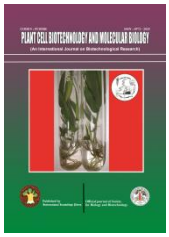

*Plant Cell Biotechnology and Molecular Biology*

***Volume 25, Issue 5-6, Page 30-42, 2024; Article no.PCBMB.12096***
***ISSN: 0972-2025***

# Advances in DNA Extraction Techniques: A Comprehensive Review of Methods and Applications

**Samilla Sheershika [a++] and Mukh Ram [a#*]**

*[a] Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Phagwara, Punjab, 144002, India.*

***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

***Article Information***

DOI: 10.56557/PCBMB/2024/v25i5-68683

**Open Peer Review History:**
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/12096

***Review Article***

***Received: 01/03/2024***
***Accepted: 04/05/2024***
***Published: 06/05/2024***

## ABSTRACT

The extraction of DNA is a fundamental process in molecular biology, underpinning a wide range of applications from genetic research to forensic science and medical diagnostics. This review aims to explore the latest advancements in DNA extraction techniques, highlighting their principles, applications, and suitability for various types of biological samples. Traditional DNA extraction methods, such as phenol-chloroform and ethanol precipitation, have served as the backbone of DNA isolation for decades. However, these techniques often involve hazardous chemicals and can be time-consuming. Recent advancements have focused on developing safer, faster, and more efficient methods, with a focus on automation and scalability. Innovations such as magnetic bead-based extraction, silica column purification, and specialized kits have greatly simplified the process, allowing for high-throughput applications in clinical and research settings. In addition to these technical improvements, new approaches have emerged to address specific challenges, such as

[++] *M. Sc. Scholar;*
[#] *Assistant Professor;*
**Corresponding author: E-mail: mukh.26422@lpu.co.in;*

extracting DNA from degraded or trace samples, as seen in forensic investigations, or isolating cell-free DNA for non-invasive prenatal testing and cancer diagnostics. Moreover, microfluidic devices and lab-on-a-chip technologies are transforming the landscape by enabling DNA extraction from minute samples with minimal reagent consumption. This review discusses the principles underlying these advanced techniques, their benefits and limitations, and the specific contexts in which they excel. It also considers future trends, including further automation, integration with sequencing platforms, and the potential for point-of-care applications. By examining the current state of DNA extraction technology, this review aims to provide researchers and practitioners with a comprehensive guide to the best methods for their specific needs.

*Keywords: DNA extraction; purification; cancer diagnostics; microfluidic devices; automation; sequencing.*

## 1. INTRODUCTION

DNA extraction is a fundamental process in molecular biology, serving as the initial step in countless scientific investigations [1]. The journey began in 1869 when Swiss physician Friedrich Miescher, while researching proteins in leukocytes, accidentally isolated a new substance from the cell nucleus, which he called "nuclein." This substance, later identified as DNA, displayed unique properties distinct from proteins [2]. Since then, the extraction and study of DNA have become crucial in various scientific fields. The information encoded in DNA is stored as a linear sequence of polynucleotides, with each strand comprising a unique order of nucleotides [3]. This sequence serves as a blueprint for biological processes, as it is transcribed into mRNA and translated into amino acids, ultimately determining the structure and function of proteins. DNA exists in different forms, with the most common being the right-handed double helix known as B-form DNA. It can also adopt the A-form, which is involved in RNA-DNA and RNA-RNA interactions, and Z-DNA, a left-handed double helix. Although DNA is typically double-stranded, some viruses contain single-strand DNA [4].

DNA extraction is essential for a wide range of applications in molecular biology, including polymerase chain reaction (PCR), quantitative PCR (qPCR), DNA sequencing, and Southern blotting. It also plays a pivotal role in creating genomic libraries and facilitating various analyses such as random amplification of polymorphic DNA, restriction fragment length polymorphism, short tandem repeat polymorphism, and single nucleotide polymorphism [5]. In the field of medicine, DNA extraction is used to diagnose genetic diseases, identify carrier status, and study pharmacogenomics. It is also invaluable in forensic science for DNA fingerprinting, enabling the identification of suspects and establishing paternity [4,6]. Given the diversity of applications, DNA extraction methods must ensure the quantity and quality of extracted DNA meet the needs of downstream processes. Critical factors to consider include extraction time, cost, yield, laboratory equipment, and specific protocol requirements [7]. This review explores various DNA extraction techniques, highlighting their principles, advantages, and limitations. At the core, DNA extraction involves three key steps: disrupting the cytoplasmic and nuclear membranes, separating and purifying DNA from other cellular components, and concentrating and further purifying the DNA for use in research and diagnostic applications.

## 2. METHODS OF DNA EXTRACTION

DNA extraction is a crucial step in molecular biology and genetic research, serving as the foundation for subsequent analyses such as PCR, sequencing, and cloning. Several methods are commonly used to isolate DNA, each with its unique approach and applicable scenarios [3, 8]. The most traditional method involves the use of phenol-chloroform extraction, which separates nucleic acids from proteins and other cellular debris through a phase separation process. This method, while effective, involves the use of hazardous chemicals and can be time-consuming [5]. Ethanol precipitation, often combined with phenol-chloroform, allows for the recovery of DNA in a more purified form by precipitating it out of solution with ethanol or isopropanol, a conventional extraction (Fig1).

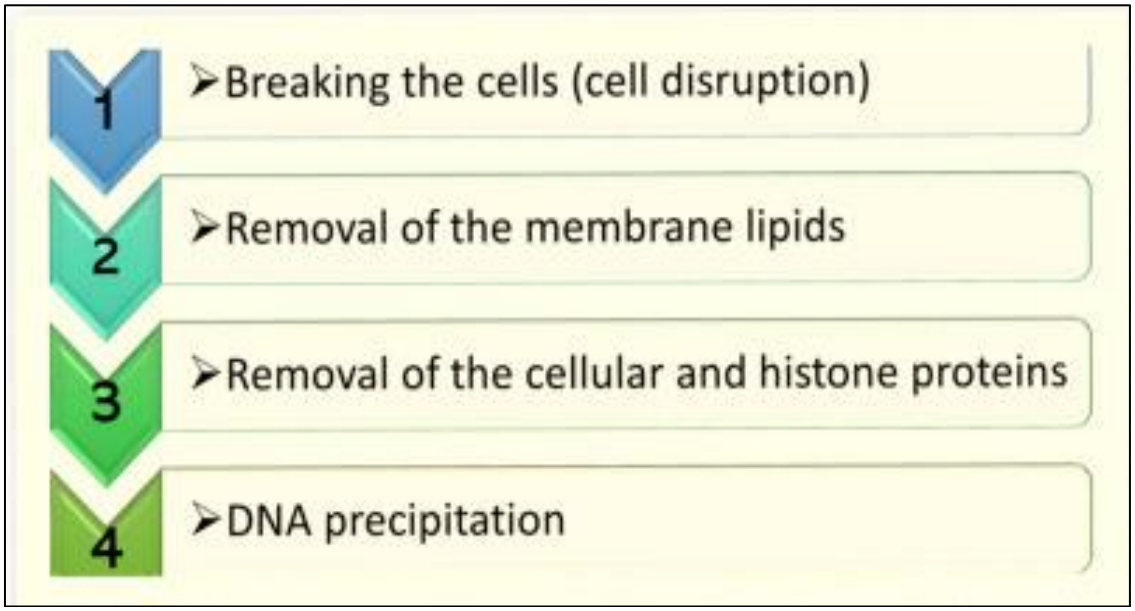


**Fig. 1. DNA extraction methods (Conventional)**

In contrast, newer methods like silica column-based extraction and magnetic bead-based techniques offer safer and faster alternatives. Silica columns, which rely on the principle that DNA binds to silica in the presence of high salt concentrations, are widely used in commercial DNA extraction kits for their simplicity and efficiency. Magnetic bead-based extraction uses magnetic beads coated with DNA-binding molecules, allowing for a straightforward and automated process, ideal for high-throughput applications [8-9]. These modern techniques are designed to improve yield and purity while minimizing the use of hazardous chemicals, making them well-suited for clinical and research laboratories. Fig 2 illustrates the workflow of a typical silica column-based extraction, highlighting the key steps: cell lysis, DNA binding, washing, and elution, emphasizing the streamlined nature of modern DNA extraction techniques.

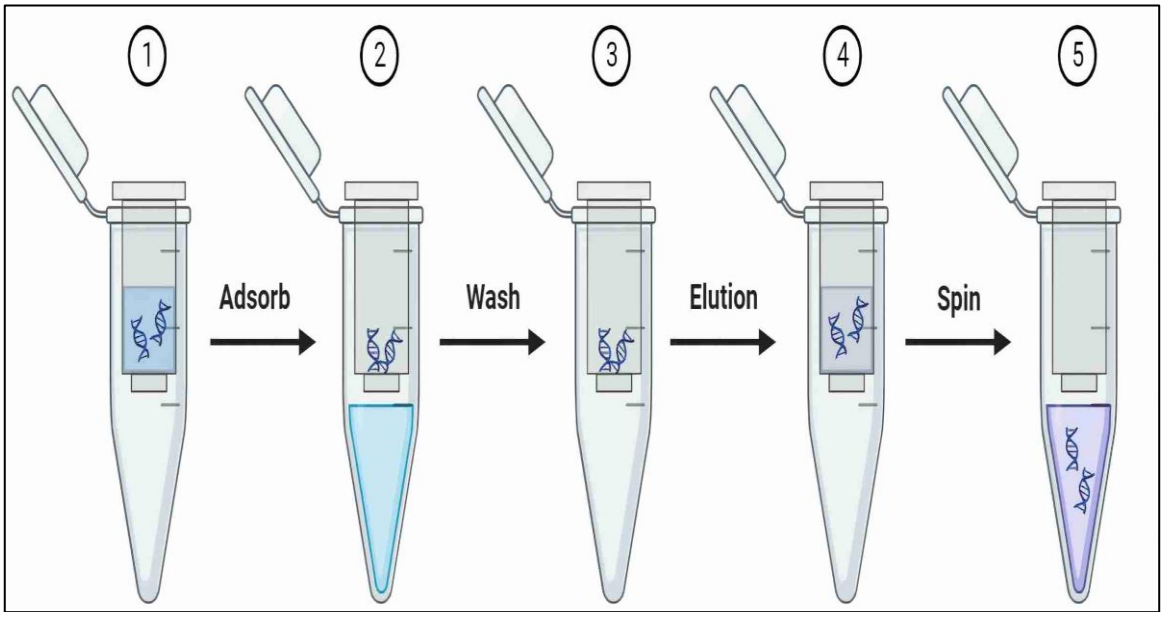


**Fig. 2. Silica spin column based nucleic acid purification**

DNA extraction is a foundational process in molecular biology, with a variety of methods available to isolate DNA from different sources [10]. These methods can be broadly classified into several categories, each employing unique principles for breaking cells and purifying DNA. The flow chart below outlines the key methods used for DNA extraction, illustrating the diverse approaches:

### 2.1 Chemical DNA Extraction Methods

This approach relies on chemical agents to lyse cells and release DNA. It is typically used when a gentle process is required to preserve the integrity of the DNA.

### 2.2 Physical DNA Extraction Methods

Physical methods use mechanical forces to break cell membranes and release DNA. Techniques such as bead beating and sonication fall into this category.

### 2.3 Inorganic DNA Extraction Methods

These methods use non-organic chemicals to extract DNA. The salting-out method, for example, employs high salt concentrations to precipitate proteins, allowing DNA to remain in solution.

### 2.4 Organic DNA Extraction Methods

This category includes the phenol-chloroform DNA extraction method, a traditional technique that uses organic solvents to separate nucleic acids from proteins. This method is effective but requires careful handling due to toxic chemicals.

### 2.5 Silica Matrices Extraction Method

A more modern approach, silica matrices use silica columns to bind DNA in the presence of high salt concentrations. This technique is popular due to its simplicity and safety, as it avoids the use of hazardous organic solvents [11].

### 2.6 Proteinase K DNA Extraction Methods

This method involves the enzyme Proteinase K to digest proteins, facilitating the release of DNA. It is often combined with other methods to improve purity [12].

### 2.7 Magnetic Bead DNA Extraction

Magnetic bead-based extraction employs magnetic beads coated with DNA-binding molecules. This method is suitable for automation and high-throughput applications, allowing for quick and efficient DNA isolation.

### 2.8 Paper DNA Extraction

In this method, DNA is bound to a paper matrix for transport and storage, later eluted for further processing. This approach is used for fieldwork and sample collection in remote areas.

These diverse methods offer various advantages depending on the application, sample type, and scale of operation. Fig 3 provides a visual representation of these DNA extraction methods, showing the pathways and connections among them, offering a clear perspective on the broad spectrum of techniques available for DNA extraction.

To extract DNA from homogenized tissue, begin by transferring the sample into a polypropylene tube, which provides a stable environment for further processing. For every 100 mg of tissue, add 500 µl of CTAB buffer, ensuring the sample is fully mixed by vortexing [13]. After thorough mixing, place the tube in a 60°C water bath for 30 minutes, which helps to lyse the cells and release the DNA into solution. Following this initial processing, centrifuge the mixture for 5 minutes at 14,000 x g to separate the cellular debris from the DNA-containing supernatant. Transfer the supernatant to a new tube, then add 5 µl of RNase solution and incubate at 37°C for 20 minutes. This step removes contaminating RNA, ensuring the purity of the DNA. To further purify the DNA, add an equal volume of alcohol to the supernatant, vortex for 5 seconds, and then centrifuge for 1 minute at 14,000 x g to separate the phases. Carefully transfer the upper aqueous phase to a new tube, repeating this step until the upper phase is clear. For DNA precipitation, transfer the clear supernatant to a new tube and add 0.7 volumes of cold isopropanol. Incubate this at -20°C for 15 minutes to precipitate the DNA. Afterward, centrifuge at 14,000 x g for 10 minutes to form a DNA pellet, then carefully decant the ethanol and use a speed vacuum to remove any remaining alcohol without overdrying the DNA [14]. Finally, dissolve the DNA pellet in 20 µl of TE buffer, warming if necessary to ensure complete dissolution. This process yields high-quality DNA suitable for further molecular biology applications, such as PCR, sequencing, or cloning [15].

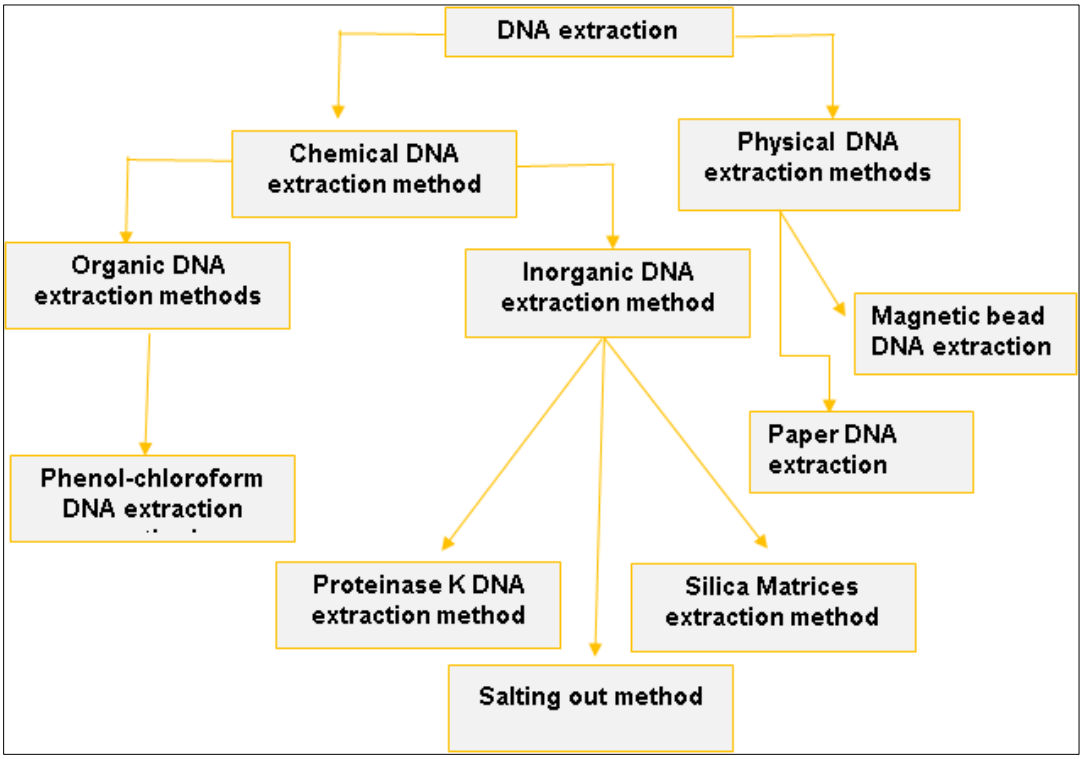


**Fig. 3. Different methods of DNA extraction, showing the pathways and connections**

## 3. PHYSICAL DNA EXTRACTION

Chromatography-based DNA extraction encompasses several techniques designed to purify DNA from various biological samples. This approach uses physical properties such as size, charge, or specific binding affinities to separate DNA from other cellular components [16]. The three primary methods are size exclusion chromatography, ion-exchange chromatography, and affinity chromatography.

**Size Exclusion Chromatography (SEC):** relies on the physical size and shape of molecules for separation. The process involves a gel-filtration mechanism, where an aqueous solution carries the DNA-containing sample through a column packed with porous beads. As the sample moves through the column, smaller molecules enter the beads' pores, while larger ones, including DNA, are excluded and pass through the matrix more quickly. This method is particularly useful for sensitive substances, as it avoids drastic pH changes and high metal ion concentrations [17]. SEC is a gentle technique that preserves the integrity of the DNA.

**Ion-Exchange Chromatography (IEC):** separates molecules based on their charge. In this method, the DNA binds to a positively charged di-ethyl aminoethyl (DEAE) cellulose resin, retaining it in the column while other cellular components, like proteins, carbohydrates, metabolites, and RNA, are washed out with medium salt buffers. This method provides high-quality DNA similar to that obtained with more complex techniques, such as CsCl-gradient centrifugation [18]. The advantage of IEC lies in its simplicity and ease of use, making it a popular choice for DNA purification.

**Affinity Chromatography:** involves the use of specific substances that have a high binding affinity for DNA. This method resembles IEC but with modifications to accommodate the unique binding interactions between DNA and other substances, such as oligo(dt) or other DNA-binding groups. Affinity chromatography is commonly used to isolate specific types of nucleic acids, such as mRNA [19]. The use of specific ligands allows for targeted DNA extraction, often resulting in a higher yield and purity of the desired nucleic acid.

**EtBr-CsCl Gradient Centrifugation Methods:** Developed in 1957 by Matthew Meselson, Franklin W. Stahl, and Jerome Vinograd, the EtBr-CsCl gradient centrifugation method has been a crucial technique in DNA extraction. This

process begins with DNA being mixed with cesium chloride, followed by ultracentrifugation at speeds of 10,000 to 12,000 rpm for about 10 hours. The high-speed centrifugation creates a gradient, where substances separate based on their densities. DNA's density varies depending on its supercoiling and intercalation with ethidium bromide (EtBr) [20]. Ethidium bromide acts as an intercalating agent, preferentially incorporating into non-supercoiled DNA, causing these molecules to accumulate at lower densities [21]. The resulting bands of DNA can be visualized under ultraviolet light, allowing researchers to isolate the desired DNA. Despite its effectiveness in extracting DNA from bacteria, this method requires a substantial amount of material and is relatively complex and time-consuming due to the need for high-speed ultracentrifugation and the use of potentially hazardous chemicals like EtBr and CsCl [22,20].

**Cetyltrimethyl ammonium Bromide (CTAB) Extraction:** The CTAB extraction method, discovered by Doyle et al. in 1990, is another widely used technique for extracting DNA, particularly from plants and bacteria that contain high levels of polysaccharides. In this method, DNA-containing samples are mixed with a 2% solution of CTAB at an alkaline pH, creating a buffer with low ionic strength. This buffer helps to separate DNA from acidic polysaccharides and other cellular components. A high-concentration salt solution is then used to further isolate DNA by forming a precipitate with CTAB (Fig 4). This method is especially useful for plant DNA extraction, as it effectively removes polyphenols and polysaccharides that can interfere with DNA isolation [23]. However, CTAB extraction can be more time-consuming and involves toxic organic solvents like phenol and chloroform, raising safety concerns. Nonetheless, the use of CTAB and related additives, such as polyvinylpyrrolidone, plays a key role in the purification of DNA from plant tissues, offering a reliable solution for DNA extraction despite its limitations [24].

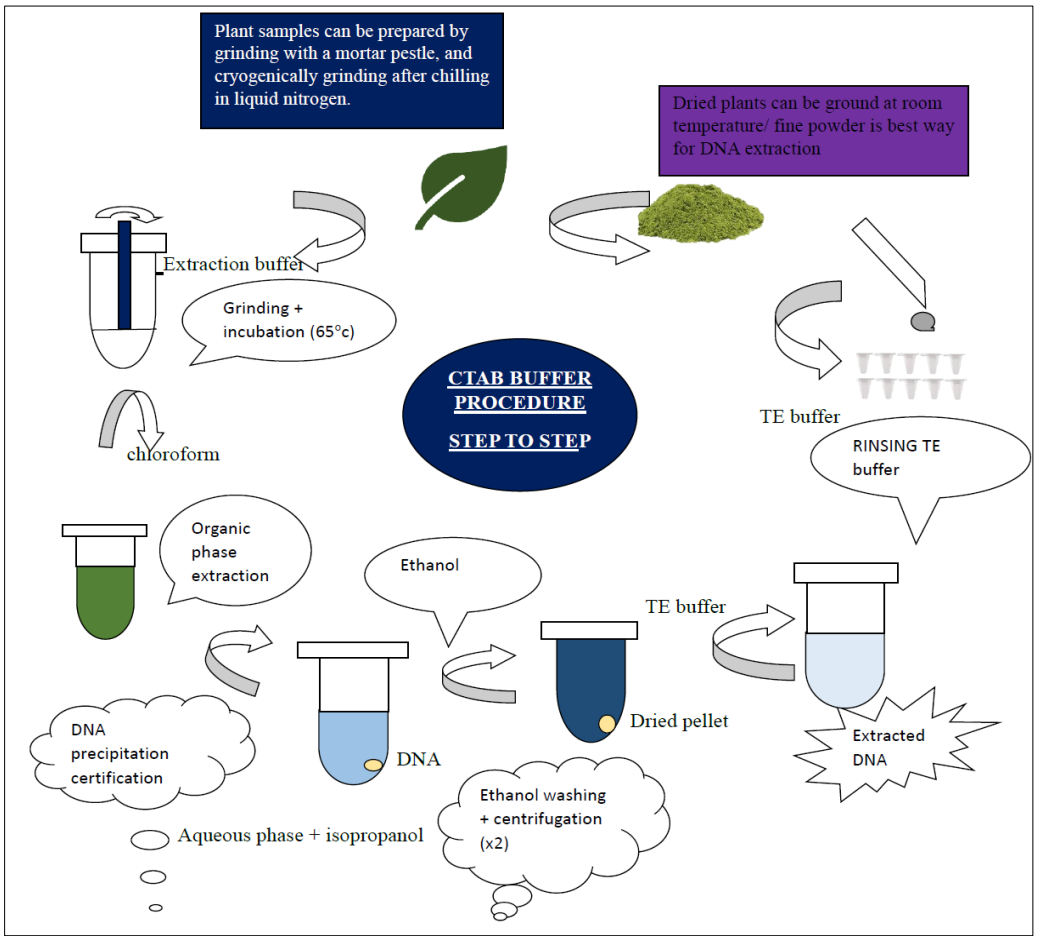


**Fig. 4. CTAB buffer procedure for extraction of DNA**

Phenol-chloroform extraction is a chemical method for isolating DNA, discovered by Barker in 1998. It involves breaking the cell wall, a process known as cell lysis, to release DNA into a solution [21] [25]. The method employs a combination of grinding and lysis buffer to disrupt cell membranes and remove contaminants. To assist in this process, detergents are used to dissolve lipids, and protease enzymes to remove proteins. Sodium dodecyl sulfate (SDS) plays a crucial role in cell lysis, but it does not break the nuclear membrane. The extraction method relies on several key components. Ethylene diamine tetraacetic acid (EDTA) acts as a chelating agent to bind divalent cations like magnesium (Mg2+), which are cofactors for DNase enzymes that degrade DNA. Proteinase K is used to digest proteins, including proteolytic enzymes, and is especially active against denatured proteins [26]. Phenol and chloroform are employed to separate proteins and other contaminants from DNA, while Tris-EDTA (TE) buffer is used for DNA storage, with Tris acting as a buffering agent to maintain a stable pH [22].

The extraction process begins with cell lysis, where SDS and proteinase K are used to break down cell membranes and digest proteins. The released DNA is then separated by using phenol-chloroform extraction, which helps to remove proteinaceous material. The organic phase contains contaminants like proteins and lipids, while the aqueous phase contains the DNA. The DNA is then precipitated from the aqueous layer by adding ice-cold 95% ethanol and salt [27]. The precipitated DNA is washed with 70% ethanol to remove any residual impurities, dried in a vacuum, and finally resuspended in TE buffer for storage and further use. The SDS-Proteinase K method, developed by Ebeling et al. in 1974, is an organic DNA extraction method initially used to extract DNA from the fungus Engyodontium album. This method relies on sodium dodecyl sulfate (SDS), a powerful detergent that disrupts cellular and nuclear membranes, combined with Proteinase K, an enzyme that digests proteins [28]. The process begins with lysis of the cells using SDS to break open the membranes, followed by Proteinase K to break down proteins and release DNA into the solution [29]. This method has proven effective in extracting high-quality DNA for various molecular biology applications.

The Salting-Out method, described by Miller, Dykes, and Polesky in 1988, offers a non-toxic alternative to DNA extraction. Unlike other methods that use organic solvents, Salting-Out relies on high salt concentrations to separate proteins and other contaminants from DNA [30]. The process involves lysing the cells and then adding high concentrations of salt, which causes proteins to precipitate while leaving the DNA in solution [31]. The DNA can then be precipitated from the solution by adding ethanol or isopropanol. This method is considered safer and more environmentally friendly because it does not use toxic chemicals like phenol or chloroform, and it can yield large amounts of DNA suitable for various downstream applications. Disruption of cytoplasmic and nuclear membranes is the first step in DNA extraction, crucial for releasing DNA from the nucleus [24]. This process involves breaking the cells mechanically to access the DNA within them. To achieve this, tissues are typically cut into small pieces and subjected to physical disruption using blenders, mortar and pestle, or other mechanical tools. Given that plant cells have rigid cell walls, the disruption process is particularly significant for them. For softer cell walls, detergents and enzymes like Proteinase K can be used to separate cellular proteins from the DNA. After the DNA is released from the nucleus, it is mixed with other cell components, necessitating further separation and purification. Alcohol is added to precipitate DNA from the aqueous solution because DNA is insoluble in alcohol. The alcohol used is typically isopropanol or ethanol [32].

Following disruption and initial separation, the focus shifts to purification of DNA from other cellular components. The goal is to clean the DNA from unwanted materials such as proteins, lipids, and saccharides. The purified DNA is then dissolved in water or a buffer for easy storage and handling [33] [25, 27]. The process includes several critical steps, such as creating the lysate, clearing the lysate, binding, purification, and washing. Lysate creation involves the physical, chemical, or enzymatic breakdown of cells to release DNA. Physical methods involve grinding and freezing, while chemical methods use detergents like SDS and chaotropic agents like guanidine salts. The enzymatic method uses specific enzymes, such as Proteinase K, to break down cellular components [34]. Lysate clearing is essential to remove cellular debris through centrifugation or filtration. The purification matrix and washing steps focus on removing contaminants while binding DNA to specific matrices, such as silica, cellulose, or ion-exchange columns, which allow for selective binding of nucleic acids [31]. The final washing

step typically uses alcohol to remove residual salts and proteins, ensuring the DNA is clean and ready for downstream applications [35].

## 4. CHROMATOGRAPHY-BASED DNA EXTRACTION METHODS

Chromatography-based DNA extraction methods are versatile techniques used to isolate DNA from a variety of biological materials. These methods leverage the principles of chromatography to separate DNA from other cellular components based on different properties such as size, charge, and specific interactions. The primary types of chromatography-based DNA extraction include size exclusion chromatography (SEC), ion-exchange chromatography (IEC), and affinity chromatography (AC) [36].

### 4.1 Size Exclusion Chromatography (SEC)

Size exclusion chromatography separates molecules based on their size and shape. In SEC, an aqueous solution is used to transport a sample through a column filled with porous beads made of polyacrylamide, dextran, or agarose. Smaller molecules, like mRNA and proteins, enter the beads through small pores, while larger DNA molecules are excluded due to their size, allowing them to move through the column more quickly [37]. This characteristic makes SEC suitable for isolating DNA from other smaller biomolecules. SEC is also ideal for substances that are sensitive to changes in pH and metal ion concentrations.

### 4.2 Ion-Exchange Chromatography (IEC)

Ion-exchange chromatography relies on the principle that DNA is negatively charged due to its phosphate backbone. The column contains an anion-exchange resin with positively charged groups, such as diethylaminoethyl (DEAE) cellulose, which selectively binds DNA. Other cellular components, like proteins, lipids, carbohydrates, and RNA, are eluted using medium-salt buffers, while DNA is retained in the column [38]. To recover the DNA, the pH is lowered, or high-salt buffers are used to displace it from the resin. IEC is a relatively straightforward method that yields high-quality DNA.

### 4.3 Affinity Chromatography (AC)

Affinity chromatography exploits specific interactions to isolate nucleic acids. It involves using substances like oligo(dT) that bind selectively to nucleic acids, allowing for their separation from other cellular components. While AC is commonly used for mRNA isolation, it can also be adapted for DNA extraction. This method is time-efficient and provides a good yield of nucleic acids. Each of these chromatography-based DNA extraction methods offers unique advantages depending on the application and the type of biological material [39]. They contribute to a more refined approach to DNA extraction, enabling researchers to obtain high-quality DNA for a wide range of applications in genomics, forensics, diagnostics, and more.

## 5. APPLICATIONS OF ADVANCED EXTRACTION TECHNIQUES

Advances in DNA extraction techniques have opened the door to a wide range of applications across various fields, offering more efficient, reliable, and versatile methods for obtaining high-quality DNA. Here are some key applications where these advanced techniques have made a significant impact:

**Medical Diagnostics:** DNA extraction is fundamental in medical diagnostics, allowing for accurate detection of genetic mutations, pathogens, and other biomarkers. Techniques such as polymerase chain reaction (PCR) and quantitative PCR (qPCR) rely on high-quality DNA to diagnose diseases, including cancer, infectious diseases, and genetic disorders [40]. Rapid and efficient DNA extraction methods have made it easier to conduct these tests quickly and accurately.

**Forensic Science:** In forensic investigations, DNA extraction from evidence such as hair, blood, or tissue samples is critical for identifying suspects and solving crimes. Advances in extraction techniques enable successful DNA retrieval from degraded or limited samples, providing reliable results that can stand up in court [41]. This has revolutionized the field of forensic science, leading to more accurate convictions and exonerations.

**Agricultural Biotechnology:** DNA extraction plays a pivotal role in agricultural biotechnology, where researchers identify and manipulate genes to improve crop traits like yield, resistance to *pests and diseases, and tolerance to* environmental stresses. These advances support the development of genetically modified organisms (GMOs) and gene editing

technologies such as CRISPR, leading to more sustainable and resilient agricultural practices.

**Environmental Monitoring:** Environmental scientists use DNA extraction techniques to monitor biodiversity, track endangered species, and study ecosystems. Environmental DNA (eDNA) allows researchers to detect the presence of organisms in various environments without direct observation, providing valuable data for conservation efforts and ecosystem management [42].

**Research and Genomics:** In the field of genomics, DNA extraction is the first step in analyzing genomes and understanding genetic information. Advances in extraction methods have facilitated large-scale genomic projects, such as genome-wide association studies (GWAS), which explore the genetic basis of complex traits and diseases [43]. This has also enabled personalized medicine, where genetic information guides individualized treatment plans.

**Archaeogenetics and Paleogenomics:** Advanced DNA extraction techniques have made it possible to study ancient DNA from archaeological remains, offering insights into human evolution, migration patterns, and ancient diseases [44]. The ability to extract and analyze DNA from ancient samples has significantly contributed to our understanding of human history and evolution.

**Veterinary and Animal Sciences:** DNA extraction techniques are used in veterinary medicine to diagnose animal diseases and in animal breeding programs to identify desirable genetic traits. This supports the development of healthier and more productive livestock and companion animals [45].

These applications highlight the diverse impact of advances in DNA extraction techniques. From medical diagnostics and forensic science to agricultural biotechnology and environmental monitoring, these techniques have transformed research and practical applications, driving innovation across multiple domains.

## 6. ADVANTAGES OF MODERN EXTRACTION METHODS

Advances in DNA extraction techniques have revolutionized molecular biology and genomics, providing researchers with efficient and reliable methods to isolate DNA from a variety of samples. The following are some key advantages of these modern extraction methods:

### 6.1 Higher Yield and Purity

New DNA extraction techniques are designed to maximize yield while ensuring high purity [46]. This is especially crucial in applications like next-generation sequencing, where DNA quality significantly affects results.

### 6.2 Faster Processing Times

Contemporary DNA extraction kits and automated systems have dramatically reduced the time required to isolate DNA [47]. This efficiency allows researchers to process more samples in less time, accelerating research projects and diagnostics.

### 6.3 Versatility Across Sample Types

Advanced techniques are capable of extracting DNA from a wide range of sample types, including blood, saliva, plant tissues, microbial cultures, and even challenging sources like ancient bones or degraded samples [48]. This versatility has broadened the scope of applications in genetics, forensics, and paleo genomics.

### 6.4 Reduced Contamination Risk

New DNA extraction methods incorporate contamination controls to minimize the risk of cross-contamination between samples [49]. This is especially important in clinical and forensic settings where sample integrity is critical.

### 6.5 Scalability and Automation

Modern DNA extraction technologies offer scalable solutions that can handle small-scale lab needs to large-scale high-throughput processing [50]. Automation has further enhanced the scalability, reducing manual labour and increasing accuracy.

### 6.6 Cost-Effectiveness

Advances in extraction technology have reduced costs, making high-quality DNA extraction more accessible. This affordability has facilitated wider adoption of molecular techniques in various fields, from research to clinical diagnostics.

### 6.7 Integration with Downstream Applications

DNA extracted using modern techniques is compatible with a variety of downstream applications, such as PCR, qPCR, and DNA sequencing [51]. This seamless integration allows for a wide range of analyses from a single extraction.

These advantages demonstrate the impact of advances in DNA extraction techniques on research and clinical practice. By improving yield, speed, versatility, and reliability, modern methods are enabling breakthroughs in fields like genomics, forensic science, personalized medicine, and beyond.

## 7. CONCLUSION

The rapid evolution of DNA extraction techniques has had a transformative impact on molecular biology, genetics, and biotechnology. This comprehensive review explored the key advancements in DNA extraction, ranging from traditional manual methods to sophisticated automated systems. These developments have not only improved the efficiency and reliability of DNA extraction but have also expanded the scope of applications across various fields. Advances in DNA extraction have led to methods that deliver higher yields and purer samples, facilitating applications in medical diagnostics, forensic science, agricultural biotechnology, environmental monitoring, and more. Techniques such as magnetic bead-based extraction, column-based chromatography, and automated systems have streamlined the process, allowing for faster and more accurate DNA isolation. This progress has paved the way for high-throughput applications and large-scale genomics projects that were previously unimaginable. These advancements have also contributed to reduced contamination risks, increased versatility with various sample types, and greater scalability. The ability to extract DNA from challenging samples, such as ancient bones or degraded biological material, has opened new research avenues in archaeogenetics and paleogenomics. Despite these significant achievements, challenges remain, particularly in balancing cost, efficiency, and quality. The need for standardized protocols and rigorous quality control is crucial to ensure the reliability of DNA extraction methods. Additionally, the integration of emerging technologies, like artificial intelligence and machine learning, may further enhance the precision and customization of DNA extraction in the future. The advancements in DNA extraction techniques have not only revolutionized our approach to molecular biology but have also had a profound impact on many other scientific domains. As research continues to push the boundaries of what is possible, these extraction methods will play a pivotal role in shaping the future of science and technology. Ongoing innovation and interdisciplinary collaboration will be key to addressing current challenges and unlocking new opportunities in DNA-based research and applications.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Dairawan M, Shetty PJ. The evolution of DNA extraction methods. Am. J. Biomed. Sci. Res. 2020;8(1):39-45.
2. Gupta N. DNA extraction and polymerase chain reaction. Journal of Cytology. 2019;36(2):116-7.
3. Tanase AM, Mereuta I, Chiciudean I, Ionescu R, Milea L, Cornea CP, Vassu T, Stoica I. Comparison of total DNA extraction methods for microbial community from polluted soil. Agriculture and Agricultural Science Procedia. 2015; 6:616-22.
4. Abdel-Latif A, Osman G. Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. Plant Methods. 2017;13:1-9.
5. Manen JF, Sinitsyna O, Aeschbach L, Markov AV, Sinitsyn A. A fully automatable enzymatic method for DNA extraction from plant tissues. BMC Plant Biology. 2005; 5:1-9.
6. Scobeyeva VA, Omelchenko DO, Dyakov LM, Konovalov AS, Speranskaya AS, Krinitsina AA. Comparison of some plant DNA extraction methods. Russian Journal of Genetics. 2018;54: 576-86.
7. Ahmed I, Islam M, Arshad W, Mannan A, Ahmad W, Mirza B. High-quality plant DNA extraction for PCR: an easy approach. Journal of Applied Genetics. 2009;50:105-7.

8. Esfandani-Bozchaloyi S, Sheidai M, Kalalegh MH. Comparison of DNA extraction methods from Geranium (Geraniaceae). Acta Botanica Hungarica. 2019;61(3-4):251-66.
9. Xin Z, Chen J. A high throughput DNA extraction method with high yield and quality. Plant Methods. 2012;8:1-7.
10. Leitch IJ, Hanson L, Lim KY, Kovarik A, Chase MW, Clarkson JJ, Leitch AR. The ups and downs of genome size evolution in polyploid species of Nicotiana (Solanaceae). Annals of Botany. 2008;101 (6):805-14.
11. Albuquerque P, Mendes MV, Santos CL, Moradas-Ferreira P, Tavares F. DNA signature-based approaches for bacterial detection and identification. Science of the Total Environment. 2009;407(12): 3641-51.
12. Bauer T, Weller P, Hammes WP, Hertel C. The effect of processing parameters on DNA degradation in food. European Food Research and Technology. 2003;217:338-43.
13. Schmiderer C, Lukas B, Novak J. Effect of different DNA extraction methods and DNA dilutions on the amplification success in the PCR of different medicinal and aromatic plants. Zeitschrift für Arznei-und Gewürzpflanzen. 2013;18:65-72.
14. Ali N, Rampazzo RD, Costa AD, Krieger MA. Current nucleic acid extraction methods and their implications to point-of-care diagnostics. BioMed Research International; 2017.
15. Tan SC, Yiap BC. DNA, RNA, and protein extraction: the past and the present. BioMed Research International. 2009; 2009.
16. Doyle K. The source of discovery: protocols and applications guide. Madison: PROMEGA. 1996.
17. Demeke T, Jenkins GR. Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits. Analytical and Bioanalytical Chemistry. 2010;396:1977-90.
18. Ali N, Rampazzo RD, Costa AD, Krieger MA. Current nucleic acid extraction methods and their implications to point-of-care diagnostics. BioMed Research International. 2017;2017.
19. Csaikl UM, Bastian H, Brettschneider R, Gauch S, Meir A, Schauerte M, Scholz F, Sperisen C, Vornam B, Ziegenhagen B. Comparative analysis of different DNA extraction protocols: a fast, universal maxi-preparation of high-quality plant DNA for genetic evaluation and phylogenetic studies. Plant Molecular Biology Reporter. 1998;16:69-86.
20. Rogstad SH. Plant DNA extraction using silica. Plant Molecular Biology Reporter. 2003;21:463. Available:https://doi.org/10.1007/BF02772598
21. Porebski S, Bailey LG, Baum BR. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Molecular Biology Reporter. 1997;15:8-15.
22. Drábková LZ. DNA extraction from herbarium specimens. Molecular Plant Taxonomy: Methods and Protocols. 2014; 69-8.
23. Khan IA, Awan FS, Ahmad A, Khan AA. A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. Plant Molecular Biology Reporter. 2004;22:89.
24. Karakousis A, Langridge P. A high-throughput plant DNA extraction method for marker analysis. Plant Molecular Biology Reporter. 2003;21:95-.
25. Dilworth E, Frey JE. A rapid method for high throughput DNA extraction from plant material for PCR amplification. Plant Molecular Biology Reporter. 2000;18: 61-4.
26. Ahmed I, Islam M, Arshad W, Mannan A, Ahmad W, Mirza B. High-quality plant DNA extraction for PCR: An easy approach. Journal of applied genetics. 2009;50:105-7.
27. Lickfeldt DW, Hofmann NE, Jones JD, Hamblin AM, Voigt TB. Comparing three DNA extraction procedures for cost, efficiency, and DNA yield. HortScience. 2002;37(5):822-5.
28. Tapia-Tussell R, Quijano-Ramayo A, Rojas-Herrera R, Larque-Saavedra A, Perez-Brito D. A fast, simple, and reliable high-yielding method for DNA extraction from different plant species. Molecular biotechnology. 2005;31:137-9.

29. Nunes CF, Ferreira JL, Fernandes MC, Breves SD, Generoso AL, Soares BD, Dias MS, Pasqual M, Borem A, Cançado GM. An improved method for genomic DNA extraction from strawberry leaves. Ciência Rural. 2011;41: 1383-9.
30. Friar EA. Isolation of DNA from plants with large amounts of secondary metabolites. In Methods in enzymology. Academic Press. 2005;395:1-12.
31. Attitalla IH. Modified CTAB method for high-quality genomic DNA extraction from medicinal plants. Pak J Biol Sci. 2011; 14(21):998-9.
32. Green MJ, Thompson DA, MacKenzie DJ. Easy and efficient DNA extraction from woody plants for the detection of phytoplasmas by polymerase chain reaction. Plant Disease. 1999;83(5):482-5.
33. Stein N, Herren G, Keller B. A new DNA extraction method for high-throughput marker analysis in a large-genome species such as Triticum aestivum. Plant Breeding. 2001;120(4): 354-6.
34. Keb-Llanes M, González G, Chi-Manzanero B, Infante D. A rapid and simple method for small-scale DNA extraction in Agavaceae and other tropical plants. Plant Molecular Biology Reporter. 2002;20(3):299-300.
35. Agbagwa IO, Datta S, Patil PG, Singh P, Nadarajan N. A protocol for high -quality genomic DNA extraction from legumes. Genet. Mol. Res. 2012;11(4): 4632-9.
36. Barbier FF, Chabikwa TG, Ahsan MU, Cook SE, Powell R, Tanurdzic M, Beveridge CA. A phenol/chloroform-free method to extract nucleic acids from recalcitrant, woody tropical species for gene expression and sequencing. Plant Methods. 2019;15:1-3.
37. Scobeyeva VA, Omelchenko DO, Dyakov LM, Konovalov AS, Speranskaya AS, Krinitsina AA. Comparison of some plant DNA extraction methods. Russian Journal of Genetics. 2018:576-86.
38. Fulton TL. Setting up an ancient DNA laboratory. Ancient DNA: Methods and Protocols. 2012:1-1.
39. Tao L, Yuan H, Zhu K, Liu X, Guo J, Min R, He H, Cao D, Yang X, Zhou Z, Wang R. Ancient genomes reveal millet farming-related demic diffusion from the Yellow River into southwest China. Current Biology. 2023;33(22):4995-5002.
40. Spadoni A, Sion S, Gadaleta S, Savoia MA, Piarulli L, Fanelli V, Di Rienzo V, Taranto F, Miazzi MM, Montemurro C, Sabetta W. A simple and rapid method for genomic DNA extraction and microsatellite analysis in tree plants. Journal of Agricultural Science and Technology. 2019;21(5): 1215-26.
41. Xin Z, Velten JP, Oliver MJ, Burke JJ. High-throughput DNA extraction method suitable for PCR. Biotechniques. 2003;34 (4):820-6.
42. Stewart Jr CN. Rapid DNA extraction from plants. In Fingerprinting methods based on arbitrarily primed PCR. Berlin, Heidelberg: Springer Berlin Heidelberg. 1997;25-28.
43. Aboul-Maaty NA, Oraby HA. Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bulletin of the National Research Centre. 2019;43 (1):1-0.
44. Mahuku GS. A simple extraction method suitable for PCR-based analysis of plant, fungal, and bacterial DNA. Plant Molecular Biology Reporter. 2004;22:71-81.
45. Rogstad SH, Keane B, Keiffer CH, Hebard F, Sisco P. DNA extraction from plants: The use of pectinase. Plant Molecular Biology Reporter. 2001;19:353-9.
46. Khan IA, Awan FS, Ahmad A, Khan AA. A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. Plant Molecular Biology Reporter. 2004;22:89.
47. Aboul-Maaty NA, Oraby HA. Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bulletin of the National Research Centre. 2019;43(1): 1-0.
48. Mahuku GS. A simple extraction method suitable for PCR-based analysis of plant, fungal, and bacterial DNA. Plant Molecular Biology Reporter. 2004; 22:71-81.
49. Rogstad SH, Keane B, Keiffer CH, Hebard F, Sisco P. DNA extraction from plants: The use of pectinase. Plant Molecular Biology Reporter. 2001;19:353-9.

50. Scott KD, Playford J. DNA extraction technique for PCR in rain forest plant species. Biotechniques. 1996;20(6):974-8.

51. Webb DM, Knapp SJ. DNA extraction from a previously recalcitrant plant genus. Plant Molecular Biology Reporter. 1990; 8:180-5.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*https://prh.ikprress.org/review-history/12096*