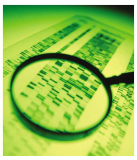
CHAPTER 12

DNA Technology

- The DNA of two people of the same sex is 99.9% identical

- Animals, plants, and even bacteria can be genetically modified to produce human proteins

- The first use of DNA fingerprinting in a murder case proved one man innocent and another guilty



- Genetically modified strains account for half of the U.S. corn crop

BIOLOGY AND SOCIETY:

HUNTING FOR GENES

- DNA technology is a set of methods for studying and manipulating genetic material
- These techniques have brought about many remarkable scientific advances
  - Genetically modified food
  - DNA fingerprinting
  - The Human Genome Project

- The goal of the Human Genome Project is to determine the nucleotide sequence of all DNA in the human genome
- Hundreds of disease-associated genes have already been identified
  - Example: Parkinson disease

Figure 12.1

RECOMBINANT DNA TECHNOLOGY

- Recombinant DNA technology is a set of techniques for combining genes from different sources into a single DNA molecule
  - An organism that carries recombinant DNA is called a genetically modified (GM) organism
- Recombinant DNA technology is applied in the field of biotechnology
  - Biotechnology uses various organisms to perform practical tasks

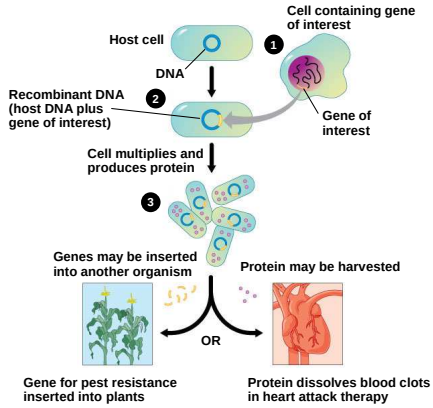


Figure 12.2

From Humulin to Genetically Modified Foods

- By transferring the gene for a desired protein product into a bacterium, proteins can be produced in large quantities

Making Humulin

- In 1982, the world's first genetically engineered pharmaceutical product was produced
  - Humulin, human insulin, was produced by genetically modified bacteria

Figure 12.3

- Prior to the development of Humulin, diabetes was treated using insulin from cows and pigs
  - These types of insulin can cause adverse reactions in recipients
- Humulin was the first recombinant DNA drug approved by the FDA

- DNA technology is also helping medical researchers develop vaccines
  - A vaccine is a harmless variant or derivative of a pathogen
  - Vaccines are used to prevent infectious diseases

Figure 12.4

Genetically Modified (GM) Foods

- Today, DNA technology is quickly replacing traditional plant-breeding programs
  - In 2002, roughly half of the American crops of soybeans and corn were genetically modified in some way

Figure 12.5a

- Corn has been genetically modified to resist insect infestation
  - This corn has been damaged by the European corn borer

Figure 12.5b

- "Golden rice" has been genetically modified to contain beta-carotene
  - Our bodies use beta-carotene to make vitamin A

Figure 12.6

Farm Animals and "Pharm" Animals

- While transgenic plants are used today as commercial products, transgenic whole animals are currently only in the testing phase
- These transgenic sheep carry a gene for a human blood protein
  - This protein may help in the treatment of cystic fibrosis

Figure 12.7

- While transgenic animals are currently used to produce potentially useful proteins, none are yet found in our food supply
- It is possible that DNA technology will eventually replace traditional animal breeding

Recombinant DNA Techniques

- Bacteria are the workhorses of modern biotechnology
- To work with genes in the laboratory, biologists often use bacterial plasmids
  - Plasmids are small, circular DNA molecules that are separate from the much larger bacterial chromosome

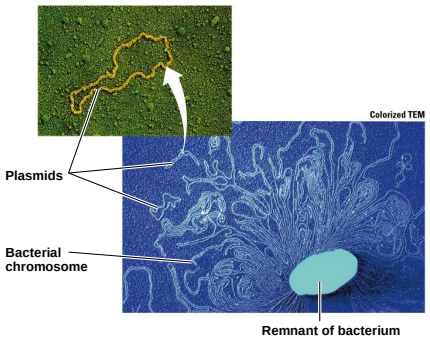


Figure 12.8

- Plasmids can easily incorporate foreign DNA
- Plasmids are readily taken up by bacterial cells
  - Plasmids then act as vectors, DNA carriers that move genes from one cell to another

- Recombinant DNA techniques can help biologists produce large quantities of a desired protein

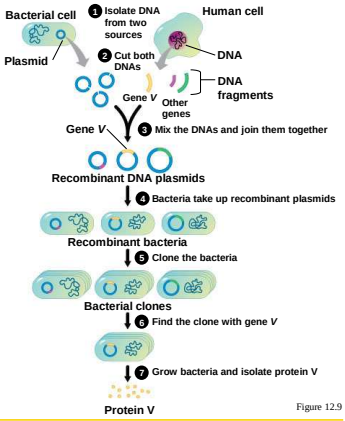


Figure 12.9

A Closer Look: Cutting and Pasting DNA with Restriction Enzymes

- Recombinant DNA is produced by combining two ingredients
  - A bacterial plasmid
  - The gene of interest
- To combine these ingredients, a piece of DNA must be "pasted" into a plasmid

- This “pasting” process can be accomplished using restriction enzymes
  - These enzymes cut DNA at specific nucleotide sequences
  - The places where DNA is cut are called restriction sites
- Many of these restriction sites leave staggered cuts that yield two double-stranded DNA fragments with single-stranded ends called “sticky ends”
  - These are the key to joining DNA restriction fragments

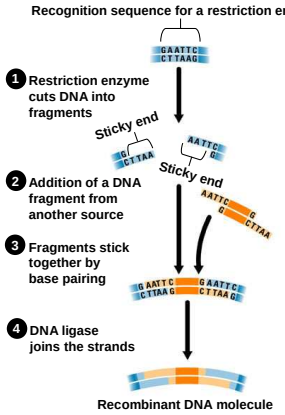


Figure 12.10

A Closer Look: Obtaining the Gene of Interest

- How can a researcher obtain DNA that encodes a particular gene of interest?
- The “shotgun” approach is one way to synthesize a gene of interest
  - Millions of recombinant plasmids containing different segments of foreign DNA are produced
  - This collection is called a genomic library

- Once a genomic library is created, biologists must identify the bacterial clone containing the desired gene
  - A specific sequence of radioactive nucleotides matching those in the desired gene can be created
  - This type of labeled nucleic acid molecule is called a nucleic acid probe

- How a DNA probe tags a gene

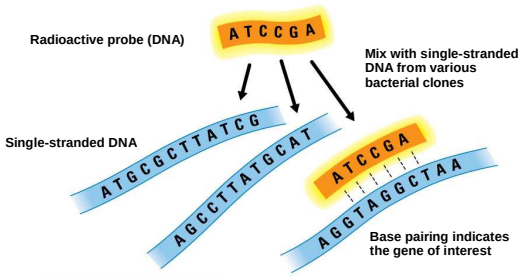


Figure 12.11

- Reverse transcriptase is another method for synthesizing a gene of interest

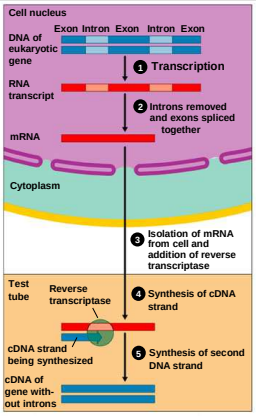


Figure 12.12

DNA FINGERPRINTING AND FORENSIC SCIENCE

- DNA technology has rapidly revolutionized the field of forensics
  - Forensics is the scientific analysis of evidence from crime scenes
- DNA fingerprinting can be used to determine whether or not two samples of genetic material are from the same individual

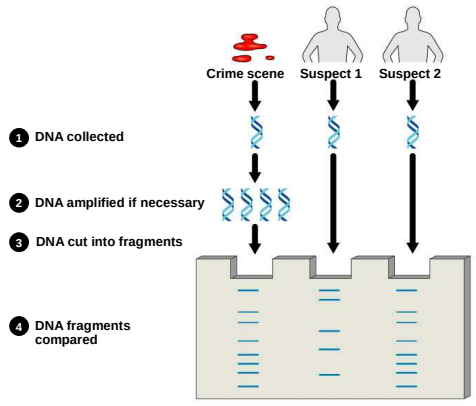


Figure 12.13

Murder, Paternity, and Ancient DNA

The First Case

- In 1983 and again in 1986, young girls were raped and murdered near Narborough, England
  - The killer left behind few clues, except for semen
  - A man confessed to the second murder, but denied committing the first

- Police turned to a professor at Leicester University who had recently developed the first DNA fingerprint identification system
  - He compared DNA from samples collected at both murder scenes and concluded that both murders had been committed by the same killer
  - Surprisingly, DNA from the suspect did not match either crime scene
- The case was finally broken using DNA fingerprinting and the killer was brought to justice

Crimes and Other Investigations

- Since its introduction in 1986, DNA fingerprinting has become a standard part of law enforcement
  - This type of evidence has been used in many cases
  - It can prove innocence or guilt

Figure 12.14

- DNA fingerprinting has also been used to identify victims of the World Trade Center attack
- In evolution research, this technique has been used to study ancient pieces of DNA, such as that of Cheddar Man

DNA Fingerprinting Techniques

The Polymerase Chain Reaction (PCR)

- The polymerase chain reaction (PCR) is a technique by which any segment of DNA can be amplified (cloned)
  - Through PCR, scientists can obtain enough DNA from even minute amounts of blood or other tissue to allow DNA fingerprinting

- DNA amplification by PCR

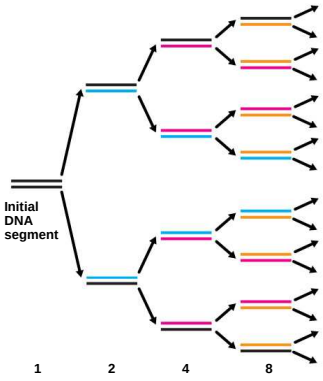


Figure 12.15

Restriction Fragment Length Polymorphism (RFLP) Analysis

- DNA fingerprinting relies on indirect methods to compare samples
  - One method is called RFLP analysis
  - RFLP analysis is the comparison of a set of restriction fragments produced by DNA from different individuals
  - RFLP stands for restriction fragment length polymorphism

- The goal of DNA fingerprinting by RFLP analysis is to determine whether or not samples of DNA contain identical markers
  - A genetic marker is a chromosomal landmark whose inheritance can be studied

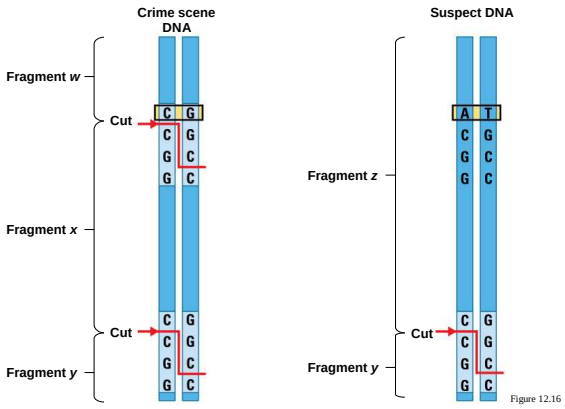


Figure 12.16

Gel Electrophoresis

- The first step of RFLP analysis is to cut up a sample of DNA with a restriction enzyme
  - This creates a mixture of restriction fragments
- The next step is to determine the number and size of fragments

– Gel electrophoresis is a method for sorting these fragments based on their length and electrical charge

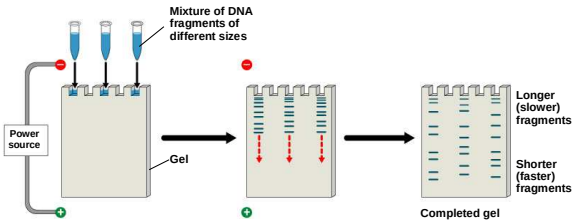


Figure 12.17

- The DNA fragments are visualized as "bands" on the gel
  - The bands of different DNA samples can then be compared

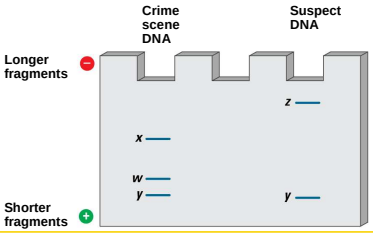


Figure 12.18

- DNA fingerprints from a murder case

Figure 12.19

GENOMICS

- Genomics is the science of studying whole genomes
  - The first targets of genomics were pathogenic bacteria

The Human Genome Project

- In 1990, an international consortium of government-funded researchers began the Human Genome Project
  - The goal of the project was to sequence the human genome

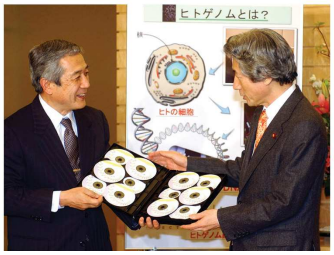


Figure 12.20

- Sequencing of the human genome presents a major challenge
  - It is very large
  - Only a small amount of our total DNA is contained in genes that code for proteins
- As of 2003, the genomes of over 100 organisms have been sequenced

Table 12.1 Some Important Completed Genomes

Organism	Date Completed	Size of Genome (in base pairs)	Approximate Number of Genes
Haemophilus influenzae (bacterium)	1995	1.8 million	1,700
Saccharomyces cerevisiae (yeast)	1996	12 million	6,000
Escherichia coli (bacterium)	1997	4.6 million	4,400
Caenorhabditis elegans (roundworm)	1998	97 million	19,100
Drosophila melanogaster (fruit fly)	2000	180 million	13,600
Arabidopsis thaliana (mustard plant)	2000	100 million	25,000
Homo sapiens (human)	2001	3.2 billion	30,000–40,000
Mus musculus (mouse)	2001	3 billion	35,000
Oryza sativa (rice)	2002	466 million	46,000-56,000

Tracking the Anthrax Killer

- In October 2001, a Florida man died from inhalation anthrax
  - By the end of the year, four other people had also died from anthrax
- Investigators analyzed the genome of the anthrax spores used in each attack

- Investigators were able to establish that the spores from all of the cases were identical
  - This suggested a single perpetrator of the crime
  - They were also able to match the anthrax with one laboratory subtype, the Ames strain
- This investigation is an example of the new field of comparative genomics

Genome-Mapping Techniques

- Human chromosomes range in size between 50 and 300 million base pairs
  - Sequencing this much DNA at once is simply not possible
- Instead, chromosomes must be chopped up into smaller pieces
  - Each piece is inserted into a vector and cloned
  - The clone is then sequenced

- The public consortium divided the genome project into three stages
  - Genetic (linkage) mapping
  - Physical mapping
  - DNA sequencing

1. Genetic (Linkage) Mapping

- Scientists combined pedigree analysis of large families with DNA technology to map over 5,000 genetic markers
- The resulting map provided anchor points
  - These enabled researchers to easily map other markers by testing for genetic linkage to known markers

2. Physical Mapping

- Researchers used restriction enzymes to break the DNA of each chromosome into identifiable fragments
  - These fragments were cloned
  - Researchers then determined the original order of the fragments in the chromosome

- Genome mapping

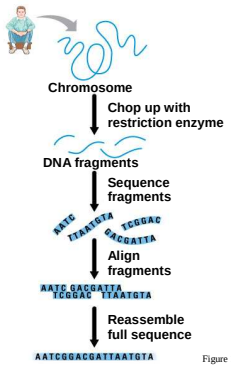


Figure 12.21

3. DNA Sequencing

- The hardest part of the project was determining the nucleotide sequences of the set of DNA fragments created in stage 2
  - Automated sequencing machines worked around the clock
  - These machines fed their results to computers that stored, analyzed, and reassembled the data

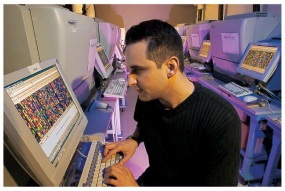


Figure 12.22

The Whole Genome Shotgun Method

- In 1998, the private company Celera Genomics entered the race to map the human genome
  - It was able to produce a draft of the human genome within three years
  - How did they do this so quickly?

- The researchers at Celera pioneered a technique called the whole genome shotgun method
  - This technique essentially skips the first two stages described and proceeds directly to the third

- In 2003, the international Human Genome Project concluded their sequencing work
  - About 99% of the human genome was complete, leaving only a few regions unsequenced
  - The DNA sequences determined by the public consortium are deposited in a database that is available on the Internet

HUMAN GENE THERAPY

- Human gene therapy is a recombinant DNA procedure that seeks to treat disease by altering the genes of the afflicted person
  - The mutant version of a gene is replaced or supplemented with a properly functioning one

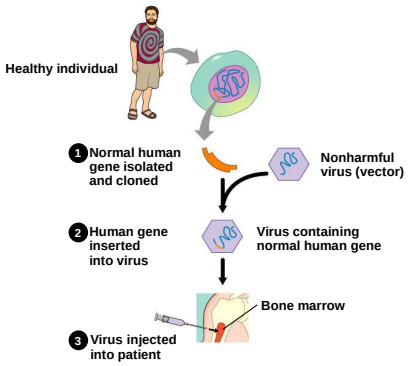


Figure 12.23

Treating Severe Combined Immunodeficiency

- SCID is a fatal inherited disease caused by a single defective gene
  - The gene prevents the development of the immune system
  - SCID patients quickly die unless treated with a bone marrow transplant

- Human gene therapy has been used to treat people suffering from SCID
  - In 2000, two infants suffering from SCID were provided with functional copies of their defective genes
  - However, the SCID study was halted in 2002, after two patients developed leukemia-like symptoms

SAFETY AND ETHICAL ISSUES

- As soon as scientists realized the power of DNA technology, they began to worry about potential dangers
  - The creation of hazardous new pathogens
  - The transfer of cancer genes into infectious bacteria and viruses

- Strict laboratory safety procedures have been designed to protect researchers from infection by engineered microbes
  - Procedures have also been designed to prevent microbes from accidentally leaving the laboratory

Figure 12.24

The Controversy Over Genetically Modified Foods

- GM strains account for a significant percentage of several agricultural crops in the United States
  - In 1999, controversy over the safety of these foods prompted protests throughout Europe

Figure 12.25

- Advocates of a cautious approach have two primary concerns
  - Fear that crops carrying genes from other species might harm the environment
  - Fear that GM foods could be hazardous to human health

- Negotiators from 130 countries (including the United States) agreed on a Biosafety Protocol
  - The protocol requires exporters to identify GM organisms present in bulk food shipments
- Several U.S. regulatory agencies evaluate biotechnology projects for potential risks
  - Department of Agriculture
  - Food and Drug Administration
  - Environmental Protection Agency
  - National Institutes of Health

Ethical Questions Raised by DNA Technology

- Should genetically engineered human growth hormone be used to stimulate growth in HGH-deficient children?

Figure 12.26

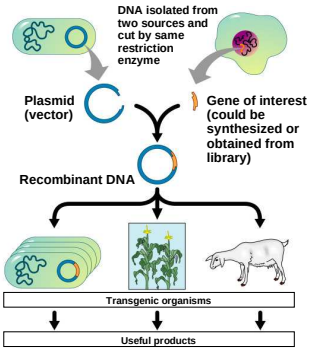
- Genetic engineering of gametes and zygotes has been accomplished in lab animals
  - Should we try to eliminate genetic defects in our children?
  - Should we interfere with evolution in this way?
- Advances in genetic fingerprinting raise privacy issues
- What about the information obtained in the Human Genome Project?
  - How do we prevent genetic information from being used in a discriminatory manner?

EVOLUTION CONNECTION: GENOMES HOLD CLUES TO EVOLUTION

- DNA sequencing has confirmed evolutionary connections
  - Such as between yeast cells and human cells
- Comparisons of DNA sequences strongly support the theory that there are three fundamental domains of life
  - Bacteria
  - Archaea
  - Eukaryotes

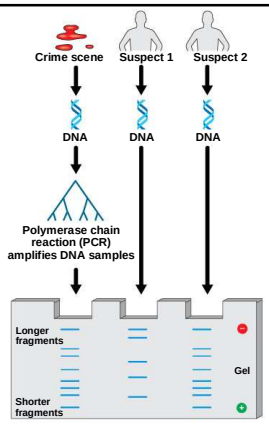
SUMMARY OF KEY CONCEPTS

- Recombinant DNA Techniques



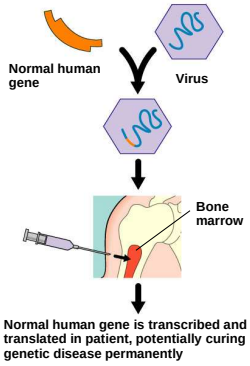
Visual Summary 12.1

- DNA Fingerprinting Techniques



Visual Summary 12.2

- Human Gene Therapy



Visual Summary 12.3