Chapter 9: DNA-Based Information Technology

- DNA Cloning
  - Methods
  - Requirements
- Site-directed Mutagenesis
- cDNA Synthesis
- Polymerase Chain Reaction (PCR)
- DNA Fingerprinting

Figure 1: DNA Cloning

- To combine DNA:
  - Hydrolyze vector (plasmid) and foreign DNA with restriction endonucleases
    - Should generate sticky ends
  - Ligate fragments together with DNA ligase
    - Sticky ends form small double helices
- Bacteria are transformed with recombinant vector
- Transformed bacteria reproduce plasmid

Table 9-2: Recognition Sequences of Restriction Endonucleases

- Usually palindromic, frequently six nucleotides long
- Restriction enzymes cleave both strands between the same two bases in palindrome

Figure 2: Restriction Enzymes and DNA

- Dimeric enzyme interacts with both strands of DNA, hydrolyzes both in the same place

Figure 3: Polylinker

- Cloning vector with multiple restriction sites allows for more flexibility in which restriction enzymes to use

Figure 4: Elements of Cloning Vector

- Origin of replication
  - DNA must have a specific sequence for replication machinery to duplicate
- Antibiotic resistance
  - Serves as a selection factor for isolating transformed bacteria

Figure 12: Site-Directed Mutagenesis

- Oligonucleotide-directed synthesis
- Utilize primers for DNA synthesis which have mutation encoded into them
- DNA synthesized with engineered primer will have encoded mutation

Figure 14: Synthesis of cDNA from RNA

- Eukaryotic mRNA is a single-stranded polynucleotide with a 3' poly-A tail.
- Poly-A tail will hybridize to a poly-T primer
- Reverse Transcriptase extends the primer, synthesizing the DNA complementary to the RNA sequence.

Figure 16: Polymerase Chain Reaction (PCR)

- DNA is heated to ~95 C to separate two strands
- Temperature is lowered to ~68 C to anneal primers to ssDNA
- Temperature is raised to ~72 C to allow thermostable polymerase to extend primer in 5'-3' direction
- Every time cycle is repeated, target sequence is doubled
- After several cycles, most copies will have only sequence flanked by primers

Box 9-1: DNA Fingerprinting

- DNA samples are digested with restriction enzymes, run on gels
- DNA fragments are probed for specific sequences
- DNA fragment length (and resulting migration distance) depends on the presence of Restriction Fragment Length Polymorphisms
  - Variation in length of tandem repeat sequences
  - Mutations which create or destroy restriction sites