Lecture Outline

I. Recombinant DNA methods grew out of research in microbial genetics
   A. Research on bacteriophages laid the foundation for recombinant DNA methods
   B. Restriction enzymes are "molecular scissors"
      1. Restriction enzymes cut DNA in areas of specific base pair sequences that are palindromic
      2. Staggered cuts in palindromic regions leave strands with complementary ("sticky") ends
      3. Segments of DNA with "sticky" ends can be joined with DNA ligases
      4. Restriction enzymes vary in the number of DNA bases they recognize
         a. A restriction enzyme that recognizes a large number of bases has a low probability of cutting in an inappropriate spot and is useful in research on entire chromosomes
   C. Recombinant DNA is formed when DNA is spliced into a vector
      1. Bacteriophages or plasmids are common vectors
      2. The vector can be introduced into the host cell by transformation
         a. Transformation involves making the bacterial cell wall permeable to the plasmid
         b. Plasmids often carry genes for resistance to antibiotics
         c. Plasmids can carry a DNA segment of smaller than 10 kb
         d. Bacteriophage vectors can carry segments of up to 15 kb
      3. Engineered viruses can also be used as vectors
   D. DNA can be cloned inside cells
      1. A genomic library contains fragments of all of the DNA in the genome
      2. Cloning techniques provide the means for replicating and isolating many copies of a specific recombinant DNA molecule
         a. After the restriction enzyme cuts the DNA to be cloned, the fragments, all with complementary ends, are of different lengths
         b. The restriction enzyme cuts both the plasmid and the DNA of the organism with the gene of interest
         c. The two types of DNA are mixed, which allows pairing of the complementary ends
         d. The gene is then cloned in E. coli
         e. The cells are incubated on a medium with antibiotics
         f. The selection scheme allows only the cells with the recombinant genes to survive, as plasmids often confer resistance to antibiotics
         g. Each recombinant bacterium gives rise to a colony which is clonal
3. A specific DNA sequence can be detected by a complementary genetic probe
   a. A probe is a radioactively labeled segment of RNA or single-stranded DNA that is complementary to the target sequence
   b. The probe hybridizes to the sequence of interest
   c. The colony with the hybridized sequence is now radioactively labeled and can be detected by x-ray film
4. A cDNA library is complementary to mRNA and does not contain introns
   a. Bacteria cannot remove introns, so reverse transcriptase is used to make a DNA copy of the mRNA
   b. The complementary DNA (cDNA) can then be inserted into the DNA of a plasmid or virus
   c. Comparison of cDNA and genomic DNA allows identification of introns and exons
   d. cDNA sequences are useful because they lack introns and bacteria are therefore able to read them and produce a functional protein product
E. The polymerase chain reaction is a technique for amplifying DNA in vitro
   1. PCR allows amplification of a small amount of targeted DNA in a short time
   2. A DNA sequence is first heated to separate the strands
      a. The solution is then cooled
      b. It is then exposed to DNA polymerase and specific primers to produce two identical strands
   3. The process is repeated over and over to produce millions of copies of the original DNA strand
   4. A DNA polymerase (Taq) from a thermophilic bacterium is used as it is not adversely affected by the heating process
   5. The use of PRC is virtually limitless
      a. One minor drawback is the sensitivity of this process, and samples can easily be contaminated
F. Gel electrophoresis is the most widely used technique to separate macromolecules
   1. DNA and RNA are negatively charged, and move through a gel at varying speeds due to different molecular sizes (lengths)
   2. DNA fragments are often transferred to a filter, denatured, and incubated with a radioactive probe
      a. This resultant “blot” is used for autoradiography and further studies
      b. This type of blot is called a Southern blot after its inventor, E.M. Southern
         1). The Southern blot can be used to diagnose genetic disorders
      c. Blots used for RNA are called Northern blots, the Western blot is used for protein molecules
G. A great deal of information can be inferred from a DNA nucleotide sequence
1. Incorporation of dideoxynucleotides allows the investigator to determine the sequence of bases
   a. Dideoxynucleotides are modified nucleotides that prevent elongation of the DNA strand
   b. Reaction mixtures are prepared containing DNA polymerase, radioactively labeled primers, 4 deoxynucleotides, and only one of each of the four dideoxynucleotides (ddATP, ddCTP, ddGTP or ddTTP)
   c. Fragments of varying length are therefore formed in each mixture, which may be separated based on length
2. Much of this sequencing is now automated, and can be done rapidly
3. The sequence of E. coli is now completely known, and the partial sequences of many eukaryotes, including humans, is being determined by many private and public groups on an international scale
4. Restriction fragment length polymorphisms (RFLPs) are a measure of genetic relationships
   a. A restriction enzyme cuts DNA into fragments, which are of different lengths in different individuals
   b. A DNA fingerprint produced by gel electrophoresis of these fragments shows a characteristic and individual pattern of banding
   c. This technology is particularly important in determination of paternity, and in forensics

II. Genetic engineering has many applications
   A. Human insulin produced by E. coli was one of the first engineered proteins
      1. Human growth hormone and many other products are now produced by E. coli
   B. Additional engineering is required for a recombinant eukaryotic gene to be expressed in bacteria
      1. Introduced genes are not necessarily expressed by bacteria
      2. Combining a strongly expressed bacterial promoter sequence with the gene of interest may increase expression
      3. Introduction of a cDNA copy may improve production
   C. Transgenic organisms have incorporated foreign DNA into their cells
      1. Viruses may be used as vectors to introduce DNA into animal or plant cells
      2. Transgenic animals are valuable in research and may have commercial uses
         a. Transgenic animals may be produced by injecting the DNA of interest into a fertilized egg cell
         b. Some transgenic animals produce a useful protein that can be extracted from their milk (e.g. sheep)
         c. Retroviruses may be used as vectors
      3. Transgenic plants are increasingly important in agriculture
         a. Agrobacterium tumefaciens (the crown gall bacterium) is often used in plant biotechnology
            1). The Ti (tumor-inducing) plasmid is used as a vector
2). The Ti vector primarily infects dicot plants
   b. Genetic “shotguns” may be used for monocots
      1). Most of our important agricultural plants are monocots
   c. Current research involves manipulation of the chloroplast DNA

III. Safety guidelines have been developed for recombinant DNA technology
   A. Concerns about the accidental release of genetically engineered microbes has been groundless to date (see Lecture Enrichment)
   B. Stringent restrictions exist in areas of biotechnology that are not well known, or where potential for hazards are known