CHAPTER 12

DNA Technology and the Human Genome

Objectives

Introduction Define and compare recombinant DNA technology, genomics, bioinformatics, and proteomics. Also note the goals of the Human Genome Project.

Bacteria as Tools for Manipulating DNA

12.1 Define and compare the processes of transformation, transduction, and conjugation. Explain why bacterial mating is not reproductive.
12.2 Describe the roles of bacterial F factors. Define a plasmid and explain why R plasmids pose serious human health problems.
12.3 Describe the overall process of using plasmids to give bacteria useful properties.
12.4 Explain how restriction enzymes are used to "cut and paste" DNA.
12.5 Describe the process used to produce many copies of a desired gene.
12.6 Describe two types of genomic libraries.

Other Tools of DNA Technology

12.7 Explain how cDNA is produced and the advantages of this process.
12.8 Explain how probes are used to identify clones carrying specific genes.
12.9 Explain how DNA microarrays make it easy to determine exactly what genes are active in any particular cell at a certain time.
12.10 Explain how gel electrophoresis is used to sort DNA.
12.11 Explain how restriction fragment analysis is used to detect differences in DNA sequences.
12.12 Explain how polymerase chain reactions work and how this process is useful to biologists.

The Challenge of the Human Genome

12.13 Describe the structure and possible functions of the noncoding sections of the human genome.
12.13 Define and discuss the significance of transposons.
12.14 Describe the three overlapping stages of the Human Genome Project. Explain why it is important to also sequence the genomes of other organisms.

Other Applications of DNA Technology

12.15 Explain how DNA fingerprinting is used in making identifications.
12.16 Describe the types and advantages of the different organisms used to mass-produce proteins.
12.17 Explain how DNA technology has helped to produce insulin, growth hormone, and vaccines.

12.18 Explain how genetically modified organisms are transforming agriculture.
12.19 Describe the recent efforts in human gene therapy. Discuss the ethical issues that these techniques present.

Risks and Ethical Questions

12.20 Describe the risks posed in the culturing of GM organisms and the safeguards that have been developed.
12.21 Describe the ethical dilemmas associated with DNA technology and increased knowledge of the human genome.

Key Terms

recombinant DNA technology
DNA technology
genomics
transformation
transduction
conjugation
F factor
plasmid
vector
R plasmid
biotechnology
restriction enzyme
DNA ligase
recombinant DNA
gene cloning
genomic library
complementary DNA (cDNA)
probe
DNA microarray
gel electrophoresis
genetic marker
restriction fragment
polymerase chain reaction (PCR)
repetitive DNA
telomere
transposon
Human Genome Project (HGP)
DNA fingerprint
vaccine
genetically modified (GM) organism
Ti plasmid
transgenic organism
gene therapy
eugenics

Lecture Outline

Introduction *From* E. coli *to a Map of Our Genes*

A. **Recombinant DNA technology** (genetic engineering) involves combining genes from different sources into new cells that express the genes.

B. Recombinant DNA technology has had—and will have—many important applications.
   1. More efficient methods of basic and applied research into molecular genetics
   2. Using bacteria to mass-produce biochemicals needed by other species
   3. Creation of new strains of plants and animals (this is raising many environmental, ethical, and health issues)
   4. Sequence analysis of the entire human genome
   5. Bioinformatics, **genomics,** and proteomics are flourishing areas of research.

C. Recombinant DNA techniques are based on bacterial mechanisms.
   1. In 1946, Lederberg and Tatum discovered that *Escherichia coli* has a sexual mechanism.
   2. They combined *E. coli* strains, each of which required a different amino acid to grow. Cells of a new strain appeared in the cultures that did not require the addition of either amino acid.

I. Bacteria as Tools for Manipulating DNA

Module 12.1 In nature, bacteria can transfer DNA in three ways.

A. *Review:* In sexually reproducing organisms, new genetic combinations are the result of meiosis and fertilization (Chapter 8). The mechanisms discussed in this module are the ways that bacteria produce new genetic combinations.

B. *Review:* Studies by Griffith showed nonpneumonia-causing strains of *Pneumococcus* becoming disease-causing in a culture medium that previously contained the disease-causing strain (Module 10.1).

C. **Transformation** is the taking up of DNA from the nonliving environment around a bacterium (Figure 12.1A). Transformation caused the results Griffith observed.

D. **Transduction** is the transfer of bacterial genes from one bacterium to another by a phage (Figure 12.1B).

E. **Conjugation** is the process by which two bacteria mate (Figure 12.1C). Conjugation is initiated by "male" cells (gene donors) that recognize "female" cells (gene recipients) by means of the male sex pili. After the initial male-female recognition, a cytoplasmic bridge forms between two cells. Replicated DNA from the male passes through this bridge to the female.

F. In all three mechanisms, the new DNA is integrated into the existing DNA in the recipient by a crossover-like event that replaces part of the existing DNA (Figure 12.1D).

G. These mechanisms are not reproductive. Sexual reproduction does not occur in bacteria, unlike the situation in plants and animals.

Module 12.2 Bacterial plasmids can serve as carriers for gene transfer.

A. The F (fertility) factor is a portion of *E. coli* DNA that carries genes for making sex pili and other requirements for conjugation.

B. The F factor may be integrated into the main bacterial DNA, or it may exist as a separate, circular DNA fragment, a **plasmid,** that is free in the cytoplasm. Plasmids replicate separately from the main DNA.

C. If the F factor is integrated into the donor's main DNA, replication begins. The replicated length of DNA is transferred from the donor to the recipient but usually breaks before the remaining F factor is transferred. Thus, the recipient does not receive the F-factor genes, and it and its descendants remain female (Figure 12.2A).

D. If the F factor exists as a separate plasmid, it replicates into a linear DNA molecule that is entirely transferred to the recipient. The recipient and all its descendants become male (Figure 12.2B).

E. When extra genes are transferred the plasmid is acting as a **vector.**

F. Plasmids that carry genes other than those needed for conjugation are called vectors. For example, **R plasmids** are a class of plasmids that carry genes for antibiotic resistance. The widespread use of antibiotics in medicine and agriculture has tended to kill bacteria that lack R plasmids and favor those bacteria that have R plasmids.
NOTE: The ease of transmission of plasmid DNA has been implicated in the rapid transfer of DNA among bacteria, even between different species. Transfers such as these are partly responsible for the spread of multidrug-resistant bacteria, particularly *Mycobacterium tuberculosis* (natural selection; Modules 13.4, 13.5, and 13.22).

G. As will be seen later in the chapter, plasmids have important places among the techniques of genetic engineers.

Module 12.3 Plasmids are used to customize bacteria: *An overview.*

A. Figure 12.3 presents a simplified version of how a plasmid can be used to custom-make a bacterium.

B. Plasmids are isolated from a bacterium.

C. DNA that encodes useful proteins or traits is removed from another organism.

D. The plasmid DNA and the gene of interest are joined and returned to the bacterial cells.

E. The bacteria are grown in culture to produce many copies of the isolated gene (the gene is cloned) or its product.

F. Such engineered bacteria play a role in the manufacture of drugs such as human insulin and human growth hormone.

Module 12.4 Enzymes are used to "cut and paste" DNA.

A. **Restriction enzymes** were first discovered in bacteria in the late 1960s.

B. In nature, bacteria use restriction enzymes to cut up intruder DNA from phages and from other organisms into nonfunctional pieces. The bacteria first chemically modify their own DNA so that it will not be cut.

C. Several hundred different restriction enzymes and about 100 different recognition sequences have been discovered.

D. DNA from two different sources is cut by the same restriction enzyme. These enzymes are cut at a specific restriction-enzyme recognition sequence (usually a palindrome). The result is double-stranded DNA sequences with single-stranded "sticky ends" (Figure 12.4).

E. DNA fragments may pair at their sticky ends. This pairing is temporary but **DNA ligase** can make it permanent. The result of this is the formation of **recombinant DNA.** *Review:* DNA ligase is normally used in DNA replication (Module 10.5).

Module 12.5 Genes can be cloned in recombinant plasmids: *A closer look.*

A. Plasmid DNA and the DNA of the cell containing the gene of interest are each cut with the same restriction enzyme. The new gene is inserted into the plasmid. The new plasmid is returned to a bacterium by transformation.

B. The example in Figure 12.5 uses a hypothetical situation where human gene V is cloned.

C. The procedure described in this module is a "shotgun" approach since the specific gene isn't targeted.

Module 12.6 Cloned genes can be stored in genomic libraries.

A. Using a shotgun approach to do this, scientists cut up all the DNA from a cell into thousands of fragments, each of which carries one or a few genes of unknown identity (one or more fragments will carry the gene of interest).

B. These fragments are temporarily stored in a **genomic library** of plasmids in separate bacterial cells (plasmid library), or in separate phages (phage library) (Figure 12.6).

II. Other Tools of DNA Technology

Module 12.7 Reverse transcriptase helps make genes for cloning.

A. A problem with cloning and bacterial synthesis of eukaryotic gene products is that bacterial genes do not contain introns.

B. Special enzymes called reverse transcriptases are found in retroviruses. These enzymes make DNA from viral genome RNA (Module 10.21).
NOTE: An example of such a retrovirus is HIV.

C. Genes that are expressed can be isolated by using mRNA that has already had its introns spliced out. When reverse transcriptase is mixed with this mRNA, double-stranded DNA coding for the gene of interest is produced (Figure 12.7).

D. These DNA fragments (called **complementary DNA** or **cDNA**) are again temporarily stored in plasmid or phage libraries called cDNA libraries.

E. These intronless DNA sequences code for whatever proteins the cell had been making and can be transcribed and translated by bacterial cells.

Module 12.8 Nucleic acid probes identify clones carrying specific genes.

A. If some of the bacterial clones in the genomic library actually produce the product expressed by the gene of interest, testing the medium in which they are growing for the product can isolate the right clone.

B. If this cannot be done, scientists use radioactive (or fluorescent) labeled single-stranded nucleic acid probes that pair with selected regions of the gene of interest. The cells or phages in the genomic library that hold onto the radioactive label contain the gene in question (Figure 12.8A).

C. The **probes** can be assembled artificially if some sequence in the target protein (and hence a corresponding sequence of nucleotides) is known.

D. A genomic library made by the shotgun approach can be screened rather quickly for a gene of interest using the DNA probe technique (Figure 12.8B). Once the clone has been identified, the gene and the product can be mass- produced by culturing the bacterial colony that contained the gene.

Module 12.9 Connections: DNA microarrays test for the expression of many genes at once.

A. **DNA microarrays** are an extension of the procedure presented in Module 12.8, a micro-method for the rapid identification of gene expression.

B. Figure 12.9 illustrates the procedure and the result of a DNA microarray assay. Fluorescence correlates with gene expression.

C. This technique has many potential applications, including but not limited to:

1. Gene activation in healthy or diseased tissues
2. Response of tissues to drug therapy
3. Gene analysis for an individual to determine the risk of certain diseases in an effort to reduce risk factors.

Module 12.10 Gel electrophoresis sorts DNA molecules by size.

A. **Gel electrophoresis** sorts proteins and nucleic acids on the basis of their size and charge.

B. Longer macromolecules move through the gel more slowly than shorter macromolecules. The result of this differential rate of movement is a pattern of bands on the gel, each band consisting of macromolecules of one particular size (Figure 12.10).

Module 12.11 Restriction fragment analysis is a powerful method that detects differences in DNA sequences.

A. Nucleotide sequences of all but identical twins are different.

B. Extracted DNA from a person's cells can be cut up into a set of fragments by exposing the DNA to a series of different restriction enzymes (Figure 12.11A; recall Module 12.4).

C. Differences in DNA sequences on homologous chromosomes produce sets of **restriction fragments** that differ in length and number between different, nonidentical-twin individuals.

D. These DNA fragments are of different lengths and will migrate different distances in an electrophoretic gel (Figure 12.11B).

E. A **genetic marker** is any DNA sequence whose inheritance can be tracked. It may or may not be a gene or a sequence within a gene (Figure 12.11C).

F. Restriction fragment analysis was used to enable workers studying Huntington's disease to find a genetic marker closely associated to the HD gene.

G. Once the genetic marker is known for a particular disease, restriction fragment analysis can be used to test for it.

Module 12.12 The PCR method is used to amplify DNA sequences.

NOTE: Tools such as restriction fragment analysis, PCR, and DNA sequencing have also been used in conservation biology. For example, are populations of an endangered species actually members of the same species (in which case they can be interbred) or are they distinct species?

A. The **polymerase chain reaction (PCR)** is a technique for copying a single DNA sequence many times.

B. A mixture of the DNA, DNA polymerase, and nucleotide monomers will continue to replicate, forming a geometrically increasing number of copies (Figure 12.12).

C. This technique has revolutionized DNA work because sequences can now be obtained from extremely small samples. Prehistoric DNA from a number of sites has been cloned into partial genomes in this way.

III. The Challenge of the Human Genome

Module 12.13 Most of the human genome does not consist of genes.

A. The human genome is all the genes present in a haploid human cell. This is approximately 3 billion nucleotide pairs of DNA.

B. Although the amount of DNA in a human cell is 1000 times that in *E. coli,* and *E. coli* has about 2000 genes, the human genome probably only has approximately 30,000 to 40,000 genes (that is, 15 to 20 times the number of genes).

C. This is because about 97% of the human genome is noncoding, consisting of sequences such as promotors and enhancers.

D. "Junk DNA" (regions of unknown function) includes introns and noncoding regions between genes.

E. The DNA found between genes mainly consists of **repetitive DNA.** Loss of repetitive DNA at the ends of chromosomes **(telomeres)** leads to cell death (Figure 12.13A). Abnormally long repeats may play a role in cancer cell immortality. Genetic disorders of the nervous system, such as Huntington's disease, are associated with repeated nucleotide triplets.

F. Longer sequences of repetitive DNA are found scattered about the genome. Little is known about the functions of these regions of DNA; however, most of them appear to be associated with **transposons.**

G. In 1940, Dr. McClintock discovered that some traits of corn change in a way implying that genes move from one chromosome location to another, even between different chromosomes. In 1983, she was finally awarded a Nobel Prize for this work (Figure 12.13B).

H. When these transposons move, they either disrupt the expression of other genes (Figure 12.13C) or change the transmission pattern of sets of genes.

I. Transposons are found in most, if not all, organisms.

J. There are two types of transposons. The cut-and-paste type moves from one location to another. There is also a copy-and-paste type that leaves a copy of itself behind when it moves.

K. Copy-and-paste transposons appear to be responsible for the dispersed repetitive DNA found in the human genome.

L. Transposons could play a role in increasing genetic diversity and in evolution. Transposons may also play a role in cancer.
NOTE: Not only do transposons seem to have great significance in natural evolution, but their properties would appear to make them suitable agents for artificially modifying the structure and expression of genes. There is some evidence that suggests that the combinatorial immune system (found in humans and other vertebrates) evolved from a transposon.

Module 12.14 Connection: The **Human Genome Project** is unlocking the secrets of our genes.

A. An internationally government-funded group of scientists began to sequence the human genome starting in 1990. There are three major stages involved in mapping the human genome.

B. Genetic (linkage) mapping: This involves constructing a map of over 5000 genetic markers that act as a set of references for other work.

C. Physical mapping: Restriction enzymes will be used to break chromosomes into identifiable fragments.

D. DNA sequencing: This is the process that will elucidate the exact order of the nucleotide pairs in each fragment and hence each chromosome (Figure 12.14).

E. A former government scientist (J. Craig Venter) decided to use the shotgun approach and founded the company Celera Genomics. Going directly to step 3, random fragments of DNA were sequenced. Due to this approach and stiff competition, 90% of the human genome was completed by February 2001 (in draft form).

F. By early 2002, over 70 species had complete genome sequence data. The most startling piece of data from the project is the relatively small number of genes that are present in the human genome—only 2 to 3 times the number in the fruit fly.

G. The project has (and already is providing) huge potential benefits: insight into embryonic development and evolution, and identification of genes that cause genetic disorders and genes that are partly implicated in more common diseases such as cancer, heart disease, diabetes, schizophrenia, alcoholism, and Alzheimer's disease. Literally hundreds of disease-associated genes have been found because of this project.

H. DNA sequences are available on the Internet.

IV. Other Applications of DNA Technology

Module 12.15 DNA technology is used in courts of law.

A. An individual's band pattern from restriction fragment analysis is called a **DNA fingerprint.** Like traditional fingerprints, DNA fingerprints are unique.

B. DNA fingerprinting uses restriction fragment analysis to identify small amounts of DNA from blood, tissue, or semen.

C. DNA lasts in the environment much longer than proteins. DNA can be extracted from dried bloodstains that are 3–4 years old.

D. In criminal investigations, restriction fragments are compared with those of other sources.

E. Increasingly, simple random repeats (SRRs) are being used for DNA fingerprinting (Figure 12.15B). SRRs are regions of repetitive DNA that vary in length among individuals.

F. PCR (Module 12.12) is used to amplify SRRs (and restriction fragments) before they are analyzed.

G. The technology of DNA fingerprinting has been shown to be reliable.

Module 12.16 Connection: Recombinant cells and organisms can mass-produce gene products.

A. Bacteria are the host of choice for making large amounts of eukaryotic gene products because bacteria are simple and can be grown rapidly and cheaply (Table 12.16).

B. The single-celled fungus *Saccharomyces cerevisiae* (bread and wine yeast) is one of the simplest eukaryotes that can be grown rapidly and cheaply. Yeast also has plasmids that can be used as vectors. In some cases, yeast does a better job than bacteria in expressing eukaryotic genes.

C. Some products are best made by mammalian cells. The genes of interest are often cloned in bacterial plasmids first and then introduced to the final host.

D. Among these mammalian cell products are monoclonal antibodies, which are glycoproteins used widely in research on cellular structures and functions. The sugar chain part of monoclonal antibodies can be added to the protein part only under the control of the eukaryotic gene expression system.

E. The use of whole animals to mass-produce a protein of interest has been explored by pharmaceutical companies. Figure 12.16B shows a sheep that produces milk containing a gene for a blood protein that may be a treatment for cystic fibrosis.

F. *Preview:* Module 24.12 offers a more in-depth discussion of monoclonal antibodies.

Module 12.17 Connection: DNA technology is changing the pharmaceutical industry and medicine.

A. Human insulin and human growth hormone were two of the first commercially produced recombinant DNA products.

B. DNA technology is currently being used to identify people with HIV; through the use of microarray assays, people will be informed of potential diseases prior to the appearance of symptoms.

C. **Vaccines** are harmless or derivative variants of proteins produced on the surfaces of pathogens. Exposing a person to the vaccine (by injection, usually) primes the person's immune system to recognize and destroy the pathogen in the case of future infection. Vaccines are particularly important in the defense against many viral diseases (Figure 12.17).

NOTE: Normally, vaccines are made using natural mutant forms of pathogens or proteins extracted from the pathogens.

D. Recombinant DNA techniques can be used to make vaccines in many ways: mass production of vaccine proteins; assembling artificial mutant pathogens; and adding proteins from several pathogens to the coat of the natural mutant smallpox virus, previously used to successfully eradicate smallpox.

E. "Genetic drugs," such as antisense nucleic acid, are a promising avenue of research for the development of treatments for viral diseases and cancers.

Module 12.18 Connection: Genetically modified organisms are transforming agriculture.

A. **Genetically modified (GM) organisms** are being developed by agricultural scientists using DNA technology in an effort to improve plants and animals. Some examples currently in use are plants that ripen slowly and resist spoilage.

B. The bacterium *Agrobacterium tumefaciens,* which is pathogenic to a number of plant hosts, is used to transfer genes between plants, in recombined form with the bacterium's **Ti plasmid.** The resulting **transgenic** plant cells are cloned in culture and grown into adult plants (Figure 12.18A).
NOTE: This process can be done much more rapidly than by depending on a full season for natural breeding to produce hybrids, and genes can be transferred between unrelated plants.

C. *A. tumefaciens* does not naturally grow in many grain-producing species. In these instances a "gene gun" is used to fire foreign DNA into cultured cells.

D. Forty percent of transgenic plants that are currently in field trials have received genes for herbicide resistance. Other plants are being engineered to be resistant to pathogens and pest insects. The hope is that this will reduce the need for the application of chemical insecticides.
NOTE: Unless care is taken, the evolution of weeds, pathogens, and pest insects that can get around these barriers is inevitable (*Preview:* Chapter 13, especially Module 13.21).

E. The nutritional value of genetically modified plants has been improved through DNA technology. A good example of this is golden rice. This GM rice has had a gene inserted that promotes the production of β-carotene, the precursor to vitamin A used in sight. Therefore people who depend on rice as a major portion of their diet will no longer be deficient in vitamin A and the rate of blindness will decrease.

F. The development of transgenic animals is progressing slowly due to problems involving reduced fertility and increased susceptibility to disease. There are also concerns over the safety of the final product.

Module 12.19 Connection: Gene therapy may someday help treat a variety of diseases.

A. **Gene therapy** holds much, as yet unrealized, promise.

B. In certain cases where a disorder is due to a single gene, it is possible to replace defective genes with normal genes.

C. An example is a trial procedure that should cure individuals with an autosomal recessive allele that causes defective functioning of the immune system and is usually fatal. Bone marrow stem cells, which are essential for blood cell formation, are removed. By means of a retrovirus, the defective gene is replaced with the normal one. The recombinant cells are cloned in culture and reintroduced in the individual, after the natural bone marrow cells have all been killed (Figure 12.19).

D. To date, most gene therapy trials have been concerned with safety and effectiveness rather than with attempted cure of a disease. There has also been a shift away from correcting gene defects and toward treating major diseases once a patient has been afflicted. Heart disease and cancer are prime candidates for this approach.

E. The expense of these techniques raises ethical questions concerning who can have access to these therapies. Further ethical questions are raised concerning the use of gene

therapy, not for treatment of disease but for enhancement of physical ability and appearance as well as intelligence. The ultimate question is what are the potential implications of genetic engineering, particularly of sex cells, on humans? Might an allele that is harmful in the current environment be essential to survival in a future environment?

V. Risks and Ethical Questions

Module 12.20 Connection: Could GM organisms harm human health or the environment?

A. American scientists have developed safety guidelines administered by the U.S. government to minimize the risks involved in genetic engineering (Figure 12.20A).
NOTE: American guidelines tend to be stricter than those of other countries.

B. The guidelines designate laboratory safety procedures for various types of experiments, including procedures normally used to protect scientists who study natural pathogens.

C. The guidelines also specify the kinds of microorganisms that can be used, often requiring that the recipient organisms be genetically altered so that they cannot live outside the laboratory.
NOTE: Certain kinds of experiments are forbidden or strictly regulated, such as working with human cancer genes or genes of extremely virulent pathogens.

D. Nevertheless, controversies have developed over the release of genetically altered organisms. For example, a certain type of GM corn contains a gene from the bacterium *Bacillus thuringiensis* that acts as a pesticide. The problem with this GM corn became apparent when it was shown that the Monarch butterfly population was being damaged when caterpillars consumed large quantities of pollen from the corn (Figure 12.20B).

E. Technological advances involve risk taking; therefore, better decisions can be made when sound scientific evidence is used in the decision process rather than basing decisions on fear or faith.

Module 12.21 Connection: DNA technology raises important ethical questions.

A. The long-term effects (including safety issues) of using recombinant products or transgenic species are not yet known.

B. There are ethical questions regarding our roles in making new organisms.

C. There are ethical questions regarding the use of these techniques to provide genetic cures of human diseases (for example, giving growth hormone to short but hormonally normal children).

D. The Human Genome Project offers some potential for misuse of the information by governments, employers, and insurers, which we as citizens must be aware of and prevent.

E. The possibility of gene therapy suggests a return to the notion of eugenics.

F. Genetic discrimination by potential employers is a concern.

Class Activities

1. I am sure you are familiar with OMIM (home page of Online Mendelian Inheritance in Man [http://www3.ncbi.nlm.nih.gov/Omim/]). Have each of your students choose a trait/disease of theirs or of their family's and use OMIM to find out what is known about the genetics of the trait/disease.

2. Class discussion can also focus on the ethical issues of the use of these techniques on humans. Should parents be allowed to genetically engineer a child to be a star athlete? a genius? Would this be acceptable as long as the gametes are left alone? Should gametes be engineered so that a disease such as Huntington's is eliminated? What about the future? A gene that is harmful in the present environment may be of benefit in a different environment.
3. Further class discussion can focus on whether or not the anticipated results of the Human Genome Project justify the expense. Have the students gather information on a genetic characteristic or disorder from Human Genome Internet sites (for example: To Know Ourselves: The U.S. Department of Energy and the Human Genome Project [http://www.ornl.gov/hgmis/publicat/tko/index.html]).

Transparency Acetates

Figure 12.1A Transformation
Figure 12.1B Transduction
Figure 12.1C Conjugation
Figure 12.1D Integration of donated DNA into the recipient cell's chromosome
Figure 12.2A Transfer of chromosomal DNA by an integrated F factor
Figure 12.2B Transfer of an F-factor plasmid
Figure 12.3 Using plasmids to customize bacteria
Figure 12.4 Creating recombinant DNA using a restriction enzyme and DNA ligase
Figure 12.5 Cloning a gene in a bacterial plasmid (Layer 1)
Figure 12.5 Cloning a gene in a bacterial plasmid (Layer 2)
Figure 12.5 Cloning a gene in a bacterial plasmid (Layer 3)
Figure 12.6 Genomic libraries
Figure 12.7 Making an intron-lacking gene from eukaryotic mRNA
Figure 12.8A How a DNA probe tags a gene by base pairing
Figure 12.8B Using a DNA probe to identify a bacterial clone (colony) carrying a specific gene
Figure 12.9 DNA microarray
Figure 12.10 Gel electrophoresis of DNA
Figure 12.11A Restriction site differences between two alleles
Figure 12.11B Gel electrophoresis of restriction fragments
Figure 12.11C A procedure for restriction fragment analysis (Layer 1)
Figure 12.11C A procedure for restriction fragment analysis (Layer 2)
Figure 12.11C A procedure for restriction fragment analysis (Layer 3)
Figure 12.12 DNA amplification by PCR
Figure 12.13A The telomeres of human chromosomes
Table 12.16 Some protein products of recombinant DNA technology
Figure 12.18A Using the Ti plasmid as a vector for genetically engineering plants
Figure 12.19 One type of gene therapy procedure
Thinking as a Scientist Question 2: Restriction fragment analysis

Media

See the beginning of this book for a complete description of all media available for instructors and students. Animations and videos are available in the Campbell Image Presentation Library. Media Activities and Thinking as a Scientist investigations are available on the student CD-ROM and web site.

Animations and Videos	File Name
Restriction Enzymes Animation	12-04-RestrictEnzymesAnim.mov
Cloning a Gene Animation	12-05-CloningAGeneAnim.mov
Scientists Working in Molecular Biology Lab Video	12-09-ScientistsWorkVideo-B.mov

Activities and Thinking as a Scientist	Module Number
Web/CD Thinking as a Scientist: *How Can Antibiotic-Resistant Plasmids Transform* E. coli*?*	12.2
Web/CD Activity 12A: *Restriction Enzymes*	12.4
Web/CD Activity 12B: *Cloning a Gene in Bacteria*	12.5
Web/CD Activity 12C: *Gel Electrophoresis of DNA*	12.10
Web/CD Activity 12D: *Analyzing DNA Fragments Using Gel Electrophoresis*	12.11
Web/CD Thinking as a Scientist: *How Can Gel Electrophoresis Be Used to Analyze DNA?*	12.11
Biology Labs On-Line: *PedigreeLab*	12.11
Web/CD Activity 12E: *The Human Genome Project: Human Chromosome 17*	12.14
Web/CD Activity 12F: *Connection: DNA Fingerprinting*	12.15
Web/CD Activity 12G: *Connection: Applications of DNA Technology*	12.18
Web/CD Activity 12H: *Connection: DNA Technology and Golden Rice*	12.20