Experiment #6 DNA Extraction from Fruits

Objective

Can we see the DNA with the naked eye?

In this experiment, our objective is to use some physical and chemical processes to extract a visible mass of DNA from a fruit tissue and learn some basic facts about the DNA molecule.

Theory

DNA (deoxyribonucleic acid) is a molecule in present in the cells of all living organisms that contains the biological instructions that make each species unique. DNA, along with the instructions it contains, is passed from adult organisms to their offspring during reproduction. In complex eukaryotic cells such as plants animal and plant cells, most of the DNA is located in the cell nucleus (chloroplasts, mitochondria, and ribosomes also carry some DNA). In simpler cells called prokaryotes, including the eubacteria and archaea, DNA is not separated from the cytoplasm by a nuclear envelope.

DNA contains the instructions needed for an organism to develop, survive and reproduce. In order to carry out these functions, DNA sequences are converted into messages that can be used to produce proteins, which are the complex molecules that do most of the work in our bodies. The Messages of the DNA are used to make proteins in a two-step process. First, the information in a DNA molecule is read by enzymes and transcribed into an intermediary molecule called messenger ribonucleic acid, or mRNA. Next, the information contained in the mRNA molecule is translated into the "language" of amino acids, which are the building blocks of proteins. This language tells the cell parts responsible of protein-making the precise order in which to link the amino acids to produce a specific protein. This is a major task because there are 20 types of amino acids, which can be placed in many different orders to form a wide variety of proteins.

DNA is made of chemical building blocks called nucleotides. These building blocks consist of three parts: a phosphate group, a sugar group and one of four types of nitrogen bases. To form a strand of DNA, nucleotides are linked into chains, with the phosphate and sugar groups alternating. There are four types of nitrogen bases in nucleotides, which are adenine (A), thymine (T), guanine (G) and cytosine (C). The order of these bases determines the biological instructions contained in a strand of DNA. For example, the sequence ATCGTT might instruct for blue eyes, while ATCGCT might instruct for brown eyes. Each DNA sequence that contains instructions to make a protein is known as a gene. The size of a gene differs from about 1,000 bases to 1 million nitrogen bases in humans. The complete DNA instruction book, or genome, for a human contains about 3 billion nitrogen bases and about 20,000 genes on 23 pairs of chromosomes.

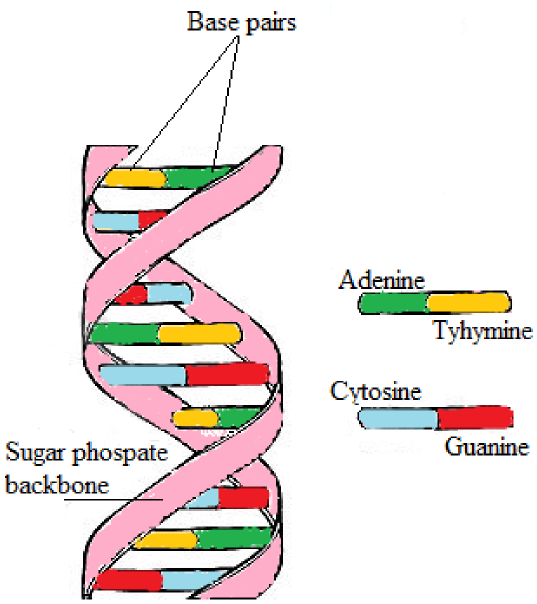

Figure 1: The DNA molecule

“Double Helix” is used by scientists to describe DNA's winding, two-stranded chemical structure. This shape gives DNA the power to pass along biological instructions with high precision. We can understand the DNA's double helix by picturing the strands of alternating sugar and phosphate groups as the sides of a twisted ladder. Each "rung" of the ladder is made up of two nitrogen bases, paired together by hydrogen bonds. Because of the highly specific nature of this type of chemical pairing, base A always pairs with base T, and base C always pairs with base G. So, if we know the sequence of the bases on one strand of a DNA double helix, it is easy to figure out the sequence of bases on the other strand. DNA molecule, for its unique structure, can copy itself during cell division. When a cell prepares to divide, the DNA helix splits down the middle and becomes two single strands. These single strands serve as templates for building two new, double-stranded DNA molecules - each a replica of the original DNA molecule. In this process, an A base is added wherever there is a T base, a C base is added where there is a G base, and so on until all of the nitrogen bases have paired once again.

History of DNA

The German biochemist Frederich Miescher first observed DNA in the late 1800s. However, nearly a century later scientists succeeded to unravel the structure of the DNA molecule and realized its central importance to biology. For many years, scientists debated which molecule carried the biological instructions of life. Most scientists had the view that DNA was too simple a molecule to play such a critical role. Instead, they argued that proteins were more likely to carry out this vital function because of their greater complexity and wider variety of forms. The importance of DNA became clear in 1953 with the work of James Watson, Francis Crick, Maurice Wilkins and Rosalind Franklin. By studying X-ray diffraction patterns and building models, the scientists figured out the double helix structure of DNA which enables it to carry biological information from one generation to the next.

DNA Extraction process

DNA is an incredibly small molecule, but in large quantities, it can be seen. In this activity, you will extract DNA from banana, strawberry and kiwi fruits. We are going to use fruits because they are soft and easy to pulverize.

There are three basic steps in DNA extraction. First, the cell must be broken open to release the nucleus. Next, the nucleus must also be opened to release the DNA. Lastly, once the DNA is released, it must be precipitated out of solution.

The reagents we require to complete the extraction procedure are salt, detergent, and alcohol. Both the cell and nuclear membranes are composed primarily of lipids.

*In order for the cell to be broken open, the lipid walls must be broken down. The manual grinding and detergent solutions accomplish this. Soap molecules mix with fats or lipids, causing structures made of lipids to break apart.

*The addition of salt solution provides the DNA with a favorable environment by contributing positively charged atoms that neutralize the normal negative charge of the DNA, allowing the DNA to clump together.

*Ethyl alcohol is used to precipitate the DNA. In water, DNA is soluble. However, when it is in ethanol, it uncoils and precipitates.

Safety

1- Ethyl Alcohol is flammable. Keep it away from heat, sparks and open flame. Avoid spilling, skin and eye contact.

Equipment

25 ml Ethyl Alcohol
5 ml detergent
5 ml NaCl
50 ml falcon tube
200 ml beaker
Plastic pipette
Glass rod
2.5 cm square piece of fruit in *Zip* bag
Spatula

Procedure

1- Mash the fruit in a zip bag.
2- Add 5 ml detergent and a spatula of NaCl (salt) to falcon tube.
3- Add 45 ml tap water in the falcon tube and cover it.
4- Gently turn the falcon tube up and down.
5- Pour the solution in the falcon tube to the fruit mash in the zip bag.
6- Gently squash the bag and wait 2-3 minutes to allow mixing the solution and fruit mash.
7- Get another falcon tube and a funnel.
8- Fit a paper towel (by wrapping it) onto funnel and water it lightly.
9- Pour the mixture in the zip bag to funnel.
10- Wait for the filtered solution to build in the falcon (20 ml of solution would be enough).
11- Add 25 ml of alcohol in the falcon tube.
12- After a few minutes, bundles of isolated DNA will float on the alcohol solution.

Questions

1- Did all of the three plants have DNA? Do you think the DNA in the different plants is mostly alike or different?
2- Are you seeing individual strands of DNA? Is that what you predicted to see before the experiment?
3- What did each reagent do to extract DNA?

References

1- Alberts, Bruce; Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts and Peter Walters (2002). Molecular Biology of the Cell; Fourth Edition. New York and London: Garland Science. ISBN 0-8153-3218-1.
2- Trefil, J., M. Hazen, R., Sciences, An Integrated Approach, 6. Ed, 2010, ISBN: 978-0-470-50581-6