REVIEW ARTICLE

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Harnessing Recombinant DNA Technology for Modern Innovations: A Review

Prasuna Kollabathula[1], Sreekar Katru[2], Prasanth Tumarada[3], Sakuntala Ravuri[1], Akhila Zainab[4], Sai Keerthi Velagala[5]

[1] Department of Microbiology, D. N. R. Degree College Bhimavaram, West Godavari, Andhra Pradesh, India.
[2] Department of Oral Medicine and Radiology, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.
[3] Department of Public Health Dentistry, St. Joseph Dental College, Eluru, West Godavari, Andhra Pradesh, India.
[4] Department of Prosthodontics, Crown and Bridge, Vishnu Dental College, Bhimavaram, West Godavari, Andhra Pradesh, India.
[5] Department of Orthodontics and Dentofacial Orthopedics, Vishnu Dental College, Bhimavaram, West Godavari, Andhra Pradesh, India.

Corresponding author:
Prasuna Kollabathula
Department of Microbiology, D. N. R. Degree College Bhimavaram, West Godavari, Andhra Pradesh, India.
Email: prasunasadgun@gmail.com

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Abstract

In the past century, controlling gene expressions to enhance desirable traits in living organisms through recombinant DNA (rDNA) technology was merely a concept. However, in recent times, this field has made significant advancements, offering unique benefits to human life. rDNA technology allows for the safe, accessible, and abundant production of crucial proteins for addressing various health issues. Through laboratory methods of genetic manipulation, scientists generate rDNA molecules by merging genetic material from different origins that wouldn't naturally occur within organisms. Although the chemical structure of DNA is the same across all organisms, the nucleotide sequences vary. The application of rDNA technology extends to diverse fields such as regenerative medicine, nanotechnology, and tissue engineering, allowing for the production of proteins with specific characteristics and effectiveness. This article explores the widespread uses of rDNA technology in basic research, highlighting its crucial role in modern efforts within biological and biomedical sciences, especially in regenerative medicine and nanotechnology fields.

Keywords: Gene expression, Genetic manipulation, Nanotechnology, Recombinant DNA, Regenerative medicine

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1. Introduction

The overall well-being of individuals is significantly influenced by three key factors, including food deprivation, health concerns, and environmental degradation. Nutrition and well-being are fundamental human needs alongside a healthy and secure environment [1]. As the global population continues to grow at an accelerated pace, the demand for food among humans is rising swiftly. People need nutritious food that is affordably priced. Numerous health problems linked to humans around the world result in a significant number of fatalities. The swift growth of industrialization has led to a rise in environmental pollution [2], and industrial waste is permitted to merge with water, which has impacted marine life and, indirectly, humans. Consequently, these concerns must be resolved through contemporary technologies. Unlike traditional approaches to overcome agriculture [3], health, and environmental issues through breeding, traditional medicines, and pollutant degradation through conventional techniques respectively, genetic engineering utilizes modern tools and approaches, such as molecular cloning and transformation, which are less time-consuming and yield more reliable products [4]. For example, compared to conventional breeding which transfers a large number of specific and nonspecific genes to the recipient, genetic engineering only transfers a small block of desired genes to the target through various approaches, such as biolistic and Agrobacterium-mediated transformation [5]. The alteration into plant genomes is brought either by homologous recombination-dependent gene targeting or

nuclease-mediated site-specific genome modification. Recombinase-mediated site-specific genome integration and oligonucleotide-directed mutagenesis can also be used.

Recombinant DNA (rDNA) technology [6] is playing a significant role in improving health conditions by developing new vaccines and pharmaceuticals. It also raises several ethical concerns and challenges, many of which have been debated since its inception. The treatment modalities are enhanced through the innovation of diagnostic tools, monitoring equipment, and innovative therapeutic techniques. The synthesis of synthetic human insulin and erythropoietin by genetically modified bacteria is one of the leading examples of genetic engineering in health. Additionally, producing new types of experimental mutant mice for research purposes is another key example [7]. Genome editing technology which is gaining popularity, encompasses a wide range of advanced techniques that enable precise manipulation of cellular DNA at specific genomic locations [8]. This is achieved by introducing targeted breaks in the DNA through nuclease-mediated cleavage, which are then repaired by the cell's natural DNA repair mechanisms, leading to altered genetic sequences [9-11]. Among the various nucleases used for genome editing, the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) proteins stand out for their simplicity, efficiency, and accuracy, making them the most commonly utilized tools [12-14]. Following the discovery and programming of the CRISPR-Cas9 system to enable RNA-guided DNA cleavage at precise locations in prokaryotes, it quickly proved to be a powerful and versatile method for editing eukaryotic genomes, as well. Genetically modified microbes are also effectively used in biomining and bioremediation.

2. Recombinant DNA technology

rDNA technology, also known as genetic engineering or gene splicing, is a method used to manipulate and modify the genetic material [15] (DNA) of an organism. The technology involves combining DNA molecules from different sources, which may be of different species, to create a new set of genes or modify an existing one.

2.1 Key Steps in Recombinant DNA Technology

2.1.1 Isolation of the Gene of Interest: The first step is to identify and isolate the gene that encodes for the desired trait or product. This involves thorough research and analysis to locate the specific gene responsible for the characteristic or function needed. For example, if the goal is to produce insulin, scientists would isolate the gene responsible for insulin production in humans or other organisms. Advanced techniques such as DNA sequencing and gene mapping are often employed to pinpoint the exact gene. Once identified, the gene is extracted from the source organism, making it ready for further manipulation or insertion into another organism. This step is crucial, as it sets the foundation for the next phases of genetic engineering.

2.1.2 Cutting the DNA: Specific enzymes, known as restriction endonucleases or simply restriction enzymes, play a crucial role in molecular biology by cutting DNA at precise locations. These enzymes act like molecular scissors, recognizing and cleaving DNA at specific sequences of nucleotides, often referred to as recognition sites [16]. Each restriction enzyme has a unique recognition sequence that is typically a short, specific series of base pairs, usually 4 to 8 nucleotides long. When the restriction enzyme encounters its recognition sequence within a DNA molecule, it cuts both strands of the DNA at or close to this sequence [17].

2.1.3 Insertion of the gene into a vector: Once the gene of interest is isolated, it is inserted into a vector—a DNA molecule specifically designed to deliver foreign genetic material into a host cell for further study or application. Common vectors include plasmids, which are small, circular DNA molecules naturally found in bacteria and viruses that have been engineered for gene delivery. Plasmids are widely used due to their ability to replicate independently within the host cell, enabling efficient amplification of the inserted gene. Engineered viruses, on the other hand, leverage their natural infective properties to introduce genetic material directly into host cells with high efficiency. Vectors are equipped with specific restriction sites—short DNA sequences recognized by restriction enzymes—that facilitate the precise insertion of the gene of interest [18].

2.1.4 Transformation or transfection: The rDNA, which is the DNA that has been combined with the gene of interest, is then introduced into a host cell through a process called transformation or transfection, depending on the type of host cell. In bacteria, the process is called transformation, where the rDNA is taken up by bacterial cells, often through methods like heat shock or electroporation, which temporarily open up the bacterial cell

membrane to allow the DNA to enter. In eukaryotic cells, the process is known as transfection, where various techniques such as lipofection, viral vectors, or electroporation are used to deliver the rDNA into the cell. The host cells are typically bacteria, yeast, or mammalian cells, which can replicate and express the inserted gene [16].

2.1.5 Selection of transformed cells: After transformation, the host cells are screened or selected to find the ones that have successfully taken up the rDNA. This can be done using antibiotic resistance markers or other selection systems included in the vector [15,16].

2.1.6 Expression of the gene: Once the rDNA is inside the host cell, the gene can be expressed, meaning the cell produces the protein or enzyme that the gene codes for. For example, bacteria might produce human insulin [18].

2.1.7 Harvesting the product: After the gene is expressed, the product (protein, enzyme, etc.) is harvested from the host cells. This could involve breaking open the cells (lysis) or collecting the protein if it is secreted into the medium [16, 17].

2.2 Applications of rDNA Technology [6,16]

2.2.1 Medicine

2.2.1.1 Production of insulin: rDNA technology has revolutionized insulin production for diabetes treatment by inserting the human insulin gene into bacteria. A study by Goeddel DV. *et al*. (1979) [19] demonstrated the first successful production of human insulin using recombinant DNA technology by inserting the human insulin gene into Escherichia coli bacteria.

2.2.1.2 Gene therapy: Introducing or altering genes within an individual's cells to treat disease. Anderson. W. F [20] reviewed and highlighted the developments in gene therapy, its mechanisms, and its potential for treating inherited genetic disorders and acquired conditions such as cancer.

2.2.1.3 Vaccines: Developing vaccines like the hepatitis B vaccine, involves using rDNA techniques. McAleer WJ. *et al*. (1984) [21] discussed the development of the recombinant hepatitis B vaccine, produced by expressing the surface antigen of the hepatitis B virus in Saccharomyces cerevisiae (yeast), and its subsequent use in immunization.

2.2.2 Agriculture [19, 22]

2.2.2.1 Genetically modified organisms (GMOs): Crops like Bt cotton or Roundup-ready soybeans have been genetically engineered to resist pests or herbicides, increasing agricultural productivity. A study by James C. (2009) [23] provided a comprehensive analysis of the global status of genetically modified crops, including Bt cotton. It discusses how the introduction of Bt cotton has led to reduced pesticide use and increased yields, benefiting both farmers and the environment.

2.2.3 Biotechnology

2.2.3.1 Gene cloning: Making copies of a gene for study, including understanding gene function and producing proteins for therapeutic purposes. Mulligan, R. C. [24] reviewed the usage of gene cloning in gene therapy research, particularly for genetic diseases. Further, they emphasized cloning specific genes associated with disease allows researchers to develop targeted treatments.

2.2.4 Forensic Science

rDNA can be used in DNA fingerprinting for identification in criminal investigations. rDNA technology is fundamental to modern DNA fingerprinting by providing the tools to amplify, clone, analyze, and characterize specific DNA sequences with unprecedented accuracy and sensitivity. This allows for the making of reliable DNA profiles that can be used in diverse applications, from forensic investigations to genetic testing [25,26]. Jeffreys *et al*. (1985) [25] introduced the concept of DNA fingerprinting and demonstrated how unique DNA patterns could be used for human identification, laying the foundation for forensic DNA profiling.

2.3 Procedure of rDNA technology

Figure 1 provides an overview of the rDNA technology procedure [27].

2.3.1 Selection and isolation of DNA insert

The first step in rDNA technology is selecting a DNA segment of interest to be cloned. This desired DNA segment is then isolated enzymatically. This DNA segment of interest is termed DNA insert, foreign DNA, target DNA, or cloned DNA.

2.3.2 Selection of suitable cloning vector

A cloning vector is a DNA molecule that can replicate itself and is used for the incorporation of a DNA insert. A suitable cloning vector is selected in the next step of rec DNA technology. The most commonly used vectors are plasmids and bacteriophages.

2.3.3 Introduction of DNA-insert into vector to form rec DNA molecule [28]

The target DNA or the DNA insert which has been extracted and cleaved enzymatically by the selective restriction endonuclease enzymes (in section 2.1.1) is now ligated (joined) by the enzyme ligase to vector DNA to form a rec DNA molecule which is often called as cloning-vector-insert DNA construct.

2.3.4 Rec DNA molecule is introduced into a suitable host

Suitable host cells are selected and the rec DNA molecule so formed (in section 2.1.3) is introduced into these host cells. This process of entry of rDNA into the host cell is called transformation. Usually, selected hosts are bacterial cells like E. coli, however yeast, and fungi may also be utilized.

2.3.5 Selection of transformed host cells

Cells that have been transformed (or recombinant cells) are host cells that have incorporated their cDNA molecule. During this phase, different methods utilizing marker genes are employed to distinguish the transformed cells from the non-transformed ones.

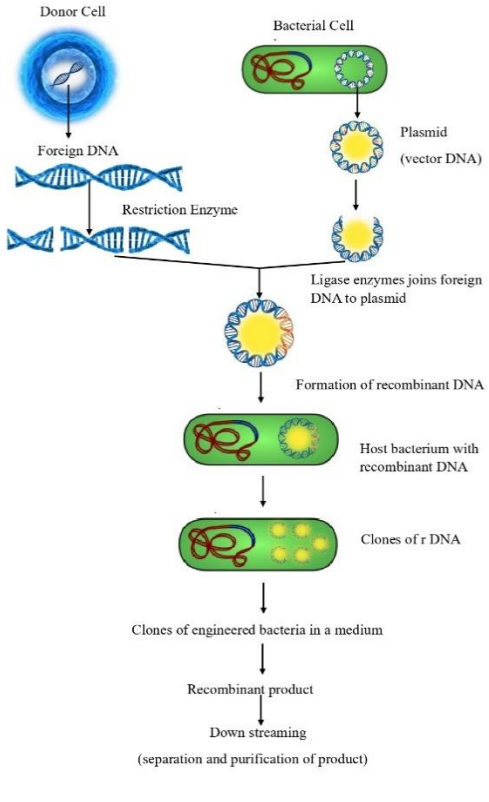

Figure 1. An overview of rDNA Technology procedure.

2.3.6 Expression and Multiplication of DNA insert in the host [24]

Finally, it is to be ensured that the foreign DNA inserted into the vector DNA is expressing the desired character in the host cells. Also, the transformed host cells are multiplied to obtain a sufficient number of copies. If needed, such genes may also be transferred and expressed into another organism.

3. Limitations and risks of rDNA technology

Recombinant DNA (rDNA) technology has transformed genetic research and biotechnology by enabling precise manipulation of genes, yet it is not without its limitations. One of the most prominent concerns revolves around ethics, particularly in the context of human genetic engineering. Editing human embryos or genes raises significant ethical questions related to consent, genetic diversity, and the potential for unforeseen long-term consequences [10]. Similarly, the environmental impact of genetically modified organisms (GMOs) is a growing concern, as the release of these organisms into natural ecosystems—whether in agriculture or the wild—could result in unintended ecological effects, including gene flow to wild populations and harm to non-target species [11].

From a technical standpoint, despite the advent of tools like CRISPR-Cas9 [12], rDNA technology does not always achieve the desired precision. Off-target effects may lead to mutations in unintended genes, causing unpredictable phenotypic changes or harmful consequences. Moreover, achieving high-efficiency editing across different organisms and tissues remains a significant challenge [12]. Legal and regulatory hurdles further complicate the landscape. Many countries enforce stringent regulations on the use of GMOs, making the approval process time-consuming and costly, which can slow the deployment of beneficial technologies [14]. Intellectual property disputes, especially those involving CRISPR tools [12,13], also pose barriers by limiting innovation and restricting access to essential technologies. Finally, the high cost of specialized equipment and reagents makes rDNA technology less accessible to smaller laboratories and institutions in developing countries [10]. Additionally, the process of constructing rDNA molecules, screening for successful modifications, and validating results can be time-intensive and laborious, potentially delaying research and product development.

4. Conclusion

Recombinant DNA (rDNA) technology has transformed health care by enabling the production of various safe, pure, and effective human proteins on a large scale. With the advances in rDNA technology, it is possible to develop mouse antibodies carrying a few human segments known as chimerical or humanized antibodies possessing higher efficacy and activity. DNA technologists can fully synthesize Human Growth Hormone using recombinant DNA (rDNA) technology. As new tools emerge and our understanding of genetics deepens, rDNA technology will likely continue to evolve, offering innovative solutions to some of humanity's significant challenges. Nonetheless, it is essential to manage these developments with careful attention to ethical, societal, and environmental factors.

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