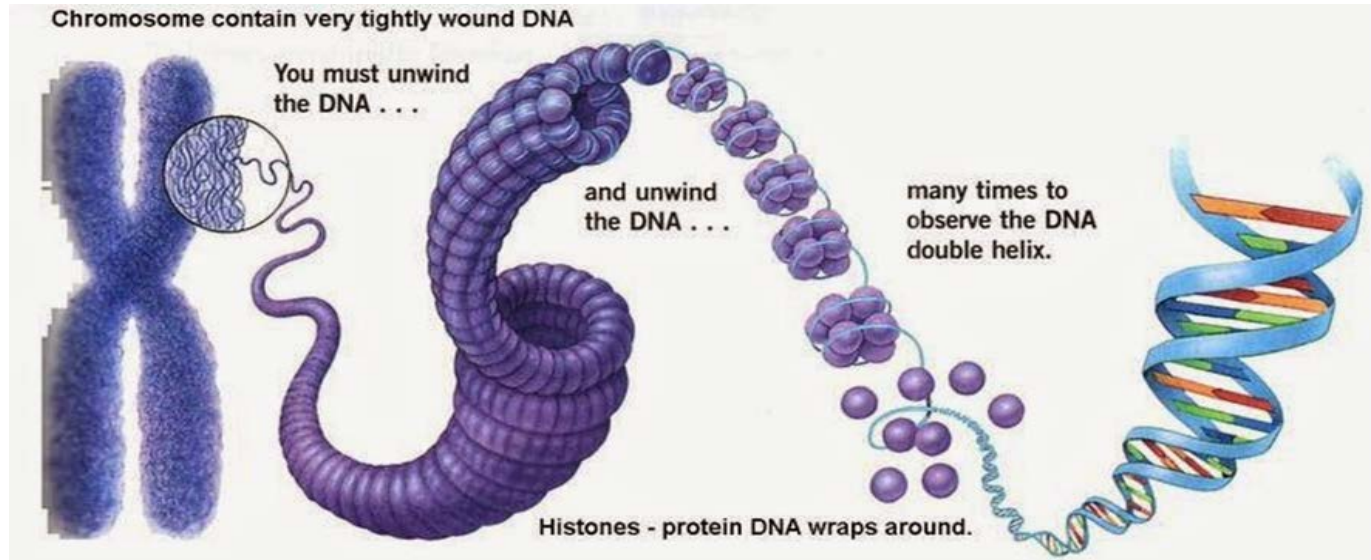


Extraction and Identification of DNA

DNA or Deoxyribonucleic acid contains all genetic information necessary for growth, functioning and reproduction of almost all living organisms. DNA molecules consist of two biopolymer strands coiled around each other to form a double helix.

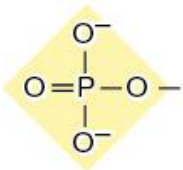


Chromosomal DNA, exists in the well-known X shape and is bound by proteins into a supercoil. In DNA,

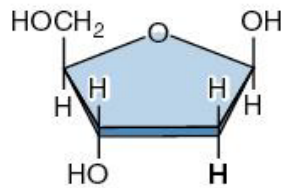
- Nucleotides form the repeating units
- Nucleotides are linked to form a strand
- Two strands can interact to form a double helix
- The double helix folds, bends and interacts with proteins resulting in 3-D structures in the form of chromosomes

STRUCTURAL COMPONENTS OF NUCLEIC ACID

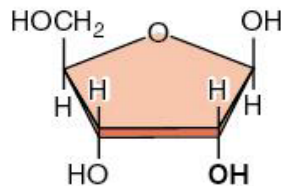
Phosphate group



Sugars

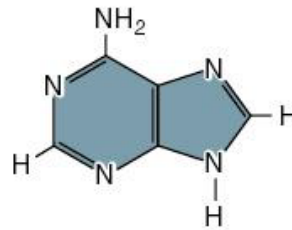


D-Deoxyribose (in DNA)

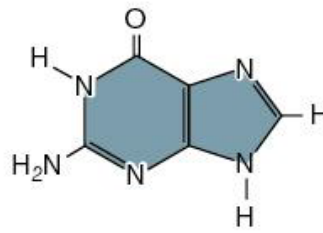


D-Ribose (in RNA)

Purines
(double ring)



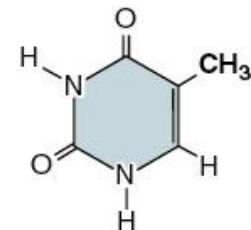
Adenine (A)



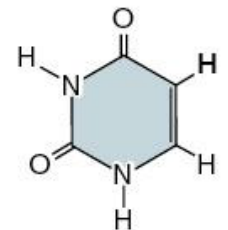
Guanine (G)

Bases

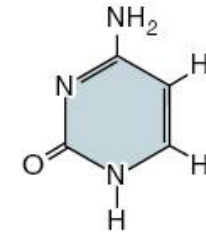
Pyrimidines
(single ring)



Thymine (T) (in DNA)



Uracil (U) (in RNA)



Cytosine (C)

STRUCTURAL COMPONENTS OF NUCLEIC ACID

➤ Base + sugar → nucleoside

➤ Example

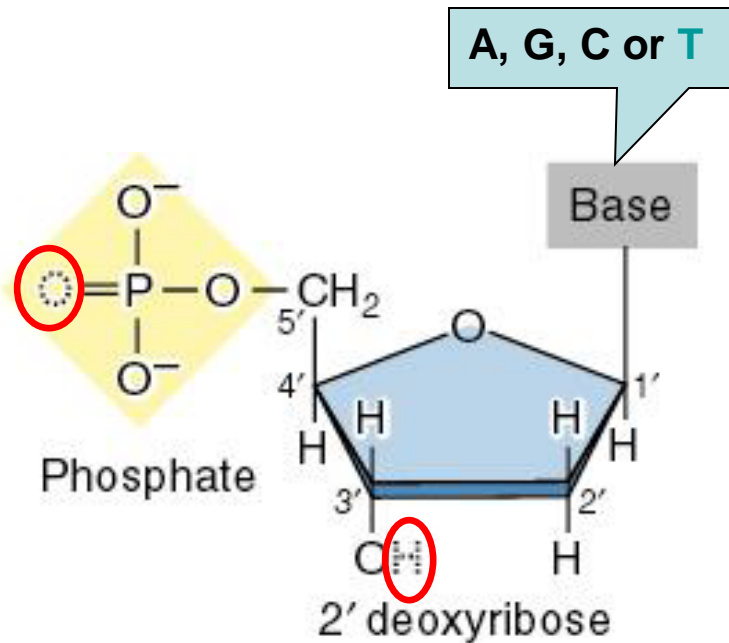
- Adenine + ribose = Adenosine
- Adenine + deoxyribose = Deoxyadenosine

➤ Base + sugar + phosphate(s) → nucleotide

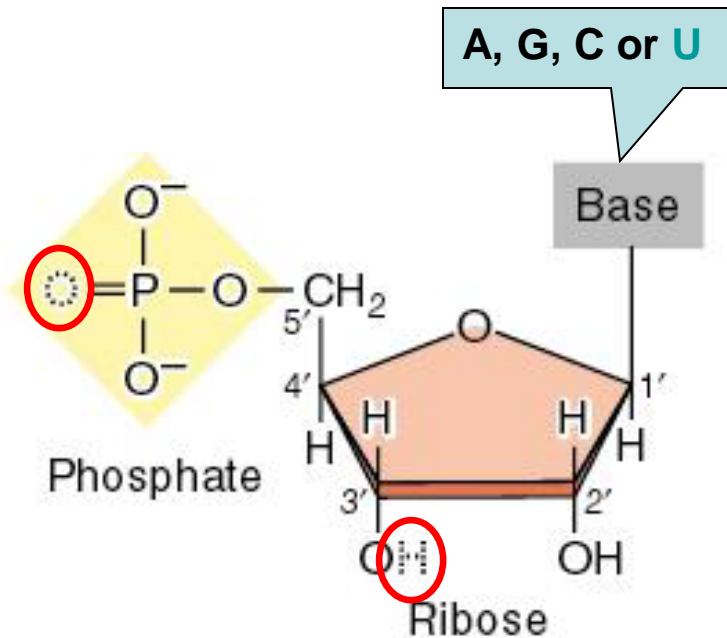
➤ Example

- Adenosine monophosphate (AMP)
- Adenosine diphosphate (ADP)
- Adenosine triphosphate (ATP)

- These atoms (red circled) are found within individual nucleotides
 - However, they are removed when nucleotides join together to make strands of DNA or RNA



(a) Repeating unit of deoxyribonucleic acid (DNA)

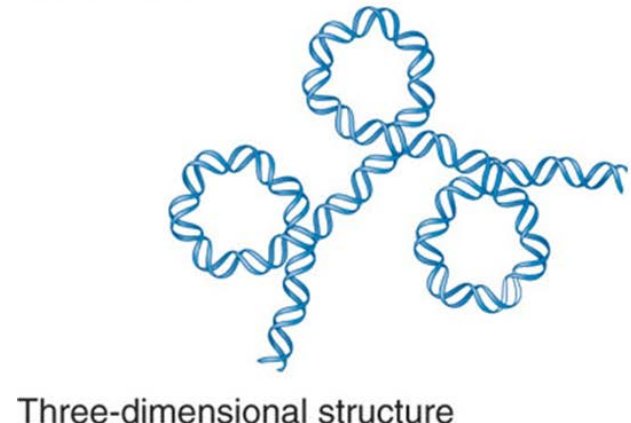
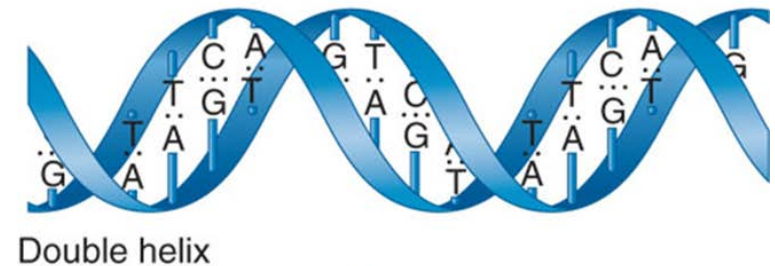
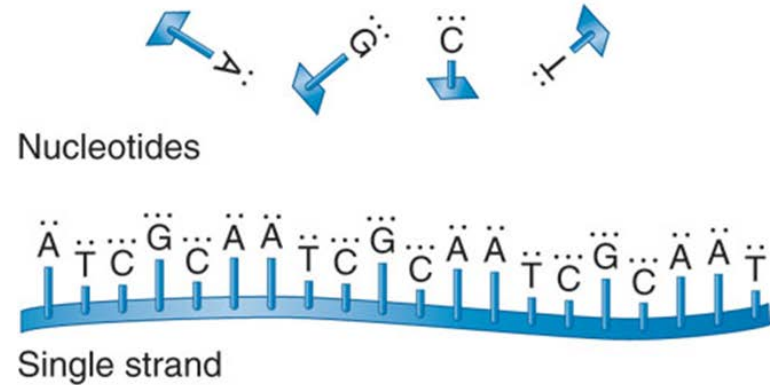


(b) Repeating unit of ribonucleic acid (RNA)

The structure of nucleotides found in (a) DNA and (b) RNA

NUCLEIC ACID STRUCTURE

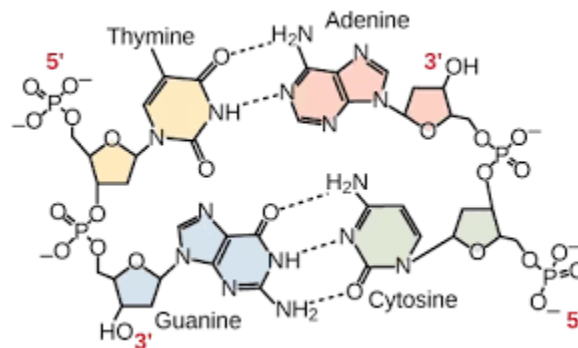
- DNA and RNA are large macromolecules with several levels of complexity
- **Nucleotides** form the repeating units
- Phosphodiester bonds link nucleotides to form a **strand**
- Two strands interact to form a **double helix**
- The double helix interacts with proteins resulting in 3-D structures in the form of **chromatin**



Structure of DNA

In 1953, James Watson and Francis Crick discovered the double helical structure of DNA. The scientific framework for their breakthrough was provided by other scientists including; Linus Pauling, Rosalind Franklin, Maurice Wilkins and Erwin Chargaff

- Two strands are twisted together around a common axis
- There are 10 bases per complete twist
- The two strands are antiparallel; one runs in the 5' to 3' direction and the other 3' to 5'
- The helix is right-handed; as it spirals away from you, the helix turns in a clockwise direction
- Hydrogen bonding between complementary bases
A bonded to T by two hydrogen bonds; C bonded to G by three hydrogen bonds

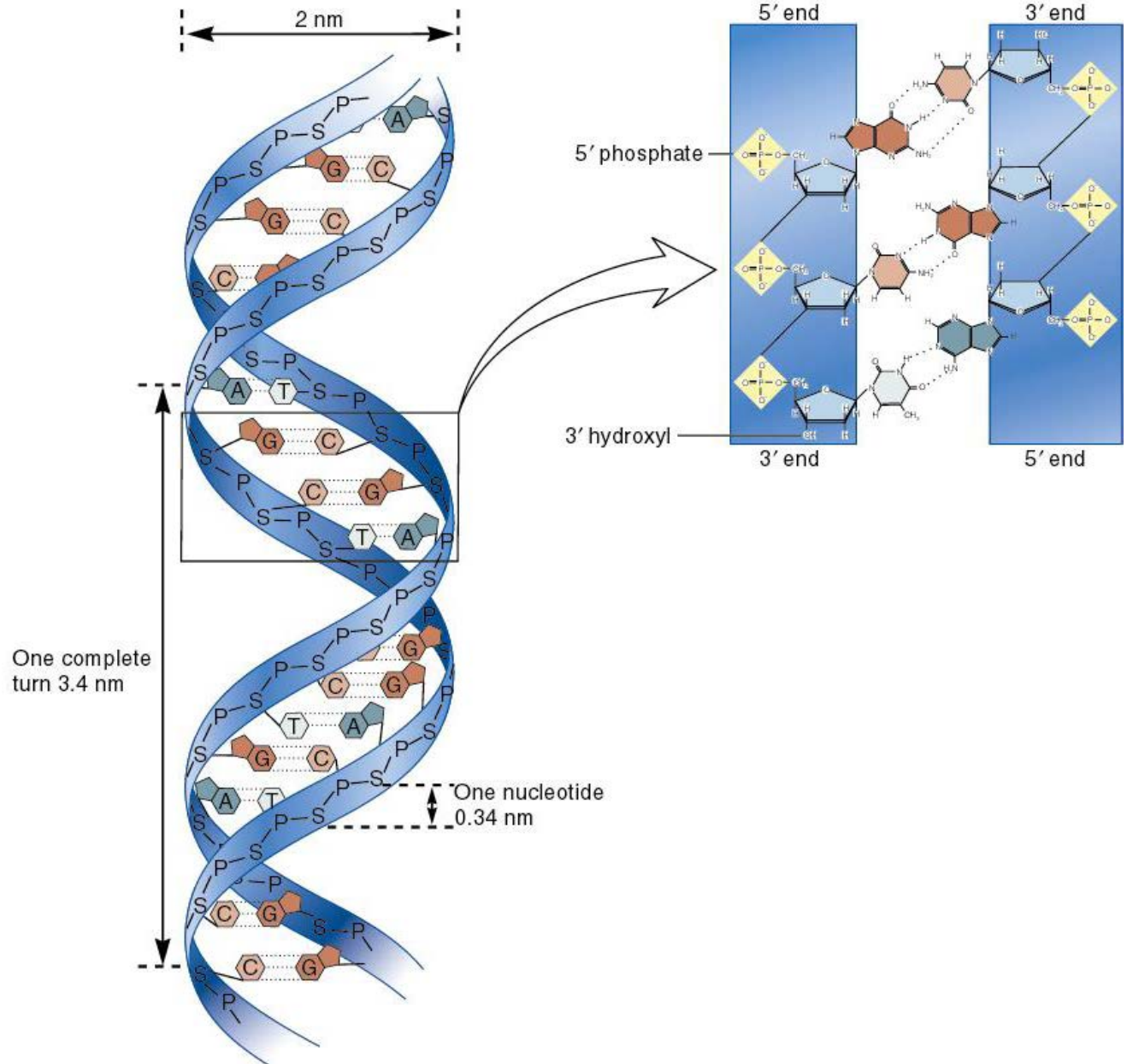


- Base stacking
Within the DNA, the bases are oriented so that the flattened regions are facing each other

Structure of DNA

Key Features

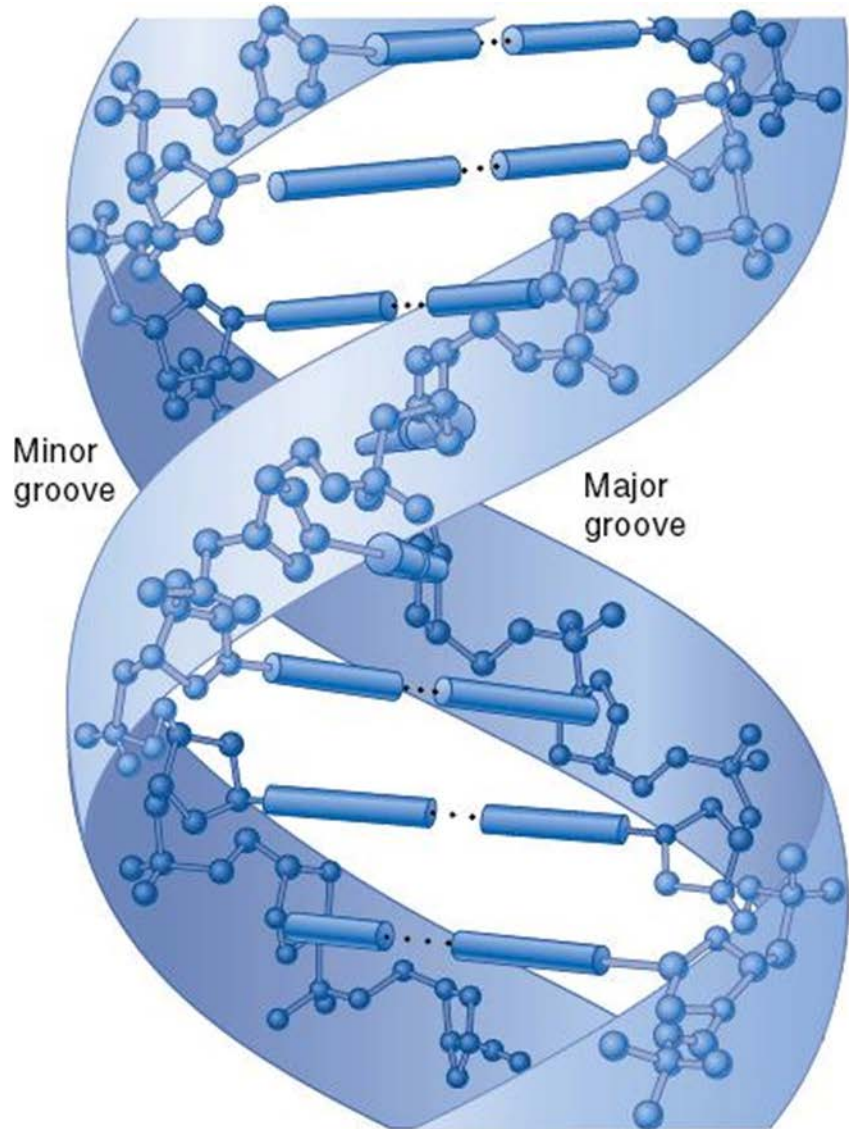
- Two strands of DNA form a right-handed double helix.
- The bases in opposite strands hydrogen bond according to the A/T and G/C rule.
- The 2 strands are antiparallel with regard to their 5' to 3' directionality.
- There are ~10.0 nucleotides in each strand per complete 360° turn of the helix.



Structure of DNA

There are two asymmetrical grooves on the outside of the helix

1. Major groove
2. Minor groove



(a) Ball-and-stick model of DNA

Principles

The main steps involved in the extraction of DNA are;

Homogenization

In this step, tissue is blended with NaCl. The cell membranes are lipid and protein in composition and must be ruptured to release the DNA. The homogenization breaks the cell wall, cell membrane and nuclear membrane to allow the release of DNA. The sodium from NaCl binds to the free end of phosphate unit and helps the DNA strands to coalesce.

Sodium dodecyl sulphate (SDS) is then added to the homogenized material. SDS is a biological detergent which interacts with the lipids and proteins, breaks the polar interaction and causes them to precipitate.

Deproteinization

A protease enzyme (enzymes that catalyze the breakdown of proteins to smaller units or amino acids by breaking the peptide bonds) is added to further denature and detach the proteins clinging to the DNA.

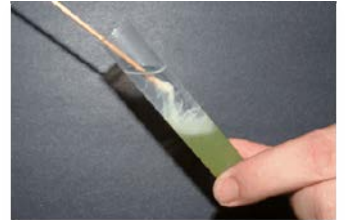
Precipitation

Addition of ethanol results in the separation of DNA from all other cellular material and the DNA moves towards the liquid interface.

Experimental Protocol

Extraction of DNA

1. Take 10 mL of the extract in a boiling tube and add 1.5 mL of the SDS solution and gently swirl. Let the mixture stand for 10 minutes in ice.
2. Add 5-6 drops of papain extract to the mixture and stir gently.
3. Now hold the boiling tube at an angle and pour very slowly 24 mL of ice cold ethanol down the wall of the test tube so that it forms a layer above the extract layer.
4. Allow the boiling tube to stand straight for a few minutes.
5. Some stringy white substance comes in the alcohol layer. This is DNA.
6. Use a hooked glass rod and place it such that its end is just below the alcohol layer. Now try to spool the DNA out of the tube.



Identification of DNA

Diphenylamine Test

1. In a test tube, add a small amount of crude DNA and 2 mL of 4% sodium chloride solution. Add 2 mL of diphenyl amine reagent and mix.
2. Place the test tube in boiling water bath for one hour and record changes. The solution turns blue.

UV-Vis Absorption

1. Dissolve DNA in 2-3 mL of TE buffer solution and determine the ratio of absorption at 260 & 280 nm.

Observations

Diphenylamine Test

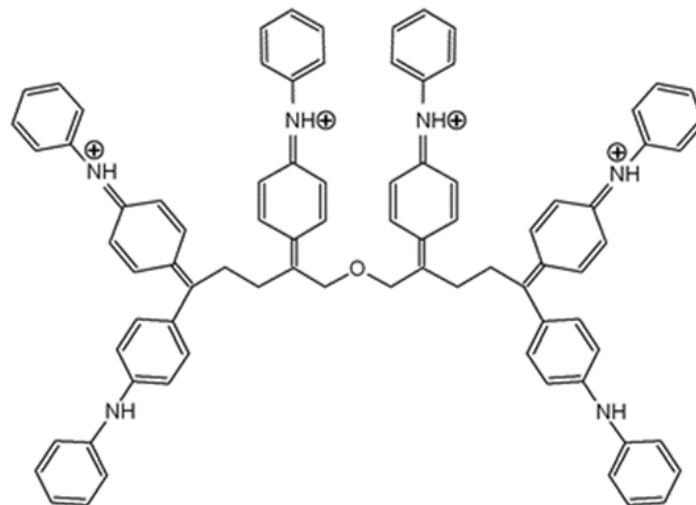
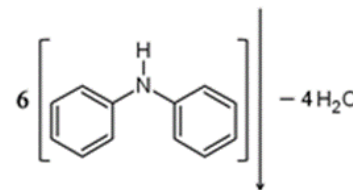
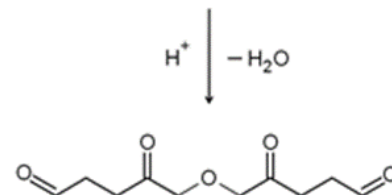
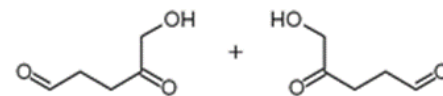
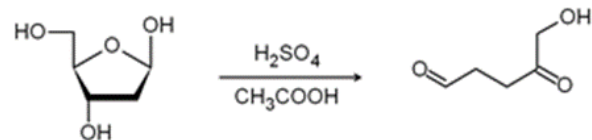
The reaction of diphenylamine with deoxyribose sugar produces a blue-coloured complex.

The DNA sample is boiled under extremely acidic conditions; this causes depurination of the DNA followed by dehydration of deoxyribose sugar into a highly reactive ω -hydroxylevulinylaldehyde.

The reaction is not specific for DNA and is given by **2-deoxypentoses**, in general.

The ω -hydroxylevulinylaldehyde, under acidic conditions, reacts with diphenylamine to produce a blue-coloured complex that absorbs at 595 nm.

RNA will not undergo this reaction.



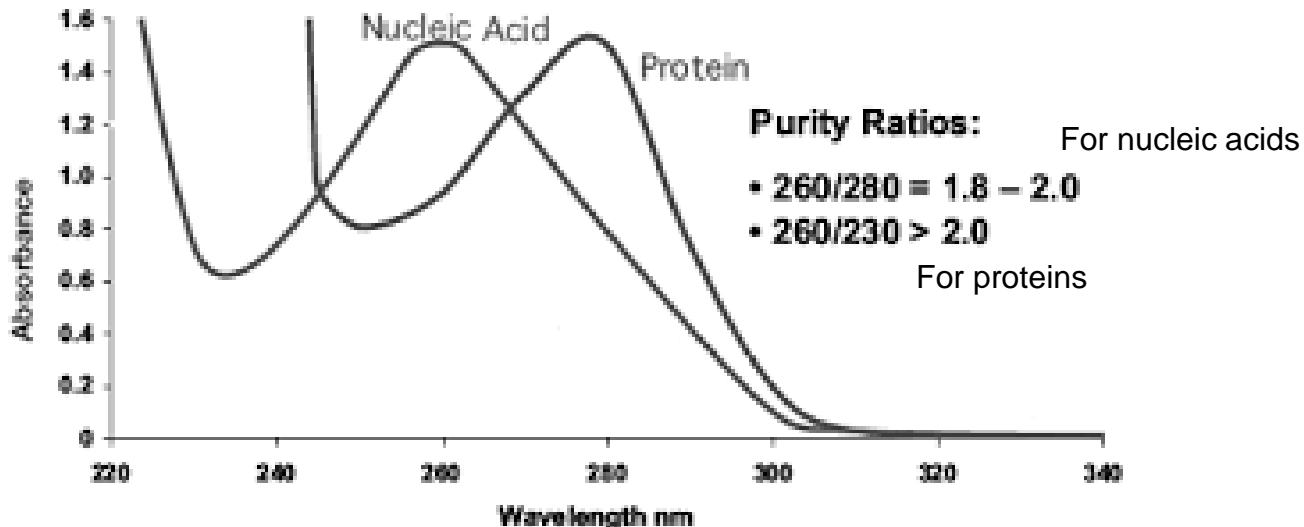
Observations

UV-Visible Spectroscopy

DNA can be identified by its UV absorption at 260 nm.

Proteins also absorb at 260 and 280 nm. The ratio of absorbance at 260- 280 nm may be used to estimate the purity of nucleic acid.

For pure nucleic acid solution, the ratio of 26-280 absorption should be 1.5 - 2.0.



Results

DNA was extracted from pea extract and identified using the diphenylamine test and UV-Visible spectroscopy.