Genetic Engineering

Enzymes for dicing, splicing, & reversing nucleic acids

- Restriction endonucleases – recognize specific sequences of DNA & break phosphodiester bonds
- Ligase – rejoins phosphate-sugar bonds cut by endonucleases
- Reverse transcriptase – makes a DNA copy of RNA - cDNA

Analysis of DNA

- Gel electrophoresis- separates DNA fragments based on size
- Nucleic acid hybridization & probes – probes base-pair with complementary sequences; used to detect specific sequences
- DNA Sequencing – reading the sequence of nucleotides in a stretch of DNA
- Polymerase Chain Reaction – A way to amplify DNA

Genetic Engineering

- Direct, deliberate modification of an organism's genome
- Biotechnology – use of an organism's biochemical and metabolic pathways for industrial production
**Sanger DNA Sequencing**

**Polymerase Chain Reaction (PCR)**

**Methods in Recombinant DNA Technology**
- Concerned with transferring DNA from one organism to another
- Cloning vectors & hosts
- Construction of a recombinant plasmid

**Characteristics of Cloning Vectors**
- Must be readily accepted by the cloning host
- Plasmids – small, well-characterized, easy to manipulate & can be transferred into appropriate host cells through transformation
- Bacteriophages – have the natural ability to inject their DNA into bacterial hosts through transduction
- Bacterial Artificial Chromosomes – Carry very large insert fragments (150,000+ nucleotides)

**Vector Considerations**
- Origin of replication
- Size of donated DNA vector will accept
- Gene which confers drug resistance to their cloning host to act as marker
Characteristics of Cloning Hosts

- Rapid turnover, fast growth rate
- Can be grown in large quantities using ordinary culture methods
- Nonpathogenic
- Genome that is well characterized
- Capable of accepting vectors
- Maintain foreign genes through multiple generations
- Produce a high yield of proteins from expressed foreign genes

Biochemical Products of Recombinant DNA Technology

- Enables large scale manufacturing of life-saving hormones, enzymes, vaccines
  - insulin for diabetes
  - human growth hormone for dwarfism
  - erythropoietin for anemia
  - Factor VIII for hemophilia
  - HBV vaccine