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# Extraction of Fruit DNA

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***Abstract*— DNA extraction and gel electrophoresis are two fundamental techniques in molecular biology that play a crucial role in genetic analysis. DNA extraction allows scientists to isolate DNA from cells or tissues, providing a starting point for various downstream applications. Gel electrophoresis, on the other hand, enables the separation and analysis of DNA fragments based on their size and charge. DNA extraction involves the isolation and purification of DNA from biological samples. Several methods exist, including phenol-chloroform extraction, silica column-based extraction, and magnetic bead-based extraction. These methods typically involve cell lysis, protein removal, and DNA precipitation steps to obtain pure DNA. Successful DNA extraction is essential for subsequent molecular biology techniques such as polymerase chain reaction (PCR), DNA sequencing, and genetic engineering. DNA extraction and gel electrophoresis form an indispensable combination in genetic research. DNA extraction provides researchers with a pure and concentrated DNA sample, while gel electrophoresis allows for the analysis and separation of DNA fragments based on their size. These techniques are pivotal in a wide range of applications, including forensics, medical diagnostics, genetic engineering, and basic research. Their continued refinement and integration with other molecular biology tools will undoubtedly contribute to advancements in our understanding of genetics and its applications in various fields.**

**Keywords: DNA extraction, gel electrophoresis, molecular biology, isolation, purification, DNA molecules, cells, tissues, analysis, separate, fragments, size, charge.**

## I. INTRODUCTION

The discovery of DNA (deoxyribonucleic acid) as the molecule responsible for carrying genetic information is a monumental achievement in the field of biology. The key discoveries and milestones leading to our understanding of DNA's structure and function are

- Early Understanding of Inheritance: In the 19th century, scientists began investigating how traits are passed from parents to offspring. Gregor Mendel's work on pea plants laid the foundation for understanding the principles of inheritance, though the underlying mechanism remained unknown [1].

- Friedrich Miescher and Nuclein: In 1869, Friedrich Miescher isolated a substance from the nuclei of white blood cells, which he called nuclein. This substance was later identified as DNA, although its significance was not fully recognized at the time[5].

Structure of DNA: DNA has a double-stranded helical structure, resembling a twisted ladder or a spiral staircase. The two strands are composed of nucleotides, which are the building blocks of DNA. Each nucleotide consists of three components: a sugar molecule called deoxyribose, a phosphate group, and one of four nitrogenous bases—adenine (A), thymine (T), cytosine (C), or guanine (G). The two strands are held together by hydrogen bonds between the nitrogenous bases: adenine pairs with thymine, and cytosine pairs with guanine. This complementary base pairing forms the rungs of the DNA ladder.[2, 3].

Functions of DNA: DNA carries the genetic information that determines the traits and characteristics of an organism. It serves as a template for the synthesis of RNA (ribonucleic acid), which in turn guides the production of proteins through a process called protein synthesis or gene expression. Proteins are essential for various biological processes, including enzyme activity, cell structure, and signaling[4].

DNA Replication: One of the crucial functions of DNA is replication, which allows for the transmission of genetic information from one generation to the next. During replication, the two strands of DNA separate, and each strand serves as a template for the synthesis of a new complementary strand. This process ensures that each daughter cell receives an identical copy of the genetic material during cell division.

Applications of DNA: The study of DNA has numerous applications in various fields. In medicine, DNA analysis is used for genetic testing to diagnose inherited diseases, identify genetic predispositions, and develop personalized treatments. In forensics, DNA profiling is employed for criminal investigations and identification of individuals. DNA sequencing techniques have revolutionized genomics

and allowed scientists to study the genomes of various organisms, contributing to advancements in fields such as evolutionary biology, biotechnology, and drug development [6].

Gel electrophoresis is a technique used to separate and analyze charged molecules, such as DNA, RNA, and proteins, based on their size and charge. It involves the migration of molecules through a gel matrix under the influence of an electric field. The principle of gel electrophoresis is since charged molecules will migrate in an electric field towards the electrode of opposite charge. The gel matrix used in electrophoresis is typically composed of a cross-linked polymer, most commonly agarose or polyacrylamide. Agarose gels are commonly used for the separation of DNA fragments, while polyacrylamide gels are used for higher-resolution separations of smaller molecules like proteins. Gel electrophoresis is a versatile technique used in various fields of research, including molecular biology, genetics, forensic science, and protein biochemistry. It allows for the separation and analysis of molecules, providing valuable information about their size, quantity, purity, and genetic or structural characteristics.[7]

The diphenylamine test is a chemical assay used to detect the presence of deoxyribose sugar in a substance. It is commonly employed in molecular biology and biochemistry laboratories to confirm the presence of DNA in samples. The diphenylamine test is based on the reaction between deoxyribose sugar and diphenylamine reagent, resulting in the formation of a blue-colored complex. Deoxyribose, a component of DNA, reacts with the diphenylamine reagent in the presence of sulfuric acid. The reaction causes the deoxyribose to undergo a series of chemical transformations, leading to the formation of a blue chromophore.[8]

## II. Method

### 2.1 ***For extraction of DNA.***

- *Preparations*
  - Start by gathering all the necessary materials and sterilize any equipment that will encounter the sample.
  - Prepare a working area, ensuring cleanliness and minimizing the chances of cross-contamination.
  - Put the ethanol or isopropyl alcohol in to freezer.
- *Mashing of the fruit*
  - Take three fruits at a time and cut into small pieces and place them in clean and contamination free petri dishes.
  - Now take clean molten and pistil and mash the fruit into it and place the pulp into zip plastic bag.
- *Extracting the DNA*:
  - Add an amount of water in the beaker 50 mL of water, add 2.5ml of Liquid detergent and add 2.5gm of NaCl and mix it properly.
  - Small amount of liquid detergent will help to break down the cell membranes and release the DNA.
  - Add extraction liquid and pulp in the plastic bag.
  - Gently mix the contents by inverting the container or using a pipette to ensure thorough mixing.
  - Let the mixture sit for 5-10 minutes at room temperature to allow the detergent to break down the cell membranes.
- *Pouring of the filtrate*
  - Carefully pour the mixture through a funnel lined with filter paper or a coffee filter into another container.
  - The filtrate that passes through the filter paper contains the DNA.

- *Visualization of DNA*
  - Collect the filtrate in a test tube or small container.
  - Slowly pour an equal volume of cold isopropyl alcohol or ethanol into the test tube, being careful not to mix the two layers.
  - The DNA will precipitate at the interface of the alcohol and the filtrate as a white, stringy substance.
  - Use a clean, sterile pipette or a glass rod to carefully extract the DNA from the interface.
  - Gently store it into the Eppendorf tubes at cold place with solute ethanol or isopropyl alcohol.

- Detection of DNA
  - We will use two methods for detection
    - Agarose Gel electrophoresis
    - Diphenylamine test.

1. Agarose Gel electrophoresis

- Preparation of the gel:
  - Take 1x Tris acetate Buffer (5ml) in a beaker and add 245ml of Distilled water.
  - Now weigh 0.4gm of agarose.
  - Take 40ml of 1x Tris acetate buffer solution and add agarose and mix it gently.

- Heat it up to 55˚C and pour it into gel casting tray.
- Add EtBr to gel while pouring.
- Place the combs at there place and allow it to solidify for 30 mins.

- Loading of Samples.
  - The samples containing the molecules of interest, such as DNA fragments or proteins, are mixed with a loading buffer that contains a tracking dye.
  - Load the sample using micro-pipette.
  - The loading dye helps monitor the progress of the electrophoresis run.

- Application of the electric field:
  - The gel is submerged in the buffer solution in the electrophoresis chamber.
  - Electrodes are placed at each end of the chamber. The negatively charged molecules, such as DNA or proteins, migrate towards the positively charged electrode (anode) when the electric field is applied.

- Separation of molecules:
  - The samples are loaded into wells made in the gel using a comb or a pipette.
  - The electric current is then applied, and the molecules migrate through the gel matrix according to their size and charge.
  - Smaller molecules move more quickly through the gel, while larger molecules migrate more slowly.

- Visualization and analysis:
  - After the electrophoresis run, the separated molecules are typically visualized by staining the gel with specific dyes or using techniques like fluorescent markers or autoradiography.
  - The separated bands or spots can then be analyzed, measured, or further processed depending on the experimental objectives.

2. Diphenylamine test.

- Preparation of the sample:
  - The sample containing the DNA is prepared by isolating DNA from cells or tissues using DNA extraction methods.

- Reaction mixture:
  - The isolated DNA is hydrolyzed by adding acid, typically sulfuric acid, to break down the DNA strands and release the deoxyribose sugar.
  - Diphenylamine reagent is then added to the mixture.

- Incubation:
  - The reaction mixture is incubated at a specific temperature, usually around 37°C, for a specific period to allow the reaction to occur.

- Color development:
  - After incubation, the reaction mixture is observed for color development. If the sample contains DNA, a blue color indicates the presence of deoxyribose sugar, confirming the presence of DNA in the sample.

## III. RESULTS

1. DNA was successfully extracted from kiwi fruit: after the extraction process, we noticed a translucent or cloudy liquid in the test tube or container. This liquid contains the extracted DNA.

2. DNA bands were seen under UV-illuminator: after electrophoresis, the gel shows distinct bands or lanes representing the separated DNA fragments. The gel itself may be transparent or stained with a dye, such as ethidium bromide to enhance the visualization of DNA bands.

3. In Diphenylamine test:

- Positive Result: the substance which we have tested contains Deoxyribose sugar, it showed its presence by turning white color into blue.

## IV. FIGURES AND TABLES

### *A. Figures*

Fig.1. Extraction liquid.

Fig.2. Kiwi fruit into small pieces.

Fig.3. Kiwi fruit after mashing into zip bag.

Fig.4. Appearance of white cloudy substance.


Fig.5. Diphenylamine test Both positive (Bluish) and negative(colorless)

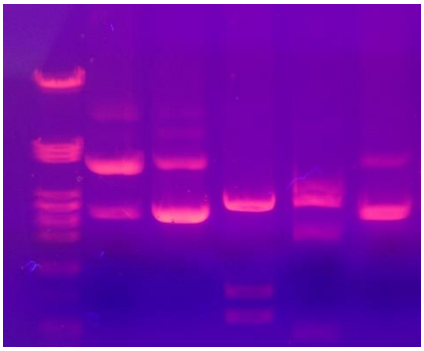
Fig.6. DNA bands seen Under UV.

TABLE I.

| | **1x TAE** | | |
|---|---|---|---|
| Sr.no. | ***Chemical*** | ***Quantity (ml/gm)*** | ***Distilled water*** |
| 1 | 1x Tris acetate | 5ml | 245ml |
| 2 | Agarose | 0.4gm | 40ml |

Table 1. Preparation of gel

## CONCLUSION

In this research we have defined a new method for DNA extraction which is chemical free, than alkaline extraction, phenol-chloroform, etc. this method is totally ecofriendly and can be performed without chemical use. During DNA extraction, detergent causes the cell to pop open, or lyse the cell so that the DNA is released into the liquid solution. Household items have been founded to be cost effective for DNA extraction. This method seems to be more ecofriendly than any other methods. DNA is the blueprint for everything that happens within the cell of associate degree organisms and every cell has a whole copy of constant set of deoxyribonucleic acid.

## REFERENCES

[1] Bahl A and M. Pfenninger Nucleic Acids Research, (1996) (24) 7, 1587–1588.

[2] Birnboim H, Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Research 24; 7(6), 1513–1523.

[3] Carpi FM, Di Pietro F, Vincenzetti S, Mignini F, Napolioni V (2011) Human DNA. Extraction Methods: Patents and Applications. Recent Patents on DNA & Gene Sequences 5(1),1-7.

[4] Clewell, D. B. & Helinski, D. R. (1969) Proc. Natl Acad. Sci. U.S.A. 62, 1159-1166.

[5] Dahm R, (2005) Friedrich Miescher and the discovery of DNA. Developmental Biology 2, 274-288.

[6] L. Buckingham and M. L. Flaws, *Molecular Diagnostics: Fundamentals, Methods, & Clinical Applications*, F.A. Davis, Philadelphia, Pa, USA, 2007.

[7] Meselson M, Stahl F, Vinograd J (1957) EQUILIBRIUM SEDIMENTATION OF MACROMOLECULES IN DENSITY GRADIENTS. Proceedings of The National Academy of Sciences 43(7), 581-588.

[8] Tan.S, Yiap B, (2009) DNA, RNA, and Protein Extraction: The Past and The Present. Journal of Biomedicine and Biotechnology: 1-10