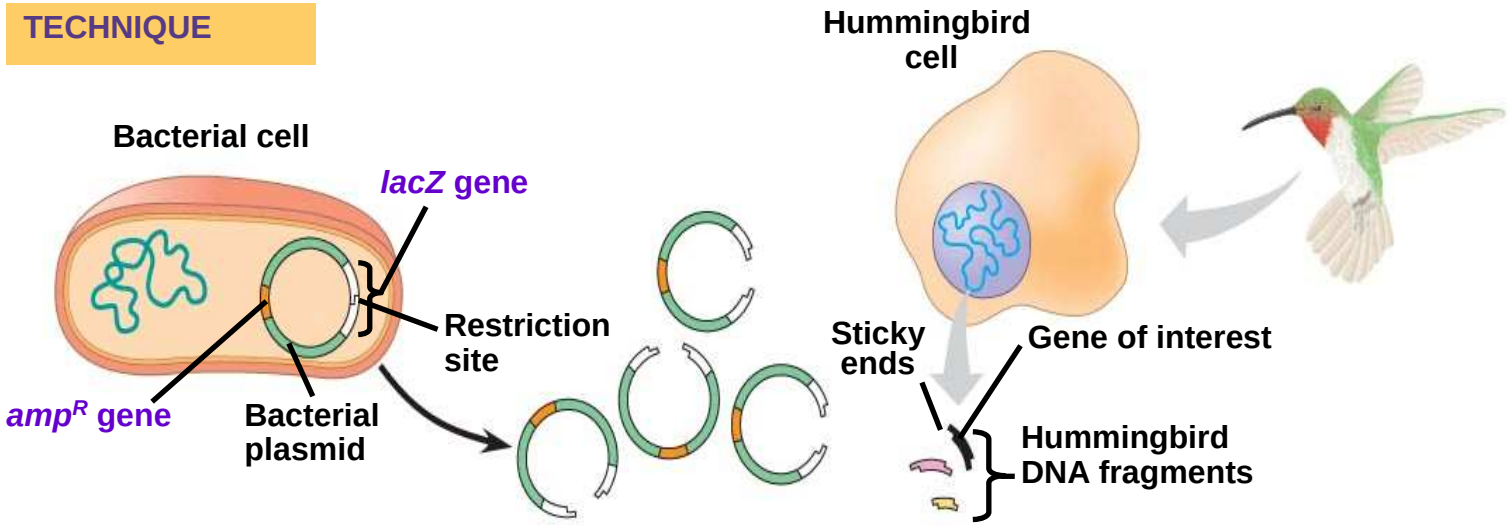
- In gene cloning, the original plasmid is called a **cloning vector**
- Cloning vector is a DNA molecule that can carry foreign DNA into a host cell and replicate there

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 digested 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 specific 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

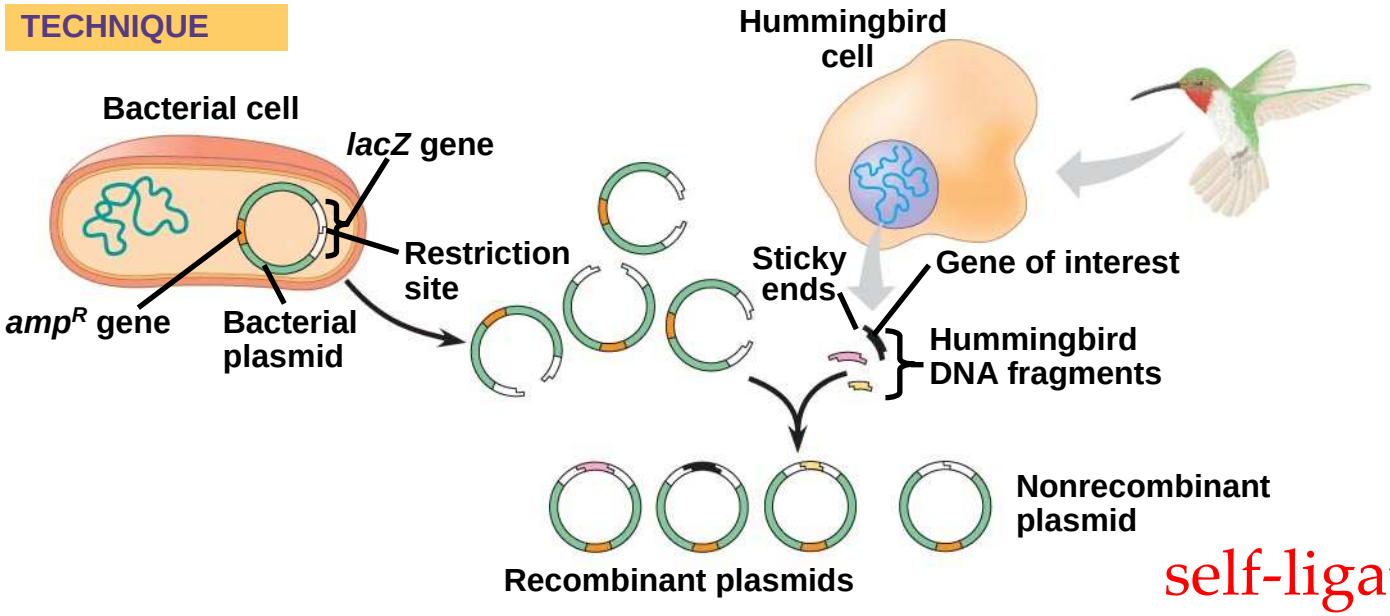
Fig. 20-4-1



amp^R gene: makes *E. coli* cells resistant to the antibiotic ampicillin.

lacZ gene: encodes β-galactosidase. This enzyme hydrolyzes a molecular mimic of lactose (X-gal) to form a blue product. (藍白篩)

Fig. 20-4-2



self-ligation

(intact *lacZ*)

Fig. 20-4-3

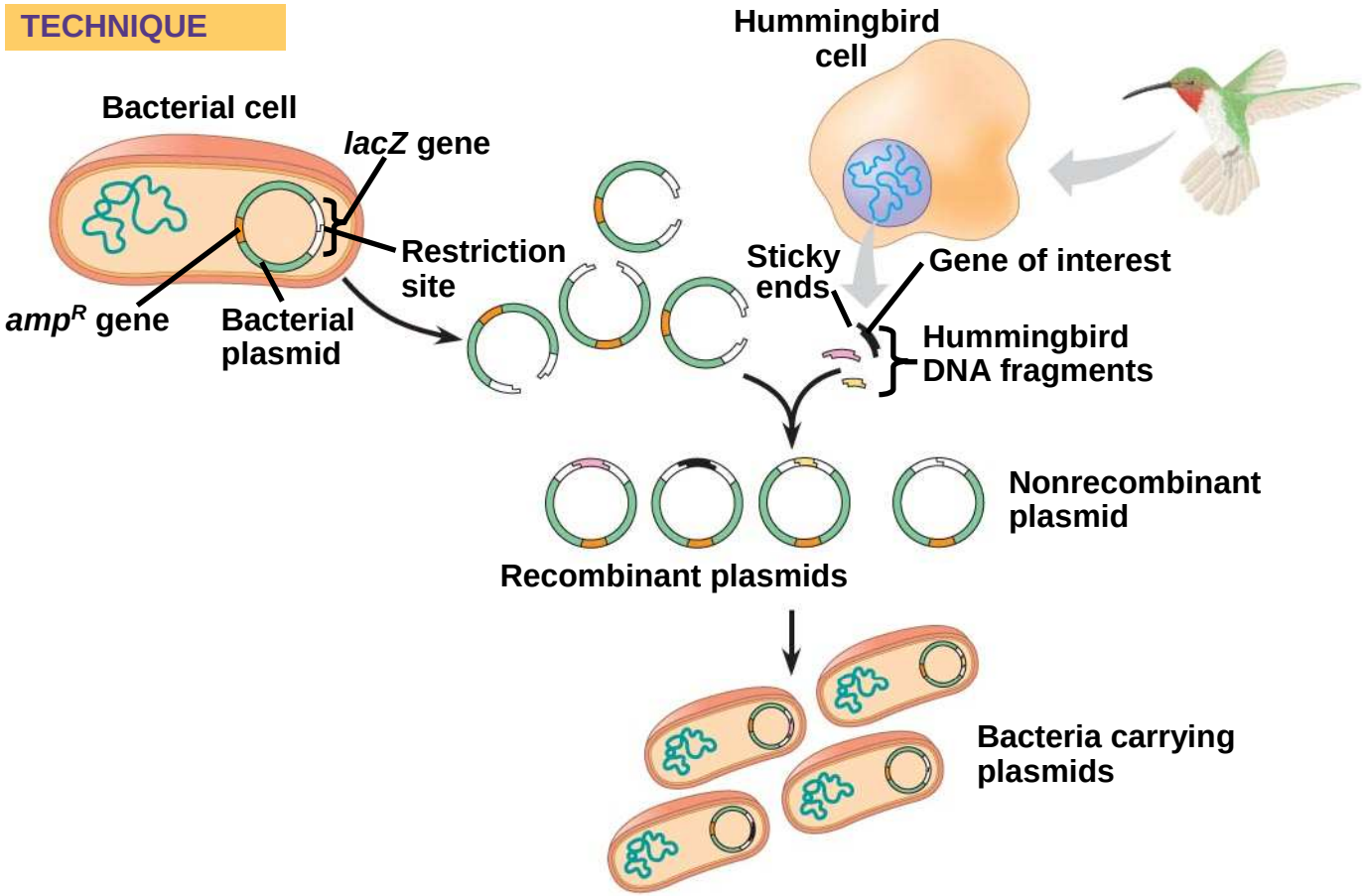
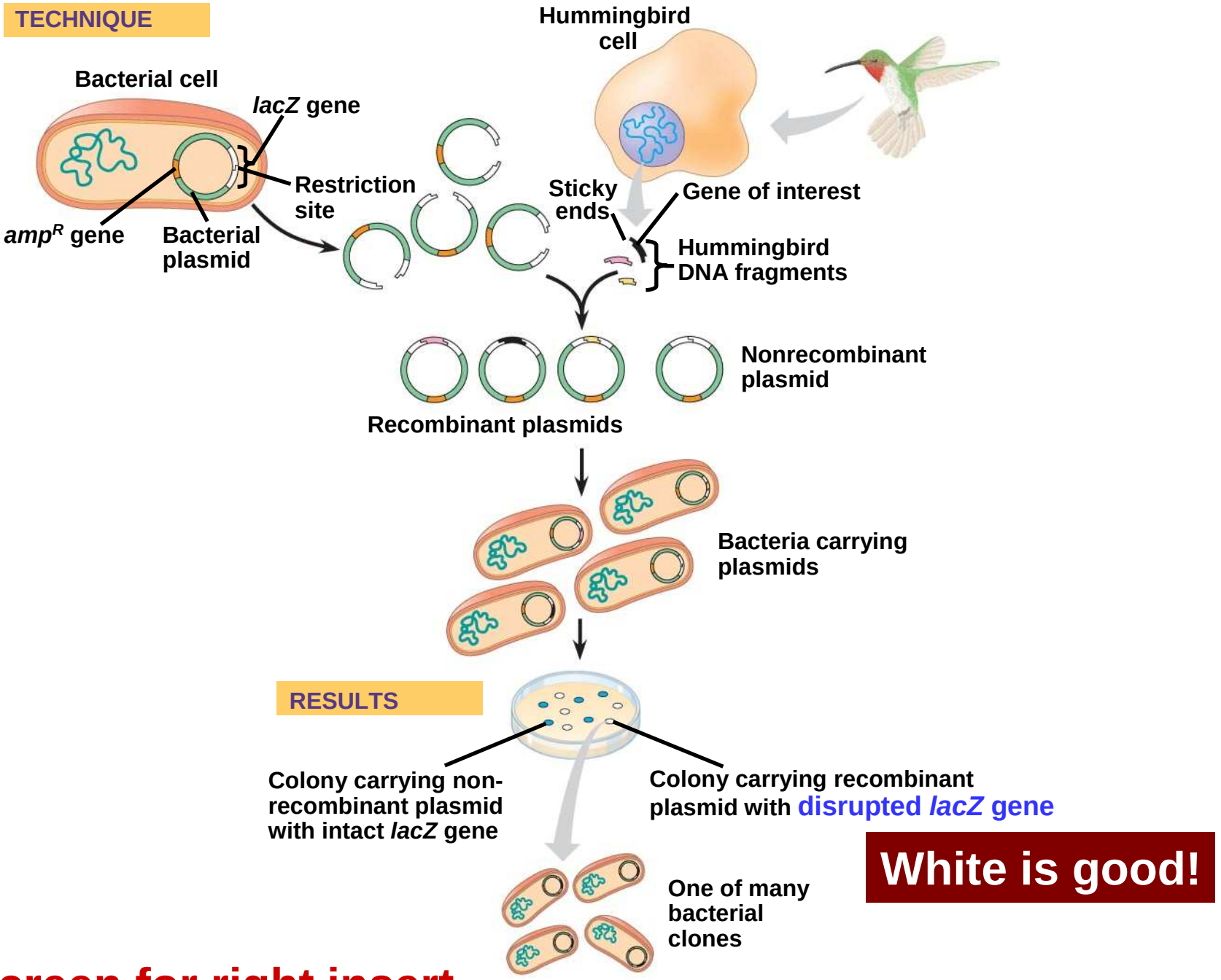
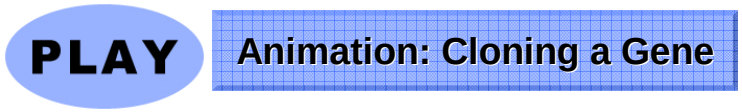


Fig. 20-4-4



Screen for right insert

Summary of cloning a gene

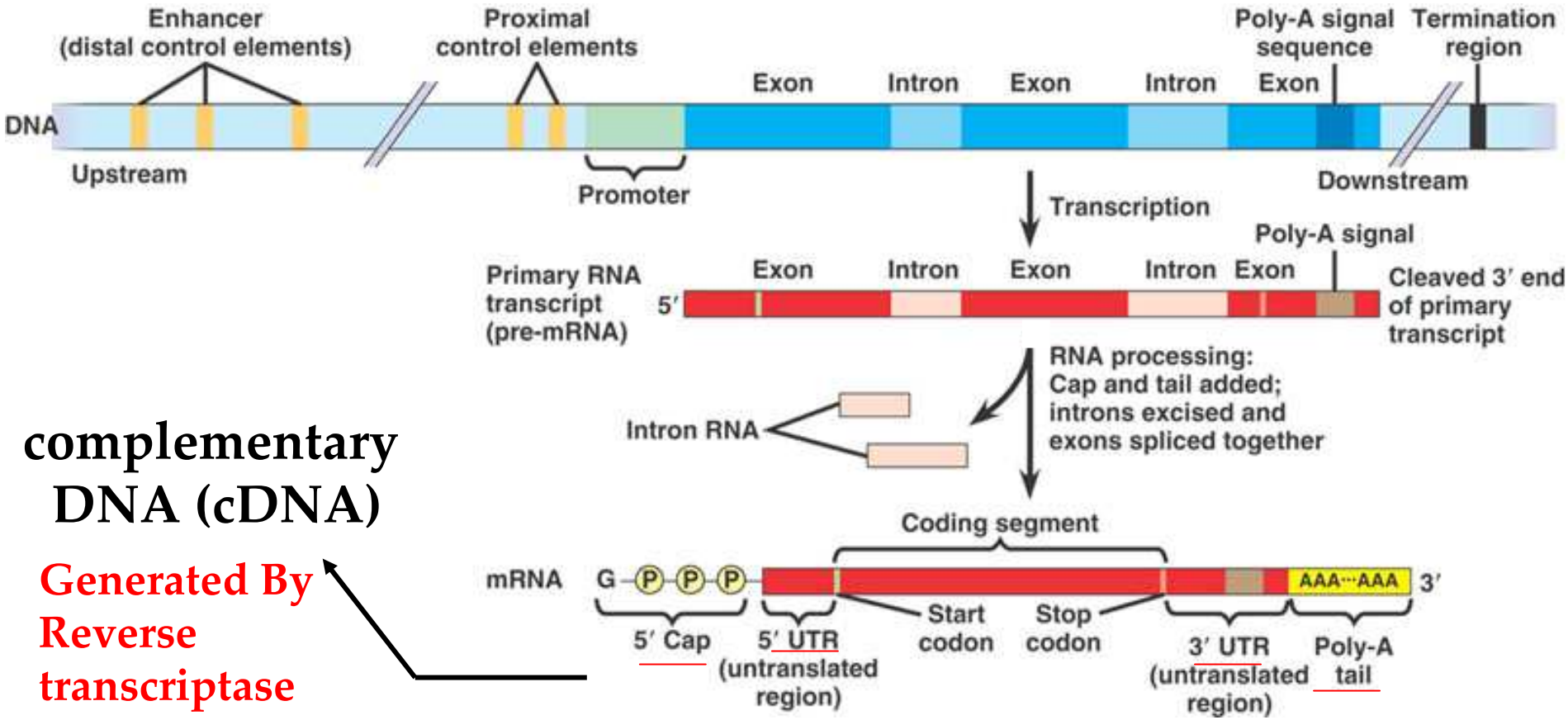


Storing Genetic information in DNA Libraries

- A **genomic library** that is made using bacteria is the collection of recombinant vector clones produced by cloning DNA fragments from **"an entire genome" -** complete sequence with regulatory region (enhancers), exon, introns, nonconding region, etc.
- A genomic library that is made using bacteriophages is stored as a collection of phage clones

Organization of eukaryotic genes: with multiple control elements

noncoding control elements ⟷ **coding region**

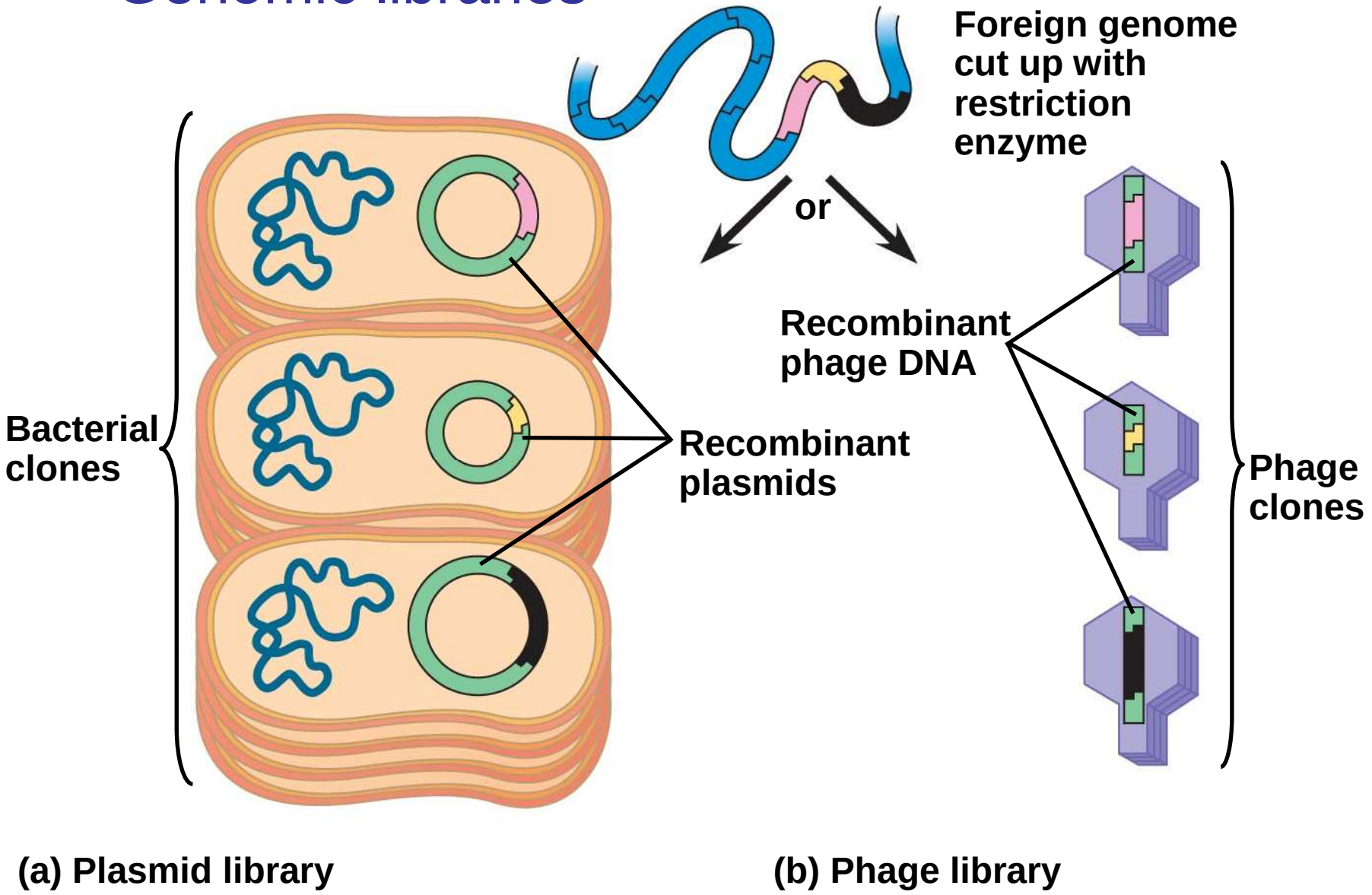


complementary DNA (cDNA)

Generated By Reverse transcriptase

Fig. 20-5a

Genomic libraries

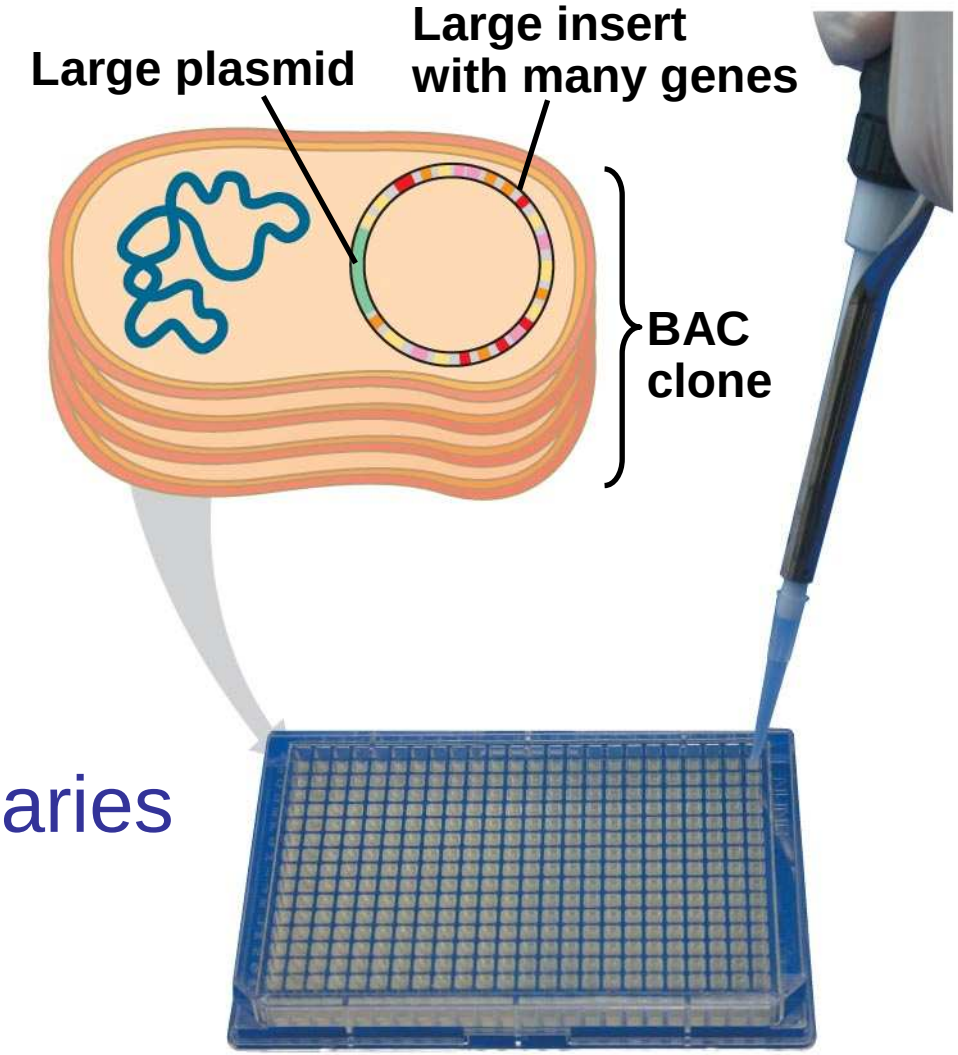


BAC clones

- 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

Fig. 20-5b

Genomic libraries



(c) A library of bacterial artificial chromosome (BAC) clones

cDNA library (from mRNAs, 只含已被表現的基因)

- 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 or tissue
- A **cDNA library** represents only part of the genome — only the **subset of genes** transcribed into mRNA in the original cells

Fig. 20-6-1

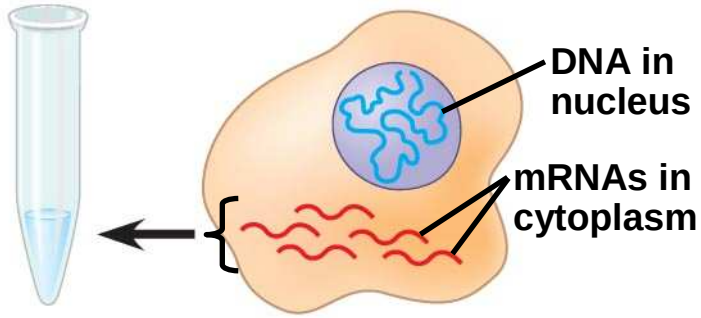
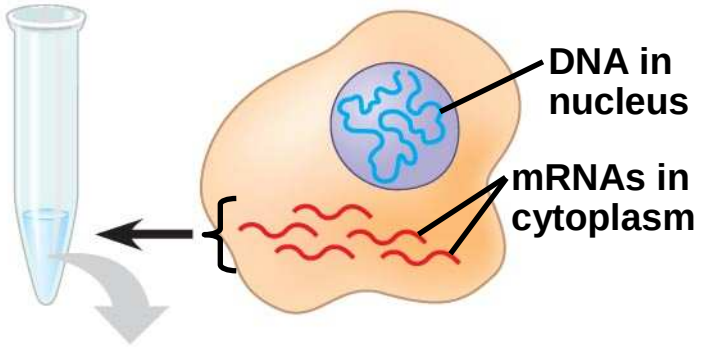
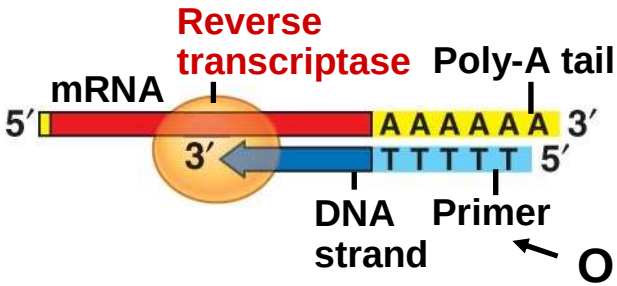


Fig. 20-6-2





Oligo dT or Random Primers

Fig. 20-6-3

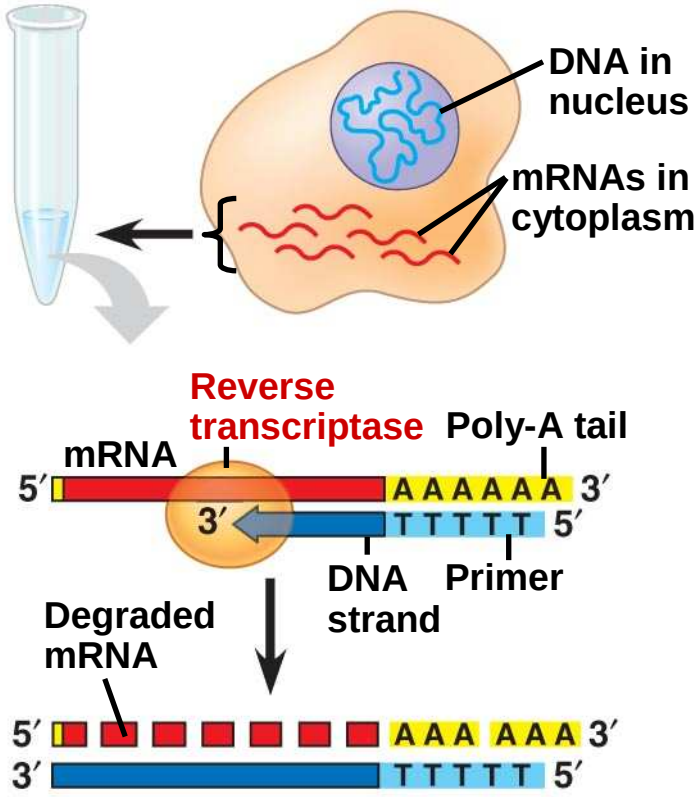


Fig. 20-6-4

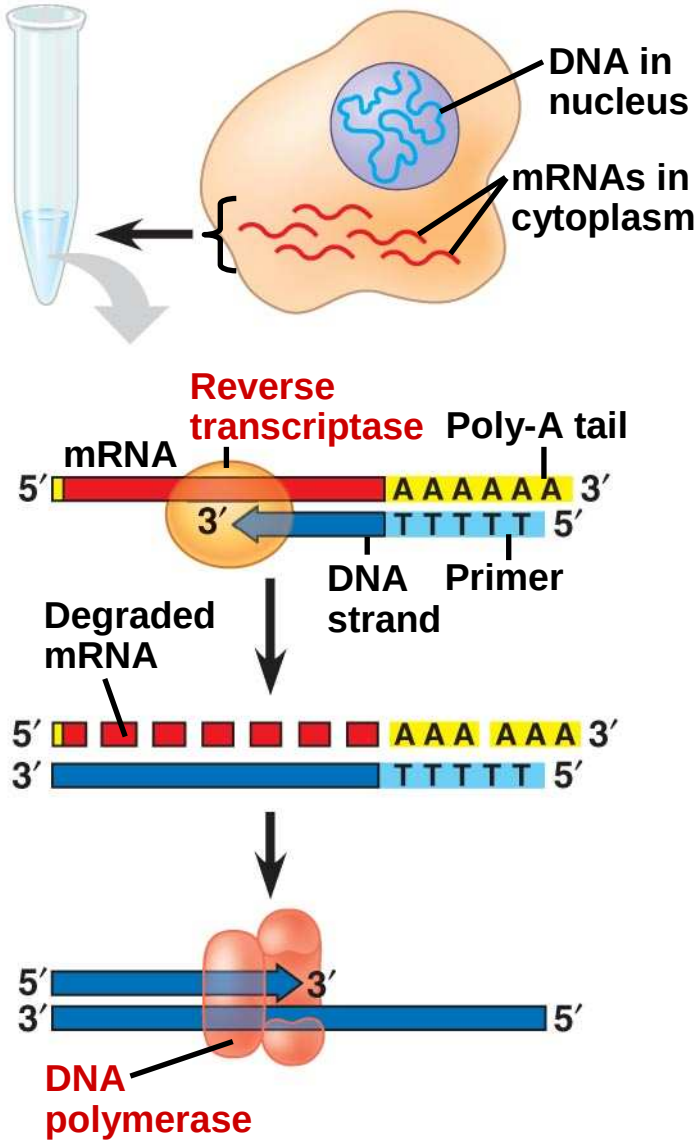
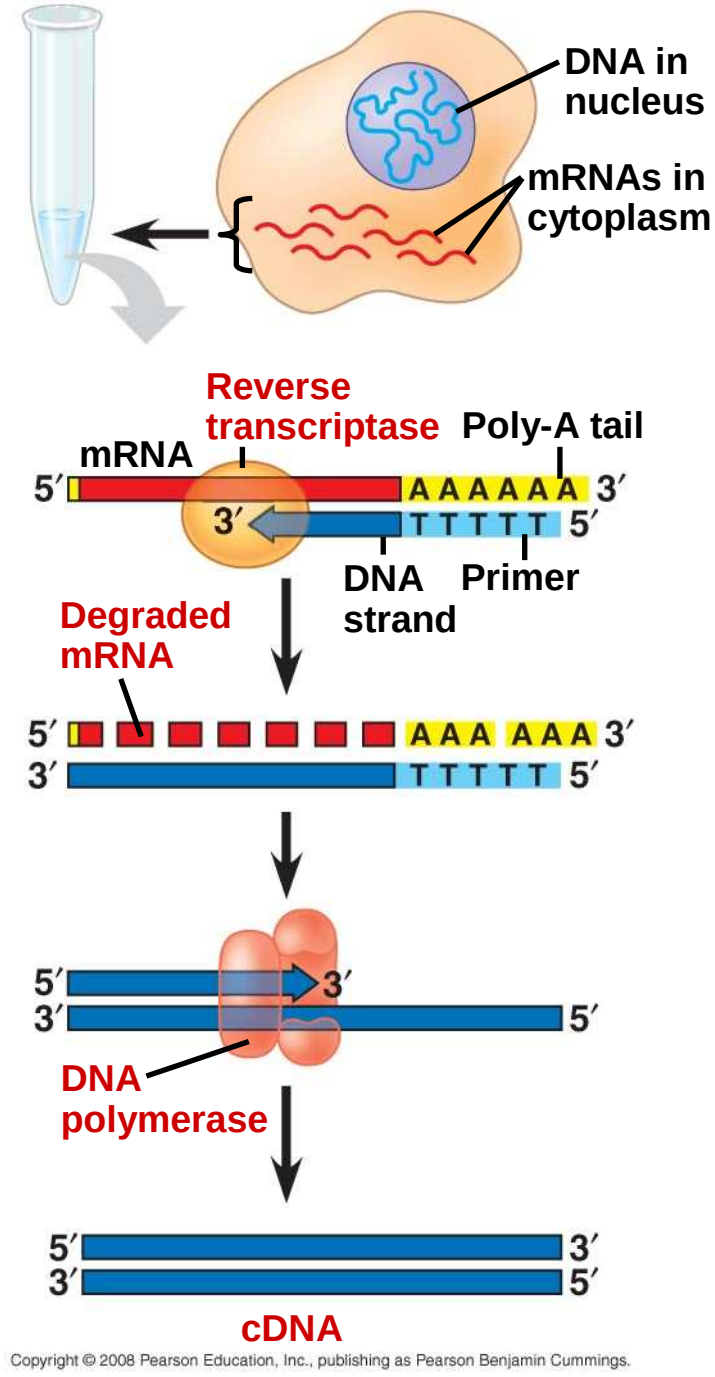


Fig. 20-6-5



Screening a Library for Clones Carrying a Gene of Interest

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

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

- – Then we would synthesize this probe

With fluorescent or radioactive label

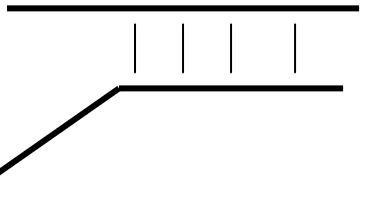
DNA denature and probe hybridization (colony hybridization)

complementary

Template 5′…GGCTAACTTAGC….3′

probe 3′…CCGATTGAATCG…5′

3′…CCGATTGAATCG…5′



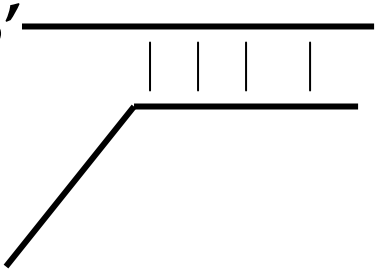
Sense vs. Anti-sense strand

Template 5′…CCATGGATACCG….3′

probe

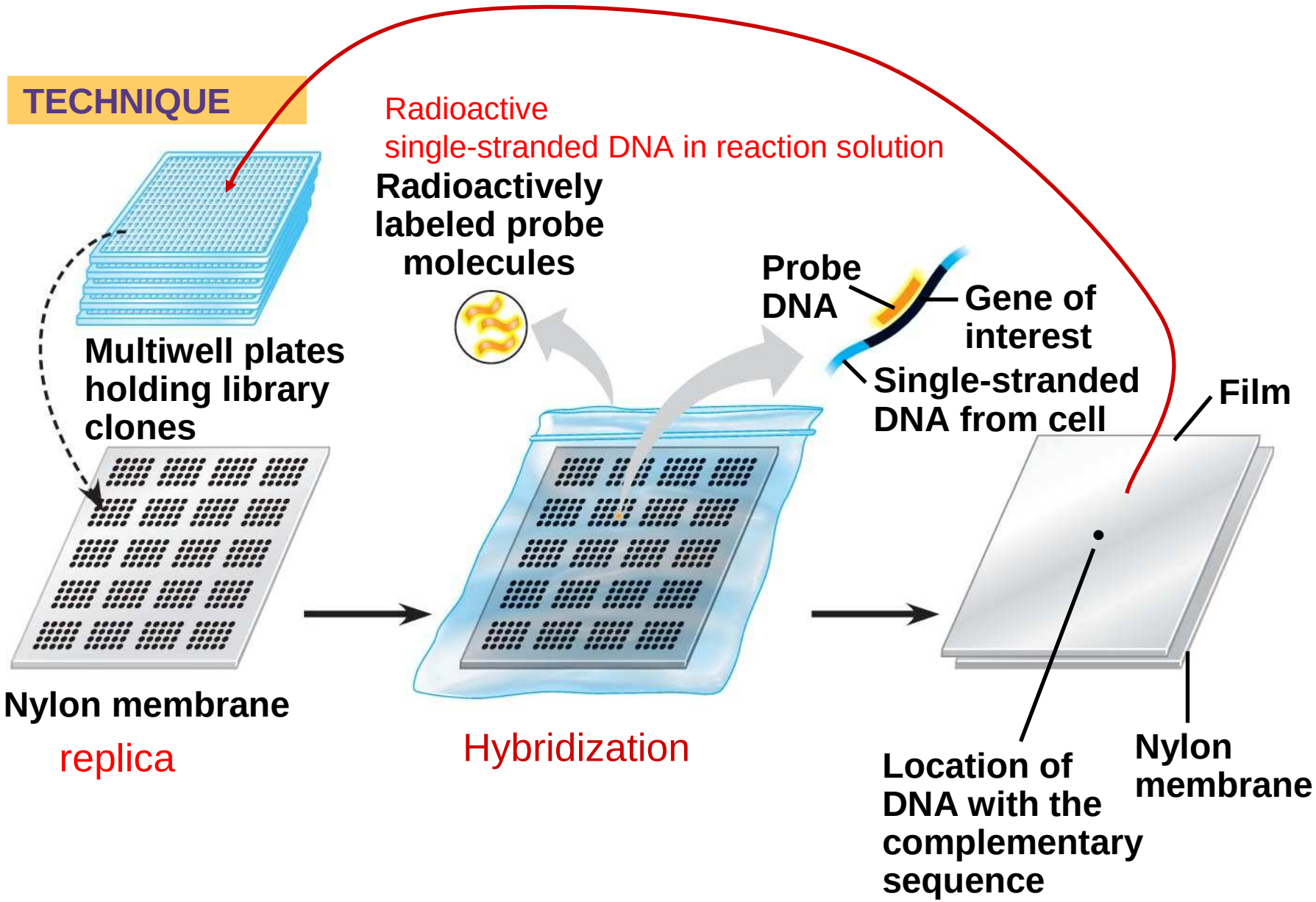
5′…CCGATTGAATCG…3′

3′…GGTACCTATGGC…5′



- 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

Fig. 20-7



Expressing Cloned Eukaryotic Genes

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

Bacterial Expression Systems

- Most popular methods to deliver plamids into *E.coli* – **heat shock** (42℃ for 45 seconds)
- Several **technical difficulties** hinder expression of cloned eukaryotic genes in bacterial host cells: no expression, not soluble, not proper folded, etc.
- To overcome differences in promoters and other DNA control sequences, scientists usually employ an **expression vector**, a cloning vector that contains a **highly active prokaryotic promoter**

Eukaryotic Cloning and Expression Systems

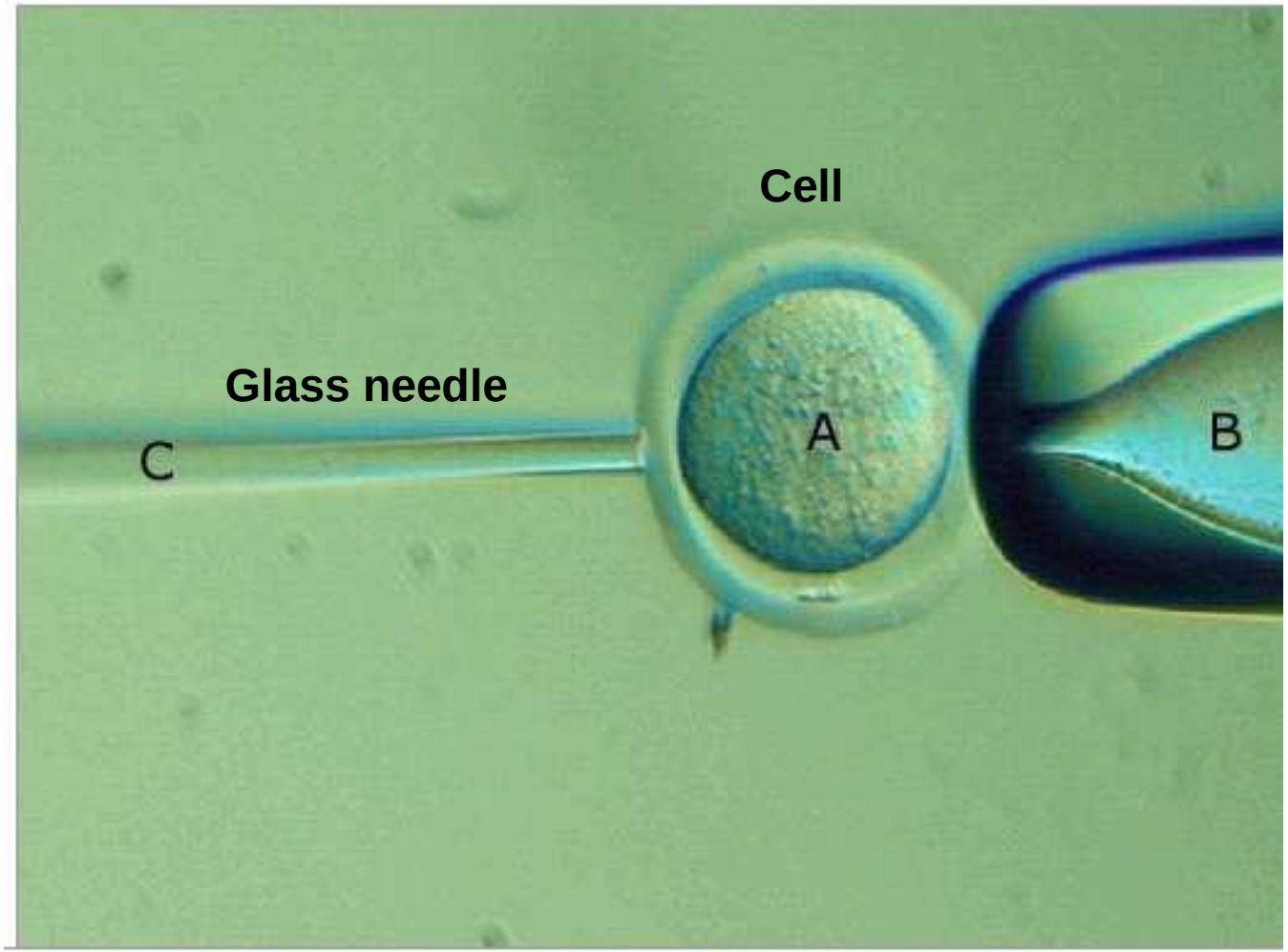
- The use of cultured eukaryotic cells as host cells and **yeast artificial chromosomes (YACs)** as vectors helps avoid some gene expression problems
- YACs behave normally in mitosis and can carry more DNA than a plasmid
- **Eukaryotic hosts can provide the post-translational modifications that many proteins require**

Delivery gene into the eukaryotic cells

- 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 (microinjection)
- Once inside the cell, the DNA is incorporated into the cell's DNA by natural genetic recombination

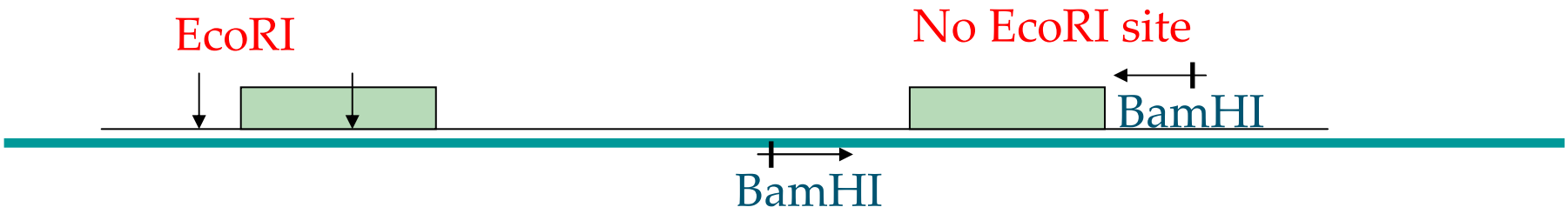
Microinjector

SCNT (Somatic Cell Nuclear Transfer)



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



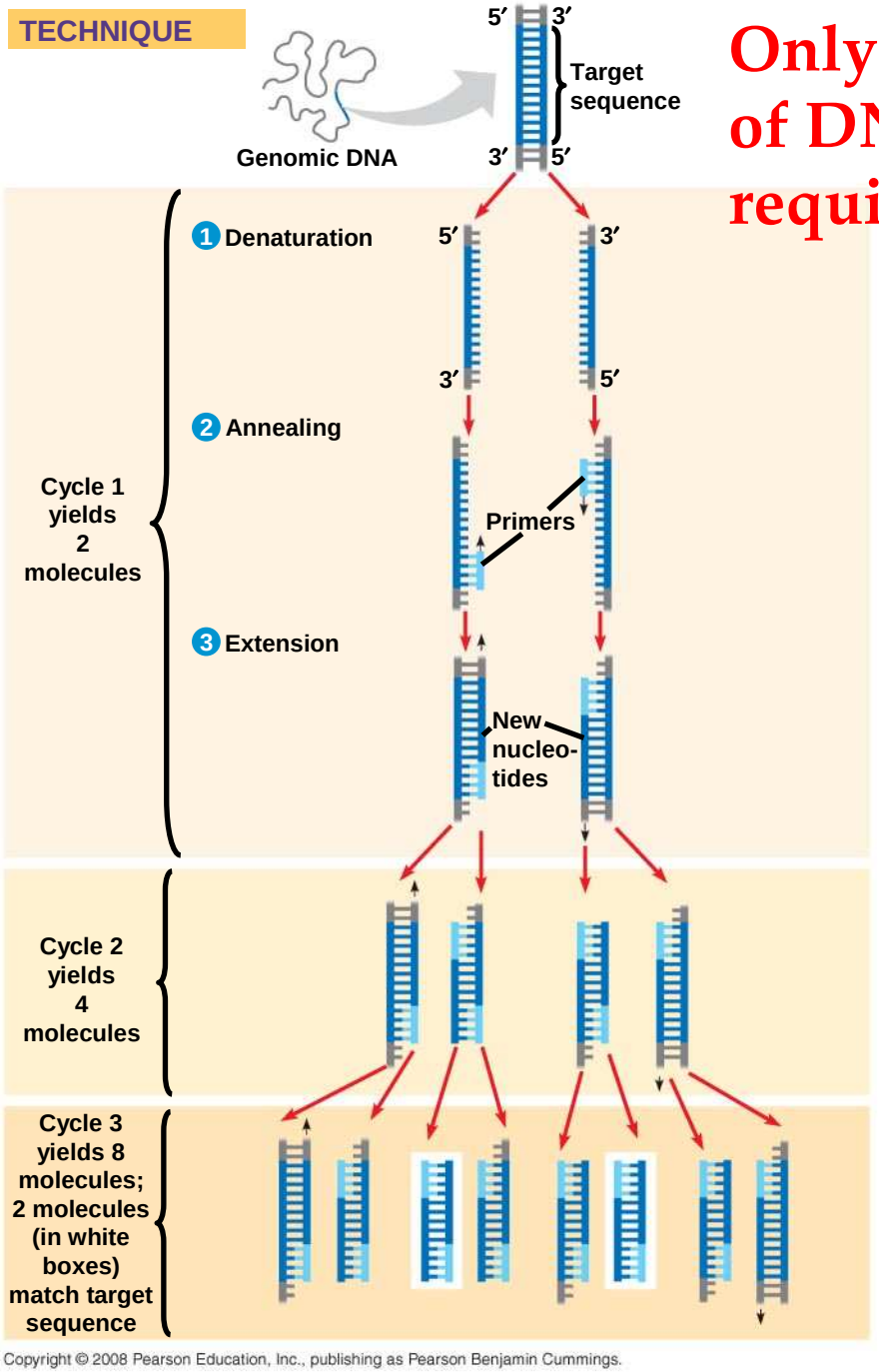
With **polymerase chain reaction (PCR)**, any specific segment (with too many or no restriction enzymes available) of the target sequence within a DNA sample can be copied many times (amplified) completely *in vitro*.

Key components in a PCR reaction:

--Uses primers (with unique restriction site) that bracket the desired sequence

--Uses a heat-resistant DNA polymerase (active at high temperature) isolated from prokaryotes living in hot spring: extend primers in 5' → 3' direction

Fig. 20-8



Only tiny amount of DNA template is required

Fig. 20-8a

The polymerase chain reaction (PCR)

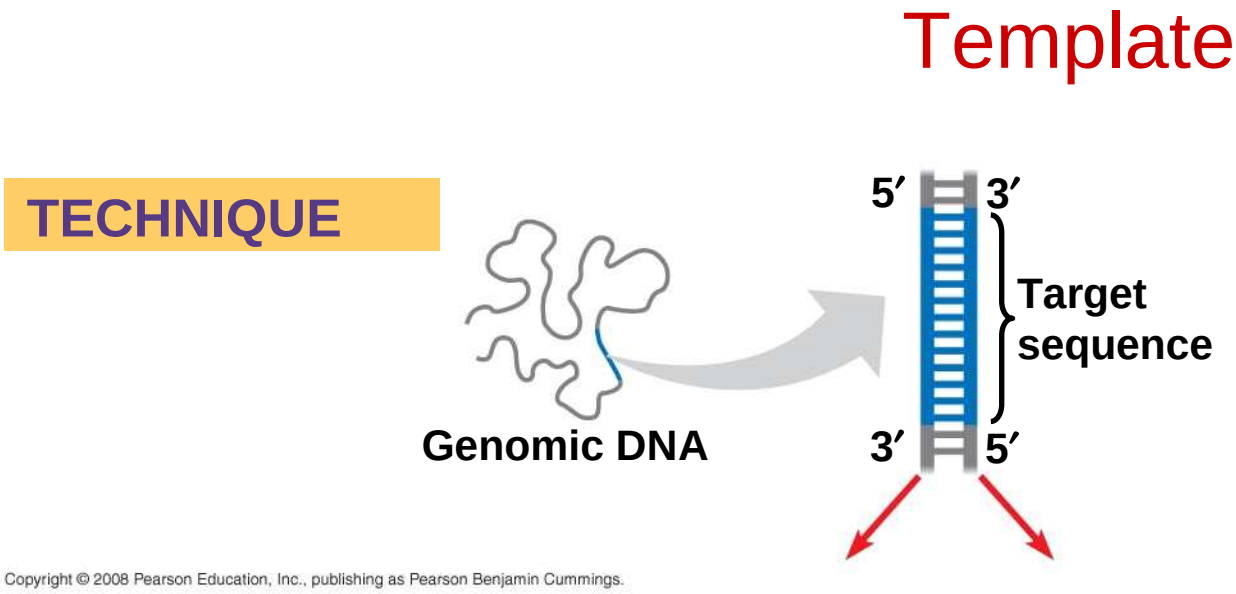


Fig. 20-8b

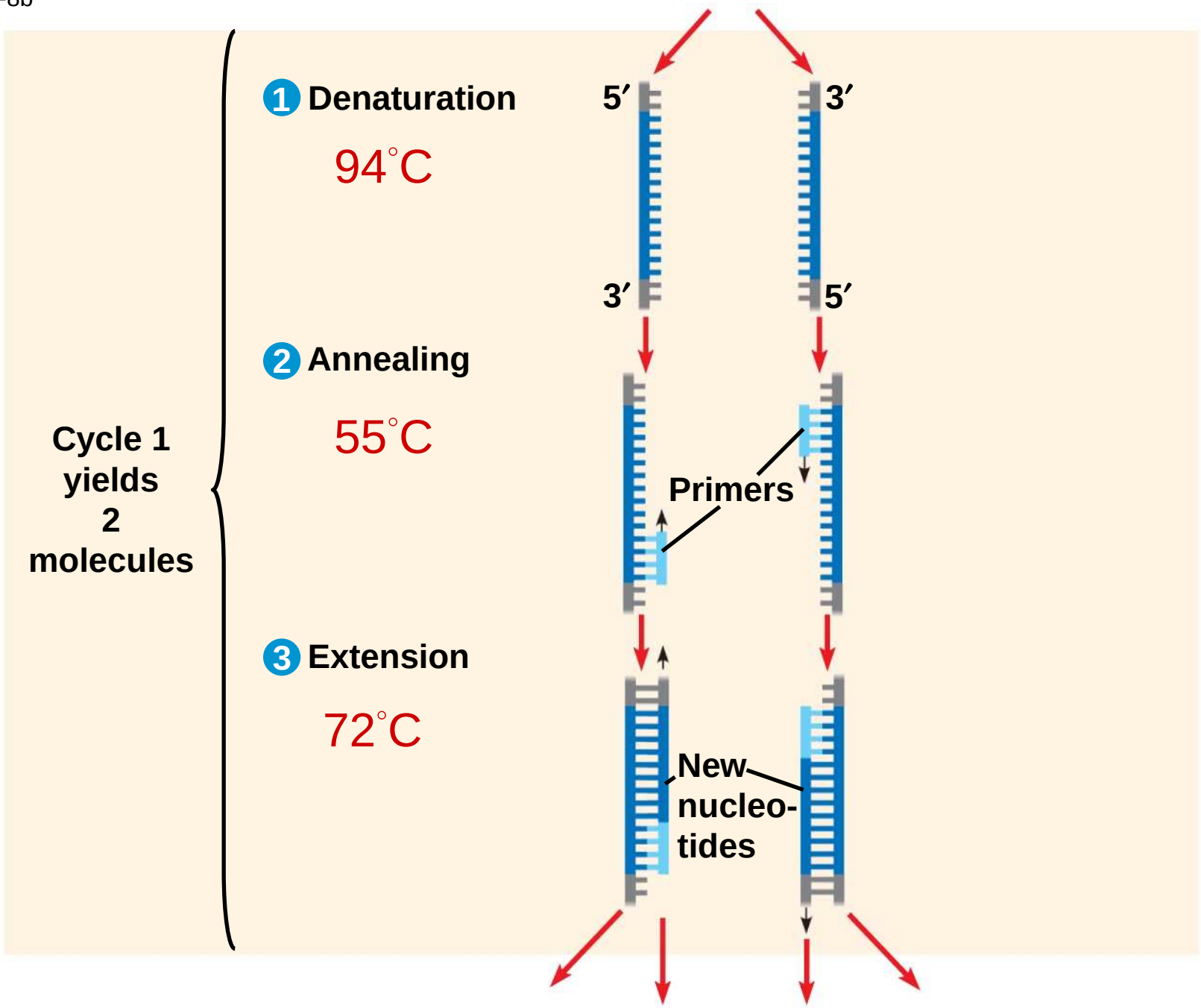
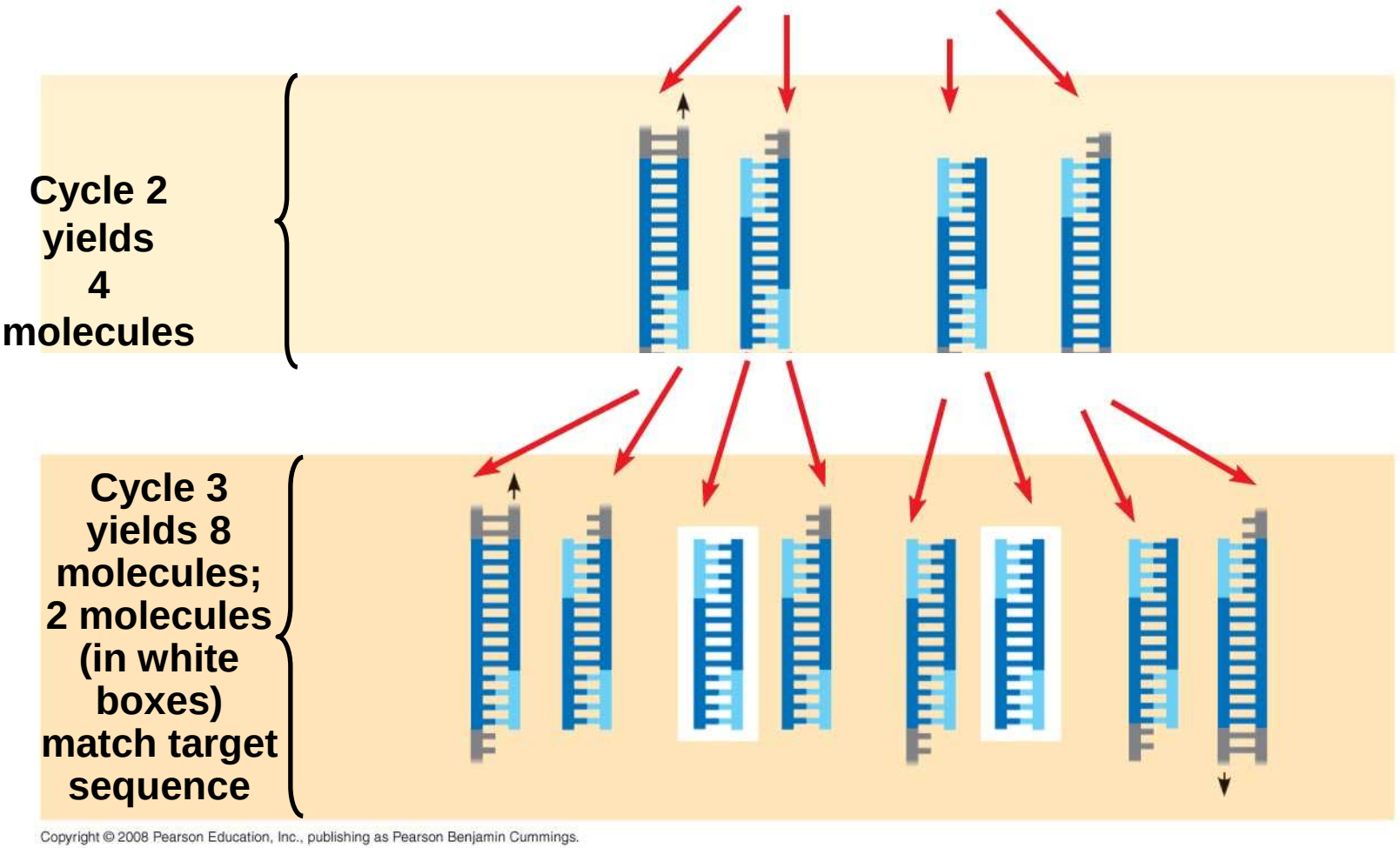


Fig. 20-8c



The polymerase chain reaction (PCR)

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

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

Gel Electrophoresis and Southern Blotting

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

Fig. 20-9a

Gel electrophoresis

TECHNIQUE

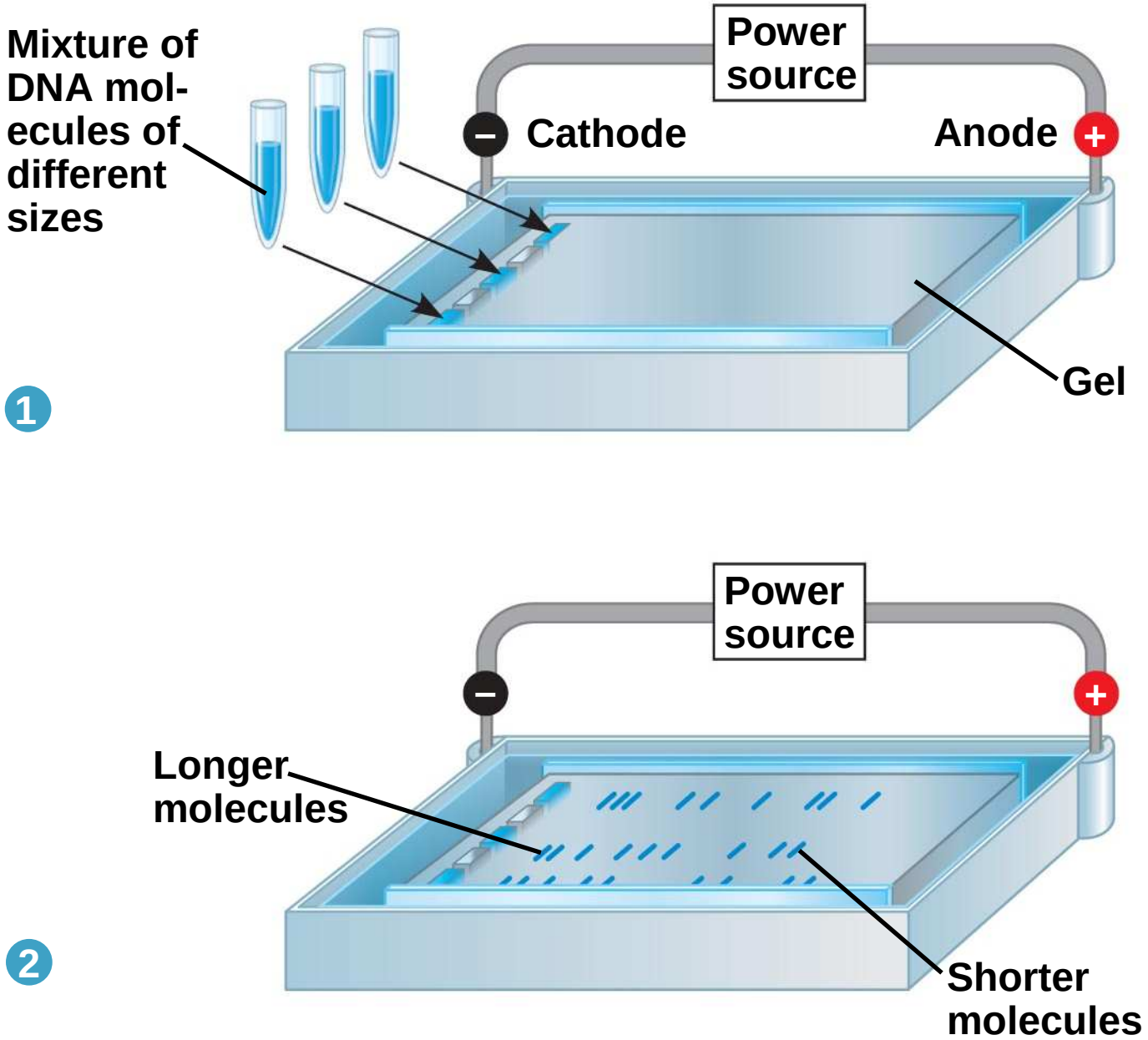
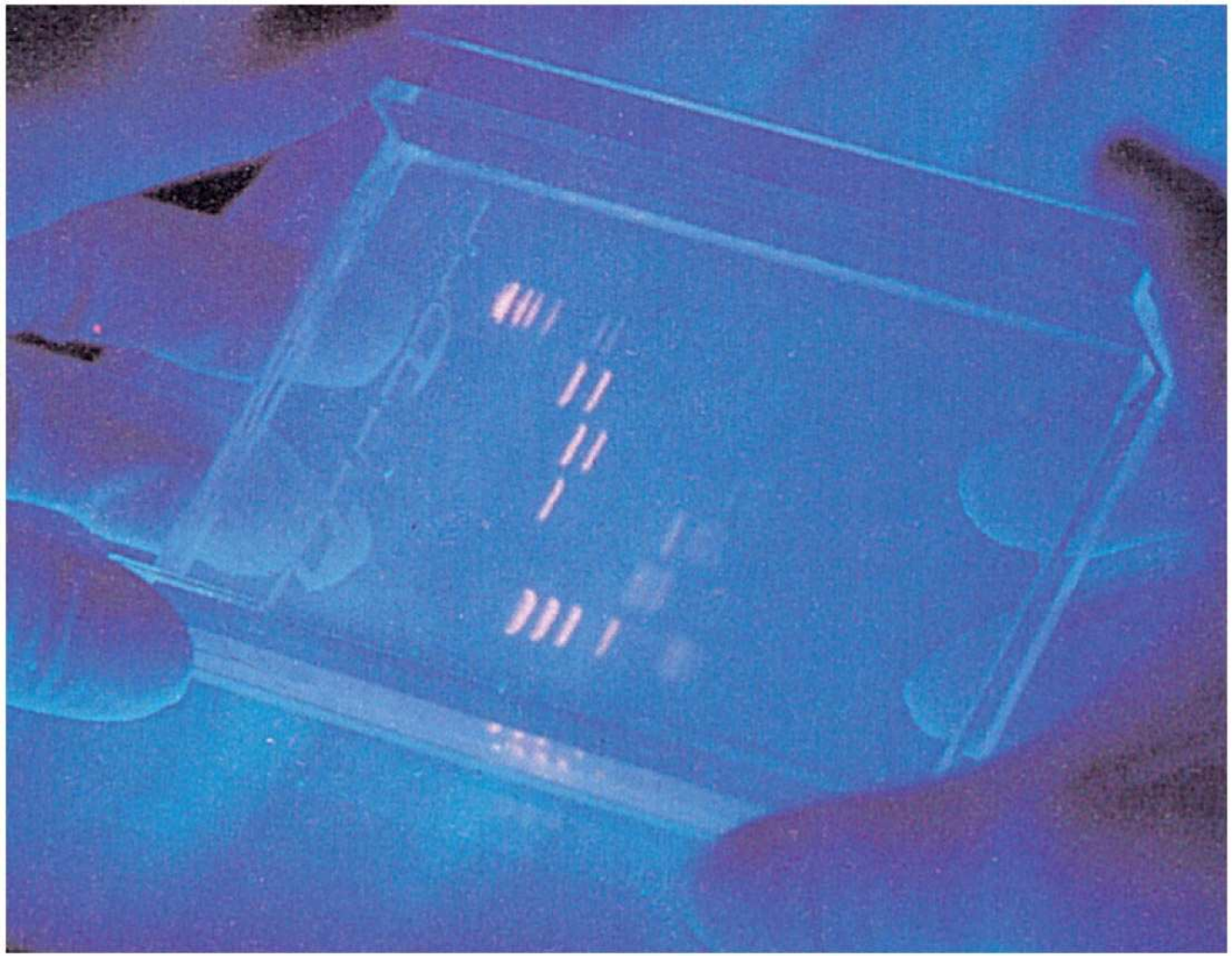


Fig. 20-9b

Gel electrophoresis

RESULTS



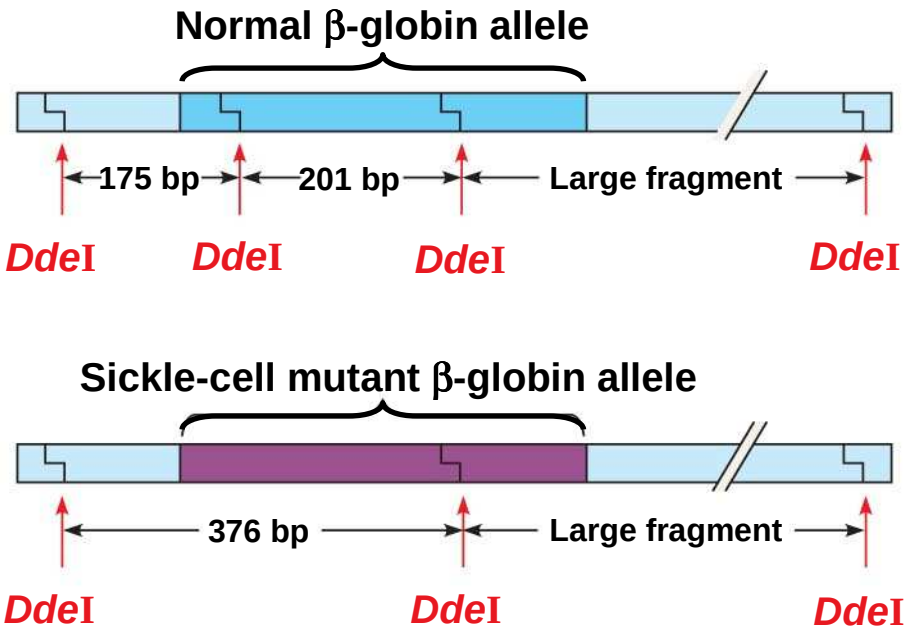
Visualization by **EtBr staining**

Restriction fragment analysis (RE digestion diagnostics)

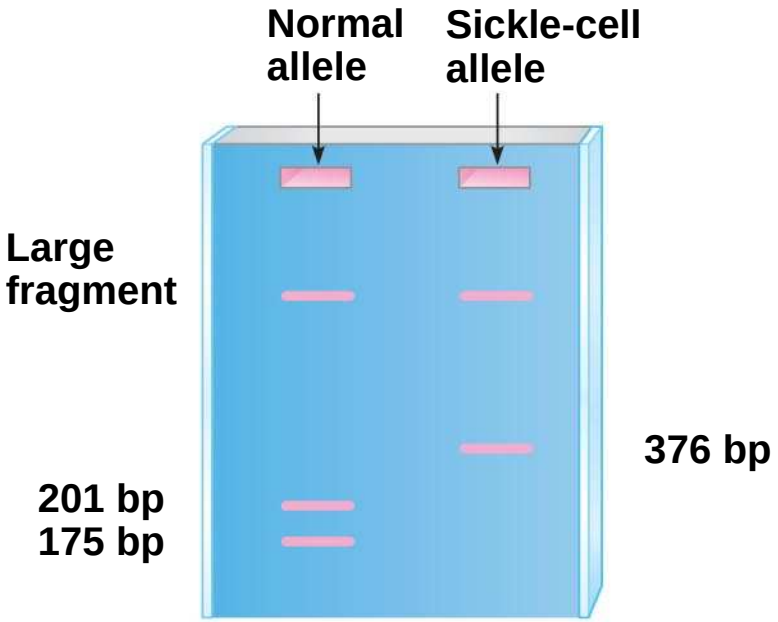
- In *restriction fragment analysis*, DNA fragments produced by restriction enzyme digestion of a DNA molecule are sorted by gel electrophoresis
  - A restriction enzyme will usually make many cuts in a DNA molecule
    - Yielding a set of restriction fragments
  - Gel electrophoresis to Separates DNA restriction fragments of different lengths:
    - Small DNA molecules from virus,plasmid– discrete bands
    - Large DNA molecules from eukaryotic chromosome --- smear
- Restriction fragment analysis is useful for comparing two different DNA molecules, such as two alleles for a gene
- The procedure is also used to prepare pure samples of individual fragments

Fig. 20-10

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

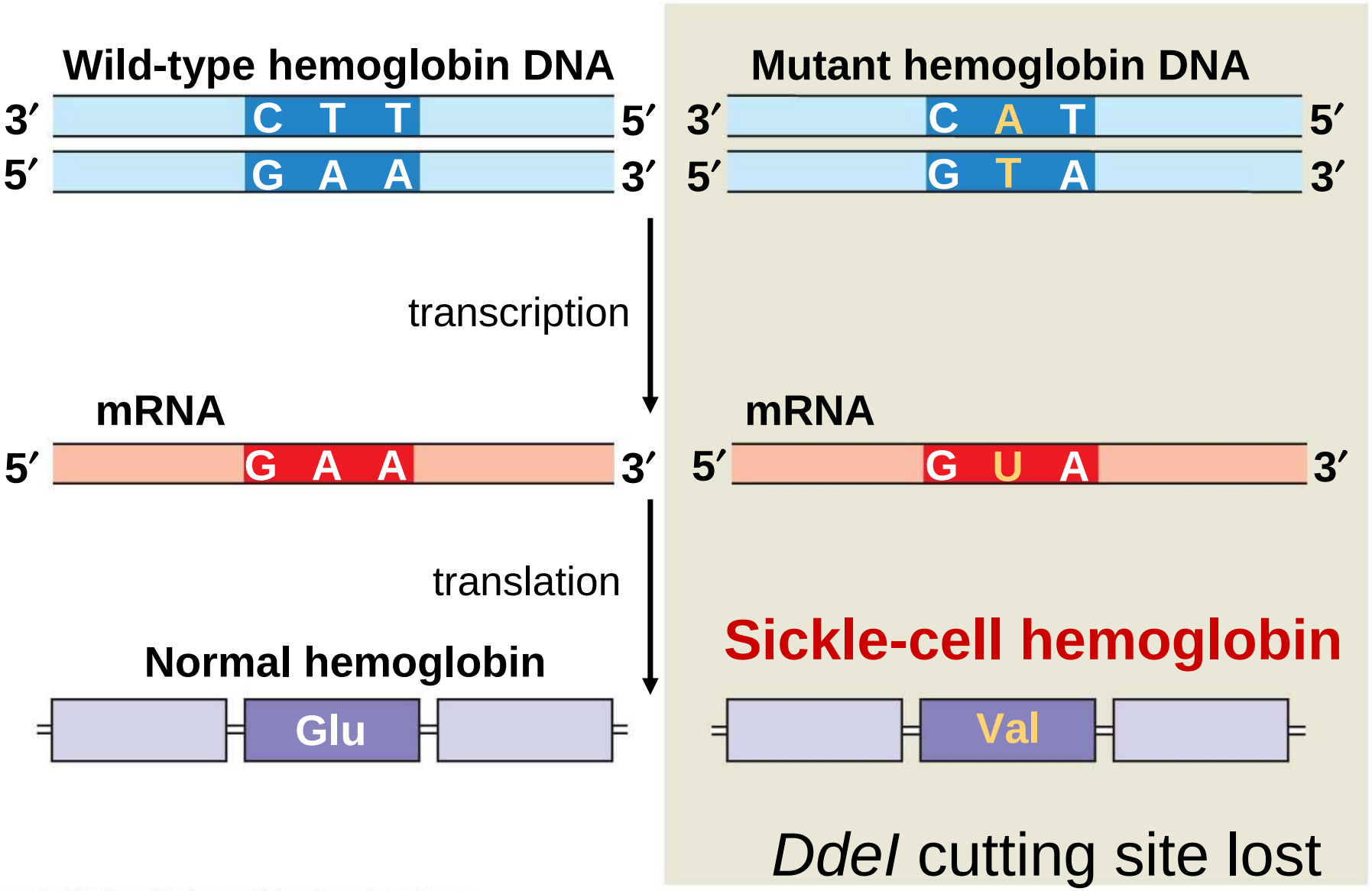


(a) *DdeI* restriction sites in normal and sickle-cell alleles of β-globin gene



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

Fig. 17-22



Southern blotting (DNA – DNA)

- 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

Fig. 20-11a

Southern blotting of DNA fragments

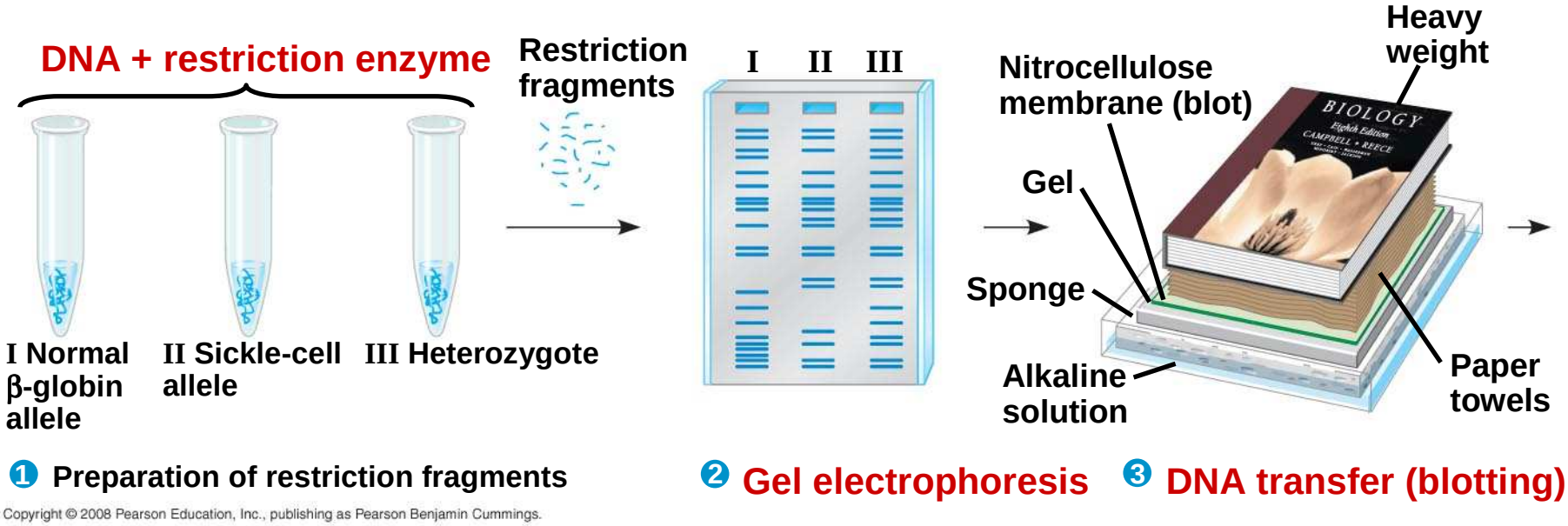
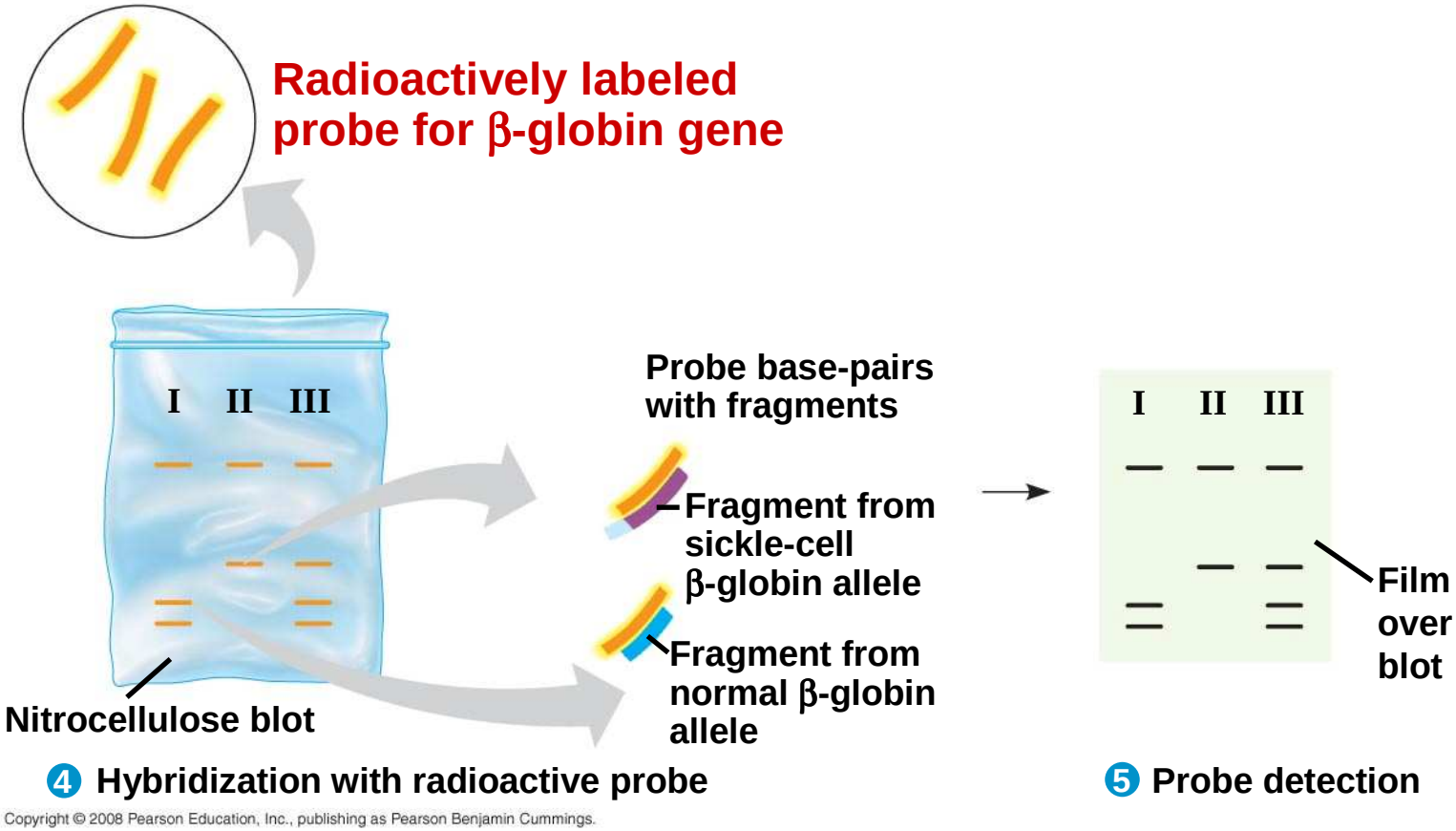


Fig. 20-11b

Southern blotting of DNA fragments



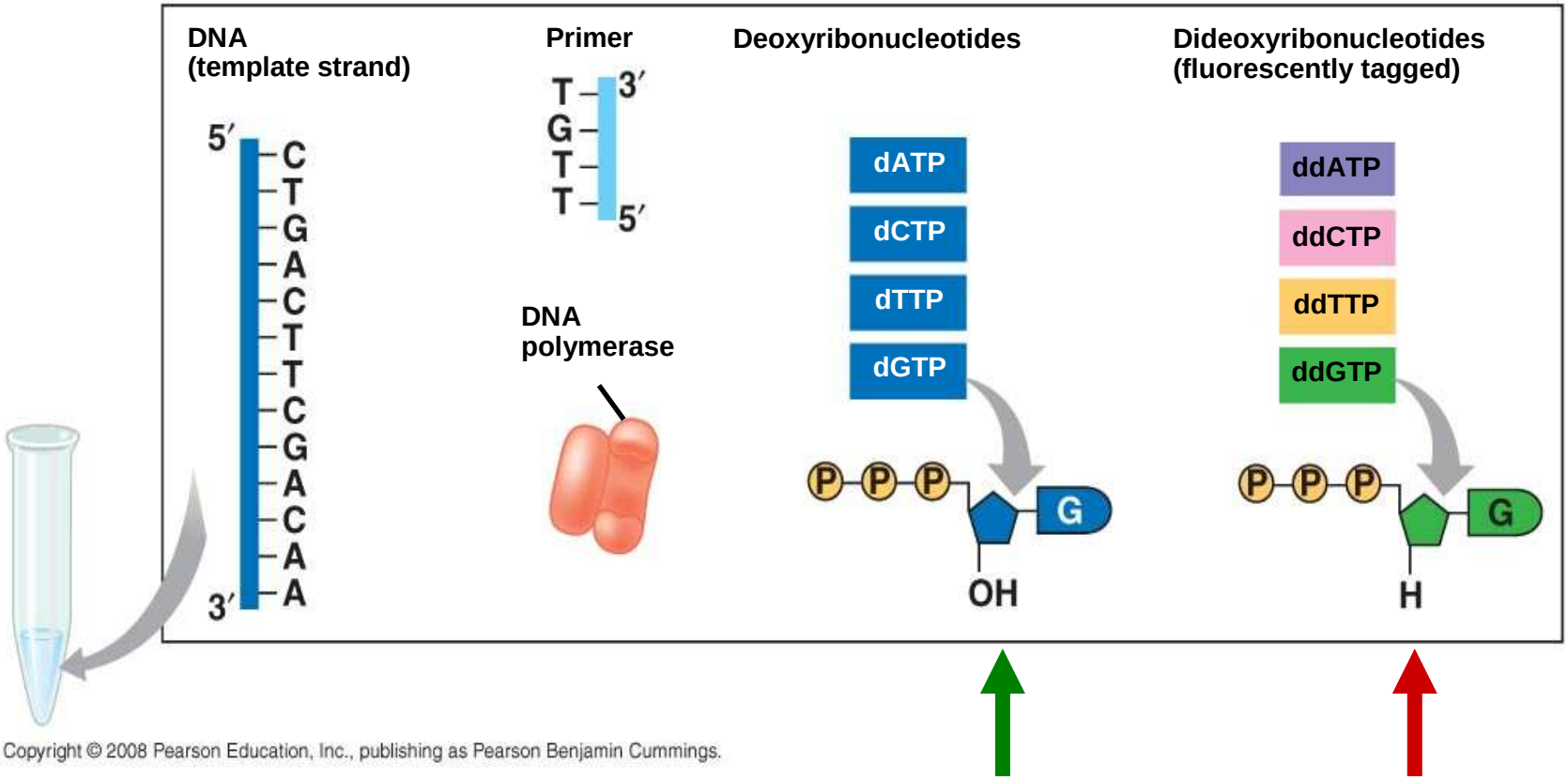
DNA Sequencing

- Relatively short DNA fragments can be sequenced by the *dideoxy chain termination method*
- 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

Fig. 20-12a

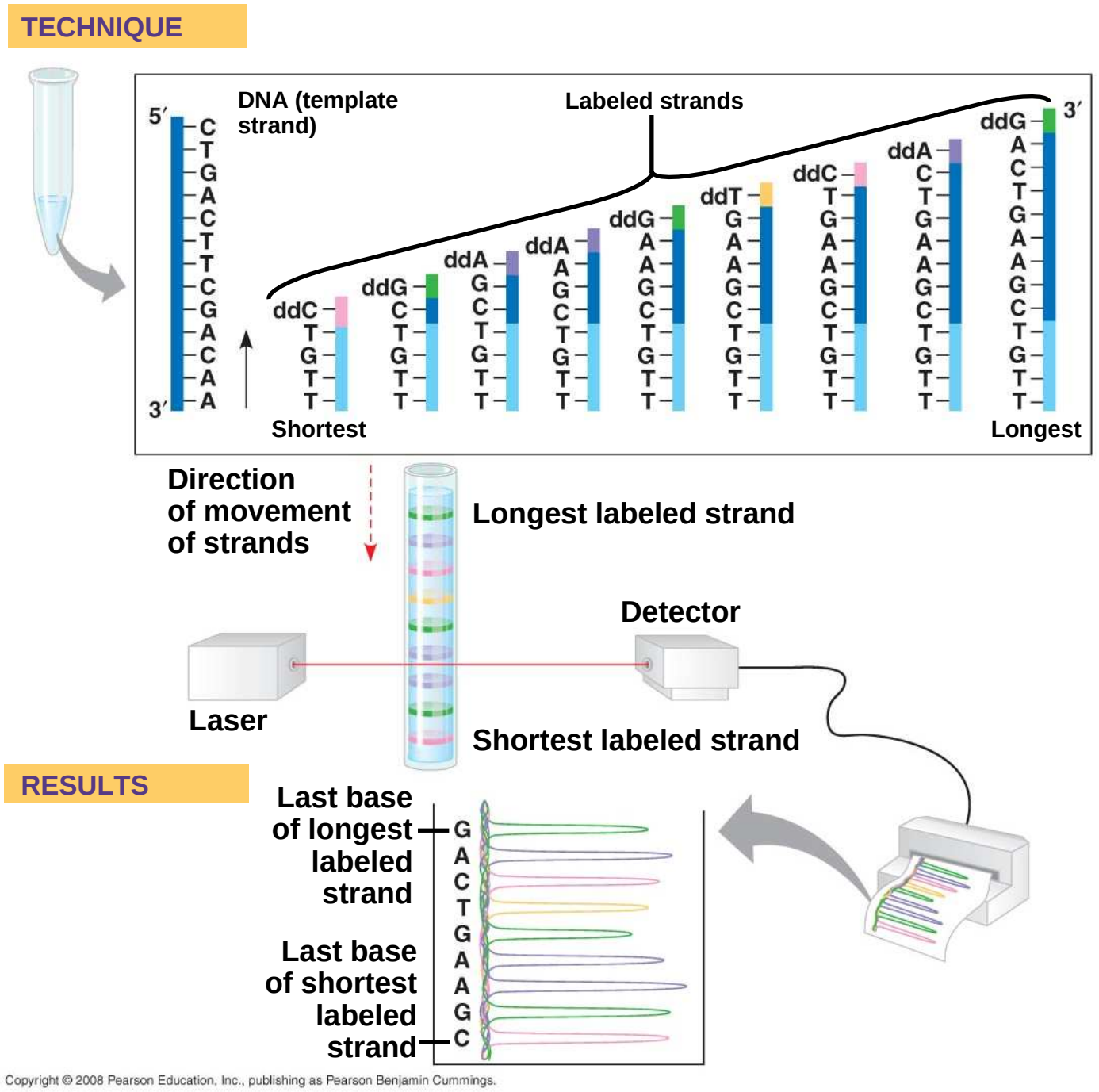
Dideoxy chain termination method for sequencing DNA

TECHNIQUE



Chain reaction terminated

Fig. 20-12b



Latest Development on

Massive & Rapid whole genome DNA Sequencing

http://en.wikipedia.org/wiki/DNA_sequencing#Large-scale_sequencing_strategies

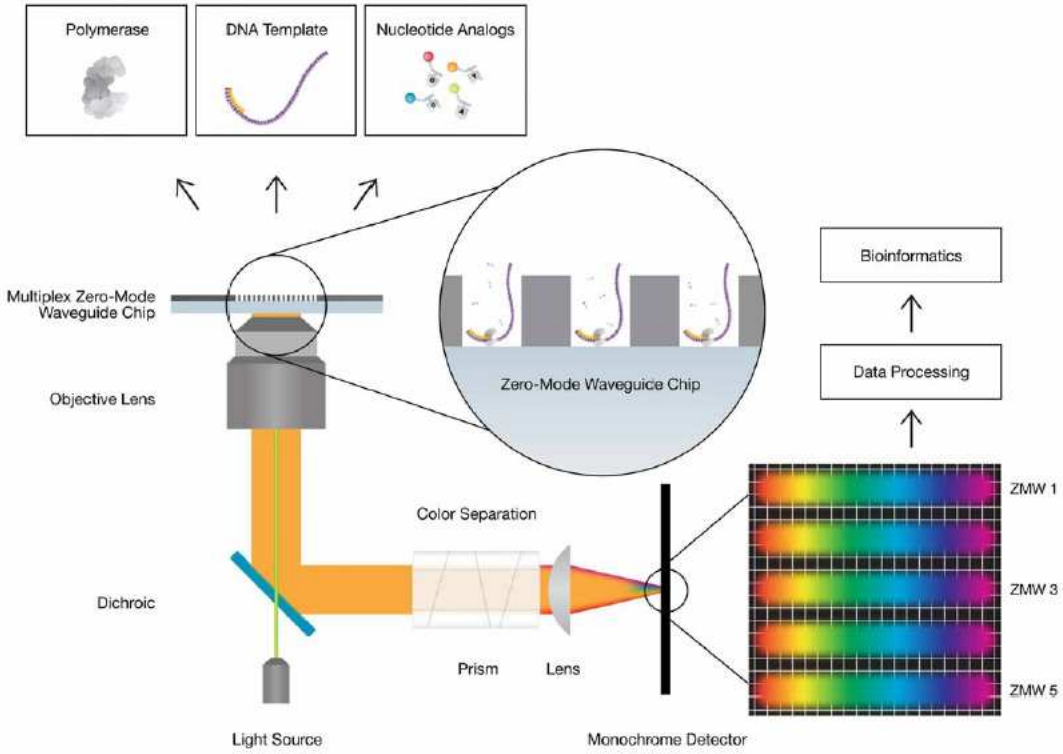
Nature Biotechnology **26**, 1135–1145 (1 October 2008)

Next-generation DNA sequencing

新世代基因定序技術

- Over the past three years, **massively parallel DNA sequencing platforms** have become widely available, reducing the cost of DNA sequencing by over two orders of magnitude

(請參考 Chapter 21 補充資料）



Analyzing Gene Expression

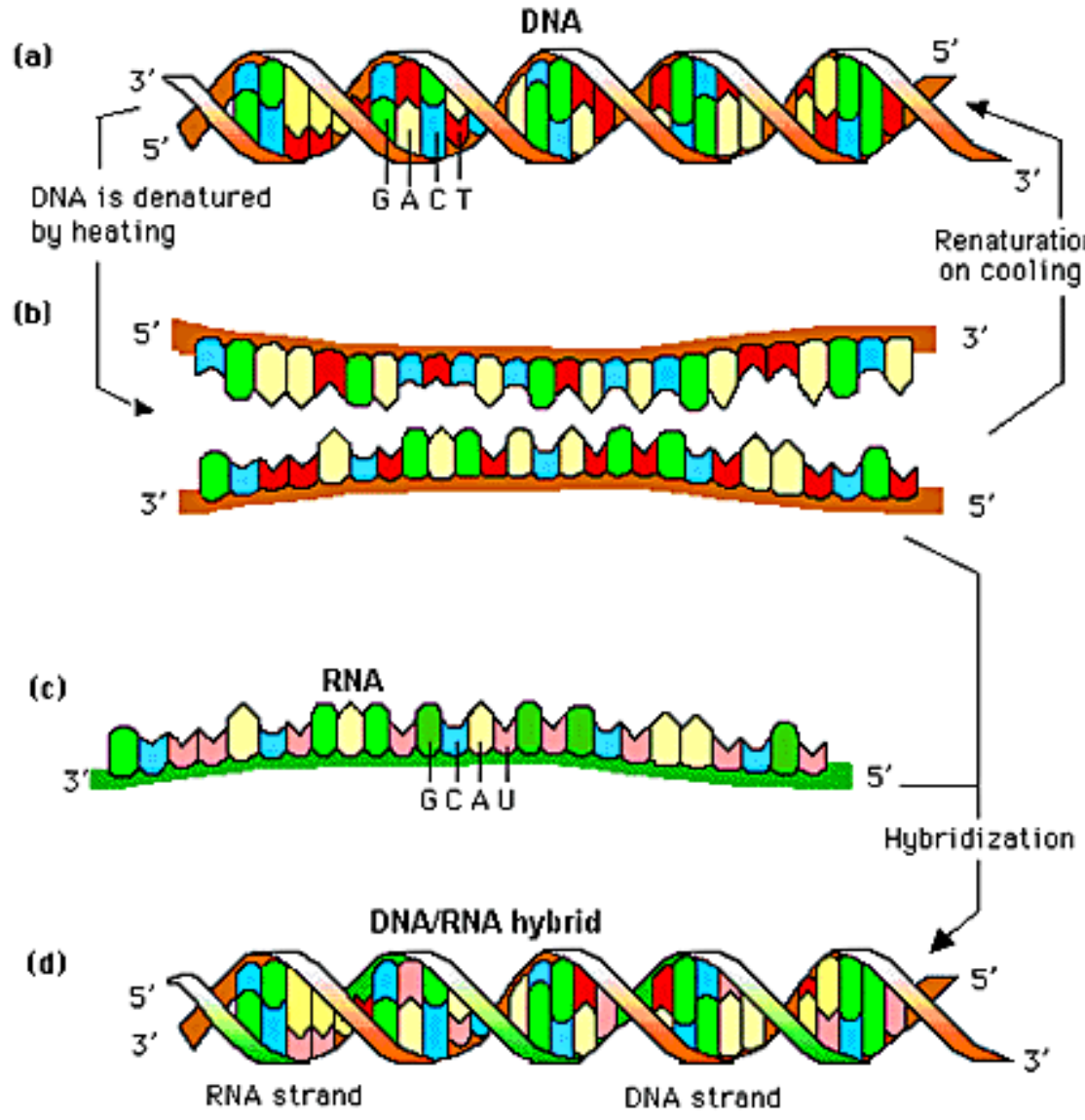
- Nucleic acid probes can hybridize with mRNAs transcribed from a gene
- Probes can be used to identify where or when a gene is transcribed in an organism

Studying the Expression of Single Genes

- Changes in the expression of a gene during embryonic development can be tested using
  - Northern blotting
  - Reverse transcriptase-polymerase chain reaction (RT-PCR)
  - Microarray
- All methods are used to compare mRNA from different stages

Northern blotting (DNA-RNA)

- **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



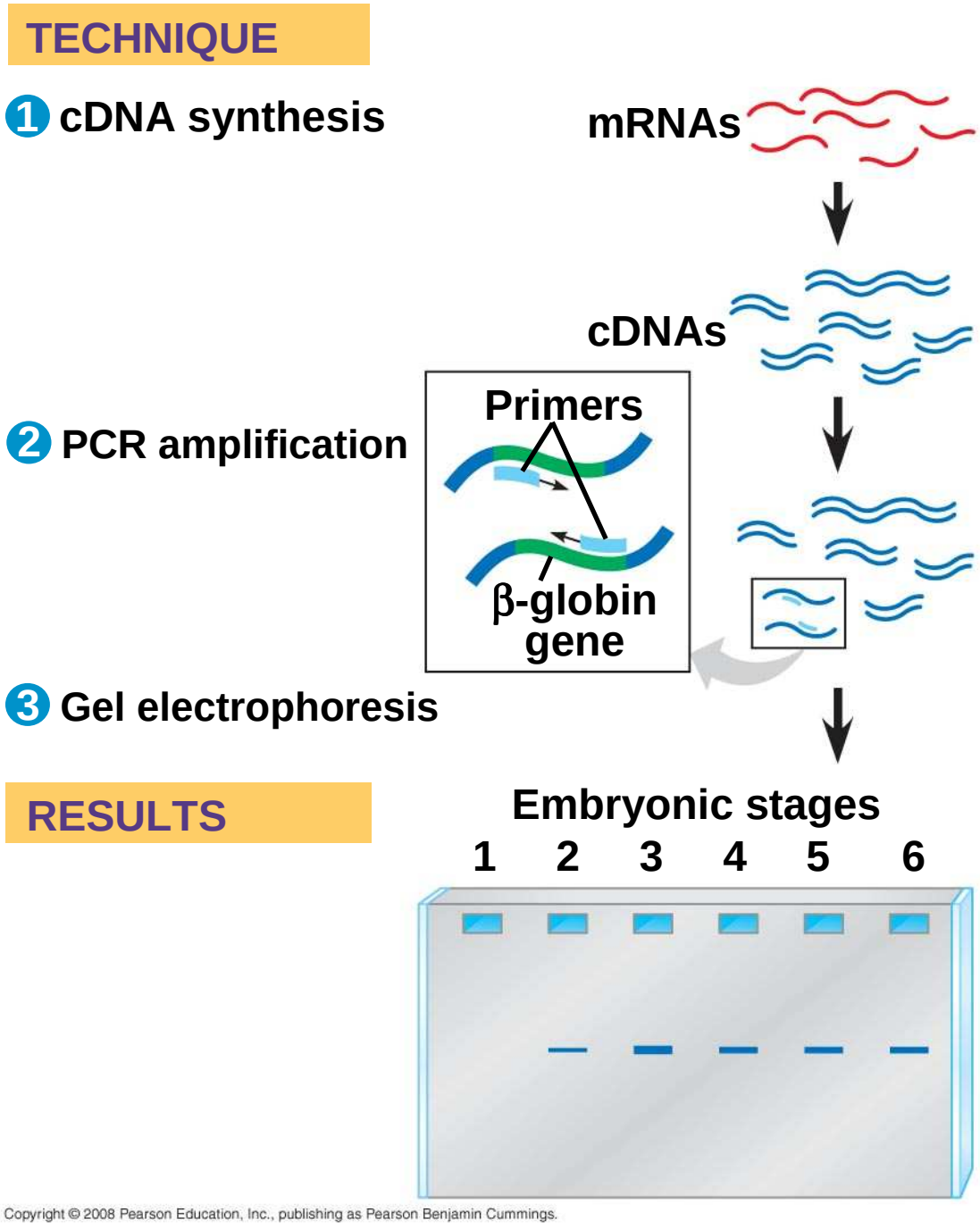
Nucleic Acid Hybridization

http://www.accessexcellence.org/RC/VL/GG/nucleic.html

RT-PCR

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

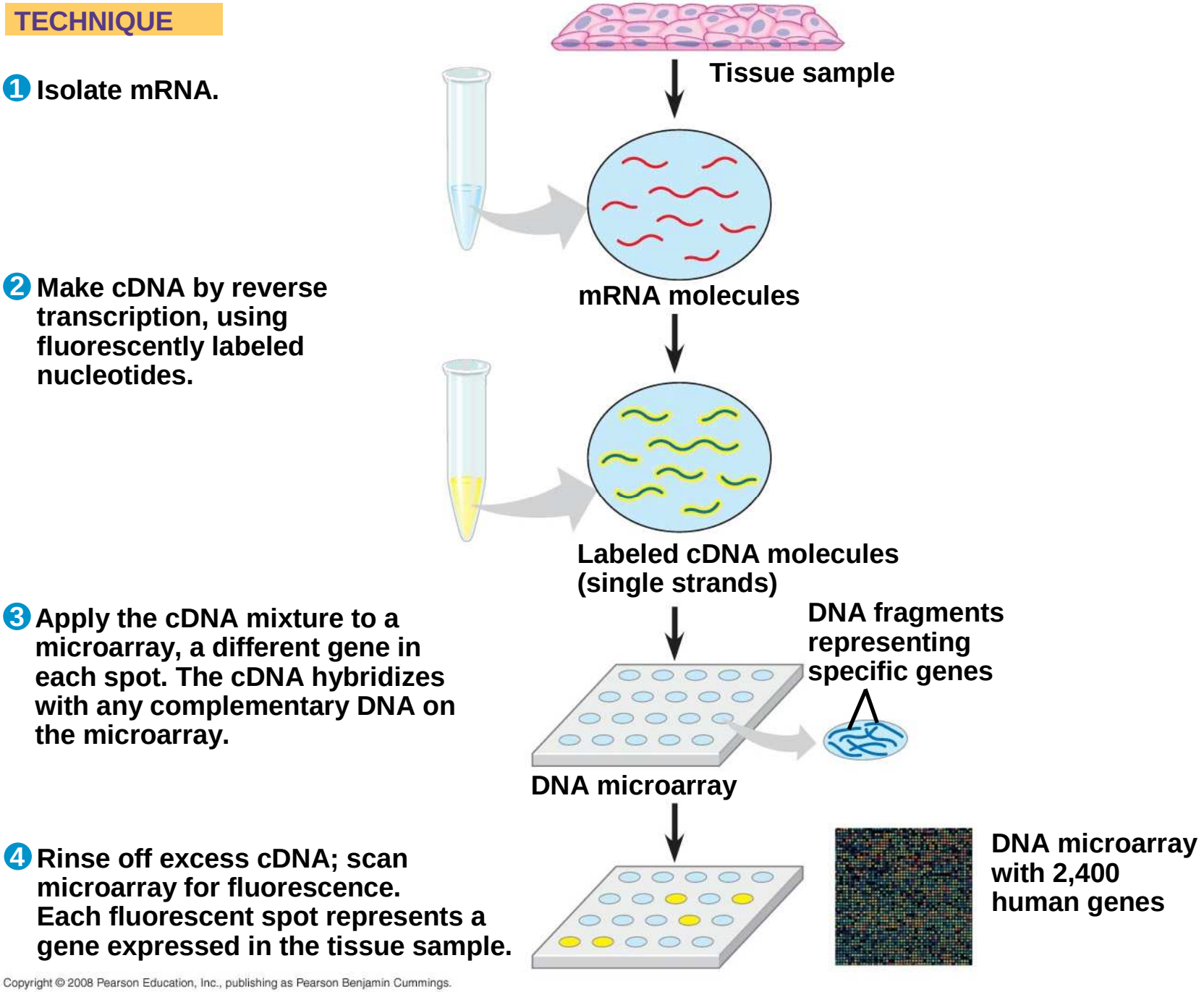
Fig. 20-13



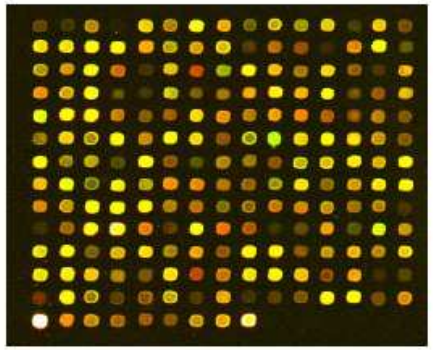
Microarray:
Studying the Expression of Interacting Groups of Genes

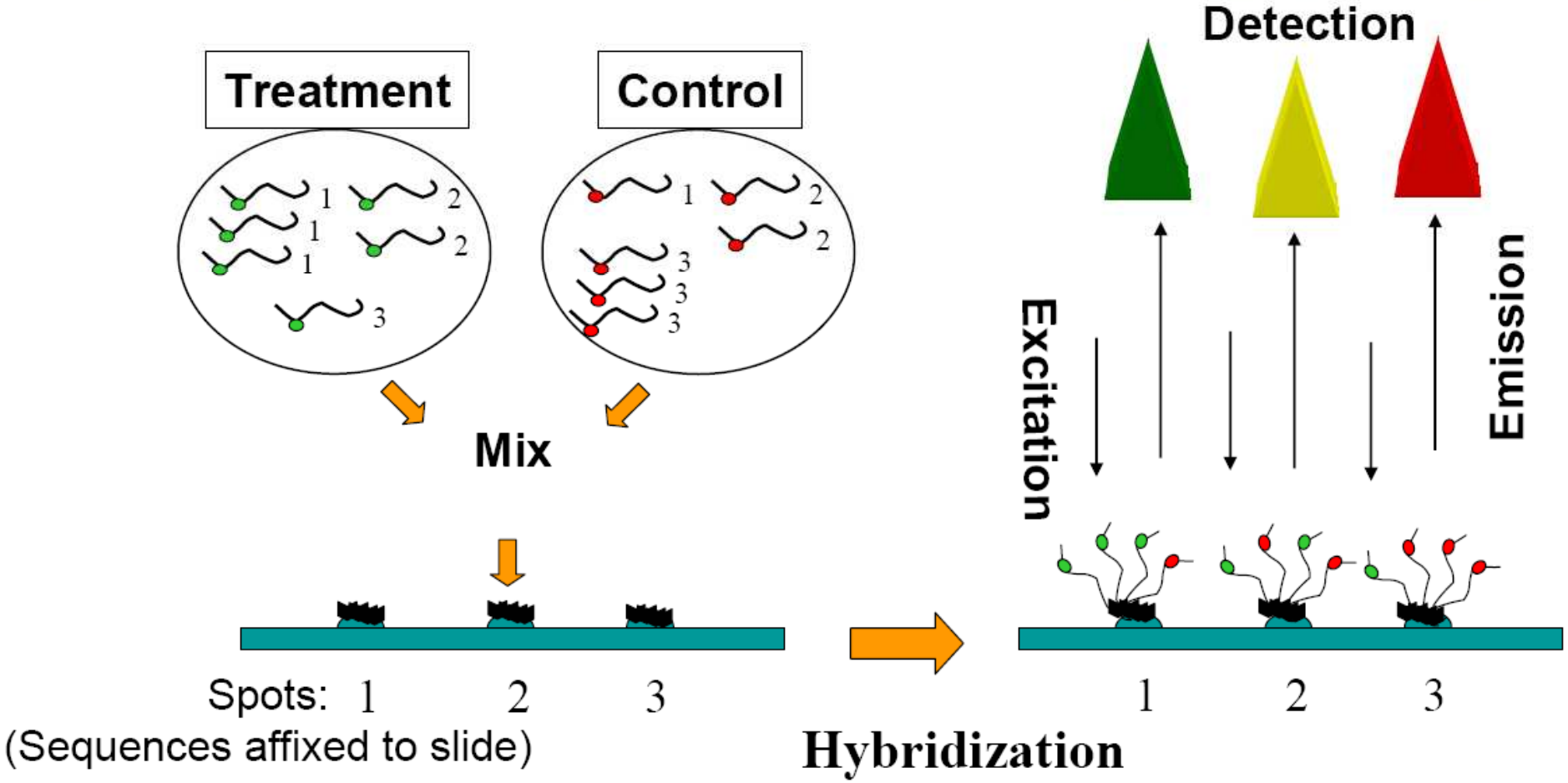
- Automation has allowed scientists to measure expression of thousands of genes at one time using DNA microarray assays
- **DNA microarray assays** compare patterns of gene expression in different tissues, at different times, or under different conditions

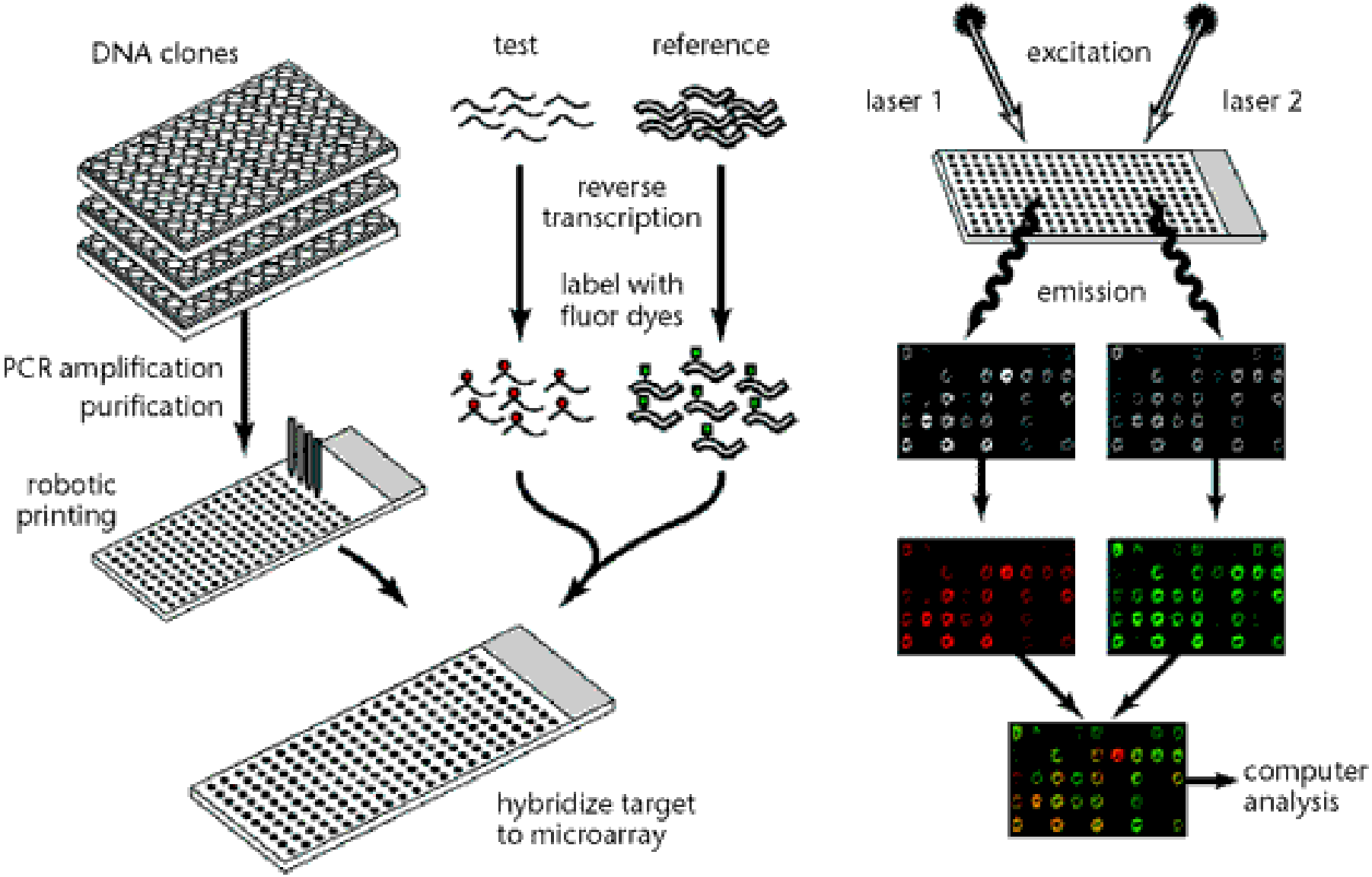
Fig. 20-15



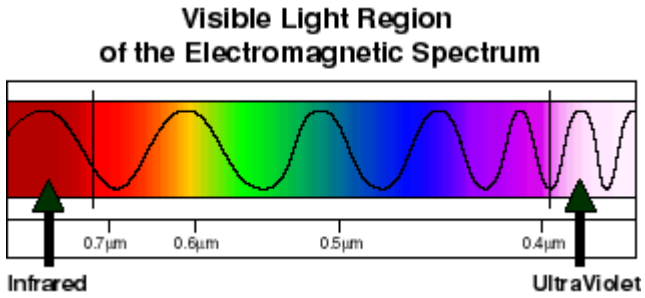
Measuring Relative Target Abundance






DNA clones
PCR amplification
purification
robotic
printing
test
reference
reverse
transcription
label with
fluor dyes
hybridize target
to microarray
excitation
laser 1
laser 2
emission
computer
analysis

Fluor Dyes
Cy3 and Cy5



In a typical cDNA microarray experiments, the samples will be visibly pink (Cy3) or blue (Cy5) in color after successful labeling.

If one looks at the visible light spectrum, the Cy3 emission filter (550-605 nM) covers the **blue-green** region, while the Cy5 emission filter (650-725 nM) covers the **orange-red** region.

Note:

- **Cy3: Three-Tree- Tree is Green!**
- **Cy5: Five-Fire- Fire is Red!**

In situ hybridization

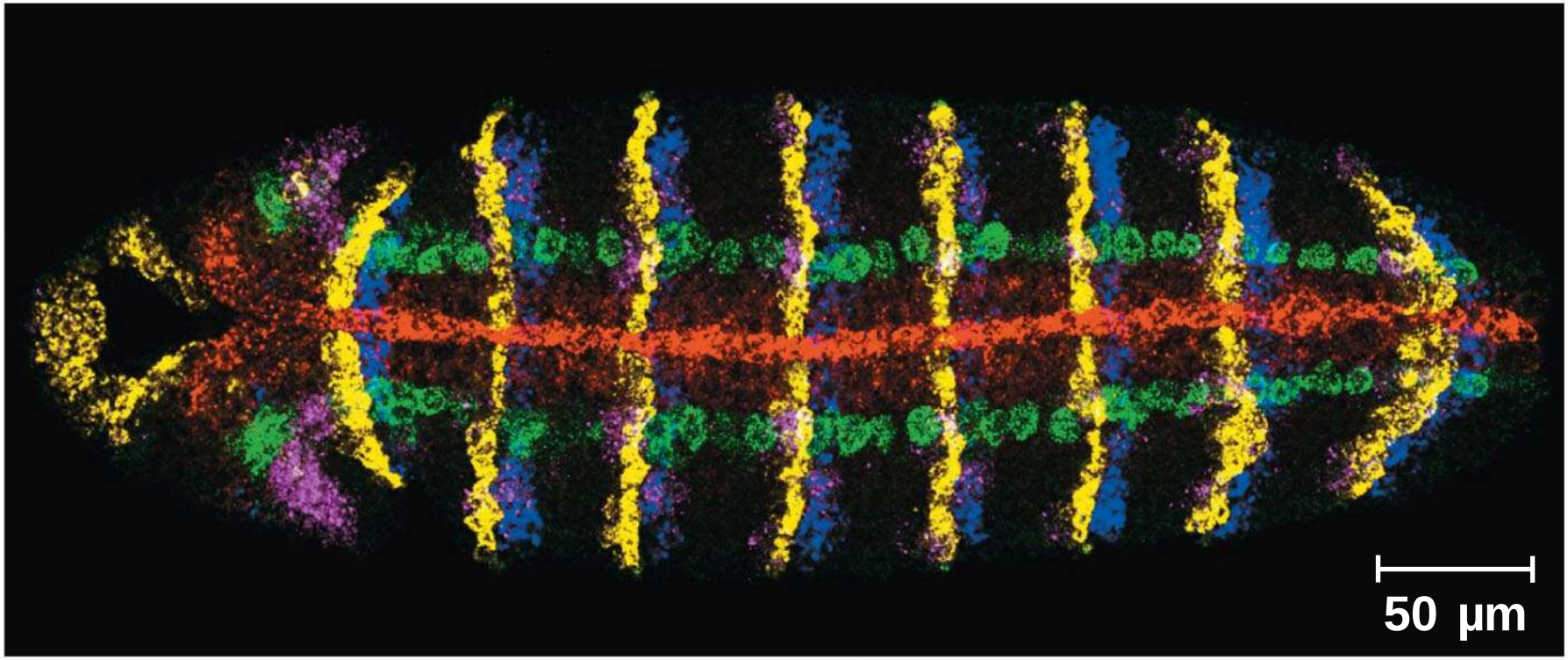
- ***In situ* hybridization** uses fluorescent dyes attached to probes to identify the location of specific mRNAs in place in the intact organism

- In situ = 在原位

In situ hybridization (ISH) 偵測完整個體上，標的基因的表現

Fig. 20-14

Determining where genes are expressed by *in situ* hybridization analysis



Determining Gene Function

- One way to determine function is to disable the gene and observe the consequences – **loss of function (vs. gain of function)**
- 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

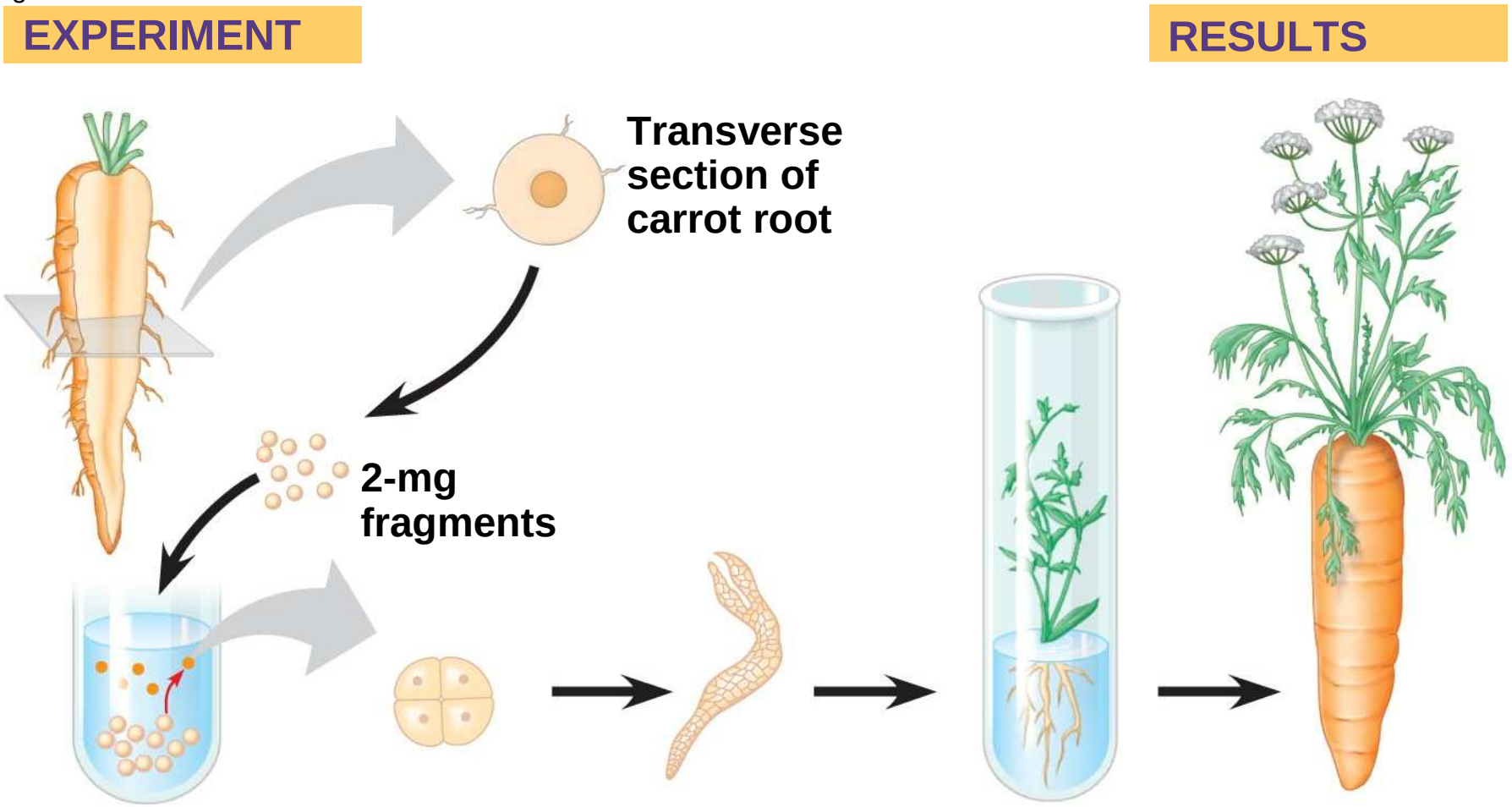
Concept 20.3: Cloning organisms may lead to production of stem cells for research and other applications

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

Cloning Plants: Single-Cell Cultures

- One experimental approach for testing genomic equivalence is to see whether a differentiated cell can generate a whole organism
- A **totipotent** cell（全能細胞）is one that can generate a complete new organism

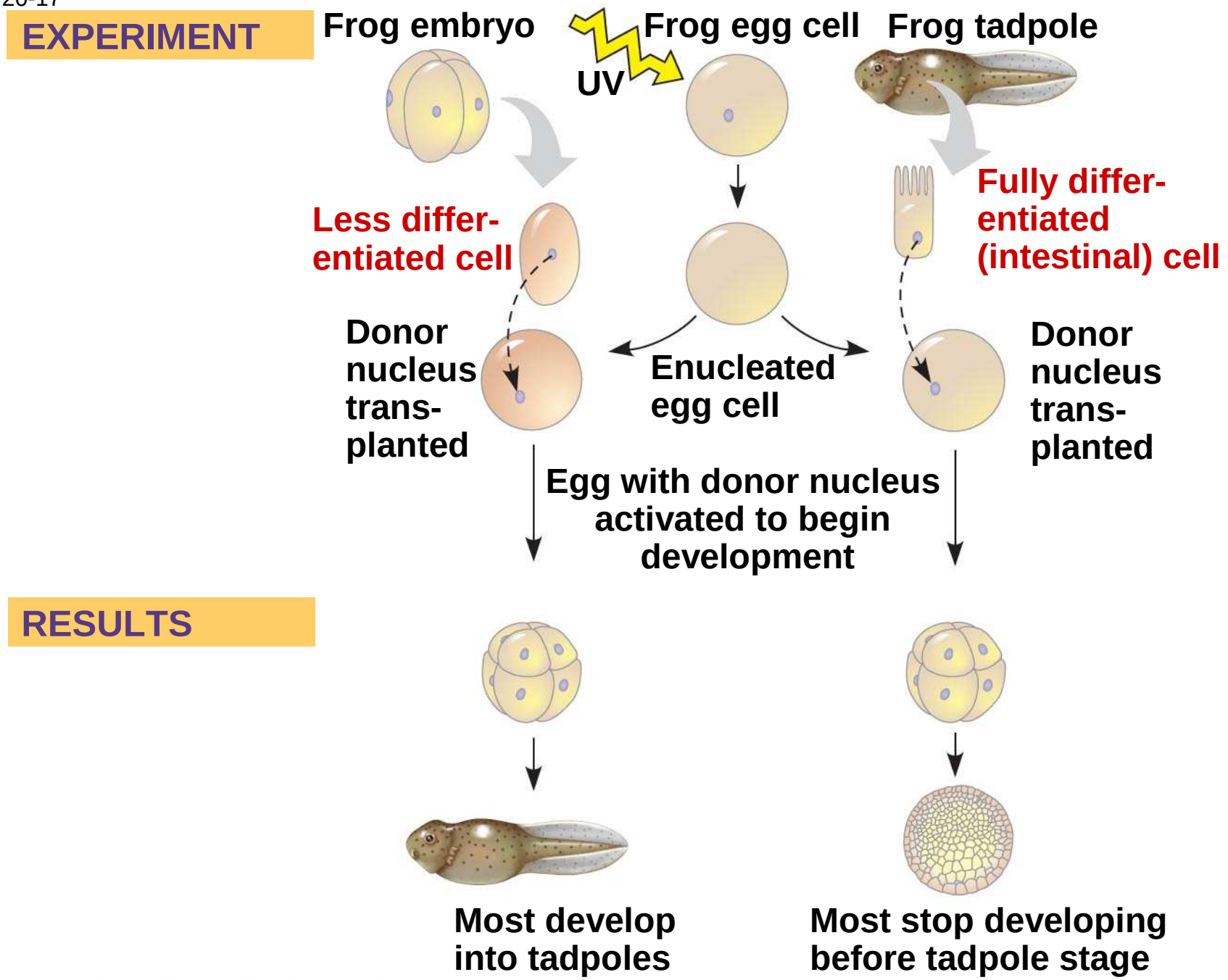
Fig. 20-16



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

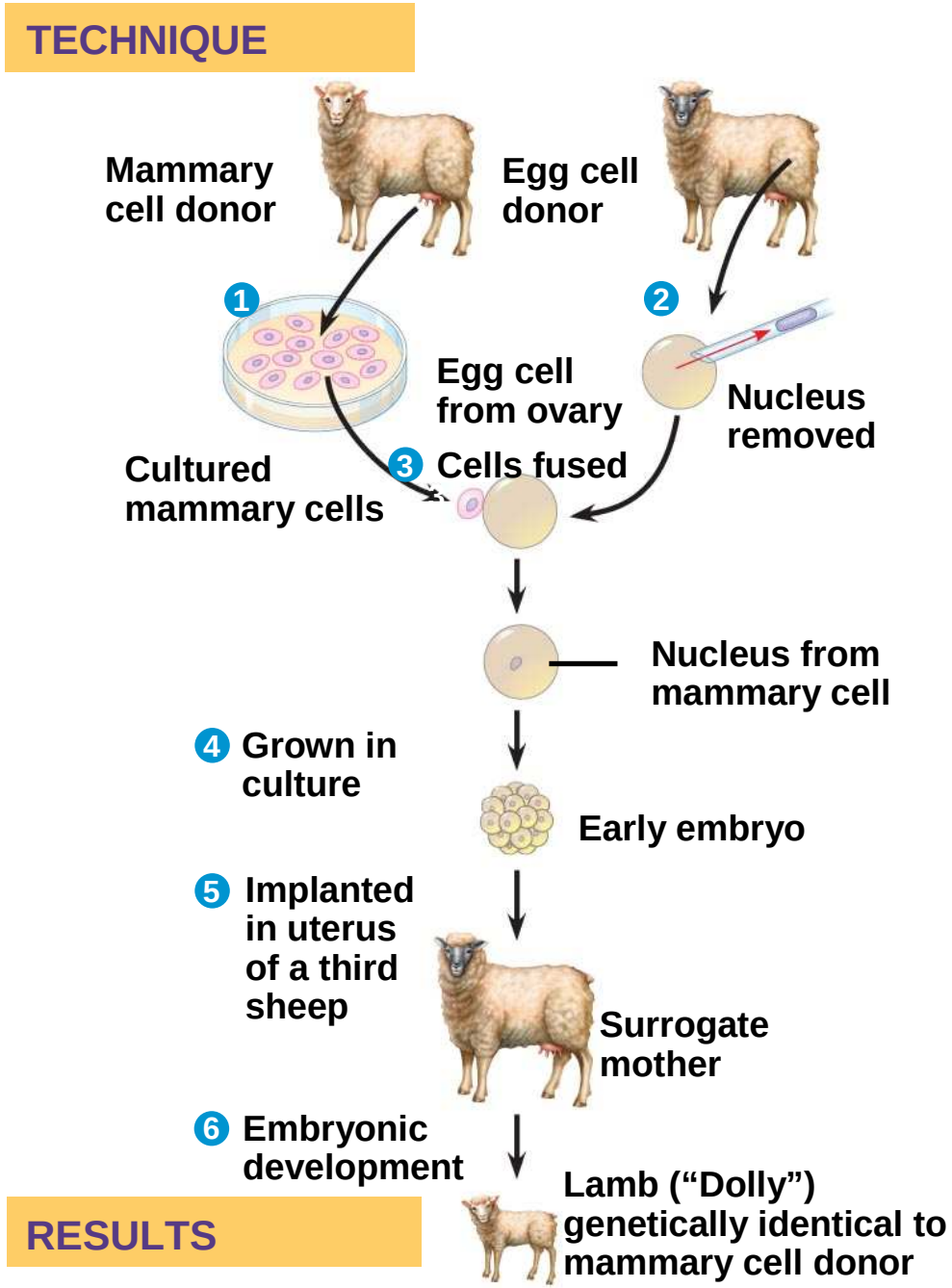
Fig. 20-17



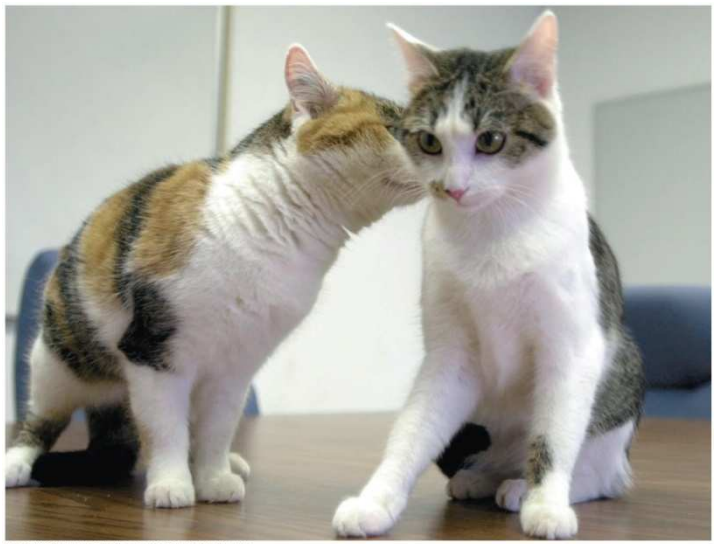
Reproductive Cloning of Mammals

- 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

Fig. 20-18



- 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”



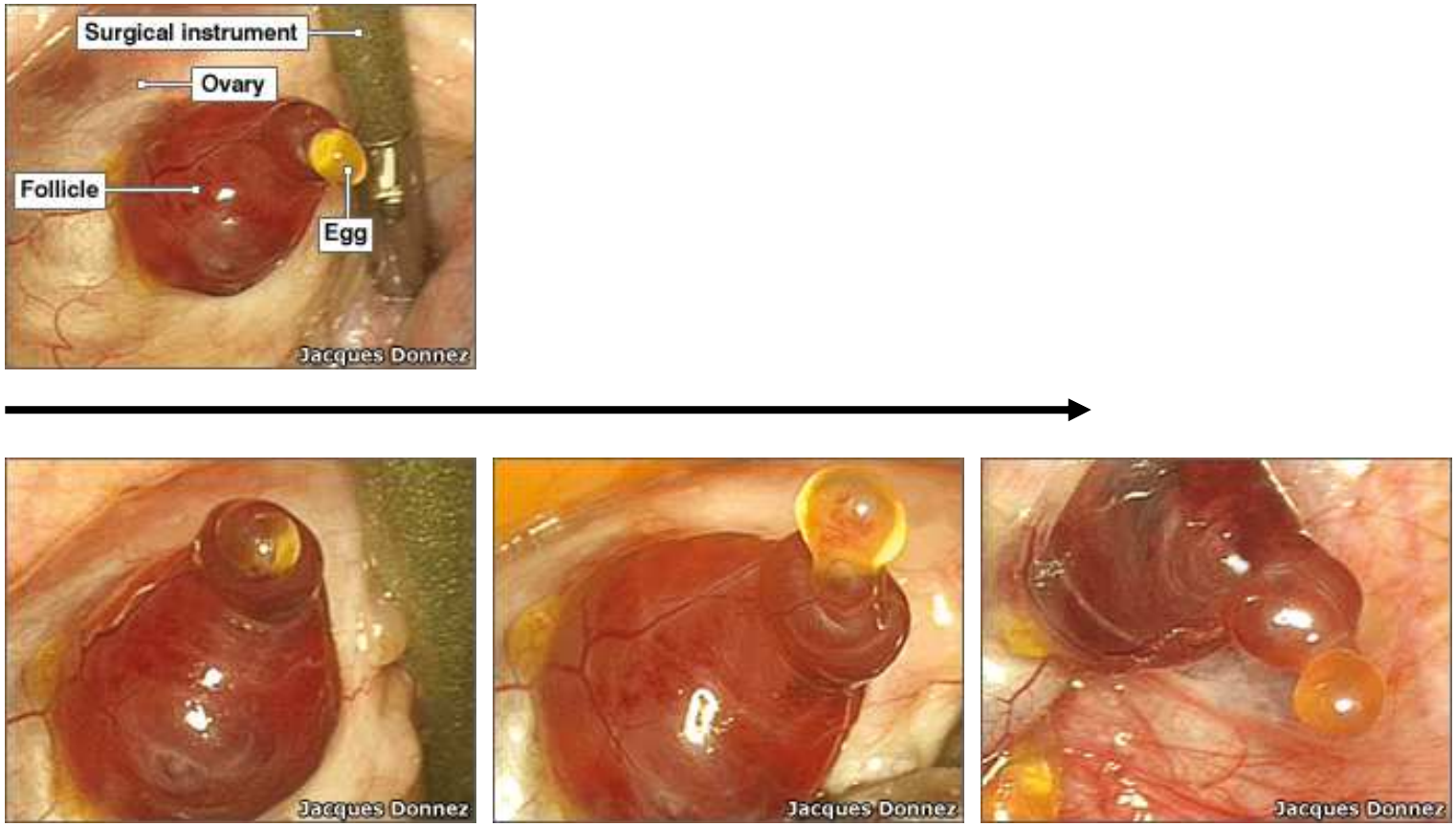
Problems Associated with Animal Cloning

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

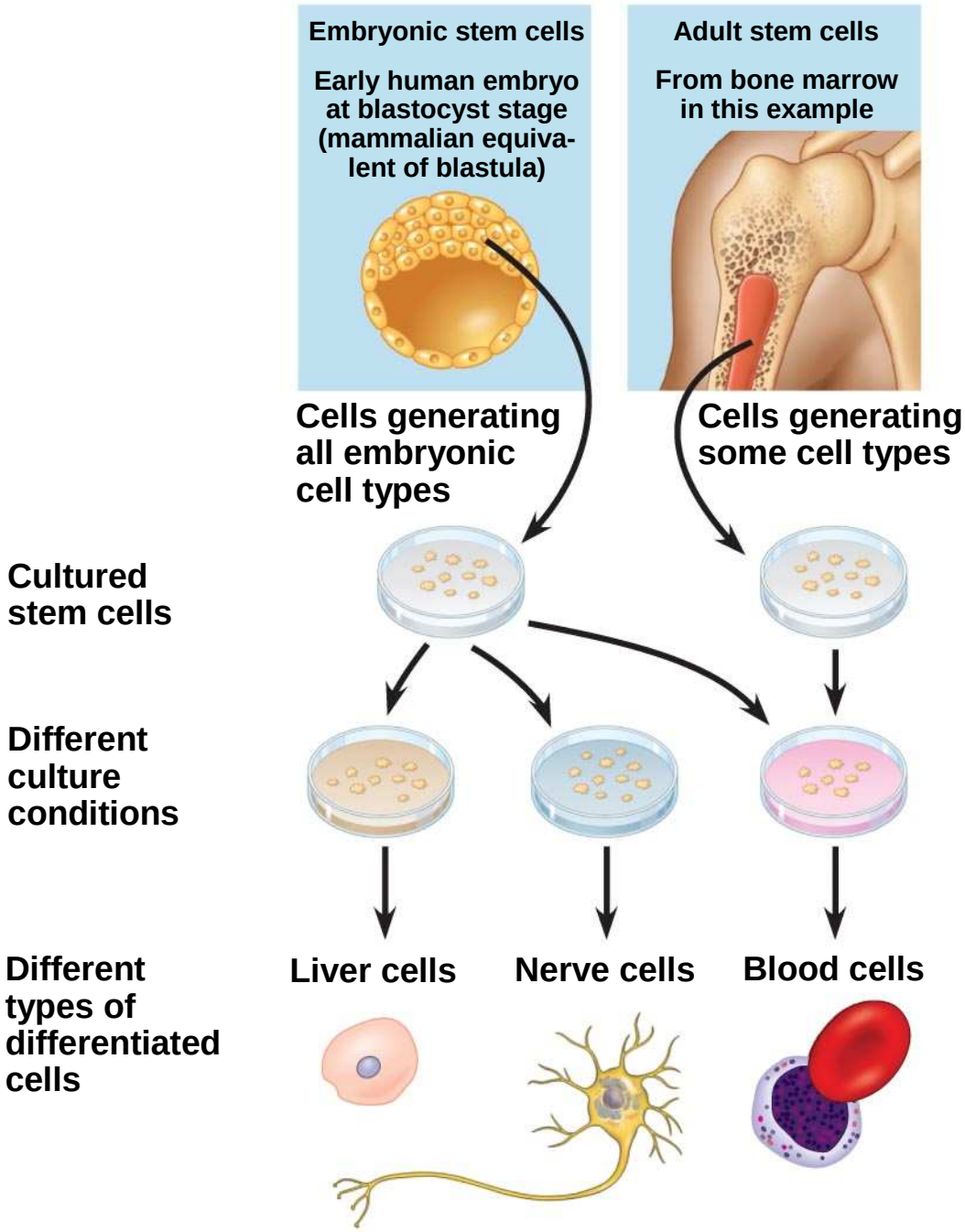
- A **stem cell** is a relatively unspecialized cell that can **reproduce itself indefinitely** and **differentiate into specialized cells of one or more types**
- Stem cells isolated from early embryos at the blastocyst stage are called *embryonic stem cells*; these are able to differentiate into all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

Human Ovulation



During a hysterectomy of a 45-year-old Belgian woman
Dr. Jacques Donnez, Catholic University of Louvain, Belgium

Fig. 20-20



- The aim of stem cell research is to supply cells for the **repair of damaged or diseased organs**

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

- Many fields benefit from DNA technology and genetic engineering

Medical Applications

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

Diagnosis of Diseases

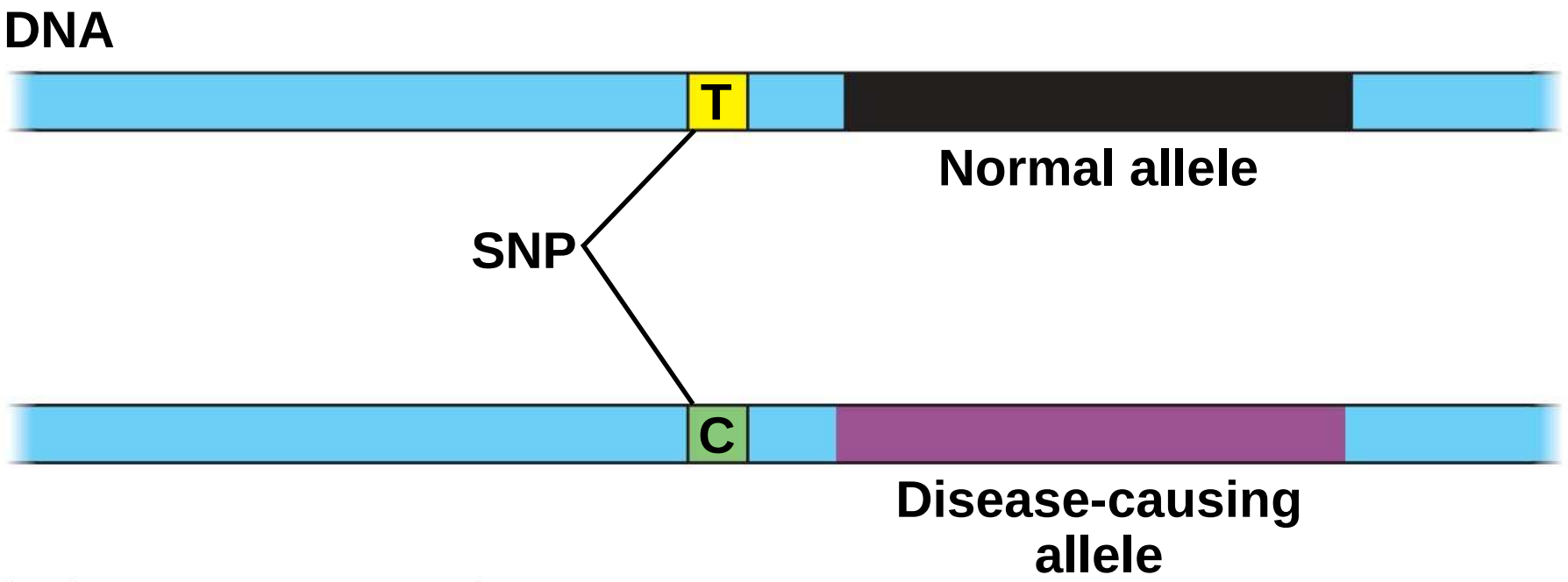
- Scientists can diagnose many human genetic disorders by using **PCR and primers corresponding to cloned disease genes**, then sequencing the amplified product to look for the disease-causing mutation
- Genetic disorders can also be tested for using **genetic markers** that are linked to the disease-causing allele

SNP 單核苷酸變異(多型性)

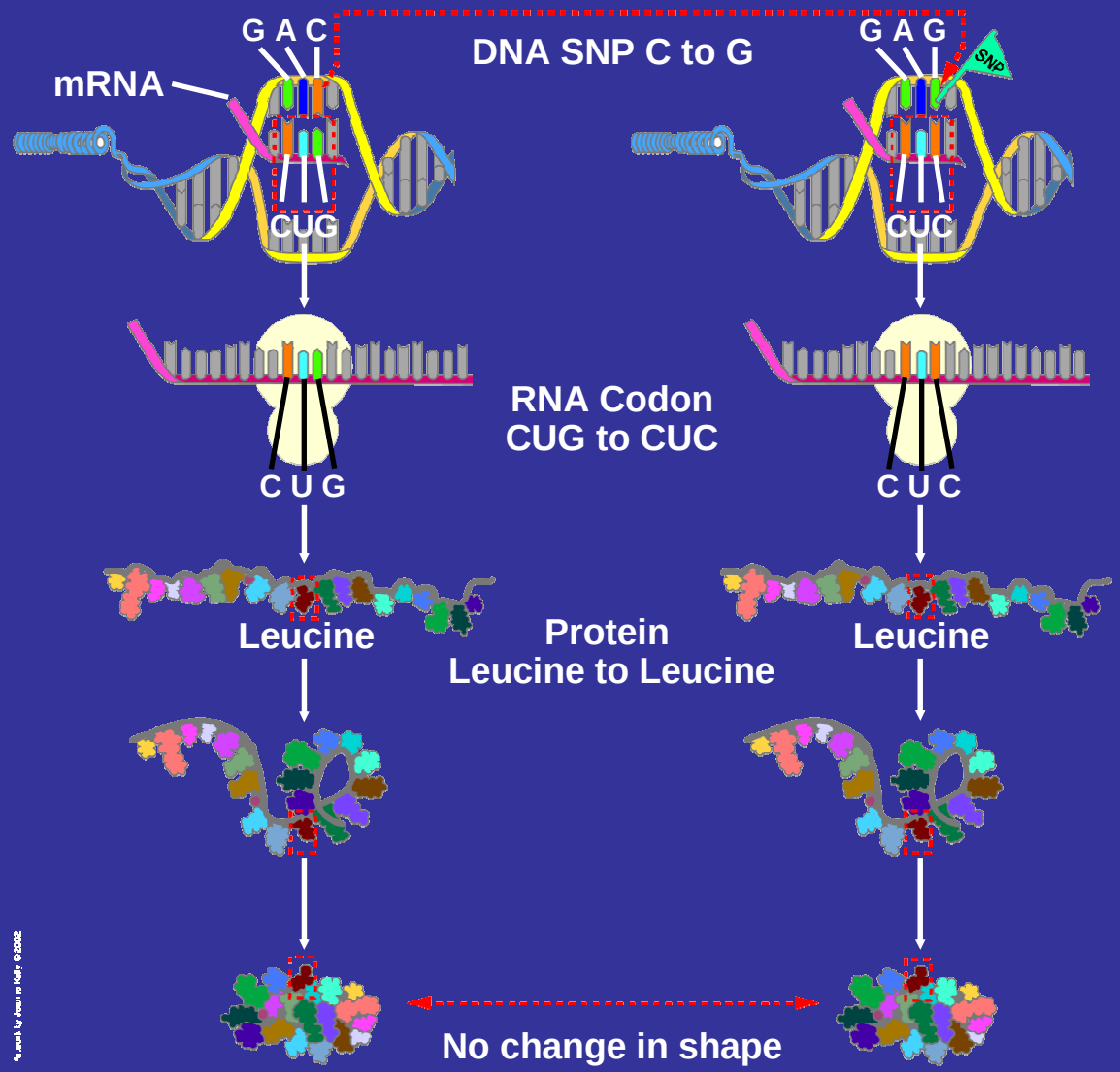
- **Single nucleotide polymorphisms (SNPs)** are useful genetic markers
- These are single base-pair sites that vary in a population
- When a restriction enzyme is added, SNPs result in DNA fragments with different lengths, or **restriction fragment length polymorphism (RFLP)**

Fig. 20-21

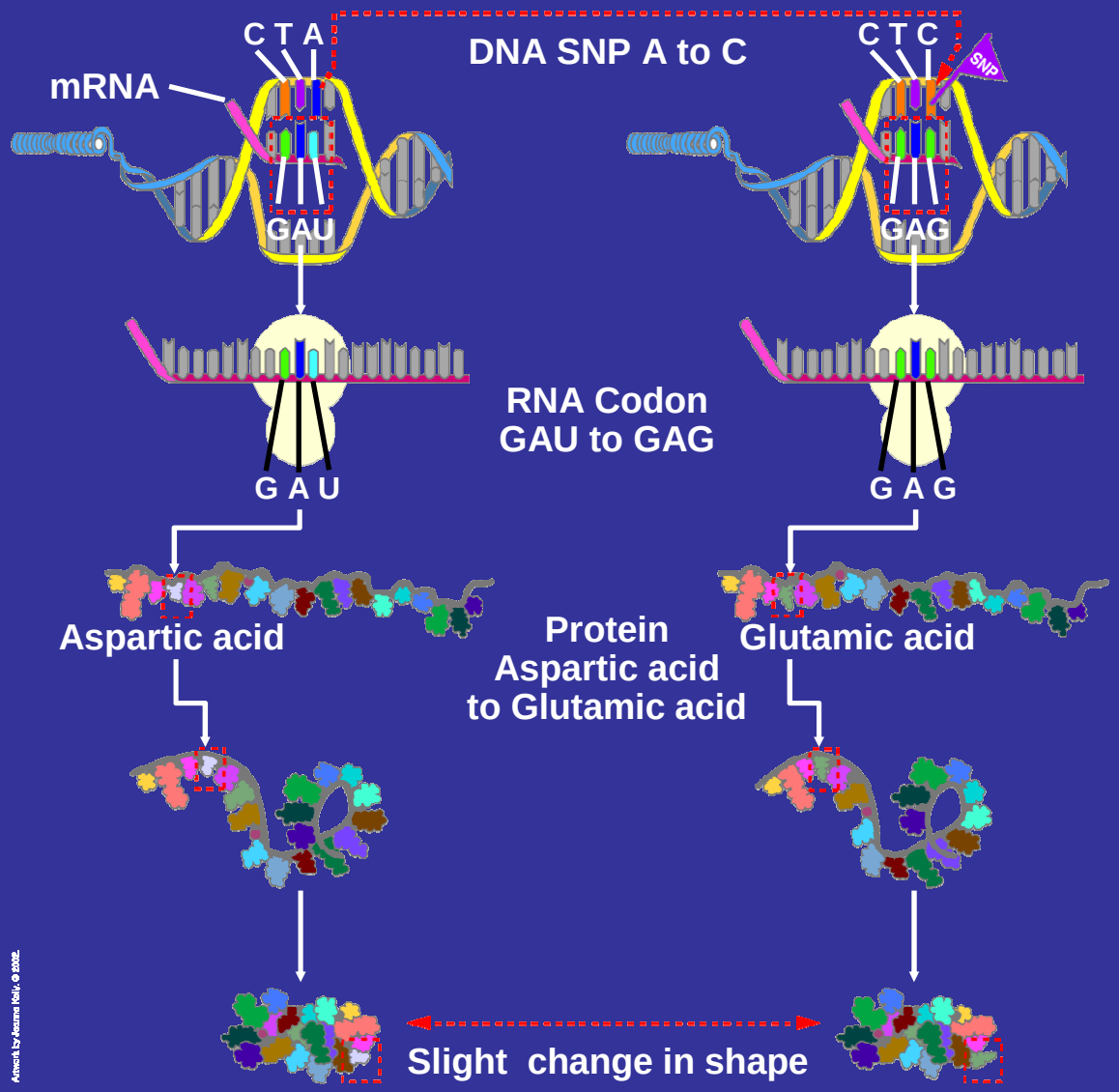
Single nucleotide polymorphisms (SNPs) as genetic markers for disease-causing alleles



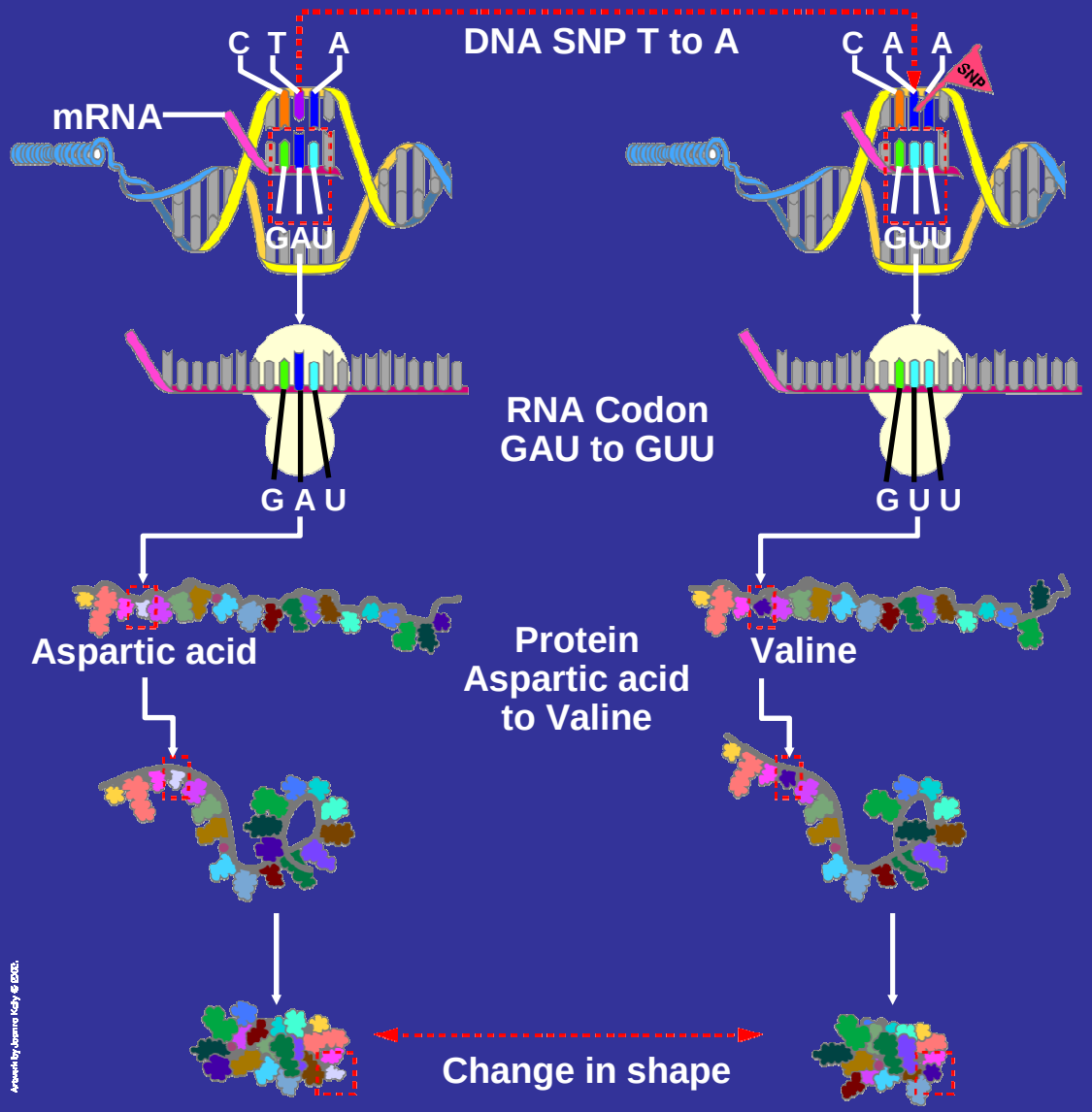
SNPs in Coding Regions –
No Changes in Protein



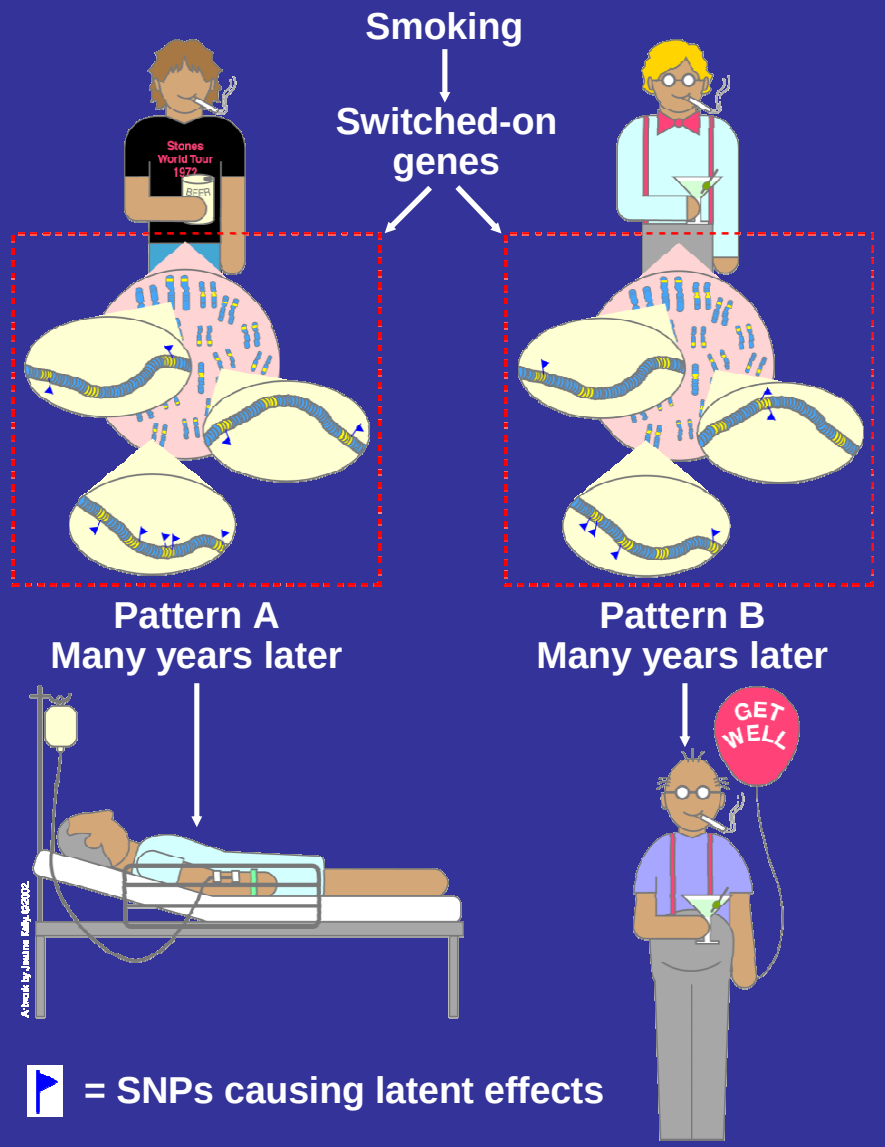
SNPs in Coding Regions – Subtle, Harmless Changes in Protein



SNPs in Coding Regions – Harmful Changes in Protein – Mutations



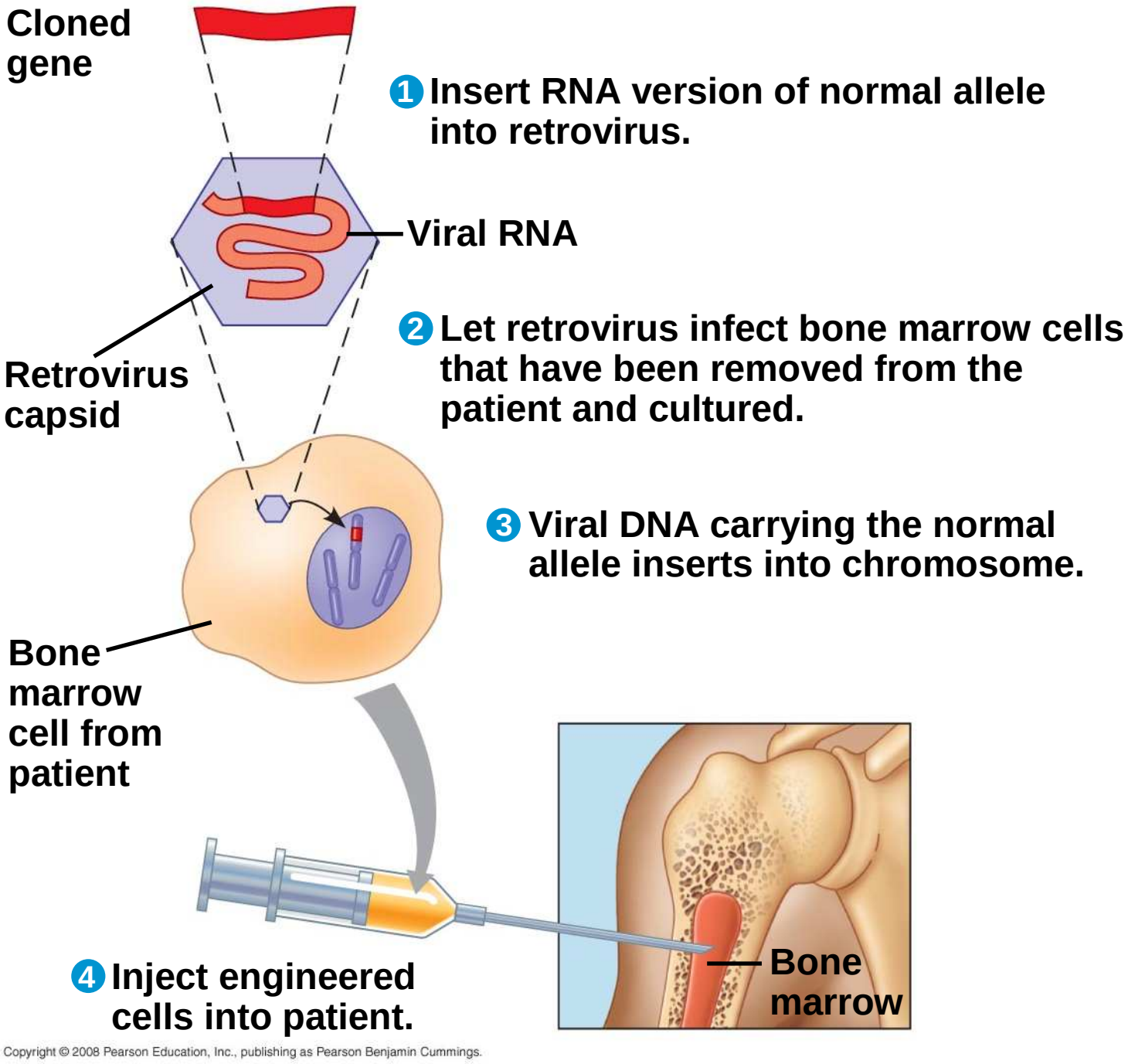
SNPs in Coding Regions –
Subtle Changes in Proteins
That Only Switch on Under Certain Conditions



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 raises ethical questions, such as whether human germ-line cells should be treated to correct the defect in future generations

Fig. 20-22

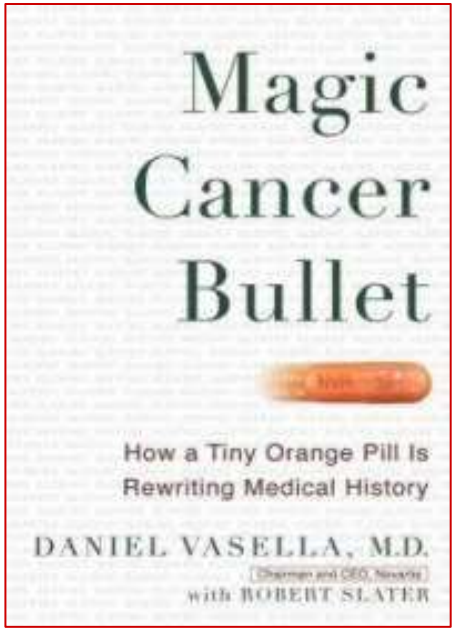


Pharmaceutical Products

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

Synthesis of Small Molecules for Use as Drugs

- The drug **imatinib** (Gleevec) is a small molecule that inhibits over-expression of a specific leukemia-causing receptor



建議閱讀
Magic Cancer Bullet: How a tiny orange pill is rewriting medical history

Protein Production in Cell Cultures

- Pharmaceutical products that are proteins can be synthesized on a large scale
- Host cells in culture can be engineered to secrete a protein as it is made
- This is useful for the production of **insulin**, **human growth hormones**, and **vaccines**

“Pharm” Animals and Plants

Protein Production by “Pharm” Animals and Plants

- **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
- “Pharm” plants are also being developed to make human proteins for medical use

Regulation of the Blood Clotting Cascade By Antithrombin

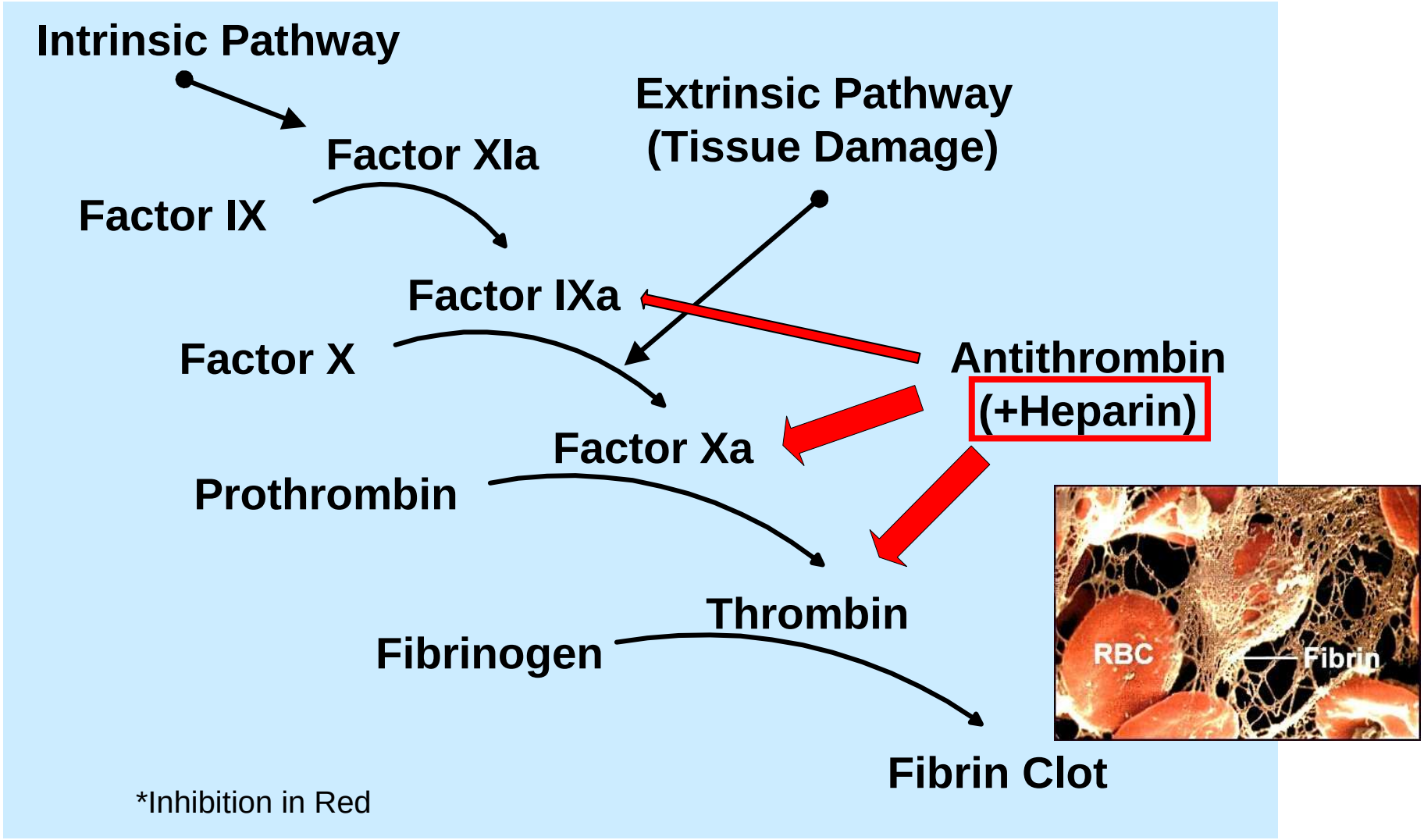


Fig. 20-23

Goats as “pharm” animals – milking “antithrombin”

Transgenic Goat

Forensic Evidence and Genetic Profiles

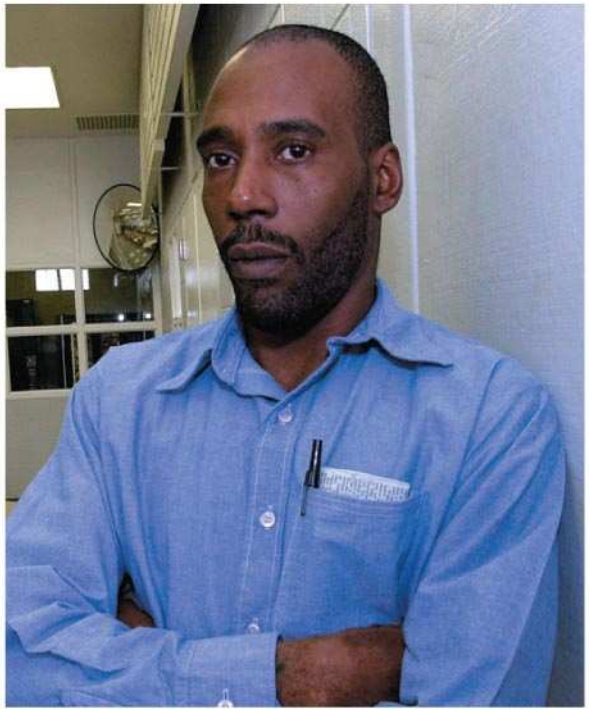
- An individual's unique DNA sequence, or **genetic profile**, can be obtained by analysis of tissue or body fluids
- Genetic profiles can be used to provide evidence in criminal and paternity cases and to identify human remains
- Genetic profiles can be analyzed using RFLP analysis by Southern blotting

Short tandem repeats (STRs)

- 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

Fig. 20-24

(a) This photo shows 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 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**
- **Biofuels** make use of crops such as corn, soybeans, and cassava to replace fossil fuels

Agricultural Applications

- DNA technology is being used to improve agricultural productivity and food quality

Animal Husbandry

- Genetic engineering of transgenic animals speeds up the selective breeding process
- **Beneficial genes** can be transferred between varieties or species

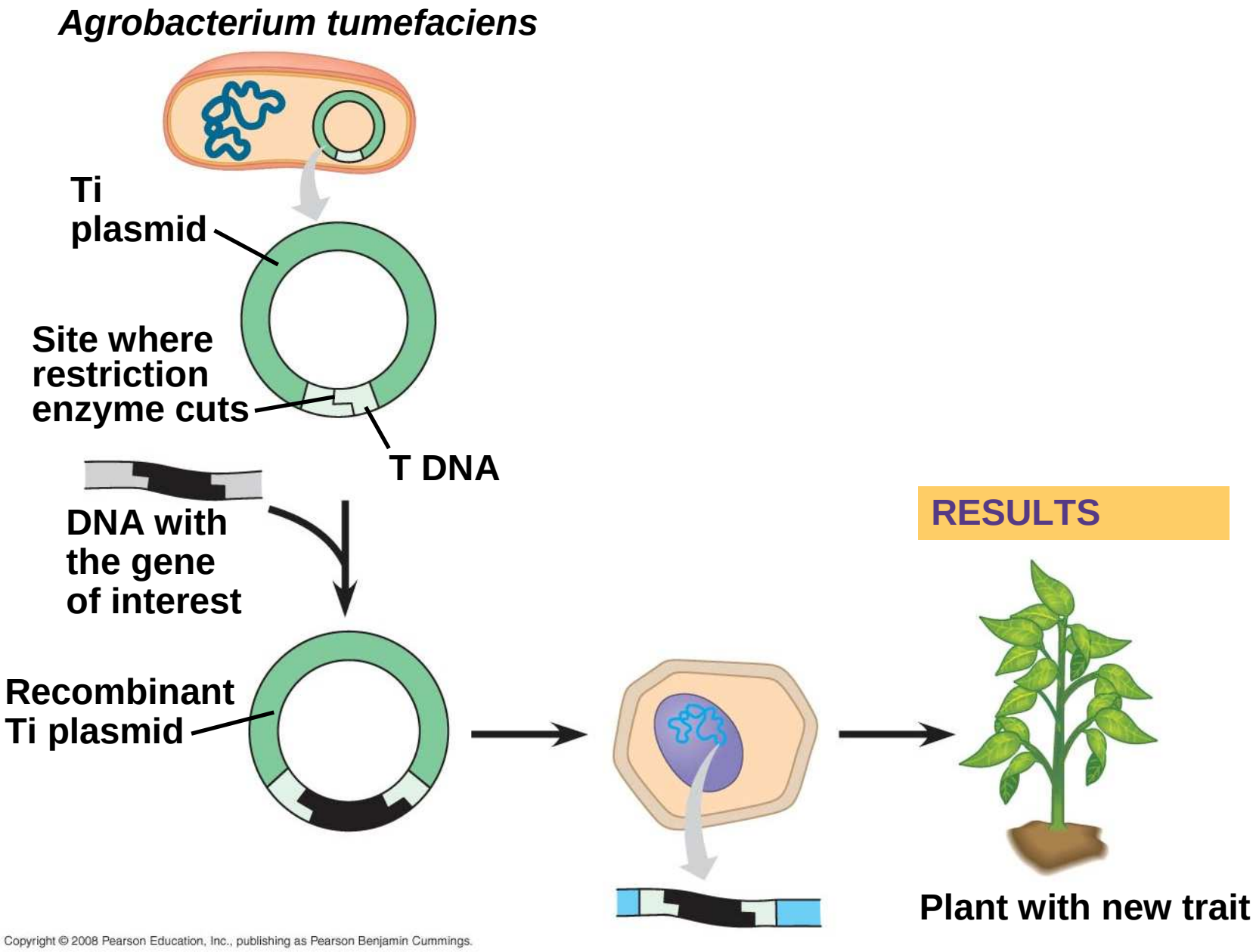
Genetic Engineering in Plants

- 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

Fig. 20-25

TECHNIQUE

Using the Ti plasmid to produce transgenic plants



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

GMO

- 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

Ethical guidelines

- 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

Solar-powered sea slug harnesses stolen plant genes

Horizontal gene transfer of the algal nuclear gene *psbO* to the photosynthetic sea slug *Elysia chlorotica*

思考題：Human Photosynthesis?
“光合人”是否可能？

PNAS (2008) Vol 18 No 46 P17867-17871

You should now be able to:

1. Describe the natural function of restriction enzymes and explain how they are used in recombinant DNA technology
2. Outline the procedures for cloning a eukaryotic gene in a bacterial plasmid
3. Define and distinguish between genomic libraries using plasmids, phages, and cDNA
4. Describe the polymerase chain reaction (PCR) and explain the advantages and limitations of this procedure

5. Explain how gel electrophoresis is used to analyze nucleic acids and to distinguish between two alleles of a gene

6. Describe and distinguish between the Southern blotting procedure, Northern blotting procedure, and RT-PCR

7. Distinguish between gene cloning, cell cloning, and organismal cloning

8. Describe how nuclear transplantation was used to produce Dolly, the first cloned sheep

9. Describe the application of DNA technology to the diagnosis of genetic disease, the development of gene therapy, vaccine production, and the development of pharmaceutical products

10. Define a SNP and explain how it may produce a RFLP

11. Explain how DNA technology is used in the forensic sciences

12. Discuss the safety and ethical questions related to recombinant DNA studies and the biotechnology industry