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Mini-Review Article

# Recombinant Deoxyribonucleic Acid Technology: A Powerful Tool for Genetic Engineering

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ABSTRACT

Recombinant Deoxyribonucleic Acid (DNA) technology which is also known as genetic engineering combines DNA molecules from different sources to produce artificial DNA. The applications of this technology in various fields of science are immense for medicine, agriculture and industry for the production of proteins for health problems. Insulin for diabetes to improve health through pharmaceutical innovations, enhancement of food resources and engaging resistance to divergent adverse environmental effects are some of the unique examples. The process starts by choosing special genes or tiny pieces of DNA, followed by mixing them into the host organism's genetic code using carriers like plasmids or viruses. This makes the organism create useful proteins, turning it into a modified organism with better features. This technique is an easy trick to make things better in pharmaceutical industry.

**Keywords:** Recombinant DNA technology, Genetic engineering, DNA isolation, Restriction enzymes, Ligase, Vectors, Polymerase chain reaction

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## INTRODUCTION

Recombinant DNA technology, which is also termed as genetic engineering or gene splicing, revolutionizes the field of molecular biology by allowing scientists to manipulate and combine DNA from different sources. This powerful technique has paved the way for numerous advancements in biotechnology, medicine and agriculture. By introducing specific genes into an organism's DNA, researchers can create novel traits, produce valuable proteins, study gene function and develop new treatments for diseases. Recombinant DNA technology has had a profound impact on scientific research and holds immense potential for the future of various fields.

## LITERATURE REVIEW

### *History and basic principles of recombinant DNA technology*

The basic principle of recombinant DNA technology involves the insertion of DNA molecules into a vector, which is then introduced into a host cell for replication and the production of multiple copies of the gene of interest (Venter M, 2007).

History of recombinant DNA technology, emanated from the pioneering work of Herbert Boyer and Stanley Cohen in the 1970s (Gill K, *et al*., 2023) that demonstrated the potential of DNA recombinant technology in medicine, industry and agriculture. Essentially, functional plasmids can be obtained by re-association of endonuclease-generated fragments of larger replicons, as well as by joining of plasmid DNA molecules of entirely different origins (Cohen SN, 2013).

### *Important tools for genetic engineering/recombinant DNA technology*

The key tools used in genetic engineering, include restriction enzymes, ligase, vectors and suitable host cells. The gene product is purified from the host cell as the initial stage of the DNA recombinant technology process (*Figure 1*).

**Restrictions Enzymes (RE):** These enzymes are naturally produced by bacteria, also termed as restriction endonucleases. Their natural function is to destroy bacteriophage DNA in bacterial cells but they cannot digest host DNA.

Endonucleases are produced by an internal cleavage in DNA molecules. A class of endonucleases that cleaves the DNA only within or near those sites which have specific base sequences. Such endonucleases are known as restriction endonucleases or restriction enzymes, and the sites recognized by them are called recognition sequences or recognition sites (Zeng F, *et al*., 2017). There are three types of RE.

- Type I
- Type II
- Type III

**Ligases:** DNA ligase is an enzyme that can link together DNA strands that have double strand breaks (a break in both complementary strands of DNA). DNA ligases are important for preserving the integrity of the genome, as they are responsible for connecting breaks in the DNA's phosphodiester backbone. These breaks can arise during processes such as replication, recombination and as a result of DNA damage and subsequent repair.

- Naturally DNA ligase has applications in both DNA replication and DNA repair
- Need Adenosine Triphosphate (ATP)

DNA ligase has an extensive use in molecular biology laboratories for genetic recombination experiments.

### *Vectors*

*Figure 1*, might depict the process of introducing the recombinant DNA into a vector, which is often a plasmid or another carrier molecule. Transformation is the step where the host organism takes up the vector containing the recombinant DNA. This is a key step in the production of genetically modified organisms or the amplification of specific DNA sequences. A vector is a DNA molecule that has the ability to replicate autonomously in an appropriate host cell and serve as a vehicle that carry DNA fragment or insert to be cloned.

Therefore, a vector must have an Origin of DNA Replication (ORI) that functions in the host cell. Any extra-chromosomal small genome example plasmid, phage or virus may be used as a vector (Zeng F, *et al*., 2017).

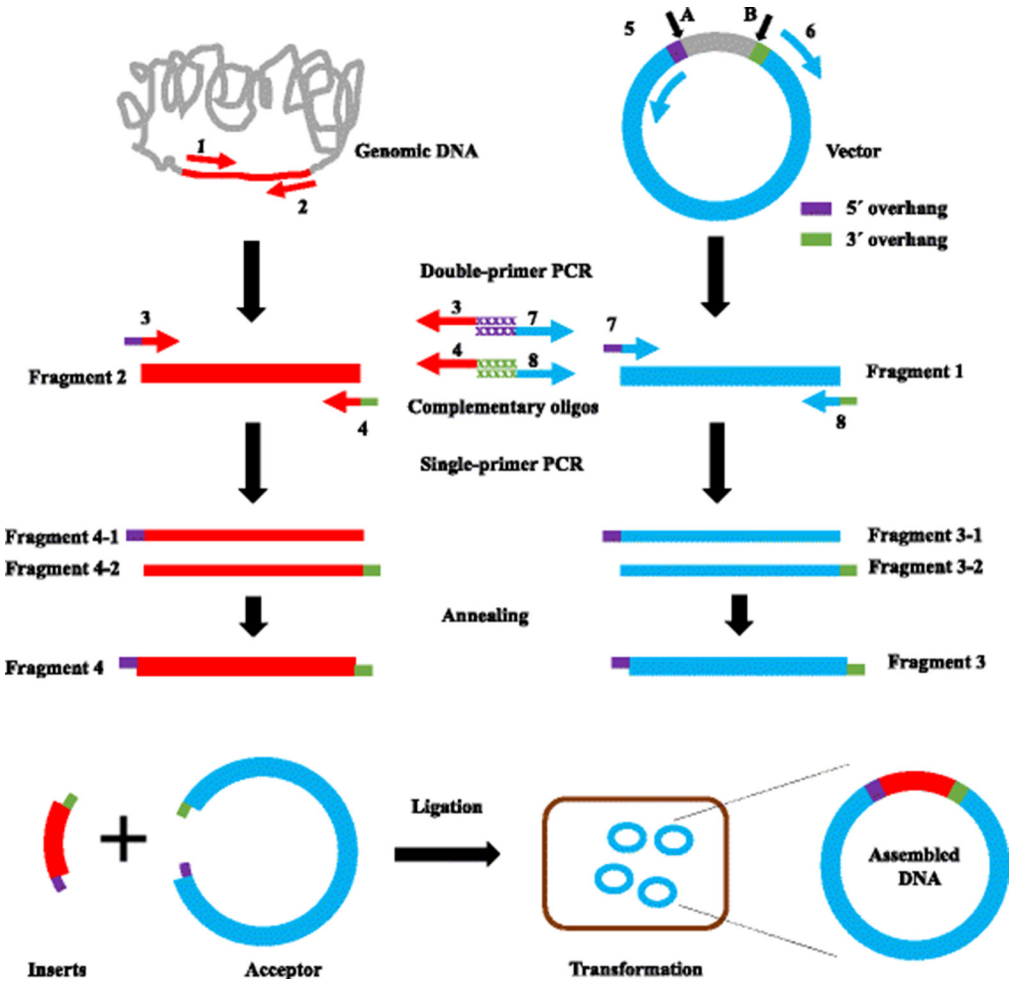


**Figure 1: Process of recombinant DNA technology**

### *Steps involved in recombinant DNA technology*

DNA isolation is a process of purification of DNA from sample using a combination of physical and chemical methods. The basic steps in a DNA extraction are mentioned below-

To get DNA for our study, we gathered the cells required for examination. Once we have these cells, we lysed the cells, thereby revealing the DNA inside. This was carried out using detergents to break down the fatty substances in the cell and nuclear membranes. Following this, we introduced a protease to break down proteins that might be holding onto the DNA.

After treating the mixture, we performed centrifugation, a process where we spin the sample rapidly. This separates the denatured proteins, leaving them in one part known as the organic phase, while the aqueous phase contains the vital nucleic acids-the DNA. This separation step is important as it allows us isolate the DNA for further analysis (Zeng F, *et al*., 2017).

### *DNA with restriction enzymes*

Restriction enzymes (endonuclease) recognize specific bases pair sequences in DNA called restriction sites and cleave the DNA by hydrolysing the phosphodiester bond (Zeng F, *et al*., 2017).

### *Amplification of gene of interest using Polymerase Chain Reaction (PCR)*

Polymerase chain reaction is a process to amplify the gene once the proper gene of interest which has been cleaved, using the restriction enzymes (Zeng F, *et al*., 2017).

### *Insertion of DNA into the vector/host*

By digesting both the plasmid and DNA with the same restriction enzyme thousands of DNA fragments can be created, one fragment with the gene that we require, and another with compatible sticky ends on bacterial plasmids. After mixing, the desired fragments and cleaved plasmids form complementary pairs that are then joined by DNA ligase. This creates a mixture of recombinant DNA molecules (Zeng F, *et al*., 2017).

### *Obtaining the foreign gene product*

In almost all recombinant technologies, the ultimate aim is to produce a desirable protein if any protein encoding gene is expressed in a heterologous host, it is called a recombinant protein (Zeng F, *et al*., 2017).

## DISCUSSION AND CONCLUSION

### *Applications of recombinant DNA technology*

Scientific applications of recombinant DNA technology have revolutionized our understanding of DNA and its functions. The ability to produce multiple copies of DNA, has paved the way for various scientific breakthroughs. Researchers can now amplify DNA segments, allowing for detailed analysis and investigation of genetic material. This technique has been instrumental in identifying mutations in DNA, aiding in the diagnosis and treatment of genetic disorders. Moreover, by altering the phenotype of organisms, scientists can manipulate specific traits and study their effects, further enhancing our knowledge of genetics (Almeida H, *et al*., 2011) (*Figure 2*).

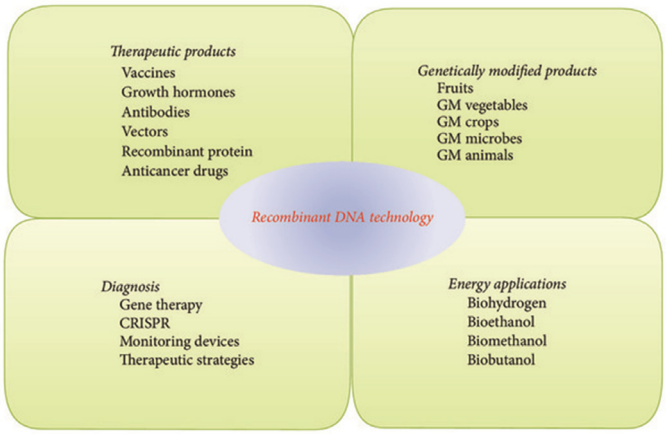


**Figure 2: Applications of recombinant DNA technology**

Recombinant DNA techniques have found invaluable applications in genetic fingerprinting identification, particularly in forensic and medical fields. DNA fingerprinting enables the identification of the source of bacterial or viral pathogens, facilitating the investigation and containment of disease outbreaks. In case of bioterrorism attacks, such as the infamous Anthrax incidents in USA, DNA fingerprinting plays an important role in tracing the origins of the pathogens. Similarly, in medical negligence cases, this technique has been employed to link the transmission of diseases like Human Immunodeficiency Viruses (HIV) to specific healthcare providers. Furthermore, during outbreaks of foodborne diseases, DNA fingerprinting aids in identifying the sources of contamination, allowing for targeted interventions to ensure public safety (Black WJ, 1989).

In the field of agriculture, recombinant DNA technology has brought significant advancements. By cloning cells from plants with desirable traits, scientists can generate identical copies and subsequently grow whole plants from them. This technique allows the production of genetically uniform crops with improved characteristics, such as increased resistance to pests or enhanced nutritional value. Moreover, it enables the harvesting of seeds from these cloned plants, ensuring the availability of superior varieties for future cultivation.

Therapeutic applications of recombinant DNA technology have revolutionized the production of essential human proteins. By introducing recombinant DNA into host cells, scientists can instruct these cells to produce specific proteins, including hormones and enzymes. This has proven particularly vital in the production of important proteins like insulin, human Growth Hormone (hGH), and various interferons such as Interferon-Alpha (INF-α), INF Beta (β) and INF-Gamma (γ). These recombinant proteins have transformed the treatment of numerous diseases, such as diabetes, growth disorders, and certain viral infections (Bazan-Peregrino M, *et al*., 2013).

Additionally, recombinant DNA technology has revolutionized the development of vaccines. By modifying cells and viruses, scientists can instruct them to produce the surface proteins of pathogens. This approach has been successfully employed in the production of vaccines for diseases such as influenza, hepatitis B, and cervical cancer. By utilizing recombinant DNA techniques, the production of these vaccines has become more efficient and reliable, contributing to global efforts in preventing and eradicating infectious diseases.

Recombinant DNA technology has revolutionized the field of genetic engineering, offering countless possibilities in science, medicine, agriculture, and industry.

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