RECOMBINANT DNA TECHNOLOGY: TECHNIQUE AND APPLICATIONS IN MODERN ERA

Abstract

The world population is ever-increasing and demands a lot more production than today to feed the globe. Besides, numerous health hazards are causing a large number of deaths all over the globe. One other factor of concern is the soaring environmental pollution caused by rapid industrialization. Though extensive efforts have been made to combat against these issues, the outcome is still far less than the need. It is of utmost importance to identify, characterize and ultimately manipulate genes involved in biological pathways to deal with these dilemmas. Deliberate alteration in the genetic material of any organism by direct modification of the nucleic acid is referred to gene manipulation or gene cloning or genetic engineering and is achieved by numerous techniques collectively termed as recombinant DNA technology. Recombinant DNA technology offers us with a set of techniques to accomplish this aim. This chapter describes the basic techniques of recombinant DNA technology and focuses on its applications in the modern day.

Keywords: Biological pathway; Genetic material; Gene manipulation; Recombinant DNA

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I. INTRODUCTION

Three factors critically affect human life: environment-related issues, health problems, and deficiency of food. Besides a safe and clean environment, health and food are the basic requirements of human beings. Human requirements for food are increasing rapidly with the rapid-growing global population. The world population is approaching to 9.7 billion by 2050, which would necessitate a 50–70% rise in the production of food grains to ensure global food security (Barman and Singh, 2021; Singh *et al.*, 2022). What all humans need is safe food at a reasonable charge. In other side if we focus, there are a numerous human related health hazards causing a large number of deaths all over the globe and each year nearly 36 million people pass away suffering from various communicable and non-communicable diseases, like malaria, tuberculosis, AIDS, diabetes, cancer etc. In spite of extensive efforts being made, the present food production is significantly lesser than human needs. In the majority of third-world countries, the available health facilities are below standard. Rapid industrialization upsurge has ascended environmental pollution. One most harmful effect includes industrial wastes to be directly thrown in natural water sources, which in turn not only affects aquatic marines but also agricultural lands, drinking ground water and ultimately human-beings directly or indirectly. Hence, these problems need to be taken through present-era scientific technologies.

Understanding the mechanisms of life processes and implementing the acquaintance for the benefit of human health and enhancing the quality of life is the prime goal of biologists. To accomplish this, identifying and characterizing genes involved in biological pathways is imperative. The knowledge acquired from basic research is then implemented for manipulating genes that are of importance in science, agriculture, industries, and medicine.

Recombinant DNA or rDNA technology offers us with set of techniques to accomplish this target. This includes recombining genes from diverse sources in a new combination and expressing them in a host (usually *E. coli*). It is an exceedingly budding field of study with upgraded and novel techniques being discovered rapidly and steadily. In this chapter, we will briefly discuss the basic techniques of rDNA technology along with its applications and some of the recent advances in this direction.

II. DEFINITION

Deliberate alteration in the genetic material of any organism by direct modification of the nucleic acid is referred to gene manipulation or gene cloning or genetic engineering and is achieved by numerous techniques collectively termed as recombinant DNA (rDNA) technology.

It comprises practices for analysing or joining fragments of DNA from one or more organisms (Fig.1) involving the rDNA molecule introduction into a cell for its replication or incorporation into the target cell’s genome.

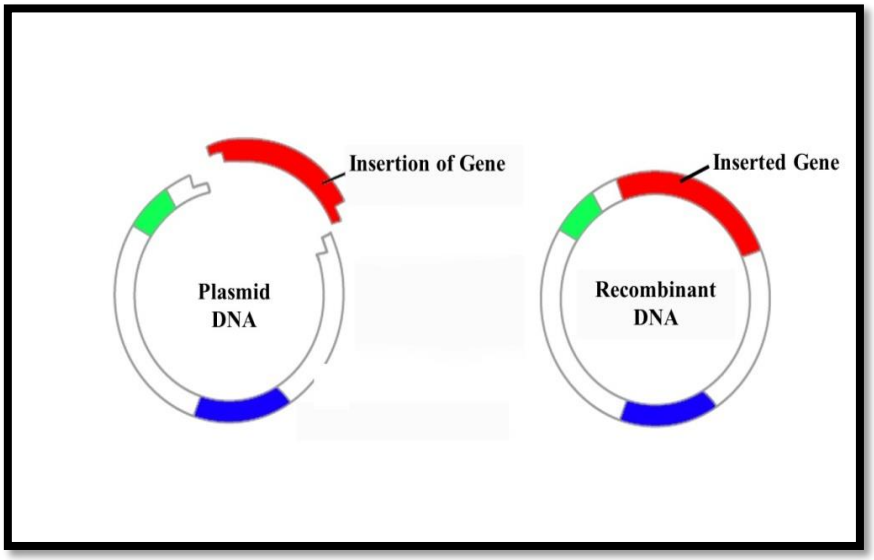


Figure 1: Basic Method of Recombinant DNA Production

III. HISTORY

For many years, through selective breeding of plants and animals, human beings have transformed living organism's genetic structure. The thoughtful alteration of the genetic material by unswervingly modifying its nucleic acids is known as genetic engineering or gene manipulation. These alterations involve countless techniques collectively designated as rDNA technology.

In the late 1960s, Werner Arber, Daniel Nathans and Hamilton O. Smith discovered restriction endonucleases in microorganisms. It is considered as the key breakthrough in the beginning of rDNA technology. Herbert Boyer, in 1969, reported that the restriction enzyme *Eco*RI (isolated from *Escherichia coli*) cuts DNA between the G and A nucleotides in the sequence GAATTC (Hedgpeth *et al*., 1972). Rapid advancement was continuously achieved in rDNA technology following this discovery. The detection of reverse transcriptase from retroviruses by H. Temin and D. Baltimore, the first recombinant DNA molecule by D. Jackson, R. Symons and P. Berg, the development of a recombinant plasmid by S.N. Cohen and H. Boyer and the uncovering of specific DNA fragments by E.M. Southern are some of the noteworthy landmarks of this technology. Some more accomplishments in this path include the DNA sequencing method by F. Sanger, G. Brownlee and B. Barrell; the first gene cloning and construction of rDNA to produce insulin by J. Baxter; first genetically modified crop (Debnath and Sadhukhan, 2014; Barman *et al*., 2021) which was an antibiotic-resistant tobacco plant; first recombinant vaccine (Hepatitis B); first mammalian clone, attained through nuclear transplantation from a non-reproductive cell of an adult animal (Dolly). Fig.2 shows the timeline of recombinant DNA technology (RDT) development.

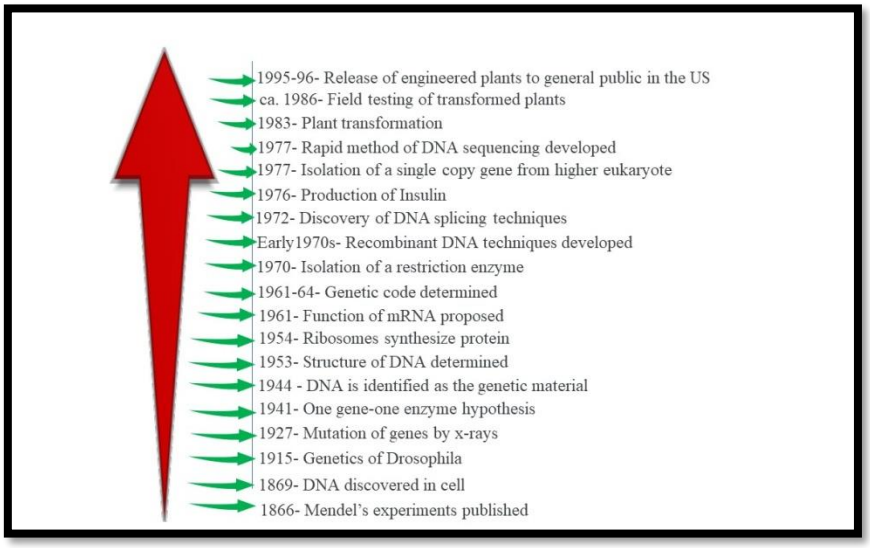


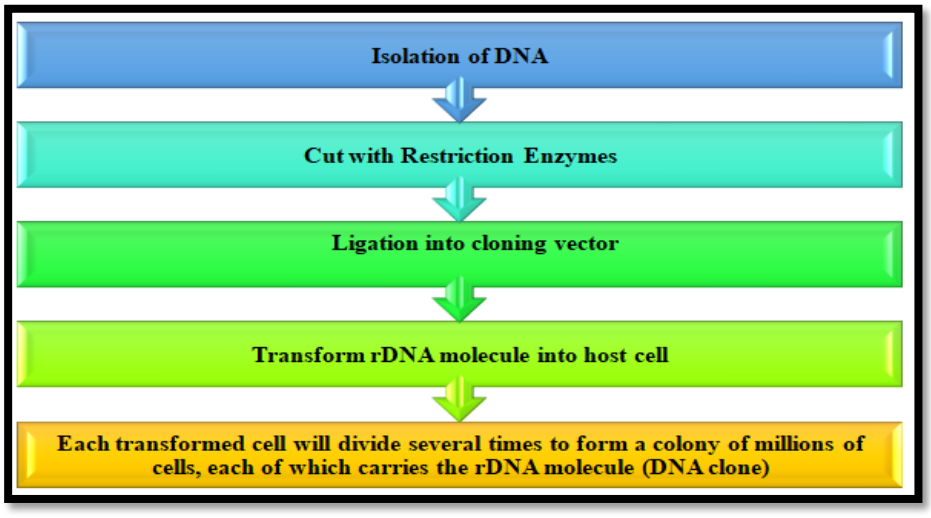
Figure 2: Timeline of Recombinant DNA Technology development

IV. BASIC PRINCIPLES OF RECOMBINANT DNA TECHNOLOGY

1. **Gene cloning and development of recombinant DNA:** Foreign DNA (gene of interest) from the source is cleaved enzymatically and ligated to another molecule of DNA, *i.e.*, cloning vector (phagemid, plasmid etc.) to produce rDNA.
2. **Transfer of vector into the host:** The cloning vector containing rDNA is transferred into a host cell and is maintained within it. This method of introducing rDNA into a host cell is called as transformation.
3. **Selection of transformed cells (host):** The host cells taken up the rDNA are recognized and selected from the pool.
4. **Transcription and translation of inserted gene:** If essential, a construct of rDNA can be formulated for confirming the protein product that is encoded by the cloned DNA sequence is produced by the host cell.

V. STEPS OF RECOMBINANT DNA TECHNOLOGY

Overview:



Step 1: Isolation of the genetic material: Nucleic acids are the genetic material and are present in all living creatures in the form of deoxyribonucleic acid (DNA). It must be of pure form, *i.e.*, free from other macro-molecules like RNA, enzymes, proteins etc., for being cut with restrictor enzymes.

Steps of genetic material isolation: Genetic material is isolated following these steps:

- As DNA is surrounded inside the membranes, so, for releasing DNA with other macro-molecules for instance, proteins, lipids and polysaccharides, bacterial cells/plant or animal tissues are treated respectively with the enzyme lysozyme (bacteria), cellulose (plant cells), chitinase (fungus).

- RNA can be eliminated by treating with ribonuclease, while treatment with protease remove proteins.

- Appropriate treatments can remove other molecules and after the addition of chilled ethanol finally purified DNA will precipitate.

Step 2: Cutting of DNA at specific locations: Incubation of purified DNA molecules with restriction enzyme is performed for restriction enzyme digestions. This step is carried out at the optimum states for that exact enzyme.

Step 3: Isolation of desired DNA fragment: Restriction enzymes' activity can be checked using agarose gel electrophoresis. As DNA is charged negatively, its movement is towards the positive electrode (anode) and DNA tends to separate in this procedure, and then the fragment of DNA looked for is eluted out.

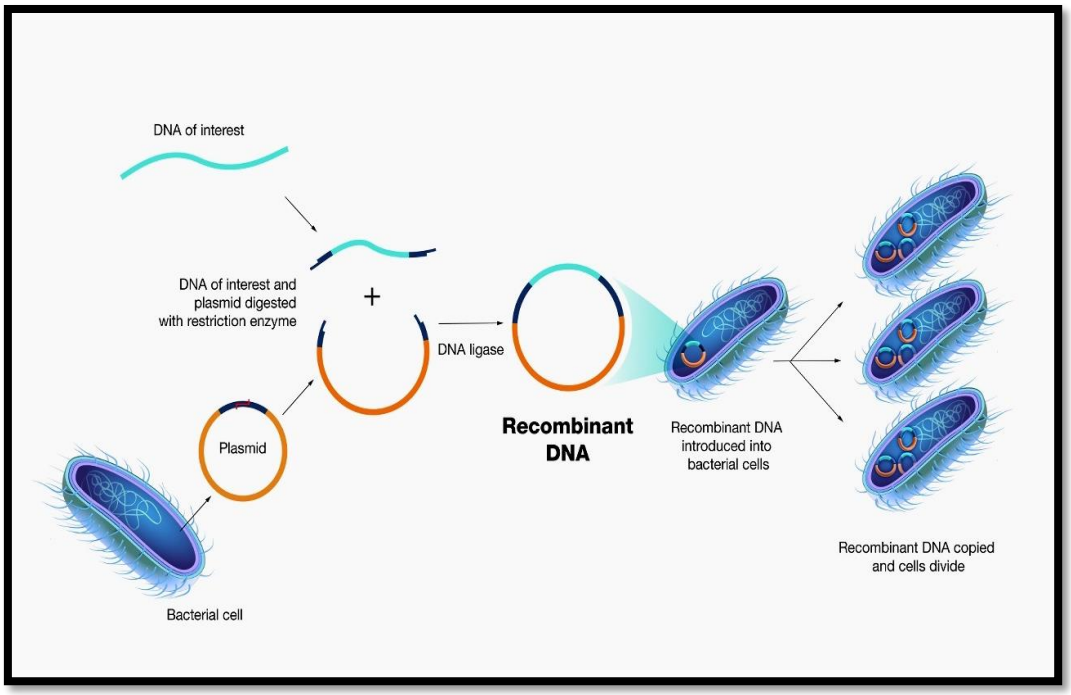
Step 4: Amplification of gene of interest using PCR: Polymerase Chain Reaction (PCR) is best defined as the in-vitro DNA replication. This is the step of cloning genes by Polymerase Chain Reaction (PCR), where at the 5′-end of the primers, restriction enzyme sites are added to ease PCR fragments' cloning. To facilitate restriction digestion of PCR products before cloning in a plasmid vector, a few additional nucleotides (~6) are usually supplemented at this 5′-end. As an alternative, PCR products without restriction digestions in *E. coli*, can directly be cloned into a T-vector.

Step 5: Ligation of DNA fragment into a vector: This course needs a source DNA and a vector DNA. Same restriction endonuclease should cut both of these DNAs to obtain sticky ends. Then gene of interest, vector DNA, and DNA ligase are mixed to ligate these both sticky ends and form the recombinant DNA

Step 6: Insertion of Recombinant DNA into the host cell/organisms: Several techniques can be adopted to achieve this step, before which the recipient cells are made competent to receive the DNA. If a recombinant DNA, bearing antibiotic (e.g., ampicillin) resistance gene, is transferred into E. coli cells, then the host cell becomes transformed into antibiotic-resistant cells. In this case the gene of ampicillin resistance is referred to a selectable marker and upon growing the transformed cells on ampicillin-containing agar plates, transformants will grow only and the rest cells will die.

Besides antibiotic use, plasmid vector systems containing the β-galactosidase encoding *lacZ* gene permit a more straightforward selection method of positive colonies harbouring the rDNA molecule of interest.

Step 7: Obtaining or culturing the foreign gene product: When we insert a piece of foreign DNA into a cloning vector and allocate it into a bacterial cell, the alien DNA starts reproducing. The eventual purpose is to get a desirable protein expression. The foreign gene(s) expression in host cells encompasses understanding numerous technical specifics.



(Image Source: National Human Genome Research Institute)

Figure 3: Steps of Recombinant DNA Technology

VI. APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology (RDT) has many applications that have made it feasible to produce unique proper enzymes for particular food processing circumstances. Due to their specialized functions and applications in the food processing industry, several significant enzymes, like amylases, lipases etc., are available for specific productions. Another considerable accomplishment made possible with RDT is the production of microbial strains. By selective engineering, numerous strains of microorganisms have been created that can form enzymes, specifically proteases. Some strains of the fungal pathogen have undergone modifications to lessen their capacity to generate lethal compounds (Olempska, 2006). Lysozymes are the most efficient tools for eliminating harmful microorganisms in the food industry. It is regarded as one of the most crucial enzymes in the food business for eliminating various zoonotic bacteria (Thallinger *et al.*, 2013). Recombinant proteins utilized as medications were recently derived from the initial plant, and many more are now ready to be employed for additional manufacturing of similar medically significant proteins. In order to be used as enzymes in industries, a wide range of

recombinant proteins have been stated in numerous plants. Additionally, novel polymeric proteins are used in both the medicinal and industrial fields (Ma *et al.,* 2003).

The searching for a rice variety containing protein kinase known as PSTOL1 (phosphorus starvationtolerance1), which aids in boosting the growth of roots in the initial stages and endures shortage of phosphorus, was made possible via traditional breeding and quantitative trait locus (QTL) analysis (Gamuyao *et al.,* 2012). In phosphorus-deficient soil, overexpression of this enzyme supports roots to absorb nutrients in appropriate amounts, ultimately increasing crop output (Hiruma *et al.,* 2016). The chloroplast genome sequences greatly influence the phylogeny and evolution of plants. It makes more of an organism's genes available for access. Genetic modification is necessary to enable the gene-by-gene introduction of well-known traits.

Plants are being developed with advantageous features, such as tolerance to the herbicide glyphosate, drought resistance, insect resistance, disease resistance, and salt tolerance. These plants include beans, squash, potatoes, sugar beet, eggplant, and others. Improvements have also been made to traits relating to ripening, nitrogen usage, and nutritional tolerance.

1. **Production of secondary metabolites:** Secondary metabolites are a class of natural compounds formed by plants and have a variety of functions, including attracting pollinators (such as colours and fragrances) and acting as defence molecules against microbes and animals. As a source of medications, perfumes, food additives and agrochemicals, these substances are also essential to humankind. Plants continue to serve as the primary source of many essential pharmaceutical chemicals despite the chemical industry's best attempts to replicate and produce specific plant secondary metabolites (Wink, 1990). Since most of these substances are derived from plants, any circumstance (such as climatic, political, etc.) that threatens the ongoing availability of these molecules will jeopardize the global supply.

   Since the plant cells could be easily cultivated and create beneficial secondary metabolites, plant cell culture was regarded as an additional or alternative technique for manufacturing these substances in the late 1970s (Alfermann and Peterson, 1995). Nevertheless, a significant obstacle to their economic use was the poor yields produced with cultured cells, which were frequently lower than the quantities found in entire plants. Several approaches have been taken to enhance the product output, such as producing segregated cell cultures known to have a greater biochemical potential (Yeoman and Yeoman, 1996). In fact, *Agrobacterium rhizogenes* infection of established hairy root cultures resulted in elevated production of those secondary metabolites that generally exist in uninterrupted roots, resulting in levels of secondary chemicals equivalent to or greater than those existing in undamaged roots (Sharp and Doran, 1990). In the past 10 to 15 years, 200 species of plants, including trees, agricultural crops, fruits, ornamentals, and vegetables, have successfully undergone genetic modification. Such genetic engineering has enhanced certain crop features, such as disease, herbicide, and environmental factor resistance, such as flood and drought (Bajaj and Ishimaru, 1999).

2. **Environmental application:** The great majority of environmental biotechnology applications detect and screen industrial waste before it is released into the environment using naturally occurring microorganisms (bacteria, fungus, etc.). Microorganisms are

currently being used in bioremediation programmes to clean up polluted air, lakes, land, and rivers. RDT helps to increase the effectiveness of these systems so that their fundamental biological processes are more effective. The creation of "bioluminescors," or microorganisms that emit light in reaction to a variety of chemical contaminants, using RDT is another use. These are employed to gauge environmental exposure to certain dangerous substances.

Trials of other genetic sensors, such as ones that can monitor how toxins naturally dissolve in groundwater, are also being conducted. These additional genetic sensors can be used to identify a variety of chemical contaminants. For instance, when living bacterial cells have genes that code for bioluminescence linked to them, such as the toluene degradation gene (tol) or the mercury resistance gene (mer), the biosensor cells can signal the presence of enormously low levels of toluene or inorganic mercury in contaminated soils and waters by releasing visible light that can be measured with fibre-optic fluro metres.

For the absorption and detection of pollutants in drinking water and other samples, RDT has been broadly applied. The plant responses to hazardous metals are enhanced by knockout engineering and fine-tuning enzyme activity. Through the attenuation of enzymatic activity, the heavy metal binding peptide synthesis enzyme, phytochelatin synthase, showed how to improve tolerance to heavy metals (Jez *et al.*, 2016). Phytostabilization, biosorption, dendroremediation, hyperaccumulation, biostimulation, cyanoremediation, mycoremediation, and genoremediation are recent biotechnology techniques for bioremediation that primarily depend on boosting or blocking specific gene activity. The difficulties in implementing the effective method, however, must be disregarded (Mani and Kumar, 2014).

3. **Energy applications:** A number of microbes, particularly cyanobacteria, facilitate the creation of hydrogen, an eco-friendly energy source. By appropriately employing the necessary enzymes, which are crucial to the development of the product, specific manufacturing is maintained. However, cutting-edge techniques, including metabolic engineering, cell-free technology, combination culture, genetic engineering, and changes in nutrition and growth conditions, have all been successful in boosting the manufacture of hydrogen in cyanobacteria and other biofuels (Ullah *et al.*, 2016). Because conventional energy sources release CO_2 and other potentially harmful compounds, keeping the environment clean will be impossible without commercialising this energy source (Tiwari and Pandey, 2012). Additionally, it is possible to modify cyanobacteria so that they can convert CO_2 into reduced fuel components. As a result, carbon-based energy sources won't affect the environment. This tactic has been effective for a wide variety of industrial compounds, mainly energy carriers like short- and medium-chain alcohols (Savakis and Hellingwerf, 2015). *Geobacter sulfurreducens* conductive biofilms are potential sources for bioelectronics, bioremediation, and renewable energy. In comparison to the wild type, the biofilm was more active when protein-encoded PilZ genes were erased from the genome of *G. sulfurreducens*.

VII. THE ROLE OF RECOMBINANT DNA TECHNOLOGY FOR HUMAN WELFARE

RDT has an extensive range of uses for curing illnesses and enhancing physical well-being. The significant advancements in RDT that have enhanced human health are discussed in the sections that follow:

1. **Edible vaccines:** Crop plants provide affordable bioreactors for the expression of vaccine-grade antigens. It is possible to separate the genes enciphering antigenic proteins from pathogens and express them in plants. These transgenic plants or their tissues can produce antigens that can be consumed for immunization/vaccination (edible vaccines). Since the fruits of crops like bananas and tomatoes can be consumed raw, the expansion of these antigenic proteins in these plants is advantageous for human immunization. When related to recombinant vaccines made by bacterial fermentation, edible vaccines made in transgenic plants have many advantages, including the elimination of storage issues, an easy feeding delivery mechanism, and a lower cost. It may soon be possible to immunize people against awful diseases like hepatitis B and cholera by giving them bananas or tomatoes, as well as to immunize animals against serious diseases like foot and mouth disease by giving them sugarbeets.

2. **Production of antibodies and their derivatives:** Many antibodies and their derivatives have recently been developed and verbalized in plant systems. Most crucially, seven antibodies and antibody derivatives have grasped the required stages in a better way. It is possible to create chimeric secretory IgA/G, also recognized as CaroRx, from transgenic tobacco plants. This antibody can identify the *Streptococcus* strains, an oral pathogen that causes teeth decay. Antigen carcinoembryonic, considered an effectively defined marker in malignancies of the epithelia, can be functionally recognized by the monoclonal antibody T84.66 (Stöger *et al.,* 2000; Vaquero *et al.,* 2002).

3. **Investigation of the drug metabolism:** For optimum therapeutic efficacy and effects, it is essential to examine the enzyme complex system involved in drug metabolism. Recent advances in RDT have contributed to its function through heterologous expression, in which the genetic material of the enzyme is manifested in vivo or in vitro by the transmission of genes (Nicholson *et al.*, 2005).

4. **Production of bio pharmaceuticals:** In order to produce biopharmaceuticals, the traditional fermentation process is being attempted to be replaced with plant-based production. Plants may be used to create a number of vaccines and therapeutic medications to treat ailments like infectious diseases, cancer, cardiovascular diseases, autoimmune diseases, and other conditions. The creation of a plant that will produce seeds with the required therapeutic protein expressed in them is made possible by plant transgenic technology. Under the appropriate circumstances, this seed can grow into plants and seed stock that will produce the desired protein. To create a biopharmaceutical, the desired protein can be isolated from the seeds. Pharmaceuticals made from maize have the advantage of long-term storage, easy purification due to the small amount of soluble seed proteins in corn seeds, low proteolytic activity, low microbial load, and tailored promoters to permit protein expression in particular plant sections.

5. **Identification of crime suspects:** DNA traces can be found in hairs, blood spots, and even typical fingerprints, allowing the polymerase chain reaction to analyze them (PCR). The analysis need not be completed immediately, and DNA testing on evidence from the past has recently helped solve several unsolved crimes and bring the offender to justice. Short tandem repeats (STRs), restrictions fragment length polymorphisms (RFLPs), and single nucleotide polymorphisms (SNPs) are among the polymorphic sites employed as DNA markers in genome mapping (Barman and Kundu, 2019). In forensic research, recombinant DNA technology plays a crucial role in paternity testing, kinship analysis, and the identification of criminals.

6. **Chinese medicines:** Traditional Chinese Medicines are an essential part of alternative medicine and are extremely significant for both diagnostics and treatments. These medications are linked to hypotheses that, to some extent, support the basic idea behind gene therapy. These medications could act as co-administered medications and sources of therapeutic genes. In addition to the Ri plasmid, the transgenic root system offers tremendous possibilities for the introduction of additional genes. In *Agrobacterium rhizogenes* vector systems, it is primarily carried with altered genes to improve properties for particular uses. The cultures developed into an important tool for researching the biochemical features and gene expression patterns of metabolic pathways.

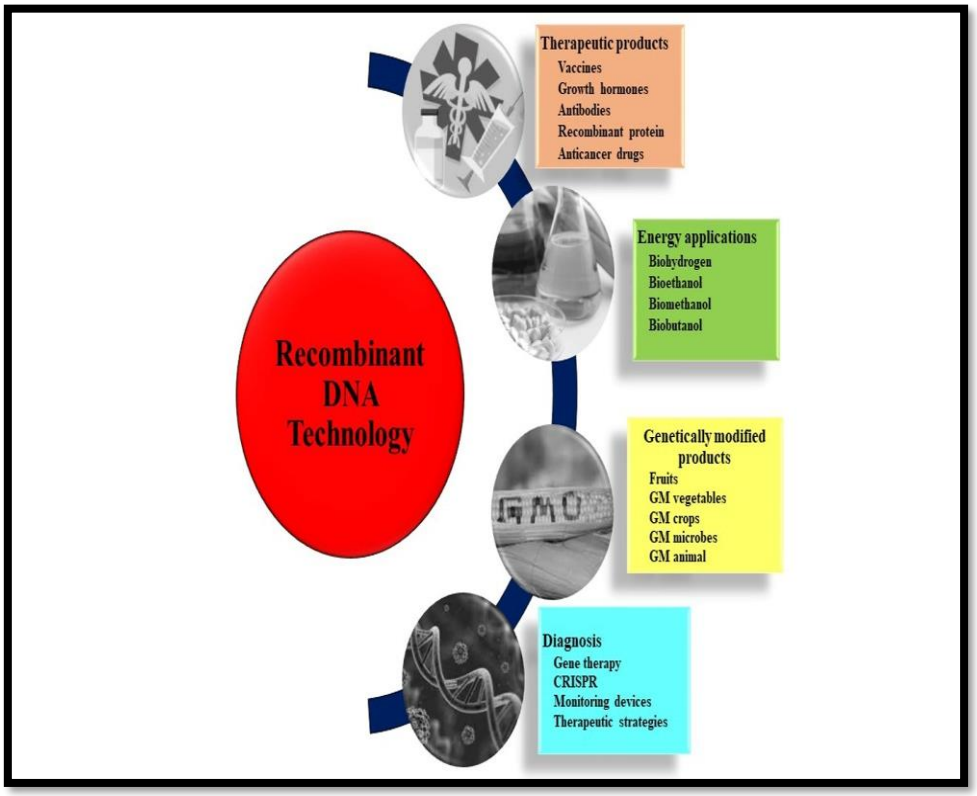


Figure 4: Applications of Recombinant DNA Technology

7. **Current challenges and future prospects:** The majority of recombinant pharmaceuticals are produced in microbial cells, which means that a number of barriers inhibit them from manufacturing functional proteins proficiently. These barriers are overcome by doing modifications to the cellular processes. Post-translational changes, activated cell stress

responses, instability of limited solubility, proteolytic activities, and resistance to the expression of supplementary genes are typical challenges that must be overcome. The genetic mutations of humans lead to shortages in the production of proteins, which can be corrected by adding foreign genes to close the gaps and restore levels to normal. *Escherichia coli* serves as a biological framework for recombinant DNA technology, enabling the producers to work in regulated ways to technically create the molecules involved through cost-effective methods (Ferrer-Miralles *et al.,* 2009).

By enabling the investigation and manipulation of yeast genes not only in the test tube but also in living yeast cells, RDT research retains considerable promise for advancing our knowledge of yeast biology. Most notably, it is now possible to get back to yeast through DNA alteration and gene cloning using a number of specially created selectable marker systems. Because of these developments in technology, it is now feasible to manipulate and analyze yeast genetic material at the molecular level as well as the traditional genetic level. RDT has been most successful in solving biological issues whose core difficulty is the structure and organization of individual genes (DeJong *et al.,* 2006; Walker, 2009).

RDT is currently undergoing rapid growth, which has drastically altered study areas and opened new, exciting avenues for investigating biosynthetic pathways through genetic alteration. Actinomycetes are utilized in the creation of pharmaceuticals, such as some helpful chemicals in the health sciences and the manipulation of biosynthetic pathways for the generation of innovative medications. These play a significant role in the creation of several biosynthetic substances and are therefore heavily taken into account when creating recombinant medications. Their compounds have demonstrated high-level effectiveness against numerous types of bacteria and other harmful germs, making them more relevant in clinical experiments. Additionally, these substances have shown immunosuppressive and anticancer properties (Méndez *et al.,* 2000).

Gene therapy using recombinant DNA technology is a method for both preventing and treating acquired genetic abnormalities. The creation of DNA vaccines is a novel method for preventing many diseases. The DNA supplied during this process comprises genes that produce toxic proteins. Human gene therapy is largely used to cure cancer in clinical examinations. High transfection effectiveness in relation to creating gene delivery systems has been the main focus of research. It is still being researched whether transfection could be used for cancer gene therapy with minimum side effects, such as in cases of lung, breast, prostate and brain cancer. Additionally, gene therapy is believed for renal transplantation, haemophilia, Gaucher disease, renal fibrosis, Alport syndrome, and several other illnesses (Misra *et al.,* 2010).

VIII. CONCLUSION

To conclude, we can say that RDT is a new vista of modern-day science with ample scope of unrevealing potential for the benefit of living beings. Till date, a bright success has already been achieved but still, it needs focus in point of public domain use. There are certain limitations of this technology in terms of cellular level biological activities that needs to be taken care in more explicit way to achieve higher success rate. In brief, this technology has opened up a new arena of science and with more scientific focus it can help us to improve our living.

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