# Chapter-09

# CHROMATOGRAPHY BASED DNA ISOLATION METHODS

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## *ABSTRACT*

*Since Friedrich Miescher's ground breaking work in 1869, DNA extraction methods have undergone remarkable progress. Initially, liquid-liquid extraction using phenol-chloroform paved the way for DNA research but came with drawbacks, such as the use of toxic chemicals. In response, safer alternatives, including physical extraction methods like magnetic beads and cellulose-based filter paper, have revolutionized the field. With the rise of gene editing and personalized medicine, there is a heightened demand for reliable DNA isolation techniques. Researchers and clinicians now seek methods that consistently yield high-quality DNA with minimal impurities for applications like gene therapy and disease diagnosis. The evolution of DNA extraction methods reflects the dynamic nature of scientific research, emphasizing the continuous pursuit of improvement and innovation in molecular biology. As technology advances and research needs evolve, we anticipate the emergence of even more efficient and sophisticated DNA extraction techniques. These advancements will play a crucial role in propelling scientific discoveries and furthering personalized medicine.*


***Keywords:*** *DNA Extraction, DNA, Phenol –Chloroform, Chromatography Based DNA Extraction.*

## 9.1 INTRODUCTION

The extraction of DNA is a fundamental step in molecular biology, genetics, and various biotechnological applications. DNA extraction methods have evolved significantly since the discovery of DNA by Friedrich Miescher in 1869. One class of techniques that has gained prominence in DNA extraction is chromatography-based methods. Chromatography, a separation technique, is used to isolate and purify DNA from complex biological samples by exploiting the differential affinities of DNA and other cellular components for a stationary phase and a mobile phase. In this chapter, we will explore the principles, steps, advantages, limitations, and applications of chromatography-based DNA extraction techniques.

## 9.2 PRINCIPLES OF CHROMATOGRAPHY-BASED DNA EXTRACTION

Chromatography-based DNA extraction techniques rely on the differential affinities of molecules for stationary and mobile phases. In the context of DNA extraction, a chromatographic column serves as the stationary phase, while a buffer solution functions as the mobile phase. DNA molecules, with their negatively charged phosphate backbone, interact with the stationary phase, which may be positively charged (anion exchange chromatography) or consist of solid-phase materials designed to capture DNA (Gaj, et. al. 2016).

## 9.3 DIFFERENT TYPES OF CHROMATOGRAPHY-BASED DNA EXTRACTION

Chromatography-based DNA extraction encompasses various techniques, each with its unique features. The primary types include:

- **Anion Exchange Chromatography:** Anion exchange chromatography relies on the electrostatic interactions between the negatively charged phosphate groups in DNA and positively charged functional groups on the stationary phase. This method selectively binds DNA while allowing other cellular components to pass through or interact differently. Anion exchange chromatography is particularly useful for the isolation of high-purity DNA.
- **Affinity Chromatography:** Affinity chromatography utilizes specific interactions between molecules. In the context of DNA extraction, it involves ligands or matrices with a high affinity for DNA. The target DNA binds to the stationary phase through a specific ligand-receptor interaction. Affinity chromatography can be employed for highly selective DNA extraction.
- **Size Exclusion Chromatography:** Size exclusion chromatography, also known as gel filtration chromatography, separates molecules based on their size. In DNA extraction, this method can be used to isolate DNA by allowing smaller DNA molecules to enter the pores of the stationary phase while excluding larger molecules. Size exclusion chromatography can be used to purify DNA and remove impurities (Peterson, E. A., & Sober, H. A. 1956, Gaj, et al. 2016).

## 9.4 STEPS IN CHROMATOGRAPHY-BASED DNA EXTRACTION

**Chromatography-based DNA extraction typically involves several key steps:**

- **Sample Preparation:** Before DNA extraction, the biological sample containing DNA is typically lysed to release DNA and other cellular components. Lysis may involve the use of detergents, enzymes, or mechanical disruption methods, depending on the sample type.
- **Column Preparation:** A chromatographic column is packed with a suitable stationary phase material. The choice of stationary phase depends on the type of chromatography being used, such as anion exchange, affinity, or size exclusion.
- **Loading the Sample:** The prepared sample is loaded onto the column. DNA molecules will interact with the stationary phase, while other components like proteins and RNA may pass through or bind differently. Loading should be done carefully to maximize DNA binding and minimize sample loss.

- **Washing:** Unbound or loosely bound molecules are washed away using a buffer solution. This step helps remove impurities and further purifies the DNA. The choice of buffer and washing conditions plays a crucial role in DNA purity.
- **Elution:** DNA is eluted from the column by changing the conditions, such as altering the pH or ionic strength of the buffer solution. This change in conditions allows DNA to be released from the stationary phase and collected in a purified form.
- **DNA Collection:** The eluted DNA is collected and can be further processed or analyzed for downstream applications, such as PCR, sequencing, or genetic analysis (Dhaliwal, A. 2022, Carpi, F. M. (n.d.).

## 9.5 APPLICATIONS OF CHROMATOGRAPHY-BASED DNA EXTRACTION

**Chromatography-based DNA extraction finds applications across various fields, including:**

- **Molecular Biology:** In molecular biology research, pure DNA is essential for techniques such as PCR (Polymerase Chain Reaction), DNA sequencing, and cloning. Chromatography-based methods provide the high-quality DNA required for these applications.
- **Genomic Research:** Genomic studies, including whole-genome sequencing and functional genomics, demand pure DNA for accurate results. Chromatography-based extraction ensures the removal of contaminants that can affect genomic analyses.
- **Forensics:** In forensic science, DNA analysis is critical for criminal investigations and identification. Chromatography-based DNA extraction contributes to the reliability of forensic DNA evidence.
- **Clinical Diagnostics:** Clinical laboratories use chromatography-based DNA extraction for diagnostic purposes, including the detection of genetic mutations and infectious agents.
- **Pharmacogenomics:** Pharmacogenomics explores the relationship between an individual's genetic makeup and their response to drugs. Chromatography-based DNA extraction provides pure DNA for pharmacogenomics studies (Carpi, F. M.et al, 2011, Gaj, et al. 2016).

## 9.6 CONCLUSION

In the realm of DNA extraction, chromatography-based methods have emerged as powerful tools for obtaining highly pure and selectively isolated DNA from complex biological samples. The principles underlying these techniques are rooted in the differential affinities of molecules for stationary and mobile phases, enabling the purification of DNA from contaminants like proteins and RNA. This chapter has provided an overview of the key principles, steps, advantages, and limitations of chromatography-based DNA extraction, offering insights into the broader landscape of DNA sample preparation.

The advantages of chromatography-based DNA extraction are evident. Its capacity for producing high-purity DNA, its selectivity for specific DNA fragments, scalability to meet diverse research needs, and versatility in handling various sample types make it an indispensable technique in molecular biology, genomics, forensics, clinical diagnostics, and pharmacogenomics. Researchers and clinicians alike rely on chromatography-based methods for their ability to deliver DNA of the highest quality, ensuring the accuracy and reliability of downstream applications.

However, it's important to acknowledge the associated limitations, including the cost of specialized equipment, the need for skilled operators, and the potential for user error. Overcoming these challenges requires appropriate training and investment in infrastructure. As technology continues to advance, we anticipate automation and integration solutions that will reduce hands-on time and lower the cost of chromatography-based DNA extraction, making it even more accessible and efficient. Looking ahead, the future of chromatography-based DNA extraction is promising.

Innovations in automation, integration, and cost reduction will further enhance the capabilities of this technique, making it a central component of advanced research and diagnostic processes. As science and technology evolve, chromatography-based DNA extraction will continue to contribute to our understanding of genetics, disease, and personalized medicine (Dairawan and Shetty, 2020).

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