CHAPTER 20
DNA TECHNOLOGY AND GENOMICS

Section A: DNA Cloning

1. DNA technology makes it possible to clone genes for basic research and commercial applications: *an overview*
2. Restriction enzymes are used to make recombinant DNA
3. Genes can be clones in recombinant DNA vectors: *a closer look*
4. Cloned genes are stored in DNA libraries
5. The polymerase chain reaction (PCR) closed DNA directly *in vitro*

Introduction

- The mapping and sequencing of the human genome has been made possible by advances in DNA technology.
- Progress began with the development of techniques for making **recombinant DNA**, in which genes from two different sources - often different species - are combined *in vitro* into the same molecule.
- These methods form part of **genetic engineering**, the direct manipulation of genes for practical purposes.
  - Applications include the introduction of a desired gene into the DNA of a host that will produce the desired protein.

- DNA technology has launched a revolution in **biotechnology**, the manipulation of organisms or their components to make useful products.
  - Practices that go back centuries, such as the use of microbes to make wine and cheese and the selective breeding of livestock, are examples of biotechnology.
  - Biotechnology based on the manipulation of DNA *in vitro* differs from earlier practices by enabling scientists to modify specific genes and move them between organisms as distinct as bacteria, plants, and animals.
- DNA technology is now applied in areas ranging from agriculture to criminal law, but its most important achievements are in basic research.

- To study a particular gene, scientists needed to develop methods to isolate only the small, well-defined, portion of a chromosome containing the gene.
- Techniques for **gene cloning** enable scientists to prepare multiple identical copies of gene-sized pieces of DNA.

1. DNA technology makes it possible to clone genes for basic research and commercial applications: *an overview*

- Most methods for cloning pieces of DNA share certain general features.
  - For example, a foreign gene is inserted into a bacterial plasmid and this recombinant DNA molecule is returned to a bacterial cell.
  - Every time this cell reproduces, the recombinant plasmid is replicated as well and passed on to its descendents.
  - Under suitable conditions, the bacterial clone will make the protein encoded by the foreign gene.

- One basic cloning technique begins with the insertion of a foreign gene into a bacterial plasmid.

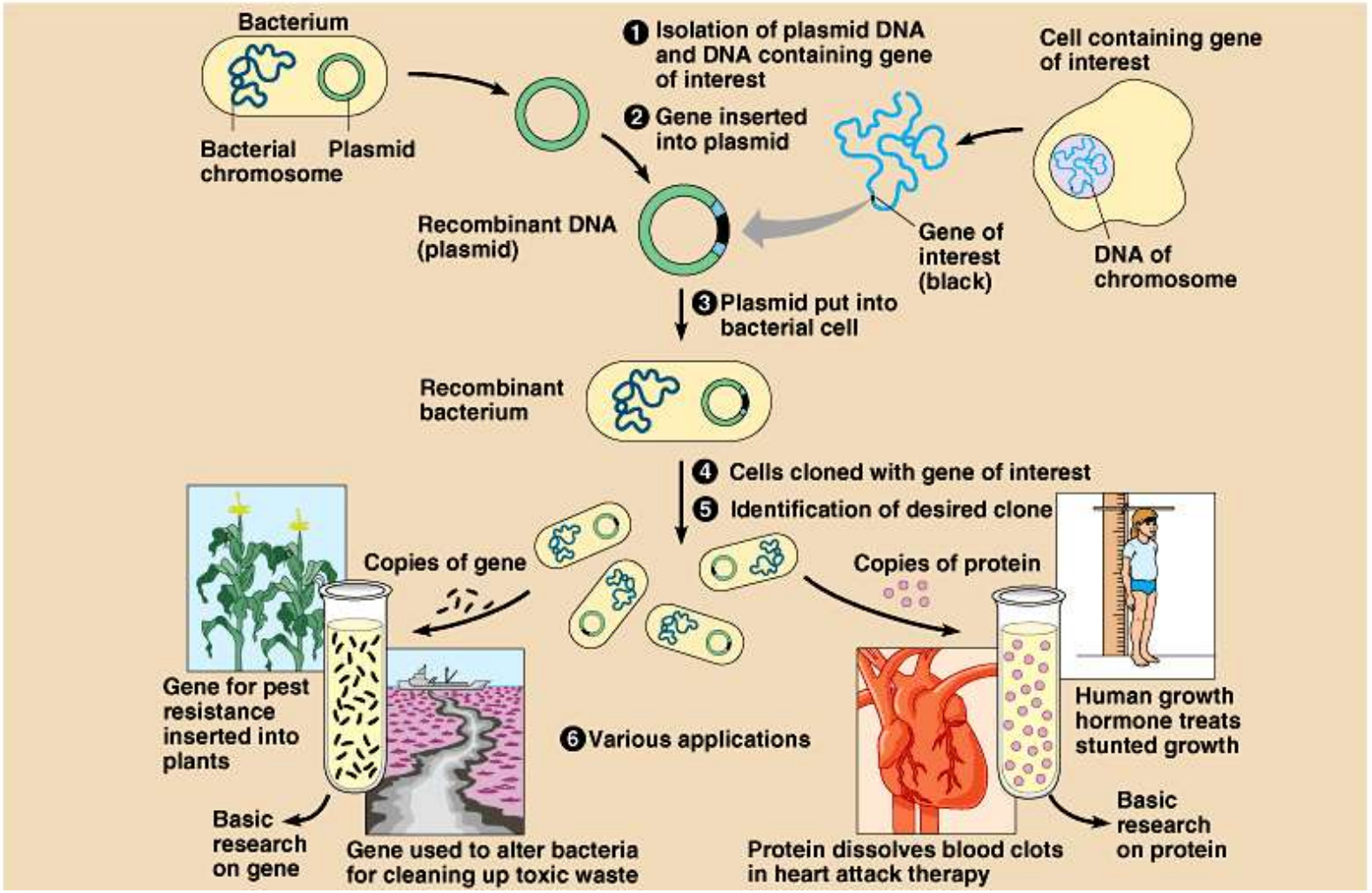


Fig. 20.1

- The potential uses of cloned genes fall into two general categories.
- First, the goal may be to produce a protein product.
  - For example, bacteria carrying the gene for human growth hormone can produce large quantities of the hormone for treating stunted growth.
- Alternatively, the goal may be to prepare many copies of the gene itself.
  - This may enable scientists to determine the gene's nucleotide sequence or provide an organism with a new metabolic capability by transferring a gene from another organism.

2. Restriction enzymes are used to make recombinant DNA

- Gene cloning and genetic engineering were made possible by the discovery of **restriction enzymes** that cut DNA molecules at specific locations.
- In nature, bacteria use restriction enzymes to cut foreign DNA, such as from phages or other bacteria.
- Most restrictions enzymes are very specific, recognizing short DNA nucleotide sequences and cutting at specific point in these sequences.
  - Bacteria protect their own DNA by methylation.

- Each restriction enzyme cleaves a specific sequences of bases or **restriction site**.
  - These are often a symmetrical series of four to eight bases on both strands running in opposite directions.
    - If the restriction site on one strand is 3'-CTTAGG-5', the complementary strand is 5'-GAATCC-3'.
- Because the target sequence usually occurs (by chance) many times on a long DNA molecule, an enzyme will make many cuts.
  - Copies of a DNA molecule will always yield the same set of restriction fragments when exposed to a specific enzyme.

- Restriction enzymes cut covalent phosphodiester bonds of both strands, often in a staggered way creating single-stranded ends, **sticky ends**.
  - These extensions will form hydrogen-bonded base pairs with complementary single-stranded stretches on other DNA molecules cut with the same restriction enzyme.
- These DNA fusions can be made permanent by **DNA ligase** which seals the strand by catalyzing the formation of phosphodiester bonds.

- Restriction enzymes and DNA ligase can be used to make recombinant DNA, DNA that has been spliced together from two different sources.

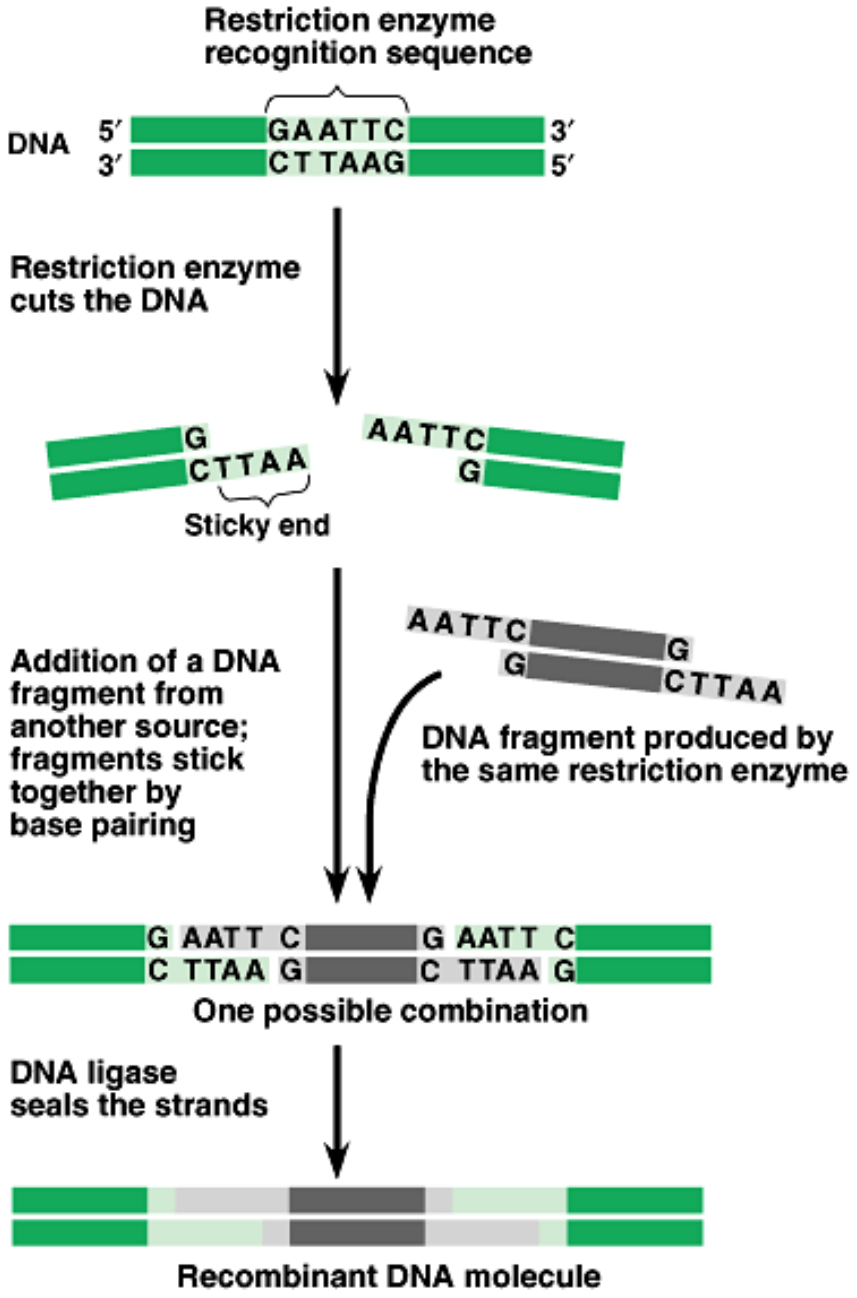


Fig. 20.2

3. Genes can be cloned in DNA vectors: *a closer look*

- Recombinant plasmids are produced by splicing restriction fragments from foreign DNA into plasmids.
  - These can be returned relatively easily to bacteria.
  - The original plasmid used to produce recombinant DNA is called a **cloning vector,** which is a DNA molecule that can carry foreign DNA into a cell and replicate there.
- Then, as a bacterium carrying a recombinant plasmid reproduces, the plasmid replicates within it.

- Bacteria are most commonly used as host cells for gene cloning because DNA can be easily isolated and reintroduced into their cells.
- Bacteria cultures also grow quickly, rapidly replicating the foreign genes.

- The process of cloning a human gene in a bacterial plasmid can be divided into five steps.

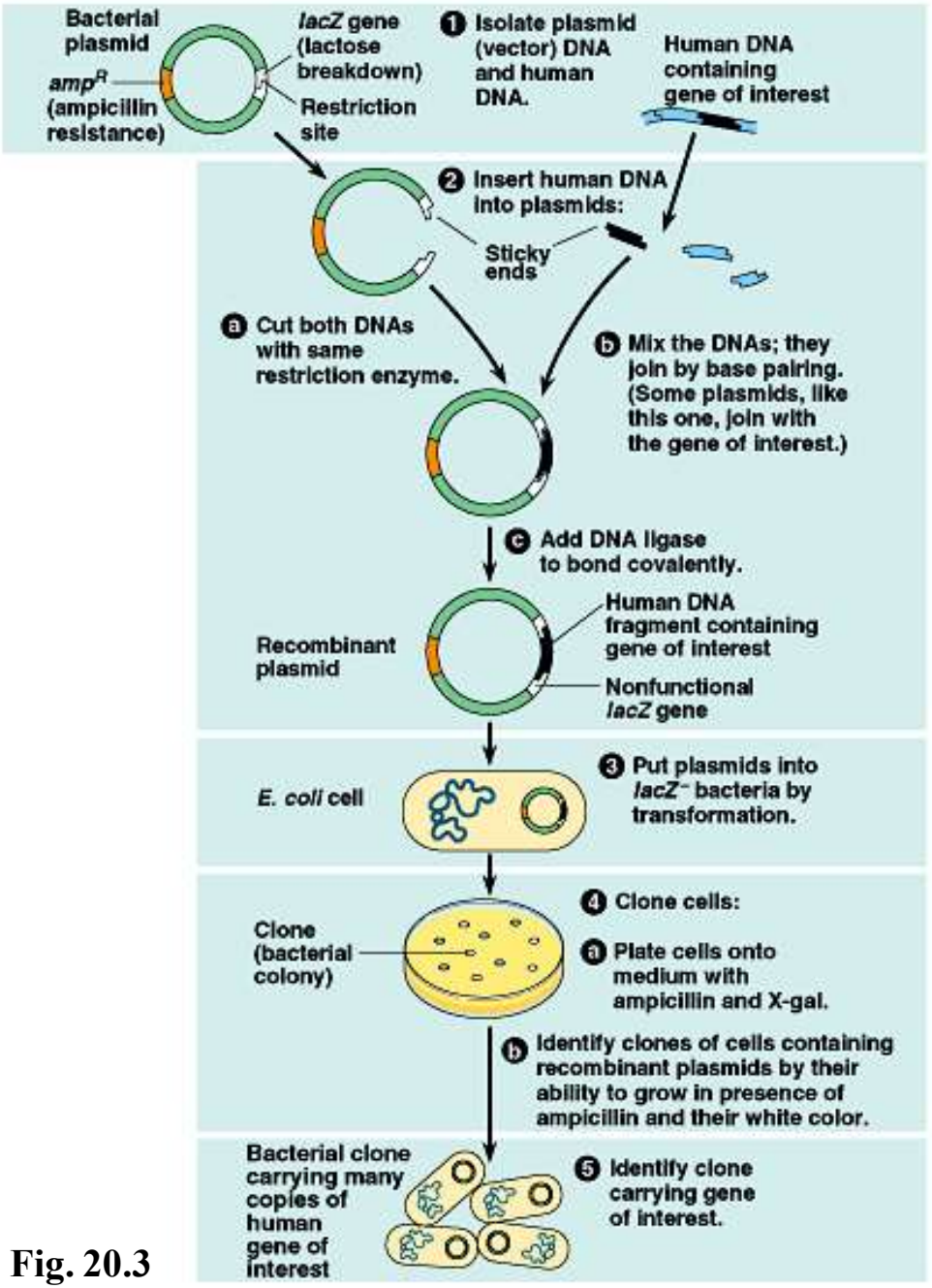


Fig. 20.3

1. Isolation of vector and gene-source DNA.

- The source DNA comes from human tissue cells.
- The source of the plasmid is typically *E. coli*.
  - This plasmid carries two useful genes, amp^R, conferring resistance to the antibiotic ampicillin and *lacZ*, encoding the enzyme beta-galactosidase which catalyzes the hydrolysis of sugar.
  - The plasmid has a single recognition sequence, within the *lacZ* gene, for the restriction enzyme used.

2. Insertion of DNA into the vector.

- By digesting both the plasmid and human DNA with the same restriction enzyme we can create thousands of human DNA fragments, one fragment with the gene that we want, and with compatible sticky ends on bacterial plasmids.
- After mixing, the human fragments and cut plasmids form complementary pairs that are then joined by DNA ligase.
- This creates a mixture of recombinant DNA molecules.

3. Introduction of the cloning vector into cells.

- Bacterial cells take up the recombinant plasmids by transformation.
  - These bacteria are *lacZ*$^-$, unable to hydrolyze lactose.
- This creates a diverse pool of bacteria, some bacteria that have taken up the desired recombinant plasmid DNA, other bacteria that have taken up other DNA, both recombinant and nonrecombinant.

4. Cloning of cells (and foreign genes).

- We can plate out the transformed bacteria on solid nutrient medium containing ampicillin and a sugar called X-gal.
  - Only bacteria that have the ampicillin-resistance plasmid will grow.
  - The X-gal in the medium is used to identify plasmids that carry foreign DNA.
    - Bacteria with plasmids lacking foreign DNA stain blue when beta-galactosidase hydrolyzes X-gal.
    - Bacteria with plasmids containing foreign DNA are white because they lack beta-galactosidase.

5. Identifying cell clones with the right gene.

- In the final step, we will sort through the thousands of bacterial colonies with foreign DNA to find those containing our gene of interest.
- One technique, **nucleic acid hybridization**, depends on base pairing between our gene and a complementary sequence, a **nucleic acid probe**, on another nucleic acid molecule.
  - The sequence of our RNA or DNA probe depends on knowledge of at least part of the sequence of our gene.
  - A radioactive or fluorescent tag labels the probe.

- The probe will hydrogen-bond specifically to complementary single strands of the desired gene.
  - After **denaturation** (separating) the DNA strands in the plasmid, the probe will bind with its complementary sequence, tagging colonies with the targeted gene.

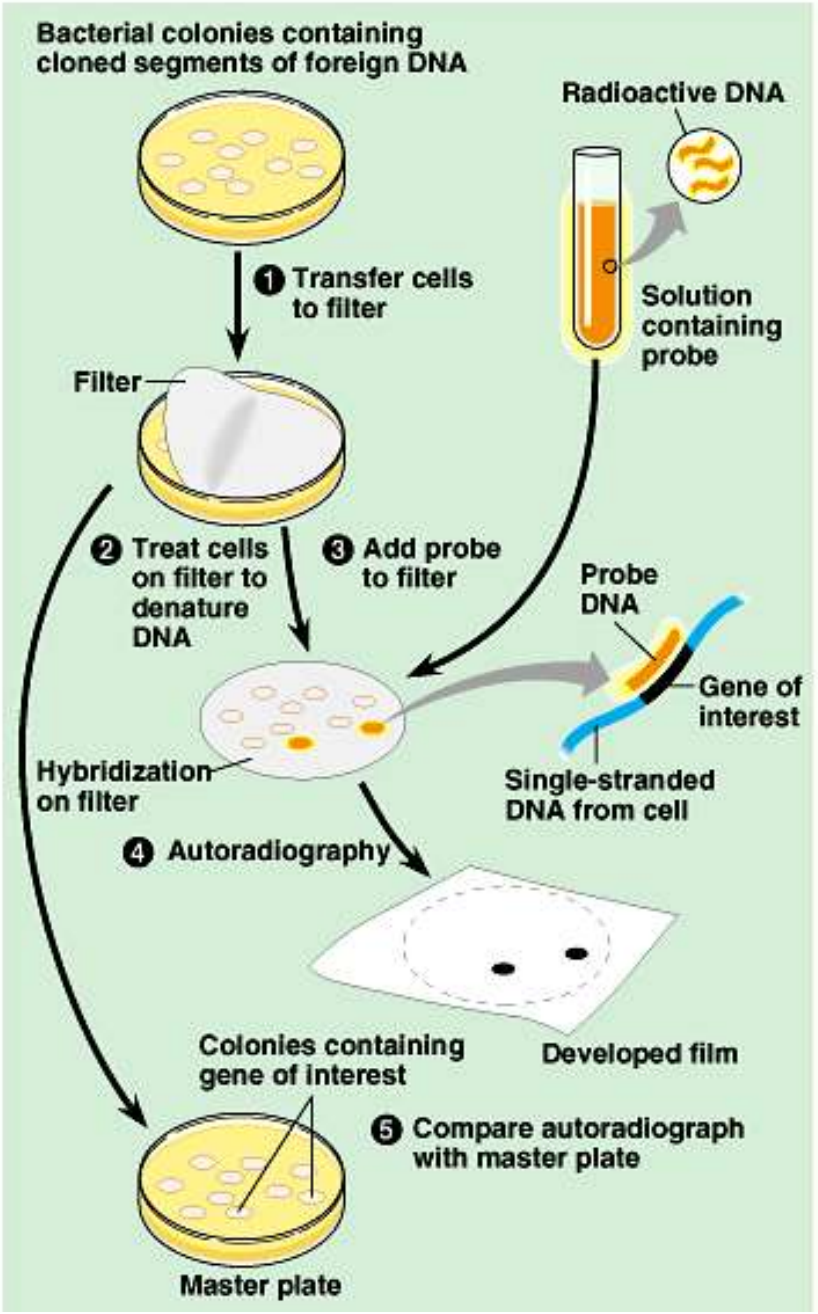


Fig. 20.4

- Because of different details between prokaryotes and eukaryotes, inducing a cloned eukaryotic gene to function in a prokaryotic host can be difficult.
  - One way around this is to employ an **expression vector**, a cloning vector containing the requisite prokaryotic promotor upstream of the restriction site.
  - The bacterial host will then recognize the promotor and proceed to express the foreign gene that has been linked to it, including many eukaryotic proteins.

- The presence of introns, long non-coding regions, in eukaryotic genes creates problems for expressing these genes in bacteria.
  - To express eukaryotic genes in bacteria, a fully processed mRNA acts as the template for the synthesis of a complementary strand using reverse transcriptase.
  - This **complementary DNA (cDNA)**, with a promoter, can be attached to a vector for replication, transcription, and translation inside bacteria.

- Complementary DNA is DNA made in vitro using mRNA as a template and the enzyme reverse transcriptase.

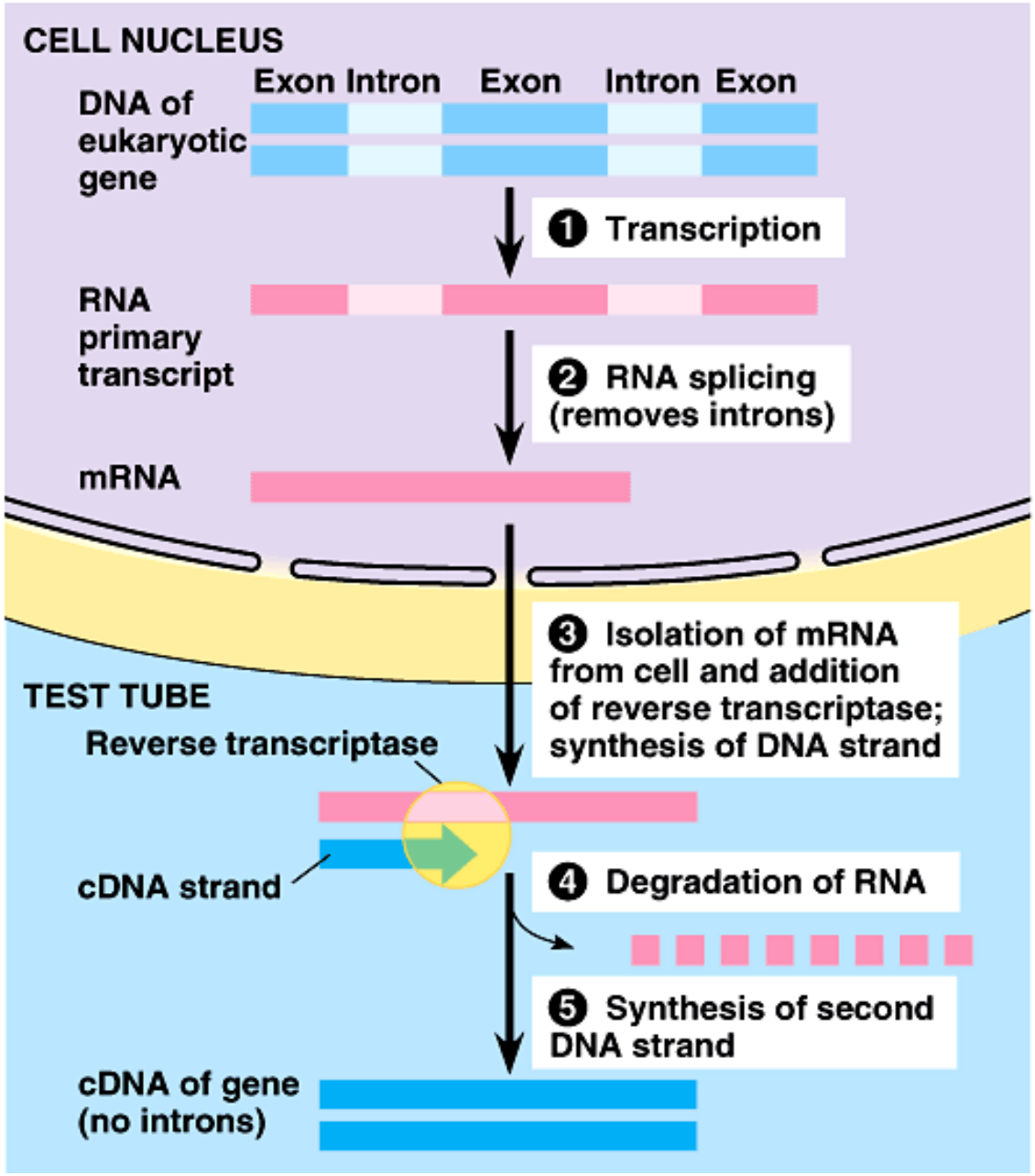


Fig. 20.5

- Molecular biologists can avoid incompatibility problems by using eukaryotic cells as host for cloning and expressing eukaryotic genes.
- Yeast cells, single-celled fungi, are as easy to grow as bacteria and have plasmids, rare for eukaryotes.
- Scientists have constructed **yeast artificial chromosomes** (**YACs**) - an origin site for replication, a centromere, and two telomeres - with foreign DNA.
- These chromosomes behave normally in mitosis and can carry more DNA than a plasmid.

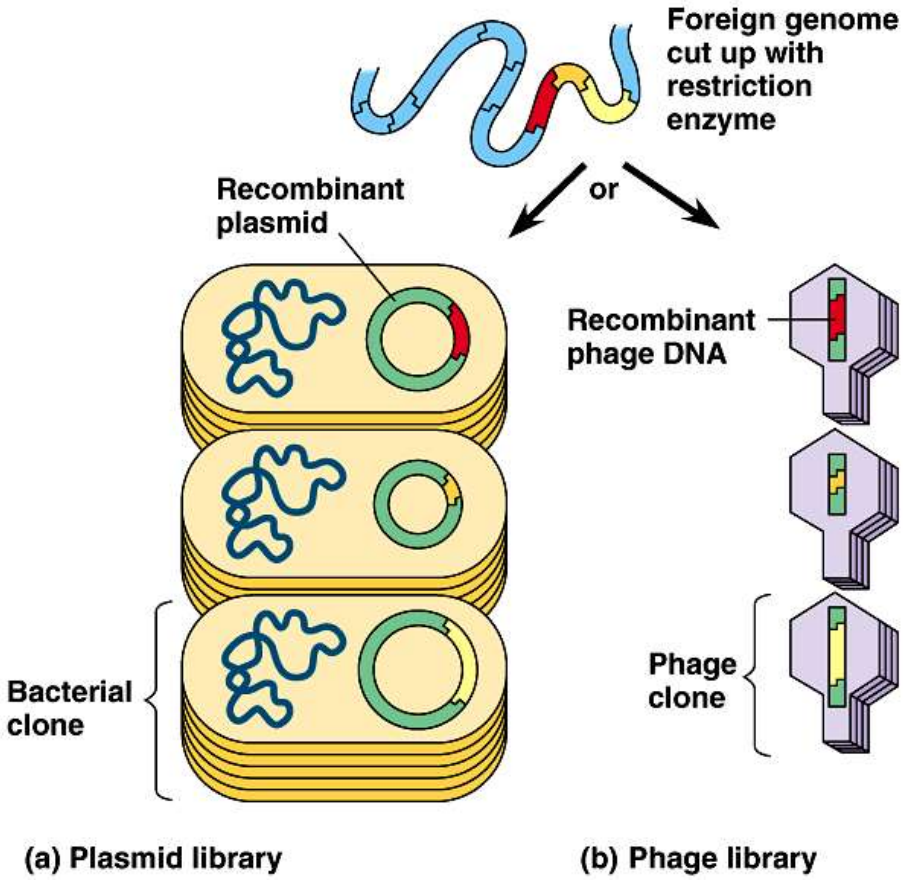
- Another advantage of eukaryotic hosts is that they are capable of providing the posttranslational modifications that many proteins require.
  - This includes adding carbohydrates or lipids.
  - For some mammalian proteins, the host must be an animal or plant cell to perform the necessary modifications.

- Many eukaryotic cells can take up DNA from their surroundings, but often not efficiently.
- Several techniques facilitate entry of foreign DNA.
  - In **electroporation**, brief electrical pulses create a temporary hole in the plasma membrane through which DNA can enter.
  - Alternatively, scientists can inject DNA into individual cells using microscopically thin needles.
  - In a technique used primarily for plants, DNA is attached to microscopic metal particles and fired into cells with a gun.
  - Once inside the cell, the DNA is incorporated into the cell's DNA by natural genetic recombination.

4. Cloned genes are stored in DNA libraries

- In the "shotgun" cloning approach, a mixture of fragments from the entire genome is included in thousands of different recombinant plasmids.
- A complete set of recombinant plasmid clones, each carrying copies of a particular segment from the initial genome, forms a **genomic library**.
  - The library can be saved and used as a source of other genes or for gene mapping.

- In addition to plasmids, certain bacteriophages are also common cloning vectors for making libraries.
  - Fragments of foreign DNA can be spliced into a phage genome using a restriction enzyme and DNA ligase.
  - The recombinant phage DNA is packaged in a capsid *in vitro* and allowed to infect a bacterial cell.
  - Infected bacteria produce new phage particles, each with the foreign DNA.



- A more limited kind of gene library can be developed from complementary DNA.
  - During the process of producing cDNA, all mRNAs are converted to cDNA strands.
  - This **cDNA library** represents that part of a cell's genome that was transcribed in the starting cells.
  - This is an advantage if a researcher wants to study the genes responsible for specialized functions of a particular kind of cell.
  - By making cDNA libraries from cells of the same type at different times in the life of an organism, one can trace changes in the patterns of gene expression.

5. The polymerase chain reaction (PCR) clones DNA entirely *in vitro*

- DNA cloning is the best method for preparing large quantities of a particular gene or other DNA sequence.
- When the source of DNA is scanty or impure, the **polymerase chain reaction** (**PCR**) is quicker and more selective.
- This technique can quickly amplify any piece of DNA without using cells.

- The DNA is incubated in a test tube with special DNA polymerase, a supply of nucleotides, and short pieces of single-stranded DNA as a primer.

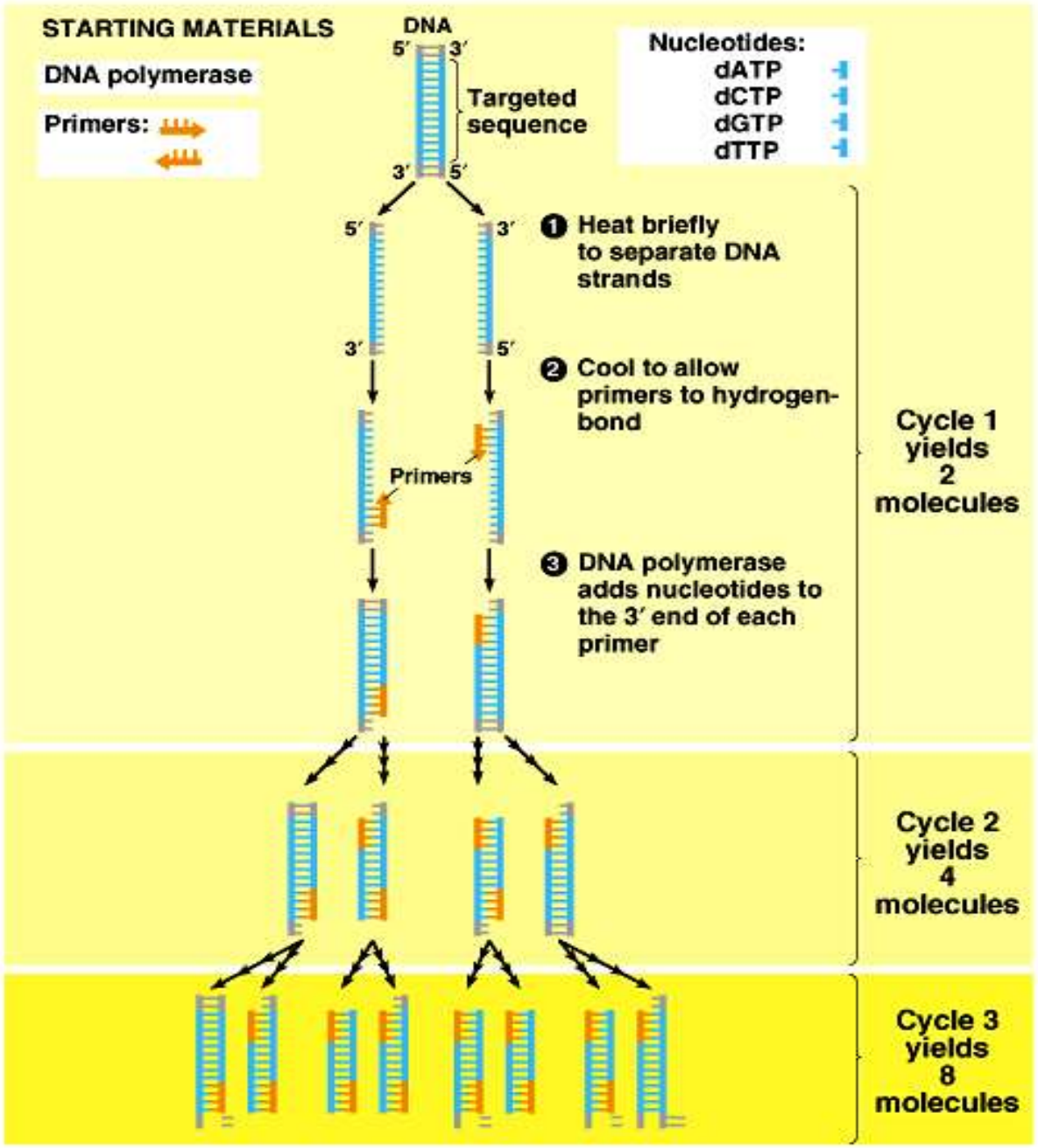


Fig. 20.7

- PCR can make billions of copies of a targeted DNA segment in a few hours.
  - This is faster than cloning via recombinant bacteria.
- In PCR, a three-step cycle: heating, cooling, and replication, brings about a chain reaction that produces an exponentially growing population of DNA molecules.
  - The key to easy PCR automation was the discovery of an unusual DNA polymerase, isolated from bacteria living in hot springs, which can withstand the heat needed to separate the DNA strands at the start of each cycle.

- PCR is very specific.
- By their complementarity to sequences bracketing the targeted sequence, the primers determine the DNA sequence that is amplified.
  - PCR can make many copies of a specific gene before cloning in cells, simplifying the task of finding a clone with that gene.
  - PCR is so specific and powerful that only minute amounts of DNA need be present in the starting material.
- Occasional errors during PCR replication impose limits to the number of good copies that can be made when large amounts of a gene are needed.

- Devised in 1985, PCR has had a major impact on biological research and technology.
- PCR has amplified DNA from a variety of sources:
  - fragments of ancient DNA from a 40,000-year-old frozen wooly mammoth,
  - DNA from tiny amount of blood or semen found at the scenes of violent crimes,
  - DNA from single embryonic cells for rapid prenatal diagnosis of genetic disorders,
  - DNA of viral genes from cells infected with difficult-to-detect viruses such as HIV.