

Experiment

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Extraction and identification of DNA from green peas and onions

Materials required

Green peas or onions
95% Ethanol (ice cold)
Conc. sulfuric acid
Papain extract from papaya
NaCl
Acetaldehyde
Sodium dodecyl sulfate (SDS) or shampoo
EDTA
Ice-cold distilled water
Tris-HCl
Diphenyl amine

Equipment and special glassware

Mixer-grinder
Ice bath
UV-visible spectrophotometer
Tea strainer/cheese cloth
Glass rod with a hook

Time required

3 hours

Key reference

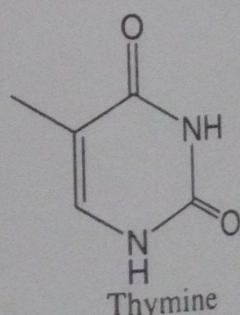
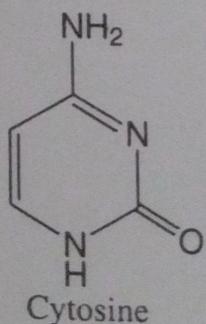
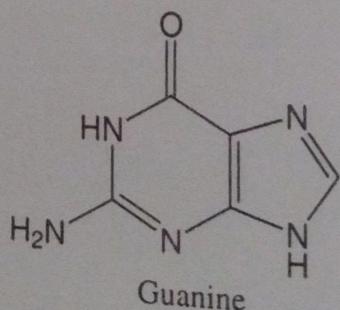
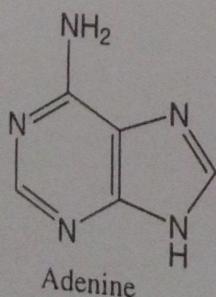
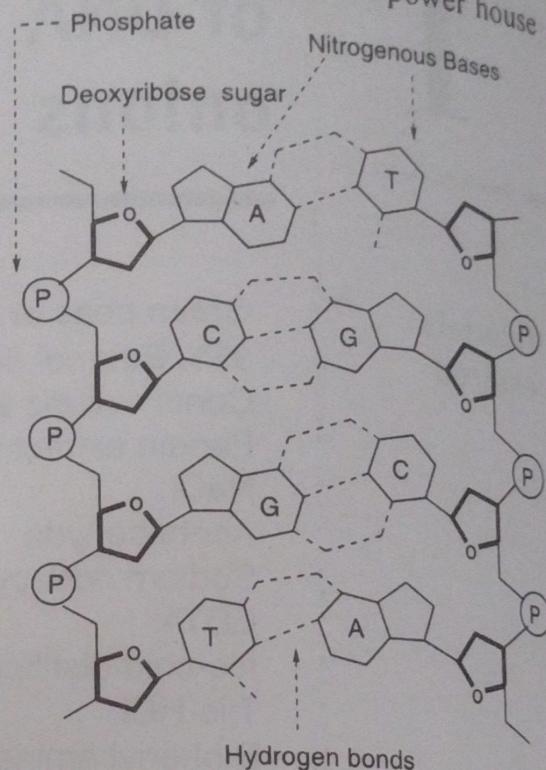
1. Nordell, K.J.; Jackelen, A.L.; Condren, S.M.; Lisensky, G.C.; Ellis, A.B., *J. Chem. Educ.*, 76, 400A, 1999.

PRINCIPLES

DNA, the molecule of life

DNA, the key to heredity, the center of all genetic information and the control center of protein synthesis is present in the cells of all living things. This nucleic acid is present in all the 23 pairs of human chromosomes as well as the mitochondria, the power house of the cells. The double helical structure of this molecule, proposed by Watson and Crick in 1953 is well known. The basic units of DNA are four nucleotides each consisting of a phosphate, a deoxyribose sugar and a nitrogen containing base. In 1960, Marshall Nirenberg, an American biochemist, and Har Gobind Khorana, an American biochemist born in India, decoded DNA. This code consists of four nitrogen containing bases, represented by the letters A (adenine), T (thymine), C (cytosine), and G (guanine). It is the sequence of these nucleotides, over the length of each individual chromosome taken together, that makes up the human genome. The bases are from complementary classes and have distinct structures with binding affinities for a specific complementary base. Adenine (A) and thymine (T) will loosely bind to one another when in opposite strands of the helix as will guanine (G) with cytosine (C). The bonding between strands is accomplished via weak hydrogen bonds between the complementary bases. Chromosomal DNA, kept tied up in the well known X shape of a dividing chromosome is bound by proteins into a supercoil.

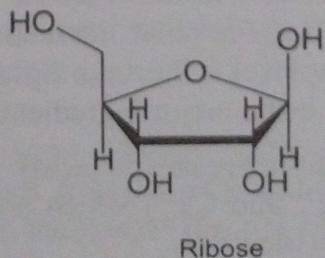
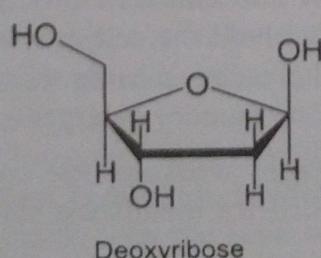
If we imagine unwinding this coil and stretching it out, the DNA molecules in a single human chromosome will have a length of approx. 1.7 cm to 8.5 cm from the smallest to the largest with the nucleotide pairs ranging from 50×10^6 to 250×10^6 in number. One can estimate that if unwound and tied together, the strands of DNA in one human cell would stretch almost six feet but would be only 50 trillionths of an inch wide. As there are about 100 trillion (100,000,000,000,000) cells in an average human being, one can extend this imaginary calculation further and say that if all the DNA in our body is put end to end, it would reach the sun and back over 600 times (100 trillion times six feet divided by 92 million miles)!



Any two unrelated strangers anywhere on the planet share 99.9 percent of the same DNA. Genomically speaking, all races are equal. In other words, you cannot tell simply by looking at someone's DNA whether they are black or white or brown. Human beings have roughly 99.1 percent of genes in common with the chimpanzee, our closest relative on earth. The overlap between mice and humans is nearly 75 percent. Scientists still do not know what more than 50 percent of the genes do. Also a lot of the DNA in our cells is "junk," that is, scientists as of now do not know exactly what the long stretches of repetitive DNA in our cells are for. The red blood cells are the only kind of cells in our body that do not have DNA as they are the only cells in our body that do not have nuclei. Ever since it was first isolated, chemists and biochemists have studied the chemistry of DNA in detail and it still remains one of the most fascinating biological molecules.

In this experiment, we will be isolating DNA from green peas or onion and identifying it by UV-visible spectroscopy or a chemical test. This will involve steps in which we carefully open up cells, deactivate the enzyme *DNAse* which cleaves the DNA molecule, separate the DNA from lipids and proteins which are bound to it and make it precipitate out into a suitable solvent. The identification by UV-spectroscopy will provide a characteristic absorption at 260 nm unique for DNA and RNA. One of the basic differences between DNA and RNA is that the former has a deoxyribose sugar unit while the latter has a ribose sugar unit. The chemical test, which depends on the concentration of the DNA solution and sometimes requires more time to observe, identifies the deoxyribose sugar unit.

Number of chromosomes present in organisms	
Female ant (<i>Myrmecia pilosula</i>)	2
Mosquito	6
Fruit fly	8
Peas	14
Onion	16
Cabbage	18
Rice	24
Earthworm	36
Cat	38
Mouse	40
Human	46
Chimpanzee	48
Rhesus monkey	48
Elephant	56
Cow	60
Goat	60
Donkey	62
Horse	64
Chicken	78
Dog	78
Pigeon	80
<i>Ichthyomys pittieri</i> (a semi-aquatic rodent [highest for a mammal])	94
Goldfish	104
Algae	148
<i>Adders tongue fern</i> [highest for a plant]	1262
<i>Aulacantha</i> (protozoa) [highest]	1600



DNA from Green Peas and Onions

The process of extracting DNA from a cell is the first step in many chemical and biochemical laboratory procedures. Although one can isolate DNA from any living cell, the common sources for isolating it with ease in the laboratory are green peas, onions, wheat germ, liver and spinach. Onion is often used as it is cheap, easily available and also has low starch content. However the color contrast between the extracted DNA which is white and the original extract (green) is more visible in case of green peas.

The main steps involved in the extraction of DNA are the following.

Homogenization involves blending with NaCl and sometimes heating the onion or pea tissue in order to break open the cells. The sodium ions bind to the free end of the phosphate units and helps the strands to coalesce as well as provide the common ion effect for it to precipitate out. The cell membranes are lipid and protein in composition and must be ruptured in order to release the DNA. The tissue is mixed in a blender and a homogenization media which is basically liquid soap or SDS with or without EDTA, is added. This process breaks down the cell wall, cell membrane and nuclear membrane allowing the release of DNA. The heat treatment can soften the two layers of phospholipids in the cell membrane and can denature the *DNAse* enzyme, which if present, will cut the DNA into small fragments making it difficult to isolate. However, in many cases, such as the procedure given here, the heat treatment is avoided as it was found to increase rupture of the DNA strands and decrease its thread character. If heated, one has to strike a delicate balance between heating and cooling as the temperature should not go beyond 60°C and it should not be heated for more than 10–12 minutes. The enzyme *DNAse*, even though present in traces will be active at 37°C and can rupture the strands. Most of the enzyme can be denatured around 60°C. DNA itself gets denatured above 80°C. Working at cold temperatures after breaking the cells by blending till isolation of the DNA, also reduces the activity of the *DNAse* enzyme.

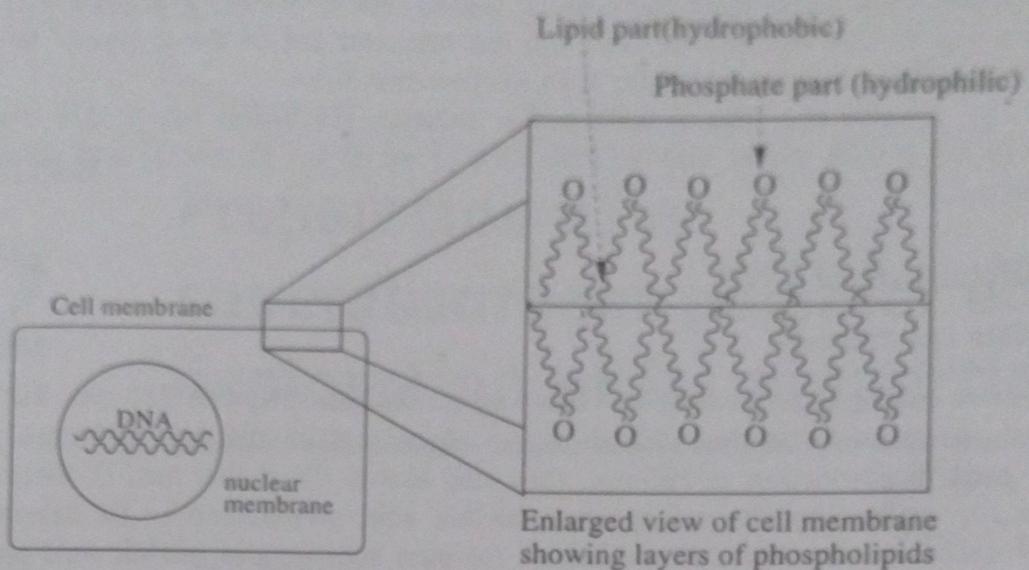
Deproteinization It involves adding a protease enzyme, papain – a common enzyme used to clean soft contact lenses. This will denature and detach the proteins clinging to the DNA, making the molecule flexible and easy to spool. Pineapple juice or contact lens cleaning solutions have also been reported to work well.

Precipitation It involves adding ice-cold 95% ethyl alcohol to form a layer above the DNA extract. This causes every component in the filtrate to stay in solution except DNA. The DNA will gather at the interface of the filtrate and the ethanol, and move into the ethanol layer and can be spooled out with a glass rod. DNA is insoluble in alcohol. One can also use cold isopropyl alcohol.

The ingredients of the onion lysis solution and their functions are as follows:

SDS (Sodium dodecyl sulfate [$\text{CH}_3(\text{CH}_2)_{11}-\text{OSO}_3-\text{Na}^+$]) is a biological detergent which causes the cell membrane to break down further and emulsifies the lipids and proteins of the cell by disrupting the polar interactions that hold the cell membrane together. The detergent forms complexes with these lipids and proteins causing them to precipitate out of the solution. SDS is the major ingredient in many laundry detergents.

EDTA (Ethylenediaminetetraacetic acid) if present weakens the cell by binding the divalent cations (Mg^{2+} and Ca^{2+}) which are needed for membrane stability. This further aids in breaking open the cells.



NaCl enables nucleic acids to precipitate out of an alcohol solution because it shields the negative phosphate end of DNA causing the strands to come closer together and coalesce so that they can precipitate out of a cold 95% ethyl alcohol solution.

PROCEDURE

1. Prepare papain extract by blending to a paste about 100g of green papaya with 50 ml of ice cold water using a mixer-grinder and filter it using a strainer into a beaker kept in crushed ice. Store the extract in the refrigerator.
2. Take 100 g fresh or frozen peas, 2 g sodium chloride (iodized or non iodized) and 100 ml of ice cold water in a clean cup of the grinder and blend at medium or high speed for 30 seconds.
3. Filter while cold, the pulverized peas through a strainer or two to three layers of cheese-cloth in to another beaker kept in crushed ice.
4. Make solution of 27 g SDS in 500 ml water. If SDS is not available one can use liquid shampoo such as Pantene Pro-V or Clinic all clear.
5. Carefully add SDS solution [a volume corresponding 1/6 th the amount of the filtered pea extract ~approx 20–25 ml] to the pea extract and gently swirl the beaker. Let the mixture stay like that for 10 minutes.
6. Take the extract in a large glass test tube up to 1/3rd of its volume and add 4–5 drops of the deproteinizer solution (papain extract). Stir gently or swirl a few times.
7. Hold the test tube with the filtered extract at an angle and very slowly and carefully pour twice the volume of ice-cold 95 % ethyl alcohol from another test tube down the wall of the test tube so that it forms a layer above the pea extract layer.

Observe what happens. You should see some stringy white substance precipitate out into the alcohol layer. This is DNA. When it looks very stringy, place a glass rod preferably with a hook, into the tube so that the end of the rod is just below the upper

layer of alcohol and try to spool the DNA. It should look very clear and glistening around the glass rod. Gently but quickly twirl the rod into and out of the 2 layers. Gently lift the DNA out of the tube and transfer it to another test tube.

One can dissolve the DNA in a TE buffer solution [TE buffer has 0.01M Tris-HCl of pH 8.0 (5 ml of 2 M stock) and 0.001M EDTA (2 ml of 0.5 M stock)] and store in the refrigerator.

Identification of DNA

UV-Visible spectroscopy

DNA in pure solution can also be identified by its UV absorption at 260 nm. Both single stranded and double stranded DNA show the absorption at 260 nm. Although proteins show a peak of absorption at 280 nm, they also absorb UV at 260 nm. In fact, a useful method for estimating the purity of a nucleic acid preparation is to determine its ratio of absorption at 260-280 nm, which for pure solutions of nucleic acid should be approximately 1.5-2.0.

Diphenylamine test

The diphenylamine method is based on treatment of nucleic acid with hot mineral acid to hydrolyze the DNA to nucleotides. Diphenylamine reacts specifically with deoxyribose sugar in DNA to form a blue-colored complex. Since diphenylamine does not react with ribose, one can assay for DNA in the presence of contaminating RNA.

The diphenylamine reaction is specific for 2-deoxypentose sugar molecules. In the presence of strong mineral acids (included in the assay reagent), 2-deoxyribose is converted to furfuraldehyde, which reacts with diphenylamine to produce a blue-colored compound. The intensity of blue color, measured at 600 nm, is directly proportional to the concentration of DNA in the sample. The reaction can sometimes be slow and heating for a longer period of time on a boiling water bath may be required.

Testing DNA with the diphenylamine reagent

1. Dissolve 1.5 g of diphenylamine in 100 ml of glacial acetic acid
2. Add 1.5 ml of concentrated sulfuric acid
3. Store the solution in a dark glass/ amber-colored bottle.
4. Freshly prepare an acetaldehyde solution before the analysis (1 cc acetaldehyde in 50 cc distilled water).
5. Add 0.5 ml of this solution to 100 ml of the diphenylamine solution.
6. In a test tube, add 3 ml of sodium chloride solution (4%) (2 g of sodium chloride to 50 ml of distilled water) and 3 ml of crude (extracted) DNA; add 3 ml of diphenylamine indicator and mix.
7. In another test tube, add 3 ml of distilled water, add 3 ml of diphenylamine indicator and mix.
8. Place tubes in boiling water bath for one hour and record the color changes. Diphenylamine indicator reacts with the deoxyribose compound of DNA to produce a blue color which will give a UV visible absorption at 600 nm.