# REVOLUTIONIZING GENETICS: EXPLORING THE POTENTIAL OF RECOMBINANT DNA

**Abstract**

The best technology for the production of artificial DNA through the combination of different genetic materials is the Recombinant DNA Technology. This technology has revolutionized biological research and has made significant contributions in the production of new varieties. This recombinant DNA technology can be considered a groundbreaking innovation in biotechnology with its applications in broad areas that helps in improving the life of human beings. It is extensively used in the field of biotechnology, medicine, agriculture and industry. Its applications have provided us with unprecedented tools to improve the quality of life. Often the term recombinant DNA technology is used as a synonym for DNA cloning or genetic engineering.

**Keywords:** Technology, Endonuclease, Vectors, Cloning, rDNA.

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## I. INTRODUCTION

The Recombinant DNA Technology is an extremely important research tool in modern biology. It was initially developed during 1970s, and is considered to be one of the greatest revolutions of the twentieth century in the field of science. In his technology the scientists first create breaks at desired places to isolate a specific DNA segment and insert that segment into another DNA molecule at a desired position to form a recombinant DNA molecule. The process of introducing the foreign DNA segment into another DNA is also called cloning. Sometimes cloning and recombinant DNA technology are used synonymously. Recombinant DNA technology comprises altering genetic material outside an organism to obtain enhanced and desired characteristics in living organisms. It involves the insertion of DNA fragments from different sources having a desirable gene sequence via appropriate vector. (Berk A. and Zipursky S. L, 2000). The application of recombinant DNA has enabled scientists to study the molecular details of the structure and function of the genes. Thus the study of recombinant DNA has contributed largely in revoulutionizing Genetics in the modern world.

## II. DEFINITION

Recombinant DNA (rDNA) molecules are the molecules formed by combining genetic material from different sources, creating new gene sequences which are of value to scientific research, production of medicines, agriculture and various industries. This process is carried out using molecular biology techniques and involves cutting of DNA at specific locations using restriction enzymes and then joining together with DNA ligases.

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Recombinant DNA Technology is based on the fact that genetic code is universal and that all life forms utilize the same DNA codons to specify the same amino acids. The first genetically modified organisms were bacteria Escherichia coli that made simple proteins like insulin, which were of pharmaceutical interest. As the technologies improved, plants and animals also became amenable for improvement by rDNA technology. In 1980, scientists began genetically engineering multicellular organisms by altering DNA at a single celled stage of development (in male and female gametes). The resulting individuals who carry the genetic alteration in every cell are called transgenic.

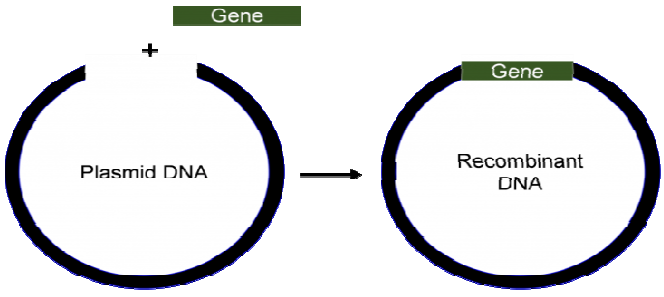



**Figure 1:** Formation of Recombinant DNA by combining DNA from different sources. Image source-Walter Suza

## III. REQUIREMENTS FOR RECOMBINANT DNA TECHNOLOGY:

In order to introduce a gene fragment into the genome of a host organism, various tools or equipments are required. The requirements of rDNA technology are as follows:

1. **Enzymes –** The important enzymes required for successful production of rDNA molecules are as follows: i. Restriction enzymes- The restriction enzymes are the important tools used in recombinant DNA technology which help in determining the location at which the desired gene is inserted into the vector genome. Endonucleases and exonucleases are the two types of restriction enzymes. The endonucleases produce internal cuts called cleavage whereas the exonucleases remove the nucleotides from the ends of the strands. The restriction endonucleases are recognize specific sequences which are usually palindrome sequences and cut the DNA double helix at or near the specific sites called the recognition sites or recognition sequences. (S. A. Shinde, Chavhan, S. B.Sapkal, V. N. Shrikhande, 2018) Endonucleases are also termed as molecular scissors.ii. Polymerase enzymes help in the synthesis of DNA. It helps in the extension of DNA strands in 5’ to 3’ direction using template DNA. iii. Ligase enzymes help to bind two DNA strands by the formation of phosphodiester bond between them. They are also known as molecular glue and are used to join genes of interest with cloning vector.

2. **Vectors** – If a DNA fragment is to be introduced permanently into a cell, a carrier molecule or a vector is required. The vectors are also called cloning vehicles that help to integrate the desired gene into the host organism. (Cohen S., Chang, A., Boyer H. and Helling R., 1973). The vectors consists of an origin of replication that allows it to replicate in the host; a selectable marker which constitute genes which show resistance to certain antibiotics like ampicillin; and cloning sites the sites recognized by the restriction enzymes where desired DNAs are inserted. Plasmids are the most commonly used vectors in recombinant DNA technology as they are small in size which makes it easy to handle and have high copy number.

3. **Host Organisms**– Host organisms are the organisms into which the recombinant DNA is introduced. The host organism takes in the vector containing the desired DNA. They are used to produce and replicate DNA molecules. They have the ability to incorporate and replicate foreign DNA, allowing the production of proteins and other molecules of interest. Common host organisms include bacteria (E. coli), yeast and mammalian cells.

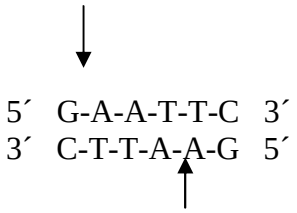
## IV. STEPS FOLLOWED IN THE PRODUCTION OF RECOMBINANT DNA:

1. **Isolation of DNA:** The first and crucial step in rDNA technology is to isolate the desired DNA from a donor cell. It is the process of purification of DNA from a sample. DNA is separated from other macromolecules like carbohydrates, RNS, proteins and fats. After that it is purified with the help of enzymes like cellulose, proteases, ribonucleases etc. Finally, DNA is precipitated out of the solution by addition of alcohol (usually isopropanol or ethanol) causing the DNA to clump together and become visible. This is then spooled out to give purified DNA. (Mark A. J. Roberts, 2019).

2. **Cutting of DNA at specific sites:** Restriction endonucleases are the enzymes used for cutting and insertion of desired foreign gene into the cloning vector. (Temesgen began,

2020). Restriction enzymes naturally occurring proteins that are isolated from bacteria and archaea, in which they act as defense mechanism against viruses. These restriction enzymes act as 'molecular scissors' which cut large DNA molecules into shorter fragments by cleavage at or near the specific sites called 'recognition sites'.
Each restriction enzyme recognizes only a short palindromic sequence, usually 4 to 6 base- pairs long. For example, the hexa-nucleotide sequence recognized by EcoRI, isolated from E.coli reads the same from 5'-3' direction to central axis and cuts DNA only at this specific sequence as follows;




The restriction endonucleases mostly make staggered cuts (sticky ends) in which the two strands of DNA double helix is cleaved at two different locations. This generates short, single stranded overhangs at their ends which are complementary to each other. If the donor DNA and the vector DNA are both cut with the same restriction enzyme, the donor and the cut vector can pair together when mixed because of the complementary sticky ends.

The restriction enzymes are named with the first letter of the genus followed by the first two letters of the species name of their bacterial source. For example, EcoRI is isolated from Escherichia coli, Hind III from Hemophilus influenza, Taq I from Thermus aquaticus etc.

3. **Amplification:** It is the process of amplifying a specific segment of DNA into millions to billions of copies of the target sequence.

Polymerase Chain Reaction or PCR is a method for producing large number of copies of a desired DNA sequence belonging to a larger piece of DNA. It helps to amplify a single copy or a few copies of DNA into millions of copies of target DNA sequence. (Jackson M., Marks L., May G.H.W. and Wilson J.B., 2018). The key components of a PCR reaction are as follows:

- Template DNA containing the desired fragments to be amplified
- Two nucleotide primers that is complementary to a region flanking the target DNA sequence.
- DNA polymerase- heat stable Dna polymerase enzyme such as Taq polymerase isolated from bacterium Thermus aquaticus
- Nucleotides in the form of deoxyribonucleotide triphosphates (dNTPs) are used by DNA polymerase to extend the primers and synthesize new DNA strands.
- Thermal Cycler- a specialized machine that cycle through different temperature steps required for each PCR cycle.

4. **Ligation of DNA molecules:** The DNA taken from both sources are treated with the same restriction endonuclease. The restriction enzyme cut both molecules at the same site. The ends of the cut have an overhanging piece of single stranded DNA called sticky ends. These sticky ends are able to base pair with any DNA molecule that contains the complementary sticky ends. DNA ligase is used to covalently link the two strands into a hybrid of two molecules. The new hybrid DNA molecule is called recombinant DNA molecule and the technology is known as the recombinant DNA technology.

5. **Insertion of Recombinant DNA into host cell:** The next step involves the introduction of recombinant DNA into a recipient host cells mostly, a bacterial cell. This process is called Transformation. Bacterial cells are treated with $Ca^{++}$ ions, or provided thermal shock or electroporation so that they become competent to accept new DNA. Once the recombinant DNA is inserted into the host cell, it gets to produce large quantities of recombinant DNA or the desired protein encoded by the inserted DNA.

6. **Selection and screening of transformed cells:** The selection and screening of transformed cells helps to identify and isolate cells that have successfully taken up the recombinant DNA. The selection process involves filtering the transformed host cells. Only the cells that have incorporated the recombinant DNA will gain resistance to the selective agent. This allows selective growth of transformed cells while inhibiting the growth of non-transformed cells.

   Once selection process is done, further screening may be required to identify the cells that have specific desired trait resulting from the inserted DNA. The combination of selection and screening process ensures identification and isolation of cells that successful carry and express the desired trait.

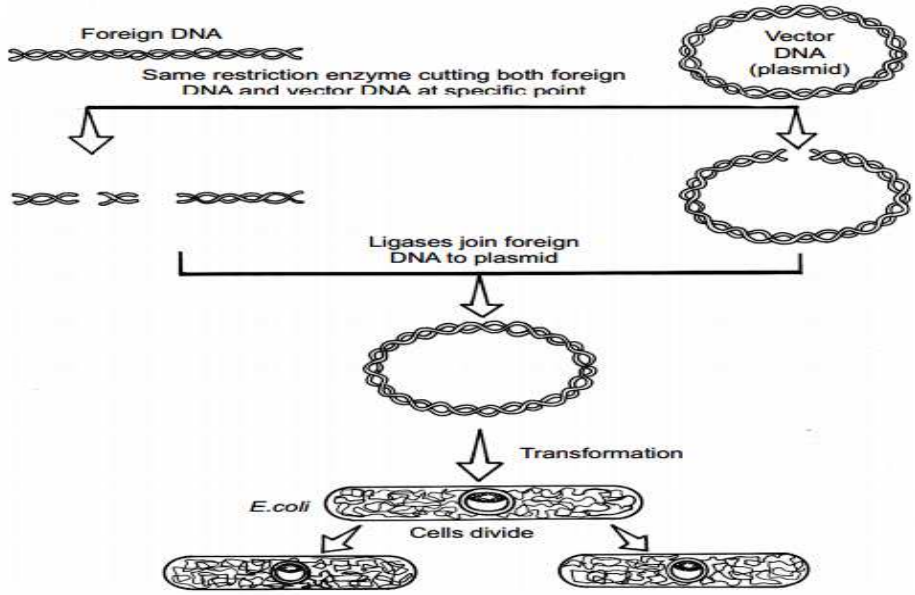



**Figure 2:** Diagrammatic representation of steps in Recombinant DNA Technology
Source: Sarthaks eConnect

## V. APPLICATION OF RECOMBINANT DNA

- Isolation, Identification, mapping and sequencing of genes.
- Production of recombinant proteins and hormones: Human insulin was one of the first therapeutic proteins that were genetically cloned. Other fine chemicals produced with the help of recombinant technology are somatostatin, somatomorphin, endorphin etc.
- In food industry: Products of rDNA are also used in food industry. (Robert L. Zimdahl., 2015). The enzyme rennin normally produced in calves' stomachs, is used to make cheese. Researchers can insert the gene coding for the enzyme into plasmids and transfer the plasmids to bacteria, which are then mass-cultured to produce large quantities of pure rennin.
- Bioremediation: r-DNA technology may help clean the environment by a process called bioremediation. This process endows harmless microbes with genes that enable them to detoxify pollutants. For example, a gene from the bacteria Pseudomonas mendocina specifies toluene mono-oxygenase, an enzyme that degrades trichloroethylene (TCE), an industrial degraders and solvent found in many polluted areas. E. coli is engineered to harbor the Pseudomonas toluene mono-oxygenase gene which effectively degrades TCE without toluene, leaving behind harmless chemicals and cellular debris. (Rohan M., 2022)
- Biofuels: rDNA technology is employed to enhance the production of biofuels. For example, genetically engineered microbes can be used to convert plant material into bioethanol of biodiesel more efficiently.
- Production of transgenic plants: rDNA technology is widely used in agriculture to develop genetically modified plants which are resistant to pests, diseases and environmental stresses. Eg.- Flavr Savr tomatoes, Golden Rice, Bt-cotton etc. (Liu W., Yuan J. S. and Stewart C. N., 2013).
- Production of transgenic animals: Transgenic animals can be engineered to express desirable traits such as disease resistance, improved growth rates or higher milk production. These modifications can lead to more efficient and sustainable agricultural practices, benefitting both farmers and consumers.
- Prevention and Diagnosis of Diseases: rDNA techniques are used to create diagnostic tests for various diseases like infectious diseases, genetic disorders and certain types of cancer. Several recombinant proteins are used for the prevention of diseases like AIDS, cholera, diabetes mellitus etc.
- Production of Vaccines: Recombinant vaccines contain either a protein or a gene encoding a protein of a pathogen origin that is immunogenic to the pathogen function. The vaccines based on recombinant proteins are also called subunit vaccines and are produced by recombinant DNA technology. Production of another vaccine called DNA vaccines involves the isolation, cloning and integration into the vector of gene coding the relevant immunogenic protein. DNA vaccines have the advantage of being relatively easy to design and they don't rely on using live or inactivated viruses.
- Production of useful biochemicals: Various useful biochemicals are produced efficiently by utilizing the methods of r DNA technology. Certain amino acids like lysine and glutamate are produced through fermentation using genetically modified bacteria. Alcohols and alcoholic beverages are also obtained through fermentation. Organic acids like citric acid, acetic acid, etc. and vitamins produced by microorganisms.

- Gene Therapy: in gene therapy, rDNA is used to introduce functional genes into a patient's cells to treat genetic disorders. This technology shows promising potential for curing or managing deadliest genetic diseases. Gene therapy has shown potential in treating certain blood disorders like Beta-Thalassemia and severe combined immunodeficiency (SCID). (Cavazzana Calvo M., Hacein Bey S. and De Saint Basile G., 2000).
- Forensic science: rDNA technology plays a critical role in forensic science. It depends on the technique called DNA fingerprinting which aids in forensic analysis. It is used in setting up of genetic data bank and helps in solving disputed problems of parentage, identity of criminals etc**.**

## VI. CONCLUSION

Various researchers all over the world are actively involved in developing new ideas in the field of rDNA technology. So far the tools that have been developed in this field have made great impact in human life by making it considerably simpler. It has transformed the landscape of modern science and medicine. In the modern world, it has expanded its applications in medicine such as cancer therapy, genetic diseases, diabetes mellitus etc. It is useful in phytoremediation and microbial remediation. It has played a crucial role in the understanding of fundamental biological processes and has contributed to the field of biotechnology.

However, the application of recombinant DNA Technology also raises ethical, social and environmental concerns that must be carefully considered and regulated. The deliberate alteration of natural genetic makeup of genetically modified organisms poses potential threats. Therefore it requires thoughtful evaluation and responsible implementation.

As we continue to explore and harness the significant benefits of r DNA technology, it is important to strike a balance between innovation and ensuring the safety and ethical implications of these advancements. Continued research, robust regulations, and open dialogue will be the key in maximizing the benefits of this technology while minimizing the risks and ensuring its responsible and sustainable use for the betterment of society.

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