Exp. 4

Aim : Extraction of DNA from green peas extract

Apparatus Required : hooked glass rod, boiling tube, measuring cylinders, droppers,

Chemicals Required : Green peas extract, Papain extract, SDS solution, ethanol, 4% NaCl solution, diphenyl amine reagent, Tris-EDTA buffer.

Principle : DNA (deoxyribonucleic acid) contains all genetic information necessary for growth, functioning and reproduction of almost all living organisms. It has two biopolymer strands coiled around each other to form a double helix.

In its extraction from green peas, basically cell membrane contains lipids, proteins, DNA etc., so to get DNA separated and released from the cell membrane, one has to rupture cell wall. For that we blend NaCl with extract, it will homogenize the mixture, and DNA, protein, lipids etc. will be released.

Now for removal of protein, lipids etc. from DNA to separate it, we first use SDS (Sodium dodecyl sulphate) solution which acts as a biological detergent and proteins get precipitated, Some proteins still remain clinging to DNA, for that we use Papaya extract (Papain) which breaks them into amino acids.

And finally ethanol is added to get DNA separated from all cellular material. And DNA is obtained as white stringy part.

Its verification is done by diphenylamine test which gives blue color
Its purity can be verified by UV spectroscopy.

Procedure :

(A) Extraction of DNA

1) Take 10ml of the extract (green peas extract blended with NaCl) in boiling tube and add 1.5 ml of the SDS solution and gently swirl.

2) Let the mixture stand for 10 mins. in ice.

3) Add 5-6 drops of papain extract to mixture and stir gently.

4) 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 without disturbing that layer.

5) Allow the boiling tube to stand straight for few mins.

6) Some white stringy substance comes in alcohol layer. This is DNA.

7) 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.

(B) Identification of DNA

(i) Diphenylamine Test

1) In a test tube, take small amount of crude DNA extracted and add 4% NaCl - 2ml and 2ml of diphenylamine reagent and mix.

2) Place the test tube in boiling water bath for one hour and record changes.

3) The solution turns blue.

4) RNA not gives this test, so it confirms DNA.

(ii) UV Visible spectroscopy

- DNA can be identified by its UV absorption at 260nm. Proteins also absorb at 260 and 280nm.
- The ratio of absorbance at 260-280nm maybe used to estimate the purity of DNA.

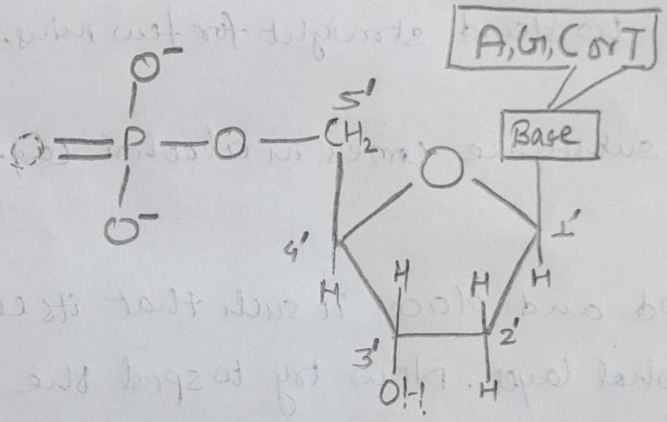
~~for pure~~

1) Dissolve DNA in 2-3ml of Tris-EDTA buffer solution.

2) Put this solution in a cuvette, put cuvette in spectroscopic machine.

3) Take readings of absorption at 260nm and 280nm.

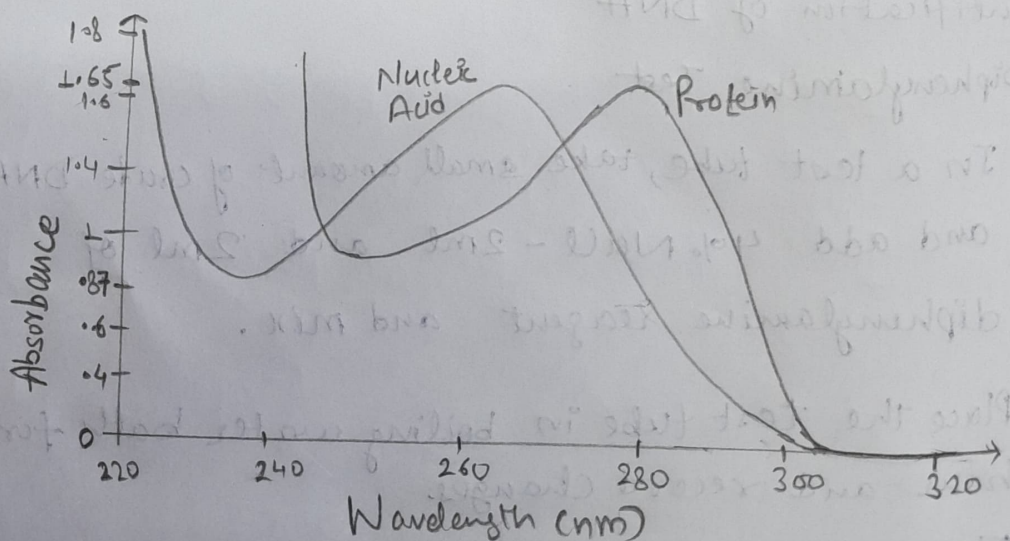
Chemical structure:



Repeating unit of DNA

Observations & Calculations:

→



for pure nucleic acid solution, the ratio 260-280 absorption should be 1.5 - 2.0

~~ratio of absorb~~

Absorption of DNA solution at 260nm = 1.65

" 280nm = 0.87

ratio of absorption = $\frac{1.65}{0.87}$ = 1.90

Results:

1) DNA was extracted from pea extract and identified using diphenylamine test.
2) In UV Visible spectroscopy, ratio of absorbance at 260 & 280nm was observed 1.9 which is in pure DNA range (1.5-2.0)