Dna extraction from chicken liver

Deoxyribonucleic acid (DNA) is the hereditary material in humans and almost all other organisms. Nearly every cell in a person’s body has the same DNA. Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA or mtDNA).

The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all people. The order, or sequence, of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences.

DNA bases pair up with each other, A with T and C with G, to form units called base pairs. Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix. The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder’s rungs and the sugar and phosphate molecules forming the vertical sidepieces of the ladder.

An important property of DNA is that it can replicate, or make copies of itself. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell.

The extraction of DNA from cells and its purification are of primary importance to the field of biotechnology and forensics. Extraction and purification of DNA are the first steps in the analysis and manipulation of DNA that allow scientists to detect genetic disorders, produce DNA fingerprints of individuals, and even create genetically engineered organisms that can produce beneficial products such as insulin, antibiotics, and hormones.

Once the DNA has been isolated, it is essential to accurately determine its concentration for subsequent manipulation such as cloning or sequence determination.

To quantify the amount of DNA that extracted by using spectrophotometry.

The aims of this experience is to:

- To use the properties of DNA to isolate long strands of DNA from liver cells.
- To determine the yield of DNA isolated from a given amount of tissue.
- To examine the light absorbing properties of purified DNA.
- To examne the relationship between the concentration of a DNA solution and the absorbnce at 595nm of DNA-diphenylamine solution.
- To generate a standrad curve relating DNA concentraton with the absorbance of DNA-diphenylamine solutions.
- To use a standard curve to determine the concentration of an unknown DNA solution.

Materials and Methods

As per lab manual.

Results

Firstly, the chicken liver cell homogenate is treated with a salt solution such as NaCl and a detergent solution containing the compound SDS (sodiumdodecyl sulfate). These solutions break down and emulsify the fat & proteins that make up a cell membrane. Finally, ethanol is added because DNA is soluble in water. After adding ethanol a relatively clear aqueous will be produced, the first layer is the milky solution that is the aqueous phase with DNA, the middle layer is the solid (precipitate proteins). The bottom layer is a clear solution (organic). The DNA can be spooled (wound) on a stirring rod and pulled from the solution at this point. The amount of DNA solution we got is 5. 4ml. Than we put the DNA solution in 2ml tube (1. 041g).

The total weight of DNA solution and tube is 1. 106g. The amount of DNA we got is 1. 106-1. 041g = 0. 065g.

Next we prepare 4 standard tubes by adding TE buffer (ml) to the DNA standard solution (ml). And also added to each of the 3 samples of my DNA. The total DNA (mg) is recorded in the table 1. The observed colour change of 4 standard tube and my 3 samples are recorded in table 2 and 3. We pipette the DNA samples and each standards tubes into separate wells of a 96 well microtitre plate. We measured the absorbance at 595nm of the DNA-diphenylamine solutions using the plate reader. Our results are shown in the

graph with the used of the reading of table 4. Form the graph we find that the concentration of undiluted DNA is 0. 23×2= 0. 46mg/ml.

Discussion and Conclusions

For this experiment we determinate the yield of the DNA isolate from given amount of tissue is:

1g -> 63mg

0. 065g -> 4. 095mg (wet weight of the DNA to dry weight)

3ml -> 4. 095mg

5. 4ml -> 7. 371mg (DNA in the entire aqueous phase is collected)

3. 4ml -> 7. 371mg

5. 3ml -> 9. 767mg

The final calculation of the dry DNA is 9. 767mg/g liver.

For the experiment we examine that the light absorbing properties of purified DNA. The wavelength is range 220-300nm. The wavelength of the DNA is 260nm.

We also calculated that the yield of DNA per g of liver from Lab 2 is:

The amount (mg) of DNA contain => 0. 46×1. 5= 0. 69mg

Aqueous from lab 1 = 5. 4mg

0. 69/2 = 0. 345mg

(0. 345×5. 4)/3 = 0. 621mg

The final value in mg of dry DNA/g liver is: 0. 621mg/g.

In the end of the experiments, we managed to complete our objectives. In summary, we learn that the alcohol can causes DNA to precipitate, or settle out of the solution, leaving behind all the cellular components that aren’t soluble in alcohol. As alcohol is less dense than water, so it floats on top forming two separate layers. We also learn that the advantage of spectrophotometry is that diphenylamine only reacts with DNA more accurate as RNA would not be determined. The disadvantage of spectrophotometry is that it always requires standard solution. The advantage of calculating of yield by its weight is that it does not require standard solution. The disadvantage of calculating of yield by its weight is that it is less accurate as RNA is counted in.