



## Unlocking the secrets of DNA - A study of Biochemical Techniques in Genetic Research

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### Abstract

The unravelling of DNA's secrets has been a cornerstone of modern genetic research. This review delves into the multitude of biochemical techniques that have paved the way for a comprehensive understanding of DNA's structure, function, and interactions. From the pioneering days of X-ray crystallography to the contemporary era of high-throughput sequencing, these techniques have revolutionized the field and empowered researchers to decode the genetic information that underpins life. This review highlights key methodologies such as polymerase chain reaction (PCR), gel electrophoresis, DNA sequencing, recombinant DNA technology, and CRISPR-Cas9 genome editing. Furthermore, it explores how these techniques have enabled breakthroughs in areas like personalized medicine, biotechnology, and evolutionary biology. As genetic research continues to evolve, a deep appreciation of these biochemical techniques is essential for navigating the intricacies of DNA and unlocking its enduring mysteries.

**Keywords:** DNA, Genetic research, Biochemical techniques, X-ray crystallography, Polymerase chain reaction, Gel

<b>CC License</b> CC-BY-NC-SA 4.0	electrophoresis, DNA sequencing, Recombinant DNA technology, CRISPR-Cas9, Personalized medicine, Biotechnology, Evolutionary biology.
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## INTRODUCTION

The genetic blueprint of life resides within the intricate structure of DNA, a molecule that has captured the imagination of scientists, researchers, and thinkers for decades. Deoxyribonucleic acid, commonly known as DNA, serves as the repository of genetic information, orchestrating the processes that govern inheritance, growth, development, and even disease susceptibility. The elucidation of DNA's structure and function has catalyzed a revolution in the field of genetics, offering insights that extend far beyond the realm of biology. This review embarks on a journey through time and innovation, exploring the remarkable biochemical techniques that have enabled us to unlock the secrets concealed within DNA's double helix[1].

### Importance of DNA in Genetics and Its Role in Heredity

At the heart of genetics lies the concept of heredity, the transmission of traits from one generation to the next. DNA, with its unique sequence of nucleotides, is the code that dictates the characteristics of living organisms. From the color of our eyes to our predisposition to certain diseases, DNA holds the key to understanding the variations and similarities among species. It is the tapestry that weaves the story of evolution, offering clues about the origins of life on Earth and the intricate interconnections between all living beings.

### The Significance of Understanding DNA's Structure and Function

The discovery of DNA's double helix structure by James Watson and Francis Crick in 1953 marked a watershed moment in the history of science. This revelation provided the foundation for comprehending how DNA stores and transmits genetic information. The structure revealed the ingenious way in which DNA's complementary strands enable accurate replication, while its sequence encodes the assembly instructions for proteins the workhorses of cellular function. Understanding DNA's structure has paved the way for manipulating and harnessing its potential for applications ranging from medicine to biotechnology [2].

## HISTORICAL PERSPECTIVE ON DNA RESEARCH

### Early Milestones in DNA Research: Discovery of DNA's Double Helix Structure

The journey to unlocking the secrets of DNA began with a series of ground-breaking discoveries that ultimately led to the revelation of its double helix structure. One pivotal moment in this narrative was the elucidation of the structure's building blocks: nucleotides. Thanks to the tireless efforts of scientists like Phoebus Levene, who identified DNA's basic components of sugar, phosphate, and nitrogenous bases, the stage was set for deeper exploration.

However, it was the collaboration between James Watson and Francis Crick in the early 1950s that marked a defining milestone in DNA research. Their remarkable model of DNA as

a double helix, elegantly structured with complementary base pairing, offered a comprehensive explanation for its ability to replicate and encode genetic information. This momentous revelation laid the groundwork for a new era of genetic understanding and manipulation.

### **Contributions of Key Figures such as James Watson, Francis Crick, Rosalind Franklin, and Maurice Wilkins**

James Watson and Francis Crick's elucidation of the DNA double helix structure earned them widespread recognition, but their achievement was built upon the foundational work of other scientists. Rosalind Franklin and Maurice Wilkins played crucial roles in this endeavor. Franklin's expertise in X-ray crystallography yielded pivotal images of DNA fibers, providing crucial insights into its helical structure. Although her work was not fully acknowledged during her lifetime, her contributions have gained rightful recognition in subsequent years. Maurice Wilkins also made vital contributions by conducting X-ray diffraction studies on DNA fibers, collaborating with Franklin and contributing to the understanding of DNA's structural characteristics. These collaborative efforts, sometimes marked by competition, were integral to the holistic understanding of DNA's architecture [3].

**X-ray Crystallography as a Foundational Technique for Understanding DNA's Structure** At the heart of the quest to unravel DNA's structure lies X-ray crystallography, a revolutionary technique that allowed scientists to peer into the molecular world with unprecedented clarity. This method involves exposing crystallized samples of molecules, such as DNA, to X-rays and then analyzing the diffraction patterns created. The analysis of these patterns enables researchers to deduce the three-dimensional arrangement of atoms within the molecule. In the context of DNA research, X-ray crystallography played a pivotal role in confirming the helical nature of DNA.

The iconic "Photo 51," captured by Rosalind Franklin and Raymond Gosling, provided the crucial diffraction image that helped reveal the double helix's dimensions and geometry. This image, along with the contributions of other researchers, provided the data needed for Watson and Crick's ground breaking model. The integration of X-ray crystallography into DNA research not only unveiled the structure of DNA but also laid the foundation for modern structural biology and contributed to the emergence of a new era of genetics. This technique exemplifies the power of collaboration, innovative thinking, and technological advancement in unlocking nature's most intricate secrets [4].

## **KEY BIOCHEMICAL TECHNIQUES IN GENETIC RESEARCH**

### **Polymerase Chain Reaction (PCR)**

#### **Principle and Significance of PCR in DNA Amplification**

The Polymerase Chain Reaction (PCR) stands as one of the most transformative breakthroughs in molecular biology, revolutionizing the way researchers amplify specific DNA sequences. Developed by Kary Mullis in the 1980s, PCR harnesses the natural process of DNA replication to create millions of copies of a target DNA segment. The technique

relies on a heat-stable DNA polymerase enzyme, such as Taq polymerase, which can withstand the high temperatures needed for DNA denaturation and replication.

The cyclic process of PCR involves three main steps: denaturation, annealing, and extension. During denaturation, the DNA sample is heated to separate its double strands. In the annealing step, primers specifically designed for the target DNA sequence bind to complementary regions. Then, the DNA polymerase extends the primers, synthesizing new DNA strands that are complementary to the template. This cycle is repeated multiple times, leading to an exponential increase in the number of target DNA copies [5].

### **Applications in Molecular Cloning, Diagnostics, and Forensic Analysis**

The significance of PCR transcends the laboratory, finding applications across diverse fields. In molecular cloning, PCR facilitates the creation of recombinant DNA by generating copies of the DNA fragment to be inserted into a vector. This technique has expedited genetic engineering, allowing scientists to manipulate DNA sequences and create genetically modified organisms with precision. In diagnostics, PCR has become an indispensable tool for detecting genetic disorders, pathogens, and mutations. The technique's sensitivity enables the identification of even minute amounts of target DNA, making it invaluable in diagnosing infectious diseases and genetic conditions. Forensic analysis has also benefited from PCR's capabilities. Amplifying DNA from trace samples like hair, saliva, or blood stains has revolutionized criminal investigations, enabling the identification of suspects with unprecedented accuracy [6].

### **Variations of PCR Techniques: RT-PCR, qPCR, Nested PCR**

As the demand for PCR grew, researchers developed variations to suit specific applications. Reverse Transcription PCR (RT-PCR) enables the amplification of RNA, allowing the study of gene expression by converting RNA into complementary DNA (cDNA) through reverse transcription. Quantitative PCR (qPCR) takes PCR a step further by incorporating fluorescent markers to monitor the amplification process in real-time. This quantitative approach provides insights into the initial amount of target DNA and its changes during the cycles, making it a cornerstone of gene expression studies and quantitative analyses.

Nested PCR involves two rounds of amplification. In the first round, outer primers amplify the target DNA region. In the second round, a portion of the first PCR product becomes the template, and inner primers amplify a smaller internal segment. This technique enhances specificity and sensitivity, making it useful for amplifying specific regions from complex DNA samples.

In essence, the polymerase chain reaction has become the backbone of modern genetic research, enabling us to amplify, study, and manipulate DNA with precision and efficiency. Its applications span the realms of discovery, diagnosis, and forensic analysis, illustrating the profound impact of this biochemical technique on our understanding of genetics and its practical implications [7].

## **GEL ELECTROPHORESIS**

### **Principles of Gel Electrophoresis and Its Role in Separating DNA Fragments**

Gel electrophoresis is a fundamental technique that allows researchers to separate DNA fragments based on their size and charge. The principle involves subjecting DNA samples to an electric field within a porous gel matrix. DNA, being negatively charged due to its phosphate backbone, migrates towards the positive electrode (anode). Smaller DNA fragments move faster through the gel, while larger fragments migrate more slowly, creating distinct bands [8].

### **Agarose vs. Polyacrylamide Gels: Applications and Differences**

Two main types of gel matrices are commonly used in electrophoresis: agarose and polyacrylamide gels. Agarose gels are versatile and suitable for separating larger DNA fragments, typically ranging from hundreds to thousands of base pairs. They are widely used for routine DNA analysis, including genotyping and plasmid DNA isolation. On the other hand, polyacrylamide gels offer higher resolution and are ideal for separating smaller DNA fragments, such as those encountered in sequencing or mutation detection.

### **Gel Imaging and Analysis Techniques: Ethidium Bromide Staining, Fluorescent Dyes**

After electrophoresis, visualizing DNA fragments is crucial for analysis. Ethidium bromide, a DNA-intercalating dye, is commonly used to stain DNA bands, which become visible under UV light. While effective, ethidium bromide poses safety concerns due to its mutagenic properties. Fluorescent dyes offer safer alternatives for DNA staining. SYBR Green and other fluorescent dyes bind to DNA without intercalating between base pairs, minimizing potential mutagenic effects. These dyes emit fluorescence upon binding to DNA, enabling visualization under UV or blue light [9].

Advanced imaging systems capture gel images digitally, allowing precise quantification of DNA fragments and eliminating the need for UV exposure. Image analysis software aids in accurately sizing and quantifying bands, facilitating research and data interpretation. Gel electrophoresis remains a cornerstone technique in genetic research, serving as the bedrock for DNA analysis. By exploiting the principles of charge and size, scientists can dissect DNA samples, revealing valuable insights into genetic variations, mutations, and even the success of genetic engineering experiments. The choice between agarose and polyacrylamide gels, along with evolving staining methods, ensures the versatility and adaptability of this technique to various research contexts.

## **DNA SEQUENCING**

### **Evolution from Sanger Sequencing to Next-Generation Sequencing (NGS)**

The journey of DNA sequencing has undergone a remarkable transformation, evolving from the pioneering days of Sanger sequencing to the revolutionary era of Next-Generation Sequencing (NGS). Sanger sequencing, developed by Frederick Sanger in the late 1970s, allowed the deciphering of DNA sequences by using chain-terminating nucleotides. While ground breaking, Sanger sequencing was time-consuming and limited in throughput. NGS

emerged as a game-changer in the early 2000s, offering the ability to simultaneously sequence millions of DNA fragments. Unlike Sanger sequencing, NGS doesn't rely on chain-terminating nucleotides; instead, it uses various methods to read the sequences of DNA fragments in parallel. This leap in technology significantly accelerated the pace of genetic research and enabled novel applications [10].

### **High-Throughput Sequencing Platforms and Their Applications**

NGS platforms, including Illumina, Ion Torrent, and PacBio, vary in their methods and capabilities. Illumina's technology, based on sequencing-by-synthesis, dominates the field due to its accuracy, scalability, and cost-effectiveness. Ion Torrent, relying on pH changes during DNA synthesis, also offers rapid and cost-efficient sequencing. PacBio employs single-molecule real-time (SMRT) sequencing, allowing longer reads and capturing complex genomic features. Its accuracy and ability to reveal structural variations have proven invaluable for genome assembly and clinical research.

### **Role of DNA Sequencing in Genome Analysis, Mutation Detection, and Evolutionary Studies**

DNA sequencing has become a cornerstone of genome analysis, offering insights into genetic diversity, gene function, and evolutionary relationships. Whole Genome Sequencing (WGS) provides a comprehensive view of an organism's entire DNA sequence, enabling the identification of genetic variations associated with diseases and traits. Mutation detection, a crucial aspect of medical genetics, relies on targeted sequencing to uncover genetic alterations responsible for disorders. Exome sequencing, focusing on protein-coding regions, is a cost-effective strategy to identify disease-causing mutations [11].

In evolutionary studies, sequencing offers a window into the history of life on Earth. Comparative genomics reveals shared and divergent genetic elements among species, shedding light on evolutionary relationships and adaptations. In essence, DNA sequencing has transformed genetics by democratizing access to genomic information. From understanding disease mechanisms to unraveling the tapestry of evolution, sequencing technologies have catapulted genetic research into an era of unparalleled discovery, redefining our understanding of life's intricacies.

## **RECOMBINANT DNA TECHNOLOGY**

### **Principles of Recombinant DNA Technology and Genetic Engineering**

Recombinant DNA technology represents a groundbreaking approach in genetic research, enabling scientists to manipulate and engineer DNA sequences from different sources. At its core, this technology involves combining DNA fragments from various organisms to create novel genetic constructs with specific traits [12].

### **DNA Cloning: Plasmid Vectors, Restriction Enzymes, and Ligases**

Central to recombinant DNA technology is DNA cloning, which involves inserting a target DNA fragment into a carrier molecule called a vector. Plasmids are commonly used vectors due to their ease of manipulation and replication within bacterial cells. This process requires



the use of restriction enzymes, which cut DNA at specific recognition sites, allowing the insertion of the desired DNA fragment. DNA ligases then seal the gaps in the DNA backbone, creating a stable recombinant DNA molecule.

### **Applications in Producing Recombinant Proteins, Gene Therapy, and Genetically Modified Organisms**

Recombinant DNA technology has far-reaching applications. In biotechnology, it plays a pivotal role in producing recombinant proteins with therapeutic, industrial, or research applications. By inserting genes coding for specific proteins into host organisms, scientists can generate large quantities of valuable proteins such as insulin, growth factors, and enzymes.

Gene therapy harnesses this technology to treat genetic disorders by replacing or correcting faulty genes. Viral vectors or other delivery mechanisms are used to introduce healthy genes into affected cells, potentially offering novel treatments for previously incurable diseases. Recombinant DNA technology has also spurred the development of genetically modified organisms (GMOs). These organisms possess altered genetic traits for improved agricultural productivity, disease resistance, or nutritional content. However, GMOs also raise ethical and environmental concerns that continue to be debated [13].

In essence, recombinant DNA technology has transformed genetic research into a realm of limitless possibilities. It empowers researchers to engineer DNA sequences and organisms to address pressing challenges in medicine, agriculture, and industry, while simultaneously prompting discussions about the ethical, societal, and ecological implications of our newfound genetic manipulation capabilities.

## **CRISPR-CAS9 GENOME EDITING**

### **Mechanism of CRISPR-Cas9 System for Precise Genome Editing**

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) have revolutionized genome editing. The CRISPR-Cas9 system is derived from the bacterial immune system, where it acts as a molecular pair of "molecular scissors" capable of precisely targeting and modifying DNA.

The system works by guiding the Cas9 protein to a specific DNA sequence using a synthetic RNA molecule. The RNA molecule is engineered to match the target DNA sequence. Cas9 then binds to the target site and induces a break in the DNA strand. Cells' repair machinery can introduce errors during the repair process, leading to gene knockout, or it can incorporate new DNA templates to insert specific sequences [14].

### **Applications in Gene Knockout, Gene Insertion, and Therapeutic Genome Editing**

The CRISPR-Cas9 system offers unparalleled versatility in genome editing. It enables gene knockout by disrupting specific genes, helping researchers understand gene function. Conversely, gene insertion involves delivering a desired DNA sequence into a specific genomic location, allowing the addition of functional genes or correcting mutations.

In therapeutic genome editing, CRISPR-Cas9 holds great promise for treating genetic diseases. The technique aims to correct faulty genes responsible for disorders, offering potential cures for conditions like sickle cell anemia and cystic fibrosis [15].

### **Ethical Considerations and Challenges of Genome Editing**

The unprecedented power of CRISPR-Cas9 raises ethical concerns and prompts careful consideration. Off-target effects, where the system unintentionally edits other parts of the genome, pose risks. Ensuring specificity and accuracy remains a challenge. Germline editing, which involves altering the genes of embryos or reproductive cells, is particularly ethically sensitive due to the potential for permanent changes to future generations. It necessitates a delicate balance between scientific progress and responsible research conduct.

Additionally, the potential misuse of genome editing for non-medical purposes, such as creating designer babies or enhancing cognitive abilities, prompts discussions about ethical boundaries, equity, and societal consequences. In conclusion, the CRISPR-Cas9 genome editing system has unleashed unprecedented potential for precision genetic manipulation. Its applications span basic research, medical advancements, and biotechnology innovations. However, alongside these opportunities, the technology invites complex ethical debates and challenges, underscoring the importance of ethical guidelines and open dialogue as we navigate the uncharted territory of genome editing [16].

## **IMPACT OF BIOCHEMICAL TECHNIQUES IN GENETIC RESEARCH**

### **Advances in Personalized Medicine and Targeted Therapies**

Biochemical techniques have catalyzed a paradigm shift in medicine towards personalized treatment strategies. The ability to sequence and analyze individual genomes empowers clinicians to tailor therapies based on patients' genetic profiles. Pharmacogenomics, for instance, identifies how genetic variations influence drug responses, optimizing medication choices and dosages. Biomarker discovery aids in early disease detection and monitoring, enhancing patient outcomes. Personalized cancer therapies, driven by genetic insights, exemplify the potential of biochemical techniques in revolutionizing medical care [17].

### **Contributions to Biotechnology: Enzyme Production, Biofuels, and Bioremediation**

Biochemical techniques have unlocked the potential of microorganisms as versatile tools in biotechnology. Through genetic engineering and DNA manipulation, microbes are engineered to produce valuable enzymes, biofuels, and other high-value compounds. Enzymes synthesized from recombinant DNA enable efficient industrial processes, ranging from textile production to pharmaceutical synthesis. Furthermore, genetically modified microorganisms play a pivotal role in biofuel production, reducing reliance on fossil fuels. Bioremediation employs microbes to detoxify pollutants, cleaning up contaminated environments [18].

### **Insights into Evolutionary Biology: Tracing Genetic Ancestry and Speciation**

Genetic research has illuminated the intricate tapestry of evolutionary history. Biochemical techniques like DNA sequencing and comparative genomics enable researchers to trace



genetic lineages across species and discern the mechanisms driving speciation. The study of ancient DNA, extracted from preserved specimens, provides glimpses into long-extinct species' genomes, offering clues about their adaptations and relationships. Genetic ancestry tracing allows individuals to explore their heritage, uncovering ancestral migrations and connections that span millennia.

In conclusion, biochemical techniques have ushered in an era of transformative possibilities across diverse fields. From the personalized realm of medicine to the frontiers of biotechnology and evolutionary exploration, these techniques continue to reshape our understanding of genetics and catalyse innovations with far-reaching implications for human health, technology, and our understanding of life's interconnectedness.

## **FUTURE DIRECTIONS AND CHALLENGES**

### **Emerging Techniques beyond the Scope of the Review Single-Cell Sequencing, Long-Read Sequencing, etc.**

The landscape of genetic research continues to evolve rapidly, with emerging techniques offering new dimensions of understanding. Single-cell sequencing delves into the genetic information of individual cells, unveiling cellular diversity and dynamics within tissues. Long-read sequencing technologies enable the sequencing of lengthy DNA segments without the need for fragmentation, promising more comprehensive genome assemblies and the detection of structural variations. These techniques hold the potential to uncover hidden intricacies and reshape our understanding of genetics at finer resolutions [19].

### **Ethical Implications and Regulatory Considerations in Genetic Research**

As genetic research advances, ethical considerations gain heightened importance. Manipulating DNA for therapeutic or enhancement purposes raises questions about safety, equity, and unintended consequences. Germ line editing, in particular, sparks debates about intervening in human heredity. Establishing international regulatory frameworks is crucial to ensure responsible research and application of genetic technologies, safeguarding both scientific progress and societal well-being.

### **Navigating Issues of Privacy, Data Sharing, and Informed Consent**

The era of big data in genetics brings forth challenges related to privacy, data sharing, and informed consent. Genetic data is sensitive and uniquely identifiable, raising concerns about unauthorized access, misuse, and discrimination. Striking a balance between sharing data for collaborative research and protecting individuals' privacy is paramount. Ensuring informed consent in the age of complex genetic information poses communication and educational challenges, requiring innovative approaches to empower individuals to make informed choices about their genetic data.

The future of genetic research promises a tapestry of discoveries and innovations, yet it's intertwined with the responsibility to address ethical, legal, and societal challenges. As we peer deeper into the genome and unlock new frontiers of understanding, the ethical compass

guiding these endeavours will play an indispensable role in shaping the trajectory of genetic research and its profound impact on humanity [20].

## CONCLUSION

In the pursuit of unlocking the secrets held within the DNA molecule, biochemical techniques have emerged as the beacon guiding genetic research towards unprecedented insights and possibilities. From the historic revelation of DNA's double helix structure to the precision of modern CRISPR-Cas9 genome editing, these techniques have revolutionized our understanding of genetics and paved the way for ground breaking applications across diverse fields. The significance of DNA in genetics and its role in heredity have laid the foundation for a journey through time, innovation, and collaboration. We have explored the contributions of key figures such as Watson, Crick, Franklin, and Wilkins, and delved into the transformative power of X-ray crystallography. Polymerase Chain Reaction (PCR) has amplified our ability to explore DNA's secrets, while gel electrophoresis has visualized its fragments, unveiling its structure. DNA sequencing technologies, from Sanger to Next-Generation Sequencing, have enabled us to decode genomes and uncover the genetic basis of life's complexities.

The ramifications of these techniques ripple across various domains. They have fuelled the rise of personalized medicine, offering tailored therapies based on individual genetic makeup. Biotechnology, powered by recombinant DNA technology, generates enzymes, biofuels, and even cleanses the environment. Insights into evolutionary biology have unlocked the mysteries of our ancestry and interconnectedness with the natural world. Yet, as we journey into the future, we must navigate the uncharted waters of emerging techniques, ethical considerations, and data challenges. Single-cell sequencing, long-read sequencing, and the promise of deeper understanding beckon. Ethical discussions about genome editing, privacy, and data sharing call for ethical rigor and regulatory frameworks that balance scientific innovation and societal well-being. In essence, the secrets of DNA are being unveiled by the collaborative efforts of scientists, researchers, ethicists, and policymakers alike. The profound impact of these biochemical techniques reshapes our understanding of life itself, offering both promise and responsibility. As we continue to explore DNA's intricacies, let us remember that every discovery, every innovation, and every ethical decision carries the potential to shape the course of science, medicine, and society for generations to come.

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