Chapter 9
Biotechnology and Recombinant DNA

Overview
- Terminology
- Tools
  - Restriction enzymes
  - Vectors
  - PCR – Polymerase Chain Reaction
- Techniques
  - DNA transfer methods
  - Obtaining DNA
  - Selecting clones
  - Making products
- Applications

Terminology
- Biotechnology: The use of microorganisms, cells, or cell components to make a product.
  - Foods, antibiotics, vitamins, enzymes
- Recombinant DNA (rDNA) technology: Insertion or modification of genes to produce desired proteins
- Vector: Self-replicating DNA used to carry the desired gene to a new cell
- Clone: Population of cells arising from one cell, each carries the new gene
- Selection: Culture a naturally occurring microbe that produces desired product
- Mutation: Mutagens cause mutations that might result in a microbe with a desirable trait
- Site-directed mutagenesis: Change a specific DNA code to change a protein
- Select and culture microbe with the desired mutation

Tools - Restriction Enzymes
- Cut specific sequences of DNA
- Destroy bacteriophage DNA in bacterial cells
- Cannot digest (host) DNA with methylated cytosines

Tools - Vectors
- Carry new DNA to desired cell
- Shuttle vectors can exist in several different species
- Plasmids and viruses can be used as vectors

Tools – PCR
- Polymerase Chain Reaction
- Enzymatic reproduction of DNA
- Used to
  - Clone DNA for recombination
  - Amplify DNA to detectable levels
  - Sequence DNA
  - Diagnose genetic disease
  - Detect pathogens

Techniques – DNA Transfer
- DNA can be inserted into a cell by
  - Electroporation
  - Transformation
  - Protoplast fusion
  - Gene gun
  - Microinjection

Techniques – Obtaining DNA
- Genomic libraries are made of pieces of an entire genome stored in plasmids or phages
- Complementary DNA (cDNA) is made from mRNA by reverse transcriptase
- Synthetic DNA is made by a DNA synthesis machine

Techniques – Making Products
- *E. coli*
  - Used because it is easily grown and its genomics are known
  - Need to eliminate endotoxin from products
  - Cells must be lysed to get product
- *Saccharomyces cerevisiae*
  - Used because it is easily grown and its genomics are known
  - May express eukaryotic genes easily
- Mammalian cells
  - May express eukaryotic genes easily
  - Harder to grow

Therapeutic Applications
- Human enzymes and other proteins
- Subunit vaccines
- Nonpathogenic viruses carrying genes for pathogen's antigens as DNA vaccines
- Gene therapy to replace defective or missing genes

The Human Genome Project
- Nucleotides have been sequenced
- Human Proteome Project may provide diagnostics and treatments
    - Reverse genetics: Block a gene to determine its function

Scientific Applications
- Understanding DNA
- Sequencing organisms' genomes
- DNA fingerprinting for identification

Forensic Microbiology
- PCR
- Primer for a specific organism will cause application if that organism is present
- Real-time PCR: Newly made DNA tagged with a fluorescent dye; the levels of fluorescence can be measured after every PCR cycle
- Reverse-transcription (RT-PCR): Reverse transcriptase makes DNA from viral RNA or mRNA

Nanotechnology
- Bacteria can make molecule-sized particles

Using Agrobacterium
- Bt toxin
- Herbicide resistance
- Suppression of genes
    - Antisense DNA
- Nutrition
- Human proteins

Safety Issues and Ethics of Using rDNA
- Avoid accidental release
- Genetically modified crops must be safe for consumption and for the environment
- Who will have access to an individual's genetic information?