**REVOLUTIONIZING GENETICS: EXPLORING THE POTENTIAL OF RECOMBINANT DNA**

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**Abstract:**

Recombinant DNA Technology is the technology used for producing artificial DNA through the combination of different genetic materials (DNA) from different sources. Recombinant DNA Technology has revolutionized biological research and has made significantly contributions to human progress in the modern age. This technology can be considered a groundbreaking innovation in biotechnology with wide range of applications and has the potential to improve many elements of human life. 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.

**Key words:** Technology, Endonuclease, Vectors, Cloning, rDNA.

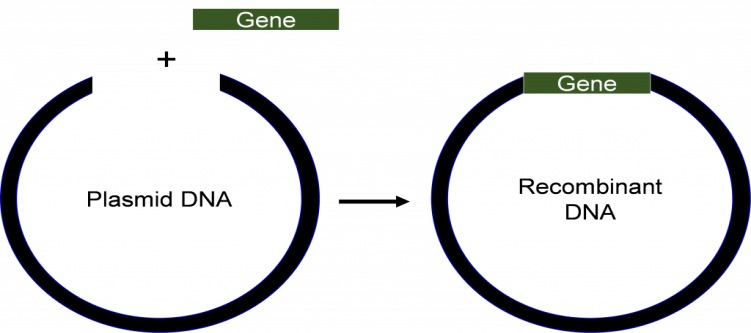
1. **Introduction:**

The Recombinant DNA Technology is an extremely important research tool in modern biology. It was initially developed during 1970s, which led to the development of a variety of gene technologies and is considered to be the greatest scientific revolution of the twentieth century. Recombinant DNA Technology provided scientists with the ability to create breaks at desired places to isolate a specific DNA segment and insert it into another DNA molecule at a desired position to form a recombinant DNA. The process of introducing the foreign gene into another organism is also called cloning. Sometimes these two words 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 thus enabled detailed molecular studies of the structure and function of eukaryotic genes, thereby revolutionizing understanding of cell biology.

1. **Definition:**

Recombinant DNA (rDNA) molecules are the molecules formed by combining genetic material from different sources, creating new genetic sequences which are of value to science, medicine, agriculture and industry. 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.

This is possible because all life forms use the same genetic code. Recombinant DNA Technology is based on the fact that all organisms 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 one cell stage of development (in a gamete or fertilized ovum). The resulting individuals who carry the genetic alteration in every cell are called transgenic.



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

1. **Tools of Recombinant DNA Technology:**

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

1. **Enzymes –** The enzymes include the restriction enzymes, the polymerases and the ligases. 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. They are two types, namely Endonucleases and Exonucleases. The endonucleases produce internal cuts called cleavage whereas the exonucleases remove the nucleotides from the ends of the strands. The restriction endonucleases are sequence-specific which are usually palindrome sequences and cut the DNA at specific points called the recognition sites or recognition sequences. (S. A. Shinde, Chavhan, S. B.Sapkal, V. N. Shrikhande, 2018). This gives rise to sticky ends in the sequence. The desired genes and the vectors are cut by the same restriction enzymes to obtain the complementary sticky notes, thus making the work of the ligases easy to bind the desired gene to the vector. Endonucleases are also termed as molecular scissors.

Polymerase enzymes help in the synthesis of DNA. It helps in the extension of DNA strands in 5’ to 3’ direction using template DNA. 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.

1. **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 in carrying and integrating the desired gene. And helps to integrate the desired gene into the host organism. Plasmids and bacteriophages are the most commonly used vectors in recombinant DNA technology as they have very high copy number. (Cohen, S., Chang, A., Boyer, H., Helling, R., 1973). The vectors consists of an origin of replication- a sequence of nucleotide from where the replication starts, 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.
2. **Host organism** –Host organisms are the organisms into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA with the help of the enzymes. The different ways in which these recombinant DNAs are inserted into the host are– microinjection, biolistics or gene gun, alternate cooling and heating, use of calcium ions, etc. (S. A. Shinde, Chavhan, S. B.Sapkal, V. N. Shrikhande, 2018).
3. **Steps in recombinant DNA production:**
4. Isolation of genetic material.
5. Cutting of DNA at specific sites.
6. Amplification.
7. Ligation of DNA molecules.
8. Insertion of recombinant DNA into host cell.
9. Selection and screening of transformed cells.
10. **Isolation of genetic material:**

The first step in rDNA technology is to isolate the desired DNA in its pure form. It is the process of purification of DNA from a sample. DNA exists within the cell membrane along with other macromolecules such as RNS, polysaccharides, proteins and lipids. Therefore, it must be separated and purified which involves enzymes such as lysozymes, cellulase, chitinase, ribonuclease, proteases etc. Ultimately, the addition of ethanol causes the DNA to precipitate out as fine threads. This is then spooled out to give purified DNA. (Mark A. J. Roberts, 2019).

1. **Cutting of DNA at specific sites:**

Cutting and insertion of desired foreign gene into the cloning vector require special enzymes known as restriction endonucleases or restriction enzymes. (Temesgen began, 2020). Restriction enzymes are extracted from different species and strains of bacteria, in which they act as defence mechanisms against viruses. These restriction enzymes act as ‘molecular scissors’ which cut large DNA molecules into shorter fragments by cleavage at specific nucleotide sequences called ‘recognition sites’.

Each restriction enzyme recognizes only one short sequence, usually 4 to 6 base- pairs long. For example, EcoRI, one of the first restriction enzymes to be isolated from *E.coli* cuts DNA only at the sequence as follows;

5´ G-A-A-T-T-C 3´

3´ C-T-T-A-A-G 5´

In this case, the target sequence is called palindrome, since it reads the same backwards as forwards. The restriction enzymes mostly make staggered cuts in which the two strands of DNA double helix are cleaved at different locations. This generates sticky ends with complementary base sequence. So , if the donor DNA and the vector DNA are both cut with the same enzyme, there is a strong possibility that the donor fragments and the cut vector will splice together because of the complementary overhangs.

In general, the names of restriction enzymes are derived from the first letter of the genus followed by the first two letters of the species name of their bacterial source. For example, EcoRI isolated from *Escherichia coli*. Hind III from *Hemophilus influenza*, Taq I from *Thermus aquaticus* etc.

1. **Amplification:**

It is the process of amplifying a single copy of DNA into millions to billions of copies once the proper gene of interest has been cut using restriction enzymes.

Polymerase Chain Reaction or PCR is a method of making multiple copies of a desired DNA sequence using the enzyme DNA polymerase in vitro. It helps to amplify a single copy or a few copies of DNA into thousands to millions of copies. (Jackson, M., Marks, L., May, G.H.W. and Wilson, J.B., 2018). PCR reactions are run on ‘thermal cyclers’ using the following components:

1. Template DNA containing the desired fragments to be amplified
2. Two nucleotide primers that is complementary to a region of the DNA.
3. Heat stable enzyme DNA polymerase. E.g. Taq polymerase isolated from bacterium *Thermus aquaticus*
4. Nucleotides- needed to extend the primers by the enzyme. The cut fragments of the DNA can be amplified using PCR and then ligated with the cut vector.

1. **Ligation of DNA molecules:**

The purified DNA and the vector of interest are cut with the same restriction enzyme generating complementary ends. This gives us the cut fragment of DNA and the cut vector that is now open. The process of joining these two pieces together using the enzyme ‘DNA ligase’ is called ligation. The result­ing DNA molecule is a hybrid of two DNA molecules – the interest molecule and the vector. In the ter­minology of genetics this intermixing of dif­ferent DNA strands is called recombination. Hence, this new hybrid DNA molecule is also called a recombinant DNA molecule and the technology is referred to as the recom­binant DNA technology.

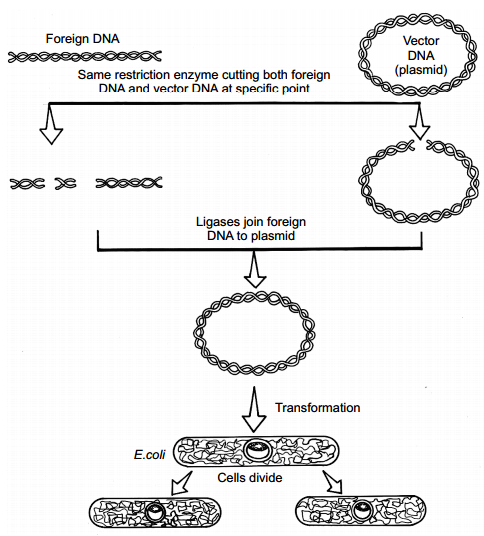
1. **Insertion of Recombinant DNA into host cell:**

In this step, the recombinant DNA is introduced into a recipient host cells mostly, a bacterial cell. This process is called Transformation. Bacterial cells do not accept foreign DNA easily. Therefore, they are treated to make them ‘competent’ to accept new DNA. The processes used may be thermal shock, Ca++ ion treatment, electroporation etc. Once the recombinant DNA is inserted into the host cell, it gets multiplied and is expressed in the form of the manufactured protein under optimal conditions.

1. **Selection and screening of transformed cells:**

The transformation process generates a mixed population of transformed and non-transformed host cells. The selection process involves filtering the transformed host cells only.

For isolation of recombinant cell from non-recombinant cell, marker gene of plasmid vector is employed. For example, PBR322 plasmid vector contains different marker gene (Ampicillin resistant gene and Tetracycline resistant gene. When pst1 RE is used it knock out Ampicillin resistant gene from the plasmid, so that the recombinant cell become sensitive to Ampicillin.



**Fig 2. Diagrammatic representation of steps in Recombinant DNA Technology**

**Source: Sarthaks eConnect**

1. **Application of Recombinant DNA:**
2. Isolation, Identification, mapping and sequencing of genes.
3. 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.
4. 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.
5. 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)
6. Biofuel Production: Biofuels are derived from biomass and these are renewable and cost effective. Genetic engineering plays an essentially important role in a beneficial and large-scale production of biofuels like biogas, bio- hydrogen biodiesel bio-ethanol etc. (Khattak W. A., Ul-Islam M., Ullah M. W., Yu B., Khan S., Park J. K W., Yu B., Khan S., Park J. K 2014). Genetic engineering helps to improve organisms for obtaining higher product yields and product tolerance.
7. Production of transgenic plants: Recombinant DNA Technology is widely used in agriculture to produce genetically modified plants such as Flavr Savr tomatoes, Golden Rice rich in proteins, and Bt-cotton to protect the plant against ball worms and a lot more. (Liu W., Yuan J. S., Stewart C. N., 2013).
8. Production of transgenic animals: By the use of r DNA technology, desired genes can be inserted into the animal so as to produce the transgenic animal. 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.
9. Prevention and Diagnosis of Diseases: Genetic engineering methods and techniques have greatly solved the problem of conventional methods for diagnosis of diseases. Several recombinant proteins are used for the prevention of diseases like AIDS, cholera, diabetes mellitus etc. Monoclonal antibodies are useful tools for disease diagnosis. Monoclonal antibodies are produced by using the technique called hybridoma technology.
10. 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. These vaccines are effective against numerous serious diseases caused by bacteria, viruses or protozoa. These include vaccines for polio, malaria, cholera, hepatitis, rabies, smallpox, etc. The generation of DNA vaccines has revolutionized the approach of treatment of infectious diseases
11. Production of useful biochemicals: Various useful biochemicals are produced more efficiently by utilizing the methods of r DNA technology. A few of them are the alcohols and alcoholic beverages obtained through fermentation; organic acids like citric acid, acetic acid, etc. and vitamins produced by microorganisms.
12. Gene Therapy: Gene therapy is the most beneficial area of genetic engineering for human beings. It involves the introduction, alteration, or removal of genetic material within a person’s cells to treat or prevent genetic disorders. The first successful report in field of gene therapy to treat a genetic disease provided a more secure direction toward curing the 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., De Saint Basile G., 2000).
13. Applications in forensic science: The applications of rDNA technology in forensic sciences largely depend on the technique called DNA profiling or DNA fingerprinting. It is used in setting up of genetic data bank and helps in solving disputed problems of parentage, identity of criminals etc**.**
14. **Conclusion:**

Recombinant DNA Technology is a rapidly evolving area, with researchers all over the world.Its significant advancement in science and medicine has made life considerably simpler for humans. It has transformed the landscape of modern science and medicine. In recent years it has expanded its applications for medicinal applications such as cancer therapy, genetic diseases, diabetes 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 potential risks associated with genetically modified modified organisms and the deliberate alteration of natural genetic makeup of living organisms requires thoughtful evaluation and responsible implementation.

As we continue to explore and harness the power of recombinant 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.

**References:**

1. Berk A., Zipursky S. L., Molecular Cell Biology. Vol. 4. New York, NY, USA: WH Freeman, 2000.
2. Cardi T., Stewart C. N., Progress of targeted genome modification approaches in higher plants. Plant Cell Reports, 2016, 35(7): pp. 1401–1416.
3. Cavazzana-Calvo M., Hacein-Bey S., De Saint Basile G., Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science, 2000, 288(5466):pp. 669–672.
4. Cohen, S., Chang, A., Boyer, H., Helling, R. "Construction of biologically functional bacterial plasmids in vitro". Proceedings of the National Academy of Sciences of the United States of America, 1973, 70 (11): pp. 3240–3244.
5. Jackson M., Marks, L., May, G.H.W. and Wilson, J.B., The genetic basis of disease. Essays Biochem, 2018, Vol 62, pp. 643–723.
6. Khattak W. A., Ul-Islam M., Ullah M. W., Yu B., Khan S., Park J. K. ,Yeast cell-free enzyme system for bio-ethanol production at elevated temperatures. Process Biochemistry; 2014, 49(3) pp. 357–364.
7. Khattak W. A., Ullah M. W., Ul-Islam M., et al. Developmental strategies and regulation of cell-free enzyme system for ethanol production: a molecular prospective. Applied Microbiology and Biotechnology, 2014, 98(23): pp. 9561–9578.
8. Kumar S., Kumar A., Role of genetic engineering in agriculture. Plant Archives, 2015, 15, pp. 1–6.
9. Liu W., Yuan J. S., Stewart C. N., Advanced genetic tools for plant biotechnology. Nature Reviews Genetics; 2013, 14(11):pp. 781–793.
10. Lomedico P. T., Use of recombinant DNA technology to program eukaryotic cells to synthesize rat proinsulin: a rapid expression assay for cloned genes. Proceedings of the National Academy of Sciences of the United States of America; 1982, 79(19):pp. 5798–5802.
11. Mark A. J. Roberts, Recombinant DNA Technology and DNA sequencing. Essays in Biochemistry, 2019.
12. Robert L. Zimdahl, Recombinant DNA; [Six chemicals that changed agriculture](https://www.sciencedirect.com/book/9780128005613/six-chemicals-that-changed-agriculture), 2015.
13. Rohan M, Current Research on Recombinant DNA Technology. Curr Synthetic Sys Biol., 2022, 10:006.
14. S. A. Shinde, S. A., Chavhan, S. B.Sapkal, V. N. Shrikhande, Recombinant DNA Technology and its Applications: A Review; MediPharm Res; 2018, 4(2): pp. 79-88.
15. Temesgen B.,Role of Recombinant DNA Technology in Agriculture. International Journal of Research in Agriculture and Forestry;2020, Vol 7(12), pp. 08-15.
16. Thompson, P.B. , Food Biotechnology in Ethical Perspective, second ed. Springer, New York, 2007, pp. 340.
17. Ullah M. W., Khattak W. A., Ul-Islam M., Khan S., Park J. K , Bio-ethanol production through simultaneous saccharification and fermentation using an encapsulated reconstituted cell-free enzyme system. Biochemical Engineering Journal; 2014, 91: pp. 110–119.
18. Ullah M. W., Khattak W. A., Ul-Islam M., Khan S., Park J. K (2015). Encapsulated yeast cell-free system: a strategy for cost-effective and sustainable production of bio-ethanol in consecutive batches. Biotechnology and Bioprocess Engineering; 20(3):561–575.
19. Van Montagu, M., The Irrational Fear of GM Food. The Wall Street Journal, 2013, pp. 15.
20. Venter M., Synthetic promoters: genetic control through cis engineering. Trends in Plant Science; 12(3): 2007, pp. 118–124.
21. byjus.com/biology/recombinant-dna-technology/.
22. Walter Suza and Donald Lee, Genetics, Agriculture and Biotechnology; Iowa State University Digital Press, 2021.