DNA

1. Evidence for DNA as the genetic material.
   a. Until the 1940s, proteins were believed to be the genetic material.
   b. In 1944, Oswald Avery, Maclyn McCarty, and Colin MacLeod announced that the transforming substance was DNA. They followed up on work done by Frederick Griffith in 1928 on the ability of one strain of bacteria which caused pneumonia in mice to transform a strain that could not cause pneumonia.
   c. More evidence that DNA was the genetic material came from studies of the infection of bacteria by viruses.
      i. In 1952, Alfred Hershey and Martha Chase showed that DNA was the genetic material of a bacteriophage.   They used radioactive S to label the protein of the bacteriophage and allowed it to infect the host bacterium.   After centrifugation, the radioactive protein was found in the supernatant.   This showed that protein is not injected into host.   Then, radioactive P was used to label the nucleic acid of the virus.   After infection and centrifugation, the radiation was in the pellet with the bacterial cells.   This showed that nucleic acid is injected into host bacterium during infection.
   d. The fact that cells double the amount of DNA in a cell just before they divide and then distribute the DNA equally to each daughter cell provided some circumstantial evidence that DNA was the genetic material.
   e. In 1947, Erwin Chargaff had made some observations regarding the composition of DNA.   He noted that:
      i. the DNA composition varies from species to species.
      ii. in any one species, the four bases are found in characteristic, but not necessarily equal, ratios.   If the bases were chosen randomly, species would be expected to have the same relative amounts of each nucleotide.
      iii. in all organisms, the amount of adenine was approximately equal to the amount of thymine (%T = %A) and that the amount of guanine was approximately equal to the amount of cytosine (%G = %C).

2. The structure of DNA.
   a. Maurice Wilkins and Rosalind Franklin used X-ray crystallography to study the structure of DNA.
   b. James Watson and Francis Crick worked out the 3D structure of DNA using molecular models made of wire
      i. The molecule consists of 2 chains wound together in a spiral (*i.e.*, a double helix).
      ii. The sides of the chains are made of alternating sugars and phosphates, like the sides of a rope ladder.
      iii. The ladder forms a twist every ten bases.
      iv. Pairs of nitrogenous bases, one from each strand, form the rungs of the ladder.   In order for the ladder to have a uniform width, a small base must be paired with a large base. A pairs with T and C pairs with G. This is called complementary base pairing.
      v. The two strands are held together by hydrogen bonding between bases.
      vi. Note that the chains have direction. Each strand has a 3= end with a free OH group attached to deoxyribose and a 5= end with a free phosphate (P) group attached to deoxyribose. This arrangement is called **antiparallel**.

3. Replication of DNA
   a. When a cell divides, the DNA must be doubled so that each daughter cell gets a complete copy.   It is important for this process to be high fidelity because any errors made would be inherited by the offspring and these errors would tend to accumulate with each generation.
   b. Because each strand is complementary to the other, each can form a template when separated. When a cell copies a DNA molecule, each strand serves as a template for ordering nucleotides into a new complementary strand.   One at a time, nucleotides line up along the template strand according to the base-pairing rules.
   c. An experiment in the late 1950s by Matthew Meselson and Franklin Stahl demonstrated that replication was **semiconservative**.
       (1) Bacteria that had been growing on a heavy isotope of N were allowed to grow for one generation (*i.e.*, DNA replicated once) on a light isotope of N.
       (2) The cells had DNA of one weight.   This meant that it was constructed from half heavy (old or parent) and half light (new or daughter) N.
       (3) After a second generation (*i.e.*, another replication) the cells had DNA of two distinct weights.   Some cells had DNA that was all light and some cells had DNA that was a mixture of light and heavy.   This showed that replication is semi-conservative.
   d. Origin of Replication
       i. The replication of a DNA molecule begins at special sites, **origins of replication.** A specific sequence of nucleotides marks the origin.
       ii. Humans have hundreds of origins from which replication proceeds on both strands in both directions.
       iii. At the origins, the DNA strands are separated, forming a replication Abubble@ with **replication forks** at each end.   An enzyme called **helicase** separates the strands.
   e. Elongating a new strand
       i. After the two strands are separated, DNA polymerase reads the bases on the template strand and attaches complementary bases to form a new strand.
       ii. DNA polymerase can only attach the 5' phosphate (P) of one nucleotide to the 3' hydroxyl (OH) of the previous nucleotide that is already part of a strand.   The enzyme can only work by building a new strand in the 5' □ 3' direction.
       iii. The new nucleotides (*e.g.*, ATP) that are attached lose two of their three phosphates which provides the energy to form the bond.
   f. Problem of antiparallel strands
       i. Remember that the DNA molecule is arranged with the strands going in opposite directions so the 3' end of one strand is aligned with the 5' end of the other.
       ii. DNA polymerase adds nucleotides only to the 3' end but can only do this on one strand, the **leading strand**.
       iii. The other strand has a 5' P at the end rather than a 3' OH like DNA polymerase needs.   This strand, the **lagging strand**, must be made in short fragments (**Okazaki fragments**) going in the direction opposite to the leading strand. Another enzyme, **DNA ligase**, then fills in the gaps by joining the fragments together.

g. Priming DNA synthesis
   i. DNA polymerases cannot *initiate* the synthesis of a new strand of DNA.
   ii. A short stretch of RNA (5-10 nucleotides) with an available 3= end is built. This short piece is called a **primer** and is built by **primase**, a RNA polymerase.
   iii. After formation of the primer, DNA polymerase can add new nucleotides to the 3= end of the RNA primer.
   iv. The leading strand requires the formation of only a single primer as the replication fork continues to separate. For synthesis of the lagging strand, each Okazaki fragment must have its own primer.
   v. A different DNA polymerase then replaces the RNA nucleotides of the primers with DNA nucleotides.
h. The problem of replicating the ends.
   i. Remember that DNA polymerase only works in the 5' to 3' direction and that it must attach each new nucleotide to a previous nucleotide. At the ends of the chromosomes this is not possible (because there is not a previous nucleotide) on the lagging strand.
   ii. Each time the cell divides, one strand of DNA cannot be completely replicated so a single-stranded section remains. Enzymes remove these single-stranded sections.
   iii. As a result, the chromosomes get shorter each time the cell divides.
   iv. To prevent the loss of genetic information at the ends of the chromosomes, long, repetitive sequences called **telomeres** are added to the ends.
i. Replication error rate, DNA damage and repair.
   i. The active site of DNA polymerase must recognize all four nucleotides. This means that it is difficult to determine if a nucleotide is mistakenly in the active site. Mistakes during the initial pairing of template nucleotides and complementary nucleotides occur at a rate of one error per 100,000 base pairs.
   ii. DNA polymerase checks for these errors by checking the width of the helix. The final error rate is only one per ten billion nucleotides.
   iii. Constant exposure to chemicals, viruses, and radiation also cause damage to DNA so human cells have about 130 enzymes which constantly check DNA for errors.

DNA Technology

1. Gel Electrophoresis is a central technique used in many applications of DNA technology.
   a. Gel electrophoresis is a technique used to separate DNA molecules on the basis of size, using this charge.
   b. The nucleic acid is loaded into a slot near the semisolid, porous gel at the end near the negative electrode. It is then pulled toward the positive electrode at the gel's opposite end.
   c. Smaller molecules move through the gel's pores faster than larger molecules. This difference in the migration rate separates the fragments on the basis of size.
   d. Researchers use molecules of known molecular weight as standards that run alongside the unknown molecules to provide a size comparison.
   e. The DNA is then stained with a fluorescent or colored dye so it can be seen.
   f. Distinct DNA fragments appear as bands at specific distances from the gel's top (the negative electrode end)

2. DNA profiling
   a. Although 99.9% of human DNA sequences are the same in every person, some sequences

are unique to each individual - like a fingerprint - so that they can be used to distinguish one individual from another using gel electrophoresis.

     i.   In violent crimes, blood, semen, or traces of other tissues may be left at the scene or on the clothes or other possessions of the victim or assailant.

     ii.   The process begins with a sample of an individual's DNA called a **reference sample**. To make a DNA profile, DNA can be collected from cheek cells, saliva, blood, semen, hair follicles, or any other tissue sample.

     iii.   The reference sample is then analyzed to create the individual's DNA profile which can then be compared to another sample to determine whether they are a match.

    b.   A **restriction enzyme** is an enzyme that cuts DNA at or near a specific nucleotide sequence known as a **restriction site**. These enzymes are found in bacteria and their natural purpose is to destroy viral DNA to defend against viral infection. Inside the bacterium, the restriction enzymes destroy foreign DNA by cutting it at restriction sites. Over 3000 restriction enzymes are known and more than 600 of these are available commercially.

    c.   To make a DNA profile, DNA is cut up into pieces by restriction enzymes. Because each person has slight differences in their DNA, restriction enzymes will cut at different places, resulting in a unique collection of fragments of various lengths, which results in a unique pattern of bands on the gel. By looking at the pattern of bands, we can distinguish the DNA of one person from that of another. These differences are called **restriction fragment length polymorphisms**.

     i.   Some of the uses of this technique include forensics, paternity determination, and screening for genetic disorders.

3.   Molecular cloning is the reproduction of sections of a genome.

    a.   A plasmid (found in bacteria and yeast) is a small circular DNA molecule that replicates independently of the chromosomal DNA. A gene of interest can be inserted into a plasmid.

     i.   Many restriction enzymes make staggered cuts in the two DNA strands, such that the cut ends have a 2- or 4-base single-stranded overhang.

     ii.   Because these overhangs are able to bond with complementary overhangs on other fragments, they are called "sticky ends."

     iii.   This allows any DNA fragment to be spliced into a plasmid as long as the two have been cut with the same restriction enzyme.

    b.   The plasmid containing the gene of interest can then be introduced into a bacterial cell. Each time the bacterial cell divides, the plasmid is copied so that every daughter cell contains a copy. Such a piece of DNA, containing genetic material from different sources, is called **recombinant DNA**.

    c.   This technique can be used for the large-scale production of important molecules such as insulin and human growth hormone, vaccines, or antibiotics.

4.   **Genetic engineering** is the alteration of an organism's genome using DNA technology to modify an organism's DNA to achieve desirable traits. The addition of foreign DNA generated by molecular cloning is the most common method of genetic engineering. The organism that receives the recombinant DNA is a **genetically modified organism** (GMO). If the foreign DNA comes from a different species, the host organism is **transgenic**.

    a.   Mutating or deleting genes can provide clues about the gene's function. Gene targeting is the alteration of a particular gene's expression, either by introducing mutations in a gene, or by eliminating a certain gene's expression by deleting a part or all of the gene sequence from the organism's genome. By observing the result in the organism, the function of the gene can be deduced.

b.   The identification of genes whose mutations are responsible for genetic diseases may lead to ways to diagnose, treat, or even prevent these conditions.

5.   Practical applications of DNA technology continue to be developed to address a wide range of medical, pharmaceutical, agricultural and environmental problems.
    a.   Medical researchers can use DNA technology to identify individuals with genetic diseases before the onset of symptoms, before treatment or even before birth. This process is called **genetic diagnosis by genetic testing**.
        i.   Depending on the inheritance patterns of a disease-causing gene, family members are advised to undergo genetic testing. For example, doctors usually advise women diagnosed with breast cancer to have a biopsy so that the medical team can determine the genetic basis of cancer development. Doctors base treatment plans on genetic test findings that determine the type of cancer. If inherited gene mutations cause the cancer, doctors also advise other female relatives to undergo genetic testing and periodic screening for breast cancer.
        ii.   Doctors also offer genetic testing for fetuses (or embryos with *in vitro* fertilization) to determine the presence or absence of disease-causing genes in families with specific debilitating diseases.
    b.   **Gene therapy** is a genetic engineering technique used to cure disease by altering the genes of an individual with a genetic disorder.
        i.   In its simplest form, a normal gene is introduced at a random location in the genome to aid the cure of a disease that is caused by a mutated gene. The good gene is usually introduced into diseased cells as part of a vector transmitted by a virus that can infect the host cell and deliver the foreign DNA. The hope is that those cells then multiply throughout the patient's life and express the normal gene, providing the missing protein. More advanced forms of gene therapy try to correct the mutation at the original site in the genome.
        ii.   Gene therapy poses many technical problems yet to be solved, including the regulation of the activity of the transferred gene to produce the appropriate amount of the gene product at the right time and place and ensuring the inserted gene does not harm other necessary cell functions.
    c.   Manipulating the DNA of plants (*i.e.*, creating GMO) has helped to create desirable traits, such as disease resistance, herbicide and pesticide resistance, better nutritional value, and better shelf-life.
        i.   We call plants that have received recombinant DNA from other species transgenic plants. Because they are not natural, government agencies closely monitor transgenic plants and other GMOs to ensure that they are fit for human consumption and do not endanger other plant and animal life.
        ii.   Because foreign genes can spread to other species in the environment, extensive testing is required to ensure ecological stability.
        iii.   Common transgenic crop plants include corn, potatoes, tomatoes and rice.
    d.   Genetic engineering has being applied to environmental work as well. The metabolism of microorganisms can be engineered to help deal with some environmental problems.
        i.   For example, genetically engineered microbes that can extract heavy metals from their environments.
        ii.   In addition to the normal microbes that participate in sewage treatment, new microbes that can degrade other harmful compounds are being engineered.
        iii.   Bacterial strains have been developed that can degrade some of the chemicals released during oil spills.

6. Ethical and social questions.
    a. Some people believe that tampering with human genes, even for those with life-threatening diseases, is wrong and could lead to eugenics, a deliberate effort to control the genetic makeup of human populations.
    b. We also must think carefully about whether we want to treat human germ-line cells which would affect future generations.
    c. It's important to recognize that removing alleles from the gene pool decreases genetic variation which is important for the survival of a species as environmental conditions change with time. Genes that are damaging under some conditions could be advantageous under other conditions, as in the example of the sickle-cell allele.