BIOTECHNOLOGY

Biotechnology

- "Application of science and engineering to the use of living organisms or substances derived from them, to generate products or to perform functions that can benefit the human condition"

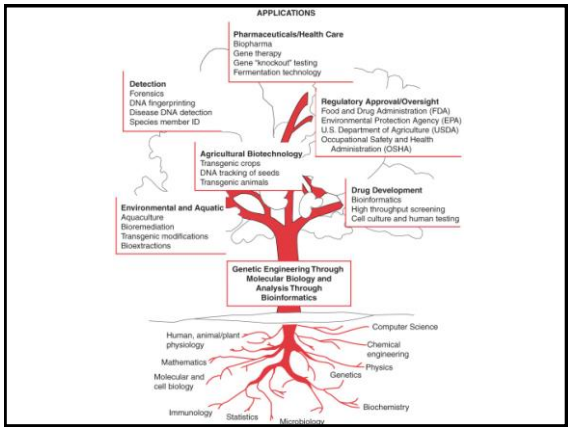


Table 1.2. *Historical development of biotechnology*

Biotechnological production of foods and beverages
Sumarians and Babylonians were drinking beer by 6000 B.C.; Egyptians were baking leavened bread by 4000 B.C.; wine was known in the Near East by the time the book of Genesis was written. Microorganisms first seen in seventeenth century by Antonie van Leeuwenhoek, who developed the simple microscope; fermentative ability of microorganisms demonstrated between 1857 and 1876 by Pasteur – the father of biotechnology; cheese production has ancient origins; so also has mushroom cultivation

Biotechnological processes initially developed under non-sterile conditions
Ethanol, acetic acid, butanol and acetone were produced by the end of the nineteenth century by open microbial fermentation processes; waste-water treatment and municipal composting of solid wastes were the largest fermentation capacity practised throughout the world

Introduction of sterility to biotechnological processes
In the 1940s complicated engineering techniques were introduced to the mass cultivation of microorganisms to exclude contaminating microorganisms. Examples include antibiotics, amino acids, organic acids, enzymes, steroids, polysaccharides, vaccines and monoclonal antibodies

Applied genetics and recombinant DNA technology
Traditional strain improvement of important industrial organisms has long been practised; recombinant DNA techniques together with protoplast fusion allow new programming of the biological properties of organisms

Biotechnology—a sustainable alternative for chemical industry

Table 1
Some well-established biotechnology products (by production volume)

Product	Annual production (tons)
Bioethanol	26,000,000
L-Glutamic acid (MSG)	1,000,000
Citric acid	1,000,000
L-Lysine	350,000
Lactic acid	250,000
Food-processing enzymes	100,000
Vitamin C	80,000
Gluconic acid	50,000
Antibiotics	35,000
Feed enzymes	20,000
Xanthan	30,000
L-Threonine	10,000
L-Hydroxyphenylalanine	10,000
6-Aminopoenicillanic acid	7000
Nicotinamide	3000
D-*p*-hydroxyphenylglycine	3000
Vitamin F	1000
7-Aminocephalosporinic acid	1000
Aspartame	600
L-Methionine	200
Dextran	200
Vitamin B12	12
Provitamin D2	5

Introduction to Biotech

- In Hawaii, a deadly pathogen called the papaya ringspot virus (PRV) had spread throughout the islands and threatened the papaya industry.
- Scientists from the University of Hawaii were able to rescue the industry by creating new, genetically engineered PRV-resistant strains of papaya.
- Today, the papaya industry is once again vibrant, and the vast majority of Hawaii's papayas are genetically modified organisms (GMOs).

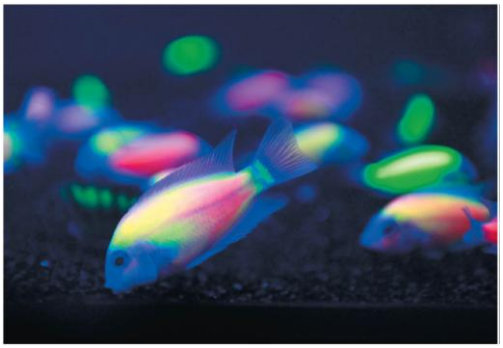
Introduction to Biotech

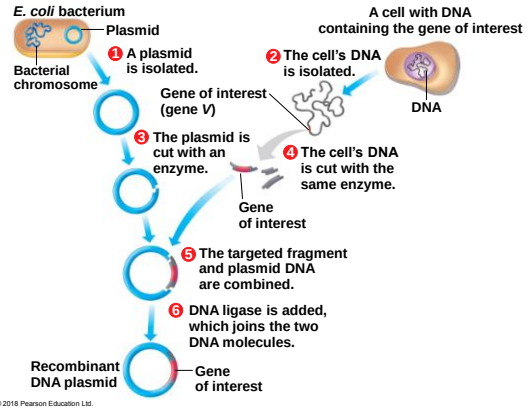
- In addition to GMOs in our diet, DNA technologies affect our lives in many other ways:
  - Gene cloning and editing are used to produce medical and industrial products.
  - DNA profiling has changed the field of forensic science.
  - Bioinformatics provides data for biological research as well as historical and evolutionary investigations.

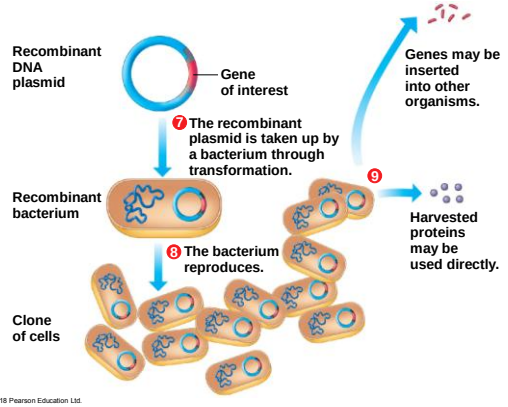
Genes can be cloned in recombinant plasmids

- **Gene cloning** is one application of **biotechnology**, the manipulation of organisms or their components to make useful products.
- Researchers can manipulate bacterial **plasmids** so that they contain genes from other organisms.
  - These **recombinant DNA** plasmids can then be inserted into bacteria.
  - If the recombinant bacteria multiply into a **clone**, the foreign genes are also duplicated and copies of the gene or its protein product can be harvested.

Glowing aquarium fish (*Amatitlania nigrofasciatus*, a type of cichlid) produced by transferring a gene originally obtained from a jelly (cnidarian)







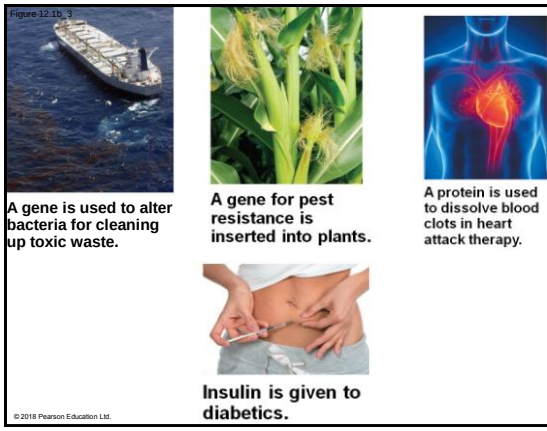
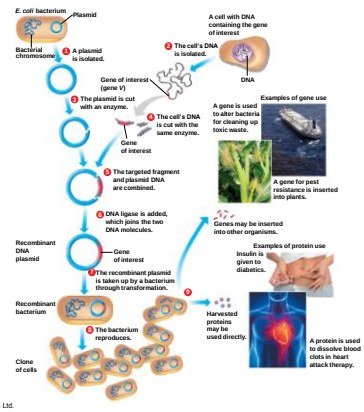
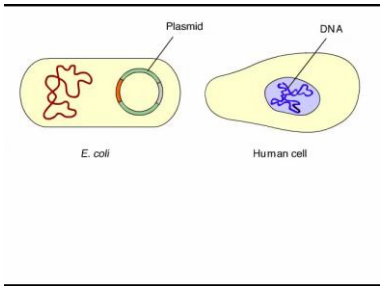


Figure 12.1b

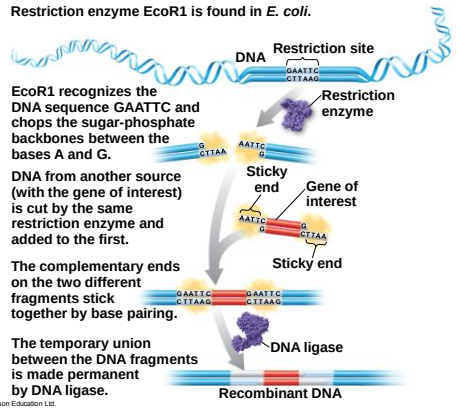


Animation: Cloning a Gene



Enzymes are used to "cut and paste" DNA

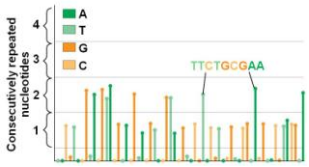
- **Restriction enzymes** cut DNA at specific sequences, forming **fragments**.
  - **Sticky ends**: Single-stranded regions of a DNA fragment whose unpaired bases can hydrogen-bond to complementary single-stranded regions of another
- DNA ligase "pastes" DNA fragments together.



GENOMICS AND BIOINFORMATICS

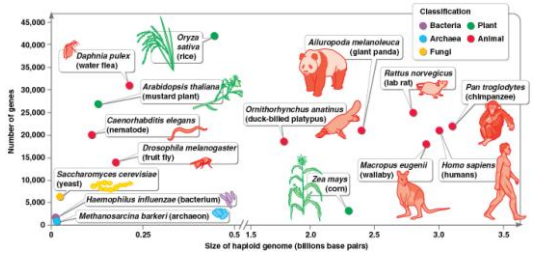
12.16 Small segments of DNA can be sequenced directly

- Sequencing machines can quickly determine the sequence of relatively short stretches of DNA.



12.17 Genomics is the scientific study of whole genomes

- **Genomics** is the study of complete sets of genes.
- Genomics researchers have sequenced many prokaryotic and eukaryotic genomes.
- Besides being of interest in their own right, nonhuman genomes can be compared with the human genome.

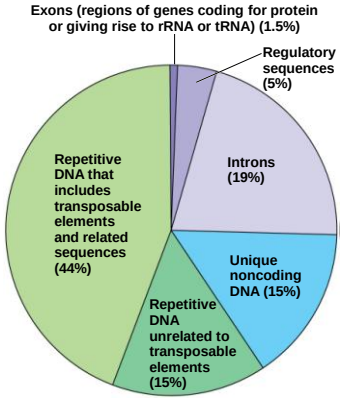


Checkpoint question Does a larger genome always correlate with more genes?

12.18 CONNECTION: The Human Genome Project revealed that most of the human genome does not consist of genes

- The **Human Genome Project (HGP)** was a massive, publicly funded scientific endeavor to determine the nucleotide sequence of all DNA in the human genome and identify the location and sequence of every gene.
- Data from the HGP revealed that the human genome contains just under 21,000 genes and a huge amount of noncoding DNA, much of which consists of repetitive nucleotide sequences.

Figure 12.18

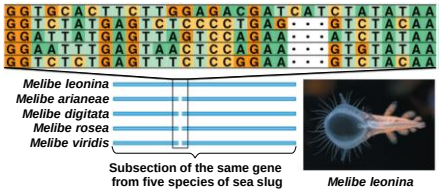


12.20 The field of bioinformatics is expanding our understanding of genomes

- **Bioinformatics**, the use of computational methods to analyze biological data, can be used to analyze large sets of data about DNA sequences and proteins.
- **Proteomics** involves similar systematic studies of the full protein sets (proteomes) encoded by genomes.

Checkpoint question Why is studying sets of proteins, rather than sets of genes, beneficial to understanding how cells work?

Figure 12.20



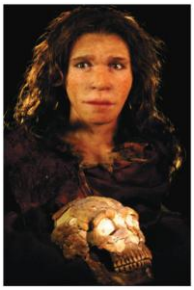
Data from J. L. Sevigny et al., The mitochondrial genomes of the nudibranch mollusks, *Melibe leonina* and *Tritonia diomedea*, and their impact on gastropod phylogeny, *PLoS ONE* 10: e0127519 (2015).

12.21 EVOLUTION CONNECTION: Genomes hold clues to human evolution

- Using databases like GenBank, researchers can now compare genome sequences from many species, allowing hypotheses about evolutionary relationships between those species to be tested.
- The comparison of genomic sequences between humans and our nearest evolutionary relatives provides insight into human evolution.

Checkpoint question How can cross-species comparisons of the nucleotide sequences of a gene provide insight into evolution?

Similarities in gene sequences correlate with evolutionary relatedness; greater genetic similarities reflect a more recent shared ancestry.



Reconstruction of a Neanderthal female, based on a 36,000-year-old skull

DNA PROFILING

The analysis of genetic markers can produce a DNA profile

- DNA technology has rapidly transformed the field of **forensics**, the scientific analysis of evidence for crime scene investigations and other legal proceedings.
- **DNA profiling** can determine whether two samples of DNA came from the same individual.

What is a DNA profile, and how can it be used?

- Samples to be tested may be derived from a variety of sources, including suspects, a crime scene, or old evidence.

DNA profiles can be prepared from ancient tissue.

As of 2015, 20 death row inmates have been exonerated based on DNA evidence.

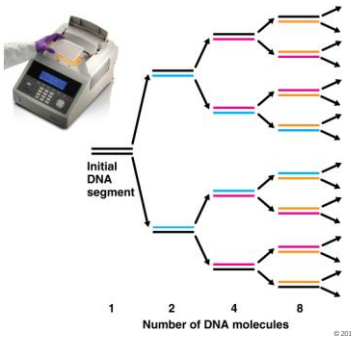
You don't need to compare everything

- The DNA of two humans of the same sex is 99.9% identical.
- It would be a waste of time to compare most of the genome.
  - there is no point in comparing regions of the genome that are identical among most humans.
- Instead, a DNA profile focuses on the few parts of the genome that do differ between people.
- This is a bit like one of those "spot the difference" puzzles—you can ignore the vast majority of the photo (the parts that are the same) and focus instead on the few differences.

The PCR method is used to amplify DNA sequences

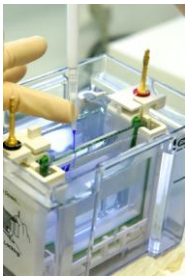
- The **polymerase chain reaction (PCR)** can be used to amplify a DNA sample.
- The use of specific **primers** that flank the desired sequence ensures that only a particular subset of the DNA sample will be copied.
- Starting with a minute sample, automated PCR can generate billions of copies of a DNA segment in just a few hours, producing enough DNA to allow a DNA profile to be constructed.

PCR: obtaining a large sample of DNA



Electrophoresis: visualizing the results

- After large samples are produced, they must be compared.
- Electrophoresis is a method of analyzing the lengths of DNA segments so that they can be measured and compared.

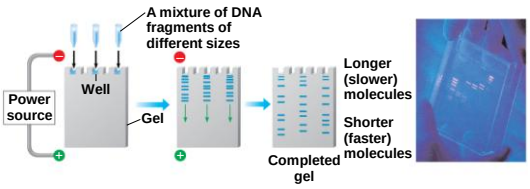


Gel electrophoresis

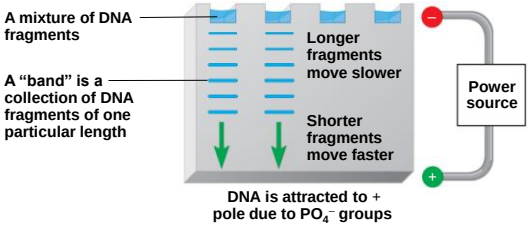
Gel electrophoresis sorts DNA molecules by size

- Many DNA technology applications rely on **gel electrophoresis**, a method that separates macromolecules, usually proteins or nucleic acids, on the basis of size, electrical charge, or other physical properties.

Checkpoint question What causes DNA molecules to move toward the positive pole during electrophoresis? Why do large molecules move more slowly than smaller ones?



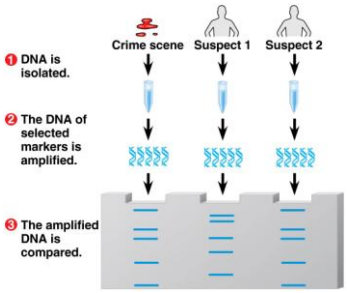
Bands that result from gel electrophoresis of DNA, each consisting of thousands of DNA fragments of the same length



DNA profiling has provided evidence in many forensic investigations

- The applications of DNA profiling include helping to
  - solve crimes,
  - establish paternity, and
  - identify victims.

Checkpoint question In what way is DNA profiling valuable for determining innocence as well as guilt?



Checkpoint question According to the data presented in Figure 12.11, which suspect left DNA at the crime scene?

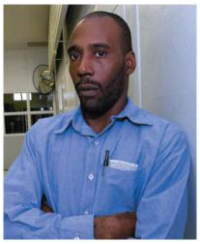
The power of DNA profiling: the first case

- The first case to be solved by DNA evidence began in 1983 and ended in 1988.
- Demonstrating innocence and guilt

Forensic investigations: guilt and innocence

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

- 1984, Earl Washington convicted and sentenced to death for the rape and murder of Rebecca Williams (1982).
- His sentence was commuted to life in prison in 1993 due to new doubts about the evidence.
- In 2000, STR analysis by forensic scientists associated with the Innocence Project showed conclusively that he was innocent.



Earl Washington, release in 2001, after 17 years in prison

The Innocence Project

Forensic investigations: identifying victims

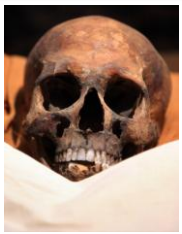
Paternity

- Genetic sequences are more likely to be a match between relatives than between unrelated individuals.
- DNA profiling can therefore definitively settle issues of paternity.
- The data table presents the number of repeats found at 5 STR sites (one per each chromosome in a homologous pair, yielding 10 numbers in total) in a hypothetical paternity case.

Marker	Mother	Child	Alleged father
D3S1358	18, 20	18, 21	19, 21
D5S818	5, 7	7, 9	9, 10
D7S820	12, 13	12, 14	13, 14
D8S1179	22, 24	22, 23	23, 27
D13S317	14, 16	16, 19	17, 19

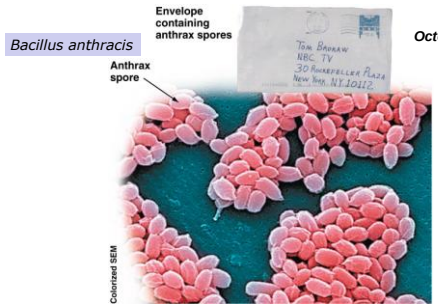
Data from a hypothetical paternity case

Historical investigations



Fighting bioterrorism: DNA profiling can be used to investigate cases of bioterrorism and other medical mysteries.

Bacillus anthracis



U.S. Army Medical Research Institute of Infectious Diseases

What issues are raised by DNA profiles?

- Advances in genetic profiling raise privacy issues.
- Self testing kits: DNA self-testing is becoming more popular. In a few weeks, the consumer can access a report that lists inherited traits, information about ethnicity, and possible hereditary risk factors.
- The FDA has ruled that genetic testing kits can be used to investigate ancestry, but they cannot be used to determine disease risks
- Discrimination: Information about disease-associated genes could be abused

GENETICALLY MODIFIED ORGANISMS

Recombinant cells and organisms can mass-produce gene products

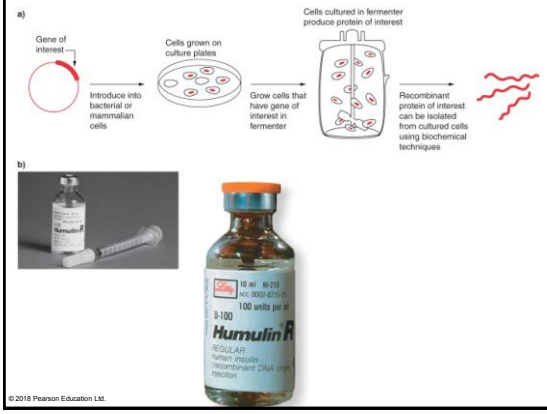
- Bacteria, yeast, cell cultures, and whole animals can be genetically modified to make products for medical and other uses.

A goat carrying a gene for a human blood protein that is secreted in the milk

DNA technology has changed the pharmaceutical industry and medicine

- Researchers use DNA technologies to
  - produce drugs,
  - diagnose diseases, and
  - produce **vaccines**, harmless variants (mutants) or derivatives of a pathogen.

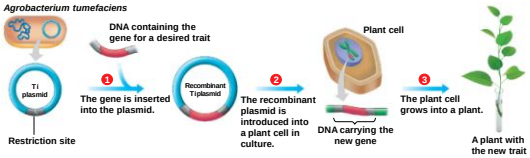
Checkpoint question If insulin and human growth hormone are both natural products, why use genetic engineering to make them?



Equipment used in the production of a vaccine against hepatitis B

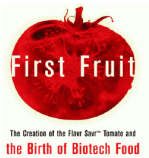
Genetically modified organisms are transforming agriculture

- Scientists have produced many different varieties of **genetically modified organisms (GMOs)**, organisms that have acquired one or more genes by artificial means.
- If a gene is transplanted from one organism into another, typically of another species, the recombinant organism is called a **transgenic organism**.
- A number of important crop plants are genetically modified.



Transgenic plants

- **The Flavr-Savr® tomato**: first GM food approved by FDA (1994), was a delayed ripening variety, stayed 2 years in the market
- **Roundup Ready Corn (1998):** spray fields of Roundup Ready corn with the herbicide Roundup, kill the weeds but not the corn
- **Golden Rice**: Swiss researchers (1999) have produced rice capable of synthesizing beta-carotene, the precursor of Vitamin A
- **Insect-resistant crops** (corn, potato, cotton): A modified version of a gene incorporated into the plant's DNA → the insect bite a leaf, it ingests the toxin and die
- **Decaffeinated tea and coffee**: The genes that lead to the production of caffeine in coffee beans and tea leaves can be "turned off" in some plants → coffee and tea trees could be developed that would produce naturally decaffeinated products with full flavor and aroma
- **Nicotine-free tobacco**: Genetically engineered tobacco that does not synthesize nicotine in the leaf (2001)



The use of genetically modified organisms raises questions and concerns

- Scientists are investigating the potential risks to human and environmental health posed by DNA technologies.

Checkpoint question Why is it often necessary to run both human and animal studies to learn about human health?

Animal diets and lifestyles can be closely controlled, but the results may not apply directly to humans.

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 safety of GM food and possible environmental consequences
- There are concerns that GM crops might transfer genes to wild plants, producing “super weeds”
- Others fear that protein products of transgenes might lead to allergic reactions

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

- As biotechnology continues to change, so does its use in agriculture, industry, and medicine
- Great benefits could result from biotechnology approaches, but unforeseen problems could arise
- We must proceed with humility and caution

Gene therapy may someday help treat a variety of diseases

- **Gene therapy**, changing a defective gene to a normal one in a living human, shows promise for curing defective genes, but actual successes are rare.

Checkpoint question Are there any safety concerns regarding the use of viruses in gene therapy?

Figure 12.10