

RECOMBINANT DNA: GENETIC TOOLKIT FOR INNOVATION AND PROGRESS

Abstract

Recombinant DNA is a type of DNA that has been combined from several sources. It can be created by genetic recombination or molecular cloning. Restriction enzymes cut DNA molecules at specific sites to create recombinant DNA molecules. These enzymes are critical tools in molecular biology and genetic engineering. Emerging developments like synthetic biology promise to engineer organisms with new functionalities, while precision genome editing through CRISPR-Cas9 offers potential solutions to genetic diseases. However, public perception, ethical considerations, and regulatory frameworks must be navigated to ensure responsible use. Environmental impact, intellectual property concerns, and global collaboration are key challenges that require attention. To harness the potential benefits of this technology while mitigating risks, society must prioritize transparent communication, ethical reflection, and sustainable practices. In summary, the chapter provides a comprehensive understanding of recombinant DNA technology's foundation, restriction enzymes' role as molecular scissors, methods of DNA preparation, and the significance of genomic libraries. The elucidation of key terms and processes lays the groundwork for comprehending the transformative impact of recombinant DNA technology in genetics and biotechnology.

Keywords: Genetic recombination, molecular cloning, restriction enzymes

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I. INTRODUCTION

Recombinant DNA or rDNA is a type of DNA that has undergone genetic recombination in a laboratory setting (by molecular cloning) to combine genetic material from several sources and produce sequences that would be absent in the genome. In 1973, Herbert Boyer of the University of California, San Francisco, and Stanley Cohen of Stanford University employed *E. coli* restriction enzymes to insert foreign DNA into plasmids.

A DNA strand that has been combined from at least two different strands is known as recombinant DNA. Recombinant DNA is feasible since all DNA molecules come from different creatures but have the same basic chemical structure and only differ in their nucleotide sequences. Because they can be created from components of two distinct species, much like the legendary chimaera, recombinant DNA molecules are frequently referred to as chimeric DNA. Palindromic sequences are used in rDNA technology, which results in the creation of sticky and blunt ends.

Recombinant DNA technology also known as gene cloning or molecular cloning can be accomplished in four simple steps:

1. A specific DNA is divided into smaller fragments.
2. The fragmented DNA is then linked to another specified section. DNA, referred to as a "vector," contains the genetic components necessary for replication within the cell.
3. A host cell receives the recombinant molecule and can afterwards, the cloned DNA fragment multiplies, creating many copies.
4. Recombinant clone-containing cells are identified and multiplied.

1. Molecular Scissors: Restriction Enzymes: Enzymes that can cut DNA molecules at precise sites are frequently referred to as "molecular scissors." These enzymes are critical tools in molecular biology and genetic engineering, allowing scientists to manipulate DNA for various purposes, such as gene editing, cloning, and DNA sequencing. One of the most common types of molecular scissors are restriction enzymes.

Restriction enzymes, also known as restriction endonucleases, are enzymes that play a crucial role in molecular biology by cutting DNA molecules at specific recognition sites. These enzymes are derived from bacteria and are part of their defence mechanism against invading viruses. By cleaving the viral DNA at specific sites, bacteria can neutralize the threat posed by the virus. The organisms from which restriction enzymes are obtained give rise to their names. The initial letter of the genus name is combined with the first two letters of the species component of the organism's Latin name. For instance, the bacterium *Escherichia coli* belongs to the *Escherichia* genus and is known by the species name *coli*. Therefore, we'll refer to restriction enzymes derived from *Escherichia coli* as Eco. For instance, the restriction enzyme *EcoRI* was the first one to be isolated from *Escherichia coli* strain R.

How restriction endonucleases work is described below:

- **Recognition Site:** Each restriction enzyme recognizes a specific DNA sequence, typically around 4 to 8 base pairs in length. This recognition sequence is usually

palindromic, meaning it reads the same on both strands when read in opposite directions. For example, a palindromic sequence could be: 5'-GAATTC-3' (reads the same on both strands: 5'-GAATTC-3' and 3'-CTTAAG-5').

- **Cutting:** Once the restriction enzyme binds to its recognition site on the DNA, it catalyzes the hydrolysis (breakage by water) of the phosphodiester bonds in the DNA backbone. This results in the cleavage of the DNA at specific positions within the recognition sequence. This cleavage can either be blunt-ended (both strands cut at the same position) or staggered-ended (creating overhanging ends, known as "sticky ends").
2. **Types of Ends:** Some restriction enzymes do not precisely cut DNA at specific locations, making them less effective instruments for recombinant DNA technology. Some of the various restriction enzymes that precisely cut DNA cause "blunt end" fragments to be formed because they simultaneously cut both strands of the double helix. Others cut and produce 'sticky' ends by staggered cutting the two strands. Because the sticky ends created by one restriction enzyme are the same despite which DNA molecule it cleaves, enzymes that make sticky end DNA fragments are particularly effective for altering DNA. Sticky ends can distinguish one another and adhere to one another.
- **Blunt Ends:** The DNA strands are cut at the same position, resulting in blunt ends with no overhangs.
 - **Sticky Ends:** The DNA strands are cut at slightly different positions on opposite strands, resulting in short single-stranded overhangs. These overhangs can base-pair with complementary sequences from other DNA fragments cut by the same enzyme, facilitating the process of DNA recombination.

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Some type II restriction enzymes specifically cut inside or near their recognition sites and do not require ATP hydrolysis for their nucleolytic action. (Welsh et al.,) These enzymes can leave DNA with blunt ends or 5' or 3' overhangs because of DNA cleavage. The majority of Type II REases work as homodimers. Each subunit interacts with one strand of the duplex DNA substrate through two cooperatively acting, non-overlapping catalytic sites, causing both strands to typically be sequentially cleaved in a single binding event.

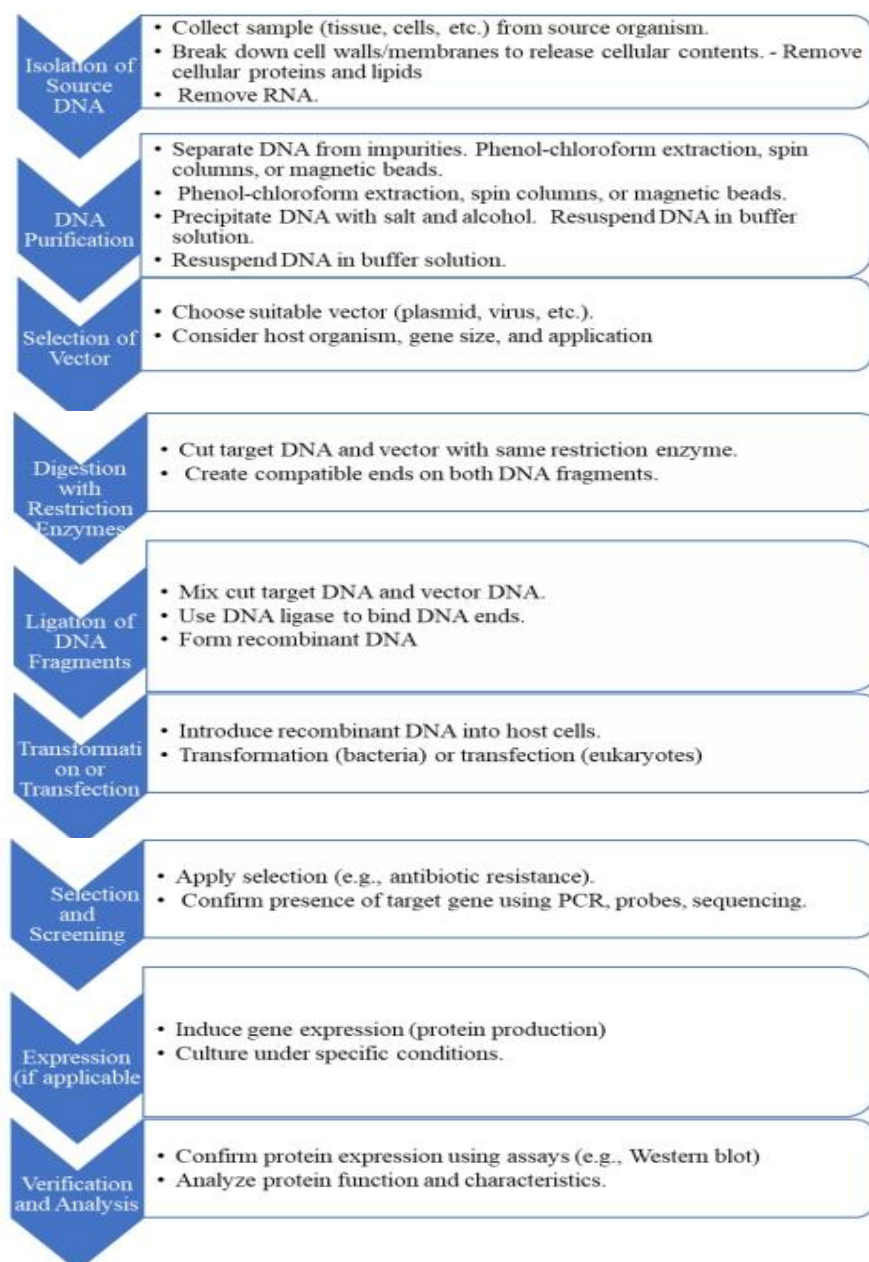
Each monomer must have structural components that correspond to the three main tasks performed by these enzymes: recognising DNA sequences, catalysing

phosphodiester hydrolysis, and monomer interaction (primarily dimerization, though some Type II enzymes form tetramers and demand a greater oligomerization interface).

II. MAKING OF RECOMBINANT DNA

The creation of recombinant DNA involves combining DNA molecules from different sources to create a new DNA sequence. This process has revolutionized genetics, biotechnology, and various fields of science. Recombinant DNA technology enables scientists to manipulate and study specific genes, produce valuable proteins, and even create genetically modified organisms.

Mentioned below are the steps involved in making recombinant DNA:



III. METHODS OF RECOMBINANT DNA PREPARATION

Recombinant DNA can indeed be created using various methods, including the three:

- 1 Transformation
- 2 Phage Introduction
- 3 Non-Bacterial Transformation.

Each of these methods involves introducing foreign DNA into host cells, resulting in the creation of recombinant DNA. An overview of these methods mentioned are as follows:

- 1. Transformation:** Transformation is a fundamental technique in molecular biology and genetic engineering that involves the introduction of foreign DNA into host cells, resulting in the creation of genetically modified organisms. This technique is widely used for various purposes, including the production of recombinant proteins, genetic research, and the creation of genetically modified organisms (GMOs).

In genetic engineering, a vector acts as a carrier DNA molecule to transport foreign DNA into a host cell through a process called transformation. Often, the host cell is a bacterium like *E. coli*. During transformation, foreign DNA carried by the vector is inserted into the host cell, allowing the desired DNA sequence to be integrated into the cell's genome for replication and expression. To prepare for transformation, host cells like *E. coli* are made competent, temporarily increasing their membrane permeability.

Methods such as calcium chloride treatment or electroporation achieve competence, facilitating the uptake of vector-delivered foreign DNA. Selectable markers, such as antibiotic resistance genes, are incorporated into the vector to distinguish transformed cells from untransformed ones. These markers confer an advantage to transformed cells during selection, aiding in the identification of successful transformations.

Vectors possess specific properties suited to different applications. They have cloning sites for DNA insertion, come in various sizes and copy numbers affecting DNA capacity, and contain promoters and expression elements for efficient gene expression. The origin of replication ensures vector replication, and additional markers help monitor processes like transformation and gene expression.

- 2. Phage Introduction:** Phage introduction also known as phage transduction is a technique used to introduce foreign DNA into host cells using bacteriophages (phages). A bacteriophage is a virus that is said to infect bacteria. Similar to transformation, where foreign DNA is introduced into bacterial cells, phage introduction involves using a phage to carry the foreign DNA into bacterial cells.

In phage introduction, foreign DNA is incorporated into a phage vector outside of living cells, a process termed "in vitro packaging." Common vectors used are lambda phage (λ phage) and M13 phage (S.A. Shinde et.al., 2018). The foreign DNA is inserted into specific regions of the phage genome. These engineered phages serve as vehicles to deliver the foreign DNA into bacterial cells.

When the engineered phage vector containing the foreign DNA is introduced to bacterial cells, it forms visible plaques on a bacterial lawn. These plaques represent areas where the bacterial cells have been infected and subsequently lysed by the phages. Inside these plaques, both recombinant and non-recombinant phages are produced.

- 3. Non-Bacterial Transformation:** This term refers to a variety of techniques for introducing foreign DNA into host cells that are not bacteria, such as eukaryotic cells. Although the basic concepts are comparable to bacterial transformation, the host species and methods are different since non-bacterial cells are more complicated.

IV. SELECTABLE MARKERS AND SCREENING

Most transformation procedures must include selectable marker genes. They are introduced along with the target gene, either on the same T-DNA/plasmid or on a different one. It is possible to use a wide variety of selectable marking regimes, and it is crucial for species with low transformation efficiency to easily chemically select transformants (H.D. Jones, 2017).

Selectable markers are specific genes or genetic elements that are introduced into host cells along with a gene of interest during recombinant DNA experiments. These markers serve to identify and select cells that have successfully taken up the recombinant DNA and incorporated it into their genomes. The main purpose of selectable markers is to distinguish transformed or genetically modified cells from non-transformed cells, allowing researchers to selectively grow and study the cells that have undergone the desired genetic modification. Selectable markers are commonly used in genetic engineering, cloning, and biotechnology applications. They often provide a survival advantage to the transformed cells, enabling their growth under specific conditions that inhibit the growth of non-transformed cells. Common examples of selectable markers include antibiotic resistance genes and genes encoding enzymes that confer resistance to toxic substances.

Screening in the context of recombinant DNA technology refers to the process of identifying and selecting cells or organisms that have successfully incorporated the desired genetic material, such as a gene of interest or a recombinant DNA construct. This process involves various methods to distinguish transformed or genetically modified cells from non-transformed ones. Screening is a crucial step in verifying the success of genetic modifications and ensuring that the desired genetic traits or functions have been introduced.

V. GENOMIC LIBRARY

A genomic library is a collection of cloned DNA fragments that collectively represent the entire genome of an organism. Genomic libraries are essential tools in molecular biology and genetic research, enabling scientists to study and analyse the complete set of genes and genetic information present in an organism's DNA. Genome size and complexity vary greatly between organisms. Since the smallest virus genomes only include a few thousand base pairs (bp), they are comparatively easy to examine. Cellular organisms' genomes, however, are far larger, making many other kinds of molecular study impossible. The human genome is 3 billion base pairs in size (around 24,000 genes), bacterial genomes typically comprise millions of base pairs of DNA (bp), and certain plants and amphibians have genomes that are over 100 billion base pairs in size (Ronald Godiska et.al., 2013). A genomic library contains

DNA fragments of varying sizes, including both large and small inserts, reflecting the complete genetic content of the organism.

- 1. Small Insert Vectors:** Plasmid vectors are usually always used to clone genomic libraries with inserts smaller than 10 kb. The pUC19 plasmid is the source of almost all popular vectors. This vector, which encodes resistance to ampicillin, is kept in *Escherichia coli* at a copy number of 300 copies per cell. The lacZ-alpha coding sequence allows one to visually distinguish between clones that have a recombinant plasmid (white) and those that have an empty parental vector (blue).

There are several small insert plasmid vectors listed below in the table:

Table 1: Types of Small Insert Vectors, their Characteristics and Applications.

Vector Type	Characteristics and Applications
pUC19 Plasmid	- Often used to clone genomic libraries with inserts < 10 kb.
	- Source of many widely used vectors.
	- Encodes ampicillin resistance.
	- Maintained in <i>E. coli</i> at ~300 copies/cell.
	- lacZ-alpha allows blue-white screening for recombinant clones.
pBluescript II	- Derived from pUC vectors, used for cloning and sequencing.
(pBSII) Plasmid	- Encodes ampicillin resistance.
	- in-vitro transcription has T7 and T3 promoters.
	- lacZ-alpha provides blue-white screening.
pGEM-T Vector System	- Designed for cloning PCR products.
	- Contains SP6 promoters for in vitro transcription.
	- lacZ-alpha enables blue-white selection.
pTZ57R/T Vector	- Used for cloning PCR products.
	- Contains M13 ori for single-stranded DNA production.
	- lacZ-alpha for blue-white screening.
pCR2.1 Vector	- Part of the TOPO Cloning system.
	- Contains lacZ-alpha for blue-white screening.
pBAD/GFP Vector	- Used for regulated gene expression studies.
	- Employs the arabinose-inducible pBAD promoter.
	- Contains GFP reporter gene for monitoring expression.

- 2. Vectors for Large Insert:** As mentioned in the above table the vectors of small insert cannot work for the DNA insert carrying more than 10kb, in order to clone longer DNA inserts, a variety of vector types have been created. One of such vectors with large DNA insert are BAC vectors which are designed to hold inserts larger than 300 kb, yet often only 100–200 kb is used because of the difficulties in preparing and putting such large DNAs into bacteria (B. Bajpai, 2014).

The table below provides an overview of various vector types that are suitable for accommodating large DNA inserts. Different vectors have their advantages and

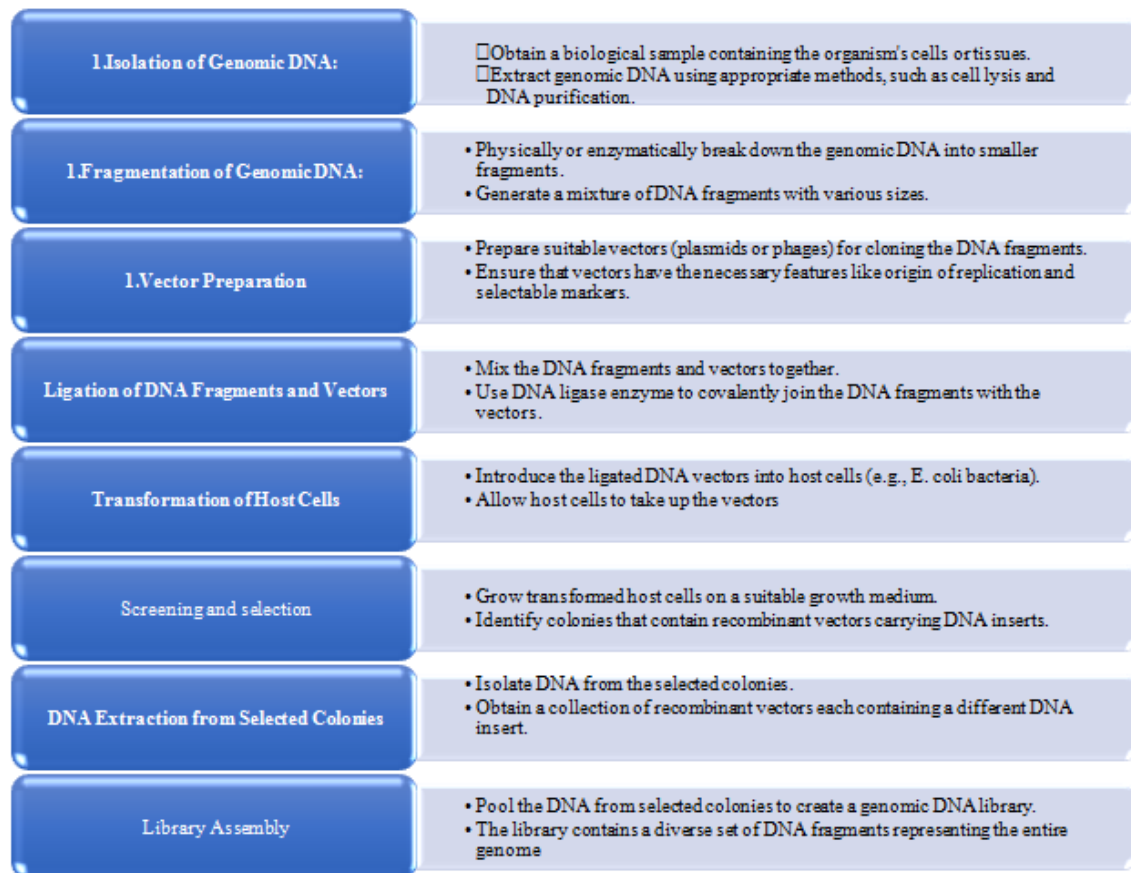
specific applications, allowing researchers to choose the most appropriate vector for their experimental needs.

Table 2 : Types of Large Insert Vectors, their Characteristics and Applications

Vectors for Large DNA Inserts	Characteristics and Applications
Bacterial Artificial Chromosome (BAC) Vectors	<ul style="list-style-type: none"> - Resemble bacterial chromosomes. - Carry large DNA inserts (up to 300 kb or more). - Suitable for genomic sequencing and large-scale studies. - Used in functional genomics and transgenic animal creation.
Yeast Artificial Chromosome (YAC) Vectors	<ul style="list-style-type: none"> - Resemble yeast chromosomes. - Can carry very large DNA inserts. - Suitable for studying chromosome structure and gene regulation. - Used in genome mapping and functional genomics.
Cosmid Vectors	<ul style="list-style-type: none"> - Derived from bacteriophages. - Can carry DNA inserts of moderate size (30 to 45 kb). - Used for cloning and manipulating large DNA fragments. - Suitable for creating genomic libraries.
P1 Artificial Chromosome (PAC) Vectors	<ul style="list-style-type: none"> - Derived from bacteriophage P1. - Accommodate DNA inserts larger than plasmids (up to 100 kb) - Used for cloning, mapping, and analyzing large genomic regions.

- 3. Construction of Genomic DNA Library:** Genomic DNA libraries are valuable resources for a wide range of genetic studies, including gene identification, functional analysis, comparative genomics, and genome sequencing. Researchers can screen the library using specific probes or primers to isolate and analyze genes or regions of interest, whether they involve large genomic segments or specific small DNA inserts.

Below mentioned are the following steps involved in constructing a genomic DNA library:



VI. DIVERSE APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY

Our understanding of genetics, health, agriculture, and industry has been completely transformed using recombinant DNA technology across a wide range of scientific disciplines. Through the use of creative methods developed from this technology, several problems have been effectively solved and ground-breaking discoveries have been made.

1. **Transgenic plants production:** It is feasible to create transgenic or genetically modified plants by using genetic engineering equipment and techniques. Numerous transgenic plants have been created that have improved traits, such as resistance to pesticides, insects, or viruses, or that express male sterility, among others (Kumar S. et. al., 2016).
2. **Transgenic animal production:** The transgenic animal can be created by inserting desired genes into the animal using rec DNA technique. Rec DNA technology helps animal farmers expand the scope and speed of their selective breeding programmes for animals. It aids in the breeding of superior farm animals to secure greater economic gains.
3. **Pharmaceutical Production:** The pharmaceutical industry has harnessed recombinant DNA technology to streamline the production of drugs and vaccines. Previously, obtaining therapeutic proteins from natural sources was arduous and often limited in quantity. With recombinant techniques, pharmaceutical companies can now produce these proteins in large quantities using engineered microorganisms or cell cultures. This has not

only increased the availability of life-saving medications but has also accelerated drug development and testing.

- 4. Agricultural Advancements:** Agriculture has greatly benefited from recombinant DNA technology, ushering in the era of genetically modified organisms (GMOs). Genes conferring traits like pest resistance, drought tolerance, and increased yield have been inserted into crops, enhancing their resilience and productivity. This technology has the potential to address global food security challenges by creating crops that can thrive in diverse and often challenging environments.
- 5. Environmental Applications:** Recombinant DNA technology has been employed to address environmental concerns. Microorganisms have been engineered to break down pollutants and contaminants in soil and water, contributing to bioremediation efforts. Additionally, genetically modified plants can be utilized for phytoremediation, absorbing and detoxifying pollutants from the environment.
- 6. Forensic and Anthropological Studies:** The analysis of DNA using recombinant techniques has revolutionized forensic science and anthropological research. DNA fingerprinting and profiling have become vital tools for criminal investigations, paternity testing, and identifying human remains. These techniques have played a pivotal role in solving numerous legal cases and shedding light on historical and evolutionary aspects of human populations.
- 7. Biotechnology and Industrial Applications:** Recombinant DNA technology has spurred the growth of biotechnology industries. Enzymes, biofuels, and biomaterials can be efficiently produced through engineered microorganisms (Liu W et. al.,2013). This technology has also enabled the development of cost-effective and sustainable methods for the synthesis of various chemicals and materials.
- 8. Research and Discovery:** At its core, recombinant DNA technology has transformed the landscape of scientific research. It allows scientists to manipulate genes, study gene expression patterns, and investigate the functions of specific proteins. Insights gained from these studies have advanced our understanding of fundamental biological processes and disease mechanisms.

VII. SUMMARY

The chapter delves into the realm of recombinant DNA technology, an innovative scientific approach that has revolutionized genetics, biotechnology, and various scientific disciplines. Recombinant DNA, or rDNA, involves the fusion of genetic material from diverse sources through genetic recombination in laboratory settings. The chapter elucidates the fundamental principles, techniques, and applications of this powerful tool.

The prospects and challenges of recombinant DNA technology are both exciting and complex. Emerging developments like synthetic biology promise to engineer organisms with new functionalities, while precision genome editing through CRISPR-Cas9 offers potential solutions to genetic diseases. However, public perception, ethical considerations, and regulatory frameworks must be navigated to ensure responsible use. Environmental impact, intellectual property concerns, and global collaboration are key challenges that require

attention. To harness the potential benefits of this technology while mitigating risks, society must prioritize transparent communication, ethical reflection, and sustainable practices.

In summary, the chapter provides a comprehensive understanding of recombinant DNA technology's foundation, restriction enzymes' role as molecular scissors, methods of DNA preparation, and the significance of genomic libraries. The elucidation of key terms and processes lays the groundwork for comprehending the transformative impact of recombinant DNA technology in genetics and biotechnology.

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