Lecture Set 1:

I. Genomics
   A. Structural Genomics – To obtain sequence information for the entire genome
   B. Functional Genomics – development and application of genome-wide experimental approaches to asses gene function that make use of the information provided by Structural Genomics
   C. Cloning Vs. Genomics
      1. Cloning – identify and study genes one at a time
      2. Genomics – study ALL genes at the same time
         a. Discovery-based science – identify all the elements of a biological system and place them in a database
         b. Systems level analyses - Use genome wide datasets to generate experimentally testable hypotheses

II. Molecular Biology Toolbox
   A. Restriction Enzymes
      1. Arber, O. Smith, Nathans
      2. Bacteriophages – those that successfully infect certain bacteria have DNA that is modified with methyl groups – this prevents them from being degraded by bacterial restriction enzymes
      3. Restriction Enzymes → Recombinant DNA technology
   B. PCR
      1. Rapid purification and cloning of specific DNA fragments
         a. 94° - Denatures Genomic DNA
         b. 50-60° - Primers base pair at sites flanking target sequence
         c. 72°- Elongation of Primers using Taq polymerase
   C. DNA Hybridization
      1. Most important uncatalyzed biochemical reaction
      2. Interaction of two DNA strands with complementary sequences
      3. Natural propensity of complementary ssDNA molecules to form double helices
      4. Necessary for many molecular biology techniques
         a. Southern & Northern blotting
         b. Microarray analysis
         c. PCR
      5. Hybridization Kinetics influenced by:
         a. Length and percent identity/homology
         b. Reaction temperature and salt conditions
         c. Concentration of the nucleic acid species
   D. RNA Retroviruses
      1. Retrovirus contains an RNA genome and Reverse transcriptase enzyme
      2. Upon entry into cell Reverse Transcriptase replicates viral RNA→Viral DNA which is incorporated into host genome

3. Reverse transcriptase is an RNA-dependent DNA Pol that also has DNA-dependent DNA Pol and RNase H Activities
4. Used to generate cDNA libraries from mRNA

E. Production of cDNA libraries:
1. lyse cells from specific tissue
2. purify mRNA
3. hybridize with poly T primer (complementary to poly A-tail)
4. Make DNA copy with Reverse Transcriptase
5. Degrade RNA with RNase H
6. Synthesize A complementary DNA strand using DNA Pol; RNA fragments act as a primer

F. Genomic Libraries vs. cDNA Libraries
1. Both start with chromosomal DNA
2. Genomic: Chromosome is fragmented using Restriction Nucleases; cDNA: mRNAs are transcribed and spliced
3. Genomic: use DNA cloning to duplicate these fragments; cDNA: Reverse Transcriptase to make DNA from RNA
4. Genomic: all DNA; cDNA: no introns (spliced out during RNA transcription)

G. Sequencing
1. Human Genome Project Goals:
   a. generate physical, genetic and sequence maps of the human genome
   b. sequence variety of model organisms
   c. Develop improved technologies for mapping and sequencing
   d. Develop computational tools for capturing, storing, analyzing, developing and distributing map and sequence information
   e. Sequence EST (expressed sequence tags) fragments of cDNAs and eventually full-length cDNAs encoding the expressed mRNAs in different cells
      i. EST- short subsequence of a cDNA sequence; used to identify gene transcripts
   f. To consider the ethical, social and legal challenges posed by genomic information
2. Sanger Sequencing Method:
   a. Uses chain-terminating, dideoxyribonucleotides – lack of OH on 3' carbon prevents extension of strand
   b. Process is easily automated; important for sequencing large genomes
   c. Manual – separate strands on gel by electrophoresis
   d. Automated each terminal nucleotide is fluorescently labeled a different color
3. Shotgun sequencing

   a. Randomly cut with four base recognition restriction enzyme
   b. Clone the fragments into vectors
   c. Sequence using vector-based primers
   d. Look for overlaps and use them to derive the order of fragments
4. Heirarchical Sequencing – used by HGP
   a. Generate genomic BAC library and identify minimally overlapping BACs that cover the entire genome
   b. Use Shotgun strategy to sequence each BAC then assemble the entire sequence
   c. Depends on prior construction of genetic and physical maps
5. Whole Genome Shotgun Sequencing – Used by Celera
   a. no physical map required; entire genome sheared and sequenced from vectors

Lecture 2

I. Positional Cloning Steps

A. Correlate Phenotypic transmission of the trait with one area of the genome:

1. Use a lower resolution genetic map to place the gene on a particular chromosomal segment
   a. Genetic Maps – indicate the level of recombination between positions on a chromosome
   b. Use DNA markers –polymorphisms to measure recombination
   c. 1cM = 1% Recombination; recombination proportional to distance between two loci
   d. Genetic Distance can be an inexact measure of physical distance between two loci: Recombination between two somewhat distant markers can be lethal, markers can be in the same gene
2. Increase the resolution of the linkage map to more precisely position the affected gene