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

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**Abstract:**

The technology used for producing artificial DNA through the combination of different genetic materials (DNA) from different sources is referred to as Recombinant DNA Technology. Recombinant DNA Technology has revolutionized biological research and has made significantly contributions to human progress in the modern age. This technology has a wide range of applications and has the potential to improve many elements of human life, such as changing health, improved food resources, and building resistance to a variety of detrimental environmental effects. Often the term recombinant DNA technology is used as a synonym for DNA cloning or genetic engineering.

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

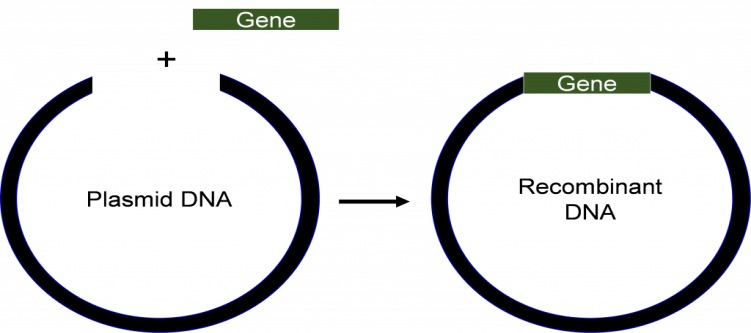
1. **Introduction:**

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 isolate sequence and manipulate individual genes derived from any type of cell. 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 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. Recombinant DNA Technology allows breakage of a DNA molecule at two desired places to isolate a specific DNA segment and then insert it into another DNA molecule at a desired position via appropriate vector. ( Berk A., 2000). The product thus obtained is called recombinant DNA.

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 that made simple proteins of pharmaceutical interest, for example, insulin. As the technologies improved, other organisms including plants 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. Recombinant DNA is made from combining DNA from different sources. Image source- Walter Suza.**

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

The tools mainly include the following:

1. **Enzymes –** The enzymes include the restriction enzymes which help to cut, the polymerases which help to synthesize and the ligases that help to bind. The restriction enzymes used in recombinant DNA technology play a major role 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 cut within the DNA strand 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. They scrutinize the length of DNA and make the cut at the specific site called the restriction site. 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.
2. **Vectors** –The vectors help in carrying and integrating the desired gene. These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism. Plasmids and bacteriophages are the most common vectors in recombinant DNA technology that are used as they have very high copy number. (Cohen, S., Chang, A., Boyer, H., Helling, R., 1973). The vectors are made up of an origin of replication- This is a sequence of nucleotide from where the replication starts, a selectable marker – 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.
3. **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. There are a number of ways in which these recombinant DNAs are inserted into the host, namely – 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).
4. **Steps in recombinant DNA production:**
5. Isolation of genetic material.
6. Cutting of DNA at specific sites.
7. Amplification.
8. Ligation of DNA molecules.
9. Insertion of recombinant DNA into host cell.
10. Selection and screening of transformed cells.
11. **Isolation of genetic material:**

The first step in rDNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules. Since DNA exists within the cell membrane along with other macromolecules such as RNS, polysaccharides, proteins and lipids, it must be separated and purified which involves enzymes such as lysozymes, cellulose, 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 plasmid (cloning vector) require special enzymes known as restriction endonucleases or restriction enzymes. Restriction enzymes are extracted from several different species and strains of bacteria, in which they act as defence mechanisms against viruses. These 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 most useful restriction enzymes make staggered cuts; that is, they leave a single-stranded overhang at the site of cleavage. These overhangs are very useful in cloning because the unpaired nucleotides will pair with other overhangs made using the same restriction enzyme. 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 thousands to millions 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 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 to be amplified
2. Primers- small, chemically synthesized oligonucleotides that are complementary to a region of the DNA.
3. Enzyme- DNA polymerase
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. 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.

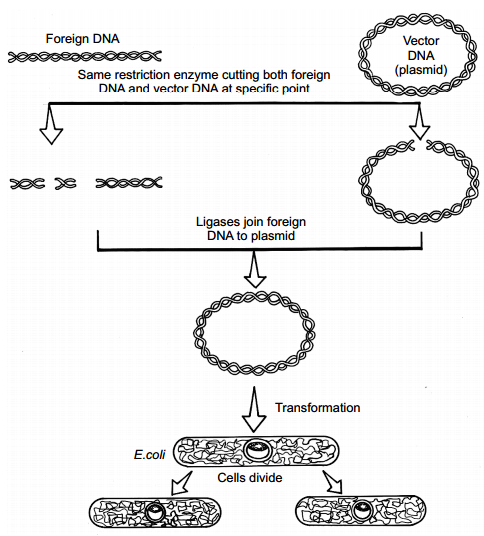
1. **Insertion of Recombinant DNA into host cell:**

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 r DNA 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. The method of r DNA technology aids the animal breeders to increase the speed and range of selective breeding in case of animals. It helps for the production of better farm animals so as to ensure more commercial benefits. Another commercially important use of transgenic animals is the production of certain proteins and pharmaceutical compounds. Transgenic animals also contribute for studying the gene functions in different animal species. Biotechnologists have successfully produced transgenic pigs, sheep, rats and cattle.
9. Prevention and Diagnosis of Diseases: Genetic engineering methods and techniques have greatly solved the problem of conventional methods for diagnosis of diseases. It also provides methods for the prevention of a number of diseases like AIDS, cholera, etc. Monoclonal antibodies are useful tools for disease diagnosis. Monoclonal antibodies are produced by using the technique called hybridoma technology.
10. Production of Vaccines: Vaccines are the chemical preparations containing a pathogen in attenuated (or weakened) or inactive state that may be given to human beings or animals to confer immunity to infection. A number of vaccines have been synthesized biologically through r DNA technology; 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. DNA-vaccine is the preparation that contains a gene encoding an immunogenic protein from the concerned pathogen.
11. Production of Commercially Important Chemicals: Various commercially important chemicals can be 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 undoubtedly the most beneficial area of genetic engineering for human beings. It involves delivery of specific genes into human body to correct the diseases. Thus, it is the treatment of diseases by transfer and expression of a gene into the patients’ cells so as to ensure the restoration of a normal cellular activity.Gene therapy is an advanced technique with therapeutic potential in health services. 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. This strategy shows good response in providing treatment for adenosine deaminase-deficiency (ADA-SCID), which is a primary immunodeficiency. At the beginning of this technology, several challenges including maintenance of patients on PEGylated ADA (PEG-ADA) during gene therapy and the targeting of gene transfer to T-lymphocytes were the reasons for unsuccessful results. (Cavazzana-Calvo M. Hacein-Bey S., De Saint Basile G., 2000). However, later on successful results were obtained by targeting haematopoietic stem cells (HSCs) by using an improved gene transfer protocol and a myeloablative conditioning regime.
13. Applications in forensic science: The applications of r DNA technology (or genetic engineering) in forensic sciences largely depend on the technique called DNA profiling or DNA fingerprinting. It enables us to identify any person by analyzing his hair roots Wood stains, serum, etc. DNA fingerprinting also helps to solve the problems of parentage and to identify the criminals.
14. **Conclusion:**

Recombinant DNA Technology is a rapidly evolving area, with researchers all over the world creating novel methodologies, gadgets and modified products for use in a variety of industries. Its significant advancement in science and medicine has made life considerably simpler for humans. In recent years it has expanded tactics for medicinal applications such as cancer therapy, genetic diseases, diabetes etc. it is useful in phytoremediation and microbial remediation. In terms of health, recombinant technology is assisting in the treatment of various diseases that cannot be treated under normal circumstances. It has played a crucial role in the understanding of fundamental biological processes and has contributed to the field of biotechnology.

However, the use 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.

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