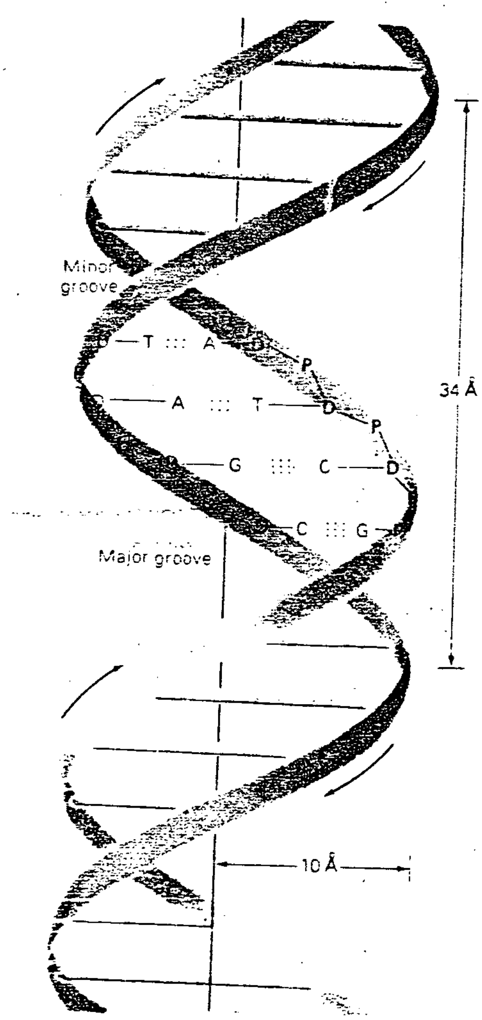
## Investigation No. 8.
# Isolation of DNA

### Background

Cells of the thymus gland posses relatively large nuclei from which large amounts of DNA can be obtained. Thymus glands can be bought from a butcher. Fresh and deep-frozen samples produce comparable results.

Over a 100 years ago it was recognized that hereditary information is located within chromosomes in the nucleus. Later, it was realized that deoxyribonucleic acid (DNA), which is a long unbranched polymer of nucleotides, carried the information in units known as genes. There are four types of nucleotides abbreviated to A, T, G and C and it is the sequence of the nucleotides in genes that code for genetic information. A, T, G and C stand for the purine and pyrimidine bases, adenine, thymine, guanine and cytosine respectively that are part of the four nucleotides. Two DNA strands are held together by hydrogen bands between A and T, G and C as shown below:



During asexual cell division, the parent DNA makes an exact copy of itself (can you see how this might be done?) and then one copy is transferred to each of the daughter cells. Genetic information stored in genes is expressed through the synthesis of proteins. Proteins as enzymes, structural proteins, etc. determine the final properties and characteristics of an organism (phenotype). As a first step, the information stored in the DNA of a gene is copied with a sequence of nucleotides in a molecule of ribonucleic acid (RNA). RNA also contains four nucleotidase, but a U instead of a T. The U pairs with A. The RNA strand is made in a manner similar to DNA replication. Matching or base pairing nucleotides corresponding to one of the DNA strands are lined up and linked together by an enzyme to make a complementary copy of the DNA sequence. The RNA is then transported to the cytoplasm of the ribosomes where the information stored in RNA as nucleotides is translated into a specific sequence of amino acids in a protein.

This experiment is designed to isolate and show some of the properties of DNA. It becomes clear that DNA is a long and fibrous molecule.

## Equipment

1 glass beaker, narrow, 100 ml
1 mortar
1 pestle
1 pair of scissors
1 scalpel
1 glass rod
1 pair of tweezers
Surgical gauze

## Material

5 to 10 g thymus glands
Abrasive sand
Tap water
Washing-up liquid
Ethanol 96% (or methylated spirit)

## Procedure

1. To produce a thymus cell suspension, cut up 5 to 10 thymuses in a mortar using a pair of scissors or a scalpel.
2. Add sand to the tissue in the mortar and grind with the pestle. Then add approximately 20 ml tap water and continue grinding until the water is completely clouded.
3. Filter the suspension through two layers of surgical gauze.
4. To liberate DNA, add approximately 5 to 19 drops of washing-up liquid to the cell suspension and shake thoroughly.
5. To obtain DNA, cover the clear, viscous suspension with approximately one and a half times the amount of cold ethanol solution in a narrow glass beaker.
6. The DNA precipitating as whitish strands can be removed with a glass rod. Rotate the rod slowly and carefully and dip it up and down occasionally to mix the suspension and the alcohol. This will precipitate a relatively large amount of nucleic acid. If the strands slip from the rod, they can be removed from the beaker using a pair of tweezers.

## Observations

Record your observations on the sheets provided.

## Discussion

1. What is the approximate stretched out length of DNA in a chromosome of man? You may deduce from what you observed that the DNA will have to be highly folded in order to fit into a thymus cell nucleus. How is this achieved?
2. Draw a sequence of 20 nucleotides and show it base pairing with a complementary strand. Illustrate how the DNA molecule that you have drawn replicates itself.
3. Twenty different amino acids make up proteins in living organisms. What is the minimum number of nucleotides needed to code for an amino acid?

Investigation No. 9.

# Conjugation in Bacteria

## Background

Genetic information coding for particular proteins such as enzymes (i.e., genes) can be transferred between bacteria. This process can take place through the simple uptake of DNA by bacterial cells (transformation), the transfer of bacterial genes that become incorporated into bacteriophages (transduction) or during plasmid or F-factor mediated conjugation. In hospitals, the transfer of resistance to antibiotics (often due to genes located within plasmids) from one bacterium to another occurs in this way.

The strain *Escherichia coli* GY767 (DSM-No. 1562) is streptomycin sensitive. The F plasmid is integrated into its chromosomal DNA and, it is able to transfer chromosomal genetic information to strains of *E. coli* that have no F plasmid. In this experiment, the recipient is *E. coli* AB1157 (DSM-No. 1563). This streptomycin-resistant strain is characterized by a large number of mutations and can no longer produce for itself the amino acids proline, leucine, arginine, threonine, or histidine, or the vitamin thiamine, which it requires to survive. These substances must therefore be present in the culture medium if growth is to occur. In the following experiment, donor cells (*E. coli* GY767,DSM-No.1562) and recipient cells (*E. coli* AB1157, DSM-No. 1563) are mixed in a ratio of 1:9. Within two hours the chromosomal genes for the biosynthesis of proline, threonine, and leucine are transferred from the donor to the recipient. Recipient cells are distinguished from the donor cells by their resistance to streptomycin. After transfer of the genes for biosynthesis of the amino acids proline, leucine, and threonine, recombinant recipient cells (now also called transconjugants) grow on minimal agar that contains arginine, histidine, thiamine, and streptomycin, but not proline, leucine, or threonine. Neither the donor, with its streptomycin sensitivity, nor the unchanged recipient, which would need the missing nutrients proline, leucine, and threonine, can grow on this minimal agar.

## Equipment

Incubator
Glass beaker, 250ml
Conical flask, 250ml
Petri dishes
Test tubes with aluminum caps or cotton wool bungs
Pipette, 5 ml, sterile
Pipettes, 1 ml, sterile
Pipette aids
Drigalski spatula
Bunsen burner

## Material

Overnight culture of *E. coli* GY767 (DSM-No. 1562)
Overnight culture of *E coli* AB1157 (DSM-No. 1563)
Nutrient broth medium for the overnight culture
Minimal agar medium
Streptomycin, 0.02 g
Distilled water
Alcohol, 70%

## Procedure

1. Using the Erlenmeyer retort, prepare minimal agar with 100 ml distilled water (see Appendix) and adjust to a pH value of 7.2 using 1 N NaOH. Seal the retort with aluminum foil and sterilize, then allow to cool to 55$^0$C. Add 0.02 g streptomycin and firmly shake the retort to dissolve it. Pour into three agar dishes.
2. Prepare 5 ml each of overnight culture of *E. coli* GY767 (DSM-No.1562) and *E. coli* AB1157 (DSM-No. 1563) (see Appendix).
3. Mix 0.3 ml *E. coli* GY767 (DSM-No. 1562) overnight culture and 2.7 ml *E. coli* AB1157 (DSM-No. 1563) overnight culture in a sterilized test tube and incubate at 37$^0$C for two hours.
4. Spread 0.1 ml of this mixture onto a minimal agar dish using the Drigalski spatula (sterilized with 70% alcohol and flamed in the Bunsen burner). Prepare two control dishes with 0.1 ml of *E. coli* GY767 (DSM-No. 1562) and *E. coli* AB1157 (DSM-No. 1563) overnight culture, respectively. Incubate all three dishes at 37$^0$C for 72 hours.

## Observations

Record your observations on the sheets provided.

## Discussions

1. Antibiotic resistance is often a convenient 'marker' for gene transfer experiments. It can select a desired small population of recombinant bacteria form a much longer population of non-recombinant bacteria. How would this concept be applied to select genetically engineered human cells?
2. Can genes be transferred between unrelated bacteria equally as well as between bacteria of the same species?
3. Outline the possible dangers of (a) the widespread use of antibiotics in animal feed (b) an incomplete course of antibiotics in treating an infection and the advantages of using a combination of antibiotics to resolve an infection.