Manipulating and sequencing DNA

Chem 210

2/9/00

Where are we so far?

- The primary component of genes is *DNA*.
- The *sequence of nucleotides* within genes encodes information.
- Most genes code for the synthesis of *proteins*--the nucleotide sequences determine amino acid sequences (according to the *genetic code*)
- DNA contains a template for its own replication.

The human genome

- A ***genome*** is the collection of genetic information contained within the cell.
- The human genome is contained on 23 chromosomes (most cells have two copies, one form each parent, to make 46 total).
- There are about 100,000 genes in the human genome.
- There are about 3.3×10^9 nucleotide base pairs of DNA.
- Some organelles also contain DNA; the mitochondrial genome has 35 genes with 16,569 base pairs.
- By comparison: the *E. coli* genome contains about 5000 genes with about 5×10^6 base pairs of DNA.

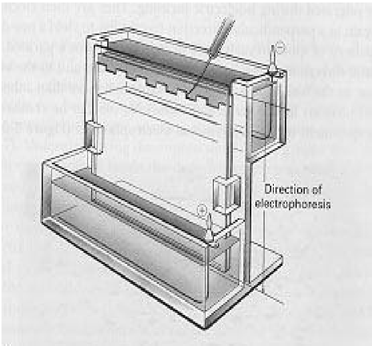
The human genome project

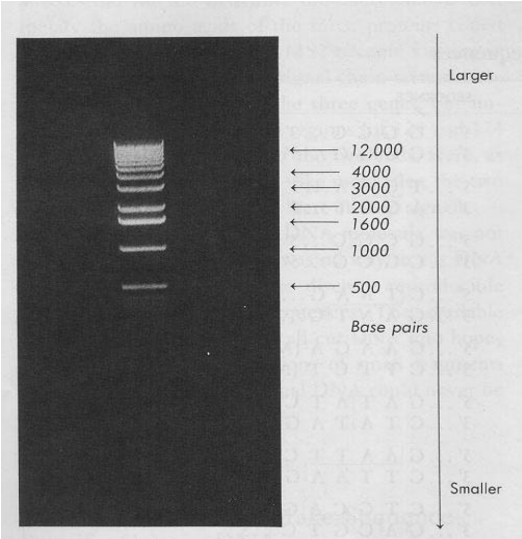
- A major research program directed mapping and sequencing all 100,000 genes of the human genome.
- The end result should be a detailed molecular blueprint.
- In the process, many genes associated with diseases have/will be identified.

Molecular biologists have developed a versatile tool box for working with DNA

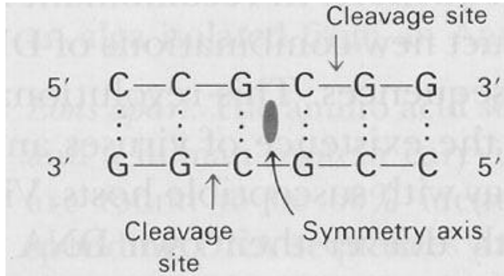
- ***Electrophoresis***: separate DNA strands based on size.
- ***Restriction endonucleases*** (***restriction enzymes***): cut DNA at specific sequences.
- ***DNA ligases***: covalently attach two different strands of DNA.
- ***Reverse transcriptase***: synthesizes a DNA strand from an RNA template.
- ***DNA polymerases***: synthesize new DNA strands from a DNA template.
- ***DNA sequencing***: determine the sequence of a DNA strand

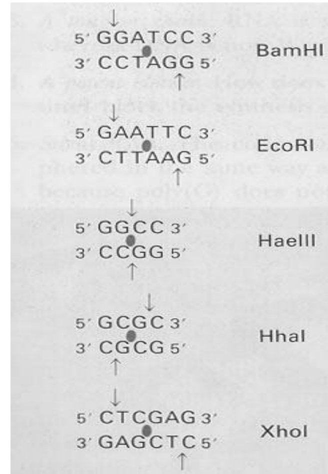
Electrophoresis can be used to separate DNA fragments by size





Restriction enzymes cleave DNA at specific sequences

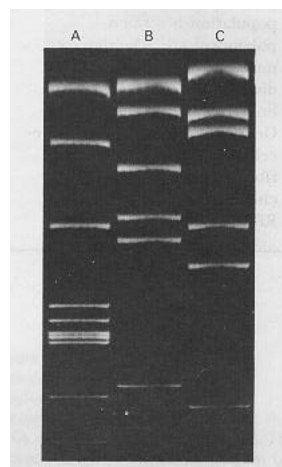




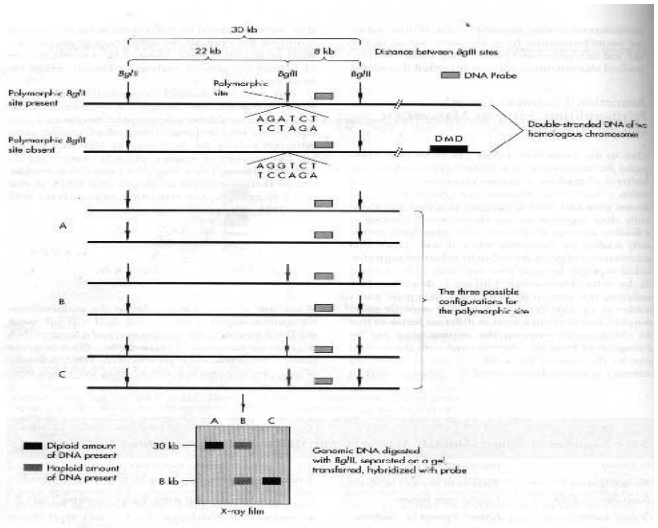
- Usually cleave at palindromic sequences.
- Can cleave to leave “sticky ends” or “blunt ends”
- Many restriction enzymes are known

Chromosomes can be analyzed by a restriction enzyme map

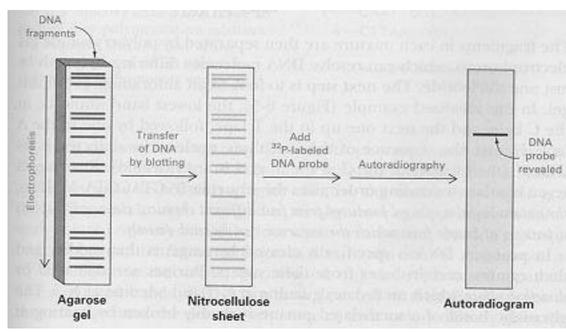
- Cut a large piece of DNA with a restriction enzyme;
- A large piece of DNA will likely contain multiple restriction enzyme cleavage sites;
- Two pieces of DNA can be compared based on the number of fragments (and size of fragments) produced by a restriction enzyme digest.
- This provides “low resolution” information on a DNA sequence.



Diseases can be detected by restriction fragment length polymorphisms (RFLPs)



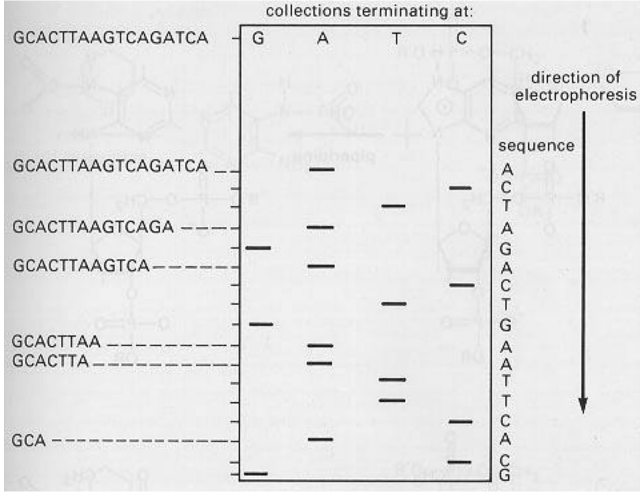
DNA fragments in gels can be probed by Southern blotting



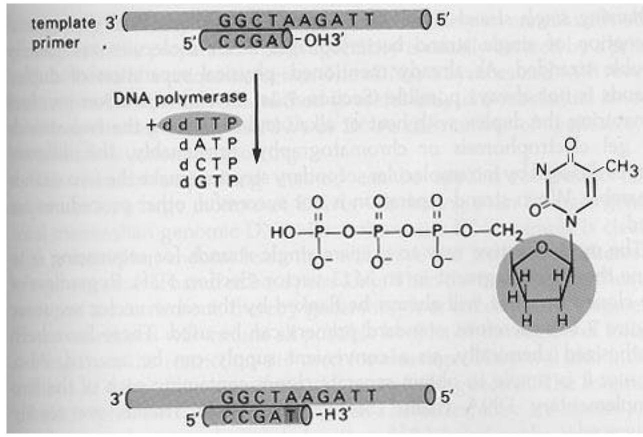
There are two major strategies for DNA sequencing

- Maxam-Gilbert method: use chemical techniques to break DNA into fragments; analyze fragments by electrophoresis
- Sanger (dideoxy- terminator) method: use enzymatic digestion to break DNA into fragments; analyze by electrophoresis

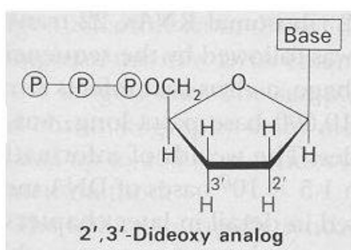
Maxam-Gilbert sequencing

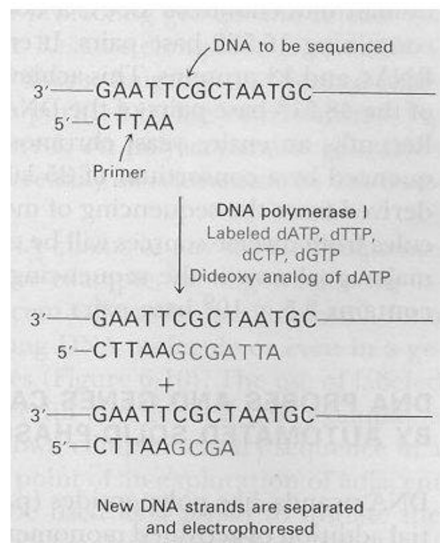


Sanger sequencing: general scheme



DNA polymerase cannot extend a chain once a dideoxy nucleotide has been incorporated





This can be exploited to create a series of DNA fragments

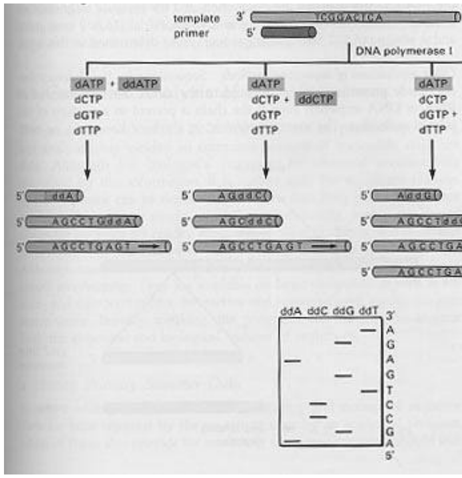
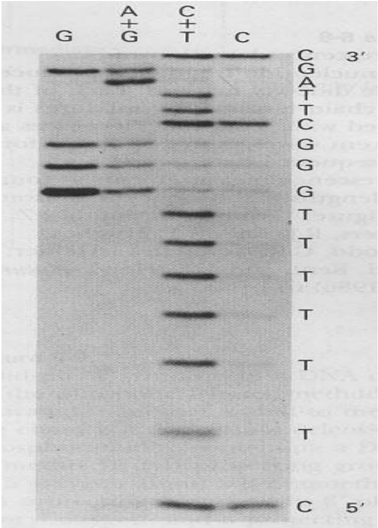
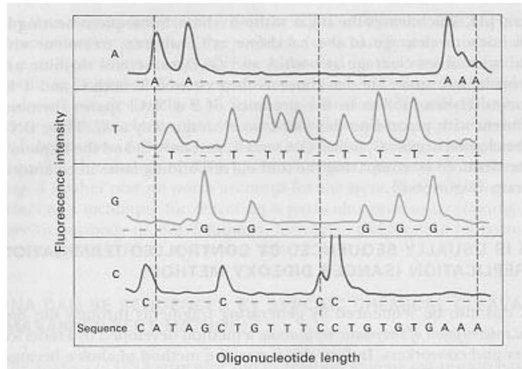


Figure 7.12
The "dideoxy" method for DNA sequencing, including a schematic diagram (below) of the resulting autoradiogram. The radiolabeled triphosphate is α-^{32}P-deoxyATP.

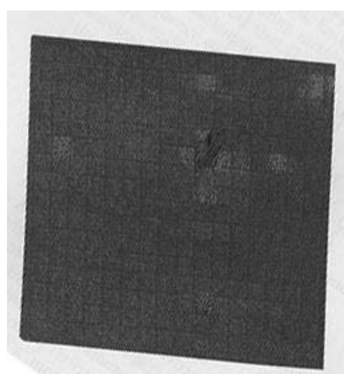
The sequence can be read from an electrophoresis gel



The Sanger method can be combined with fluorescence detection and automated



DNA chips may be a quick method of obtaining sequence information



- An array of oligonucleotides can be chemically attached to a silicon chip (like a computer chip).
- The chip is then exposed to an oligonucleotide of unknown sequence.
- An electrical or optical signal is produced if the unknown oligonucleotide binds to one of the oligos in the array.

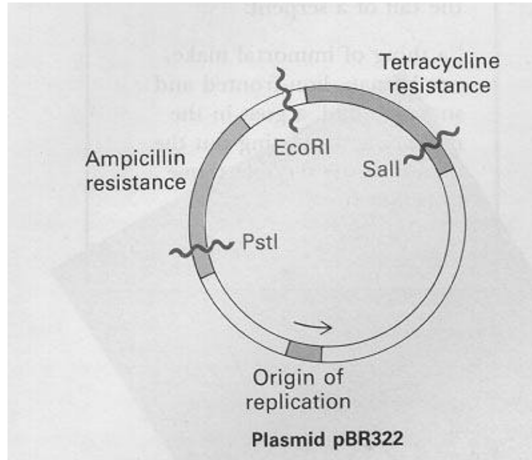
How do we get the DNA to analyze? DNA cloning: making copies of DNA

- Cloning of genomic DNA: break the chromosomal DNA into pieces, make a lot of copies of these fragments.
  - Advantage: get the "real" DNA to analyze
  - Disadvantage: a lot of that DNA can't be understood (introns, repeat sequences, etc.).
- Cloning "cDNA": the DNA which is complementary to the RNA of a cell.
  - Advantage: get the important, coding parts of the genome.
  - Disadvantage: missing all of the mysterious parts; not easy to relate to position within chromosomes.

Foreign DNA can be incorporated into vectors

- Plasmids: "extra" (non-chromosomal) DNA elements carried by bacteria.
  - Plasmids are small, circular DNA strands.
  - Bacteria can pick up plasmids from the environment (*transformation*).
  - Bacteria can pass plasmids from one cell to another (*conjugation*).
- Phage: viruses which attack bacteria
  - Contain small genome (short strand of DNA)
  - Need machinery of a host cell to reproduce
  - Infect a host cell; use the cell's machinery to make copies of itself (and its DNA).

Structure of a plasmid



Life cycle of a bacteriophage

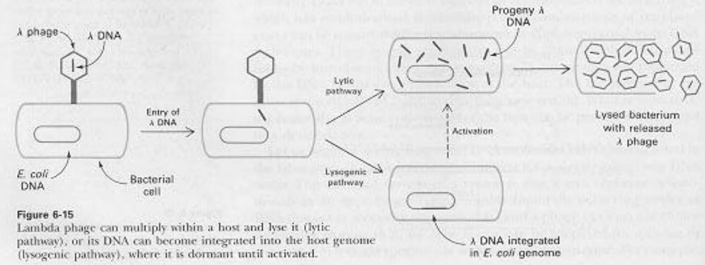


Figure 6-15
Lambda phage can multiply within a host and lyse it (lytic pathway), or its DNA can become integrated into the host genome (lysogenic pathway), where it is dormant until activated.

Cloned DNA fragments can be introduced into plasmid vectors

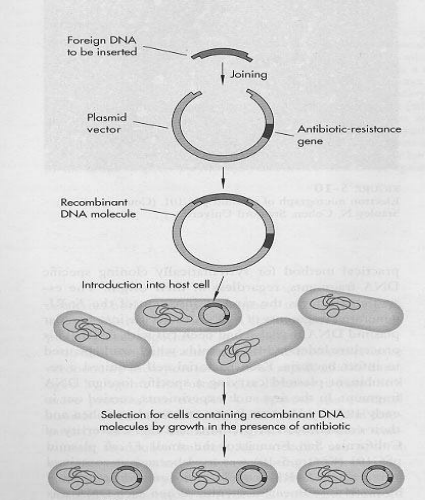


FIGURE 5–11
The cloning of DNA in a plasmid.

DNA can be incorporated into phage DNA

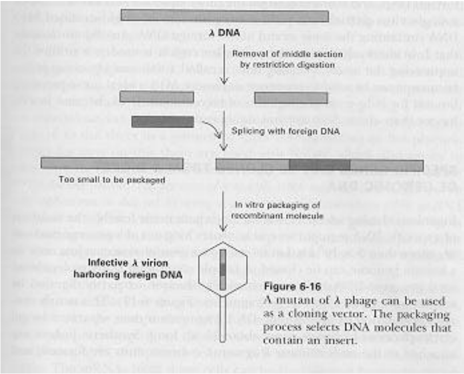
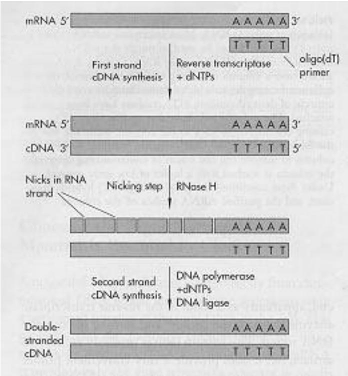


Figure 6-16
A mutant of λ phage can be used as a cloning vector. The packaging process selects DNA molecules that contain an insert.

Genome DNA can be put into libraries

- Isolate chromosomal DNA and break into fragments (using physical, chemical, or enzymatic techniques).
- Insert these fragments into vectors(perhaps taking advantage of restriction-enzyme generated “sticky ends”).
- The vectors can be used to generate a large number of copies of each of the fragments.
- These “libraries” of DNA fragments can be probed by various techniques (Southern blotting, DNA chips, etc.).
- DNA from the libraries can be sequenced or analyzed for RFLPs.

RNA can be converted to DNA using *reverse transcriptase*



• Using RNA as a template, generate a DNA strand.
• This single strand of DNA can be converted to a double strand by a DNA polymerase.

mRNA can be isolated and cloned from a cell