**RECOMBINANT DNA TECHNOLOGY**

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**ABSTRACT:**

 Recombinant DNA technology was merely a theory a century ago when it came to improving desirable traits in live organisms by regulating the expression of target genes. However, in more recent times, this field has proven to have distinctive effects in advancing human life. This technology makes it possible to securely, economically, and sufficiently generate essential proteins needed for nutritional needs and health issues. This technique has interdisciplinary applications and the ability to address significant facets of life, such as boosting food resources, enhancing health, and resistance to many harmful environmental effects. The genetically modified plants, particularly in agriculture, have increased resistance to hazardous agents, improved product yield, and demonstrated increased adaptation for better survival. This chapter mainly focuses on its importance and potential applications in daily life due to the significant advancement and wide variety of applications in the field of recombinant DNA technology.

Key words: Recombinant DNA technology, Biotechnology, Applications, Recent trends, Agriculture, Industries.

**INTODUCTION:**

 Three things have a big impact on human life: lack of food, health challenges, and environmental problems. Aside from a clean and safe environment, basic human needs include food and health. Human needs for food are growing quickly as a result of the world population's rapid growth. Humans demand wholesome food that is affordable (6). There are many deaths worldwide due to various human health conditions. Infectious and non-infectious diseases like cancer, diabetes, AIDS/HIV, tuberculosis, malaria, and several others cause about 36 million deaths worldwide each year. Despite significant efforts, the world's food supply currently falls well short of what humans need, and health facilities are even subpar in third-world nations. Environmental contamination has skyrocketed as a result of the rapid development in industry (2).

Genetic engineering uses contemporary tools and approaches, such as molecular cloning and transformation, which take less time and produce more reliable results than traditional approaches to address issues with agriculture, health, and the environment through breeding, traditional medicines, and pollutants degradation through conventional techniques, respectively (5). For instance, genetic engineering uses a variety of techniques, such as biolistic and Agrobacterium-mediated transformation, to only transfer a small block of desired genes to the target, as opposed to conventional breeding, which transfers a large number of both specific and nonspecific genes to the recipient. Homologous recombination-dependent gene targeting or nuclease-mediated site-specific genome editing are the two methods used to modify plant genomes. Site-specific genome integration mediated by recombinases and oligonucleotide-directed mutagenesis are further options (3).

By creating novel vaccinations and medications, recombinant DNA technology is significantly enhancing health conditions. By creating new therapy modalities, monitoring tools, and diagnostic kits, the treatment techniques are also improved. One of the most prominent examples of genetic engineering in health is the development of new varieties of experimental mutant mice for research purposes and the synthesis of synthetic human insulin and erythropoietin by genetically engineered bacteria (7). Similar to this, genetic engineering techniques have been used to address environmental problems, including the production of biofuels and bioethanol from waste, the cleanup of toxic wastes like carbon and oil spills, and the detection of toxins like arsenic in drinking water. The genetically altered bacteria are also useful for bioremediation and biomining(9).

Recombinant DNA technology's introduction transformed biological research and sparked a number of significant changes. By changing microbes, animals, and plants to generate medicinally valuable compounds, it provided new potential for innovators to produce a wide range of therapeutic goods with immediate effect in medical genetics and biomedicine [7,8]. Recombinant drugs, which make up the majority of biotechnology pharmaceuticals, are crucial in the fight against fatal human diseases. Recombinant DNA technology was used to create pharmaceuticals that completely altered human life. As a result, the U.S. Food and Drug Administration (FDA) approved more recombinant drugs in 1997 than it had in the years prior combined. These drugs included treatments for anemia, AIDS, cancer (including Kaposi's sarcoma, leukemia, colorectal, kidney, and ovarian cancers), hereditary diseases, and anemia. (Cystic Fibrosis, Familial Hypercholesterolemia, Gaucher's Disease, Hemophilia A, Severe Co-Infectious Disease, and Turnor's Syndrome), Diabetic Foot Ulcers, Diphtheria, Genital Warts, Hepatitis B, Hepatitis C, Human Growth Hormone Deficiency, Multiple Sclerosis. Site-specific integration and specifically regulated gene expression are essential advanced technologies [9] because plants have multigene transfer. Plant biotechnology faces several significant hurdles, including the precise control of transgenic expression, the efficiency of endogenous genes in novel environments, and transcriptional regulation of these genes .(8)

 Numerous things endanger human life, including food shortages that create starvation, various deadly diseases, environmental issues brought on by rapid industrialization and urbanization, and many others. Genetic engineering has taken the role of traditional methods and modern methods (13).

Recombinant DNA molecules can be made from DNA sequences that come from any species. For instance, human DNA and fungus DNA can be linked together, as can plant DNA and bacterial DNA. Additionally, DNA can be chemically synthesized to construct DNA sequences that do not exist anywhere in nature and then added to recombinant DNA molecules. Any DNA sequence can be produced and inserted into living things using synthetic DNA and recombinant DNA technology.(3).

 Recombinant proteins are those that can be produced when recombinant DNA is expressed within living cells. Recombinant protein is not always created when recombinant DNA encoding a protein is added to a host organism. Use of specialized vectors is required for the expression of foreign proteins. Recombinant DNA varies from genetic recombination in that the latter is a natural biological process that results in the remixing of existing DNA sequences in nearly all species, whereas the former is produced by artificial means.(4) .

 The current evaluation outlined the main difficulties faced by people and discussed how recombinant DNA technology can help resolve these problems. In keeping with this, we have outlined the genetic engineering constraints as well as potential future avenues for researchers to get beyond these restrictions by altering the genetic engineering techniques that are now being used(8).

**Background Information:**

Definition:

 Recombinant DNA is a technology of joinin together of DNA molecules from two different species, which are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture and industry.(12).

Recombinant DNA technology entails changing genetic material outside of an organism to produce living things or their products with improved and desired traits. This method entails inserting DNA fragments with acceptable gene sequences from a number of sources using the right vector. The manipulation of an organism's genome can take place either by adding one or more new genes and regulatory elements, or by recombining existing genes and regulatory elements to reduce or prevent the expression of indigenous genes (3)

History of Recombinant DNA technology:

 In 1972 Paul Berg succeeded in inserting DNA from bacterium into the virus DNA. He created the first DNA molecule made of parts from different species. This type of molecule is known as “Hybrid DNA”or “Recombinant DNA”. Paul berg is known as the“Father of genetic engineering”.In 1973 Herbert Boyer of the university in California at San Francisco and Stanley Cohen from Stanford university reported the construction of functional organisms that combines and replicate the genetic information from different species(10)

 Boyer and Cohen’s achievement represented an advance upon the ingenious techniques developed by Paul Berg in 1972 for inserting viral DNA into bacterial DNA. It was a creative synthesis of earlier researching field(4).

 Living organisms able to serve as carrier for genes to transfer from one to another organism . Enzymes helps to cleave and rejoin DNA fragments that contain such genes. DNA molecules from one organism precisely targeted and manipulated for insertion into the DNA of other organisms.(7).

IMPORTANT TOOLS FOR RECOMBINANT DNA TECHNOLOGY:

* Restriction Enzymes
* Ligase
* Vectors
* Suitable host(7)

Restriction Enzymes:

 Naturally produced by bacteria Restriction Endonucleases .It destroys bacteriophage DNA in bacterial cells. Endonucleases are enzymes that produce internal cut called cleavage in DNA molecules. A class of endonucleases that cut DNA only within or near those sites which have specific base sequences,such endonucleases are know as restriction endonucleases or restriction enzymes.(1,6).

Ligase:

 DNA ligase is an enzyme that can link the DNA strands together which have double stranded breaks. Naturally, DNA ligase has applications in both DNA replication and repair mechanism. DNA ligase has extensive use in molecular biology laboratories for genetic recombination experiments.(4).

Vectors:

 A vector is a DNA molecule that has the ability to replicate autonomously in an appropriate host cells and serve as a vehicle that carry DNA fragment or insert to be cloned. Therefore a vector must have an origin of DNA replication that functions in the host cell. Eg. Plasmids, phage or virus may be used as a vector.(4,5).

Host for DNA recombinant technology:

 1. Bacteria:

 *E .coli* is used because it can be easily grown and its genomics are well understand. Gene product is purified from host cells.(5).

2. Yeast – *Saccharomycetes cerevisae .*

It express the eukaryotic genes easily and can be collected and purified efficiently. It is easily grown and its genomics are known .(6).

3. Plant cells and whole plants:

 May express eukaryotic genes easily.

 Plants are easily grown, it produce plants with new properties.(11).

4. Mammalian cells:

 It has a predominant role in medicine and can express the eukaryotic genes easily.(2).

Process of recombinant DNA technology:

* Isolation of DNA
* Cutting of DNA at specific location
* Amplification of gene of intrest using PCR
* Inserting of Recombinant DNA into host.
* Obtaining the foreing gene products.(11).

Isolation of DNA :

 DNA isolation is a process of purification of DNA from sample using a combination of physical and chemical methods.

Steps in DNA extraction;

1.Cells which are to be studied need to be collected

2. Breaking the cell membranes to expose the DNA along with the cytolaplasm cell lysis.

3.Lipids fromm the cell membrane and the nuclear membranes are broken down with detergents.

4.Breaking protiens by adding an proteinase.

5.After centrifugation of the sample, denatured proteins stay in the organic phase while aqueous phase containing nucleic acid.(11, 13).

DNA cloning:

 DNA cloning is a group of individual cells or organisms descended from one progenitor. This means that the members of a clone are genetically identical, because cell replication produces identical daughter cells each time. The use of the word clone has been extended to recombinant DNA technology which has provide scientists with the ability to produce many copies of a single fragment of DNA, such as a gene creating identical copies that constitute a DNA clone.(10).

Human genome sequence:

 The fundamentals of gene expression were worked out in bacteria and viruses in 1950s and 1960s. At that time, prior to the development of recombinant DNA techniques learning how the genes of animals and plant cells are controlled seemed next to impossible.(8).

**APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY :**

 By creating novel medications and vaccines, recombinant DNA technology is significantly enhancing healthcare. By creating diagnostic tools, monitoring systems, and fresh therapeutic gimmicks, the treatment strategies are also improved. One of the most well-known applications of genetic engineering in health is the development of new varieties of experimental mutant mice for research purposes and the genetic modification of bacteria to produce synthetic human insulin and erythropoietin . In a similar vein, genetic engineering techniques have been used to address environmental problems like turning wastes into biofuels and bioethanol , cleaning up oil spills, carbon, and other toxic wastes, and identifying toxins like arsenic in drinking water. Additionally useful in biomining and bioremediation, genetically modified microorganisms.(9).

 Recombinant DNA technology's introduction revolutionised biological research and sparked a number of significant changes. By changing microbes, animals, and plants to generate medicinally valuable compounds, it provided new potential for innovators to produce a wide range of therapeutic goods with immediate effect in medical genetics and biomedicine . Recombinant drugs, which make up the majority of biotechnology pharmaceuticals, are crucial in the fight against fatal human diseases. Recombinant DNA technology was used to create pharmaceutical products that completely altered human life. As a result, the U.S. Food and Drug Administration (FDA) approved more recombinant drugs in 1997 than it had in the previous several years combined. These drugs include treatments for anaemia, AIDS, cancers, and hereditary disorders.(14)..

 Numerous things endanger human life, including food shortages that induce starvation, various deadly diseases, environmental issues brought on by rapid industrialization and urbanisation, and many others. Conventional approaches have been supplanted with genetic engineering, which has a larger chance of success. The current evaluation outlined the main difficulties faced by people and discussed how recombinant DNA technology can help resolve these problems. In keeping with this, we have outlined the genetic engineering constraints as well as potential future avenues for researchers to get beyond these restrictions by altering the genetic engineering techniques that are now being used.(9).

 The most convenient hosts for the production of molecular drugs are thought to be microorganisms. These cells have less resistive barriers that enable the assimilation of foreign genes, and expression can be easily regulated. Microbial systems have less complex machinery than plant and mammalian cells, which eventually improves the performance and quality of protein production. The utilization of widespread microbial species, such as yeasts and bacteria, is promising, but less widespread strains have also shown promise as cellular factories for the production of recombinant molecular medicines. If these cellular factories of microorganisms are integrated into pharmaceutical production processes, the rising demand for drugs and the need for quality can be met with improved outcomes.(8).

1. Health care:

 Recombinant DNA technology plays wide role in treating diseases to improve health conditions. In health care services gene therapy is an advanced technique providing therapeutic potential in diseases such as genetic disease, cancer and autoimmune diseases. By targeting hematopoietic stem cells using advanced gene transfer mechanism brought successful results. In HIV, a unique disorder of X-linked chromosome Adrenoleukodystrophy is treated through the expression of specific genes and are transferred by lemtiviral vector. It was the first time of treating disease by lentiviral vector and achieved successfully. There were several therapeutic mechanisms by altering specific protein expression through immunotherapy enhancement used to test diseases such as Metastatic melanoma. Sustainable cells were recombined for tumor recognition in blood by retrovirus encoding a T-cell receptor treated with infusion in regression of Metastatic melanoma lesions and for Metastatic synovial cell carcinoma. The important stratergy in the field of health care is treating cardiovascular diseases by gene therapy such as Myocardial protection, regeneration, prevention of bypass graft failure and therapeutic angiogenesis.(7).

 Polypeptide hormones include human growth hormone. In both people and animals, it is in charge of cell growth, division, and regeneration. It is produced by the pituitary glands include somatotroph cells. Before recombinant HGH was made available, cadaveric pituitary glands were the only source of HGH for therapeutic purposes. Some people who underwent this risky procedure eventually developed Creutzfeldt-Jakob disease. This issue was resolved by recombinant HGH, which is now used medically. Athletes and others have misused it as a performance-enhancing substance. In recent years, biotechnology has aided in the production of several growth hormones. With the help of this hormone, dwarfism condition can be successfully cured.(5).

2.Agriculture:

 Crops that have been genetically engineered in agriculture are done so for two reasons: high yield and pest resistance in various nations (Paoletti et al., 1996). The first genetically altered crop to receive a licence for human consumption was the tomato CGN-89564-2, which was genetically modified in 1994 (Bruening et al., 2000). Genetically modified crops are being employed in commercial situations in many different nations. And it had the moniker "FlavrSavr," and it was a flop on the market. Additionally, 88% of the corn and 93% of the soybeans in the US are genetically modified. (1).

 Among different plant species, recombinant protiens have been used to improve resistant crop varities and also to improve the nutritional value in them. In recent times more polymers of Proteins in plants were obtained to produce specific pharmaceutical products. Similarly human collagen is obtained from tobacco plants by genetic engineering technique. Furthermore in resistant variety of plants, quantitative trade locus is analysed in the specific rice variety by protein kinase known as phosphorus starvation tolerance which helps to enhance the growth of roots in earlier stages which can tolerate the phosphorous deficiency. This enzyme includes the root to absorb the nutrients sufficiently even in phosphorus deficient soil which promotes the grain yield. Dynamics in transcriptome and gene expression fluctuation can be helpful to enhance the resistant crop varities to microbial stress and environment crisis. Infections caused by fungal and bacterial species in rice variety can be inhibited by WRKY45 gene which is a class of DNA binding proteins which is induced by plant activator benzothiadiazole that activates the innate immunity in plants. Desirable characters such as resistance to insects, disease and drought resistance, tolerance of the herbicides and pesticides ,salt tolerance have been developed in sqash, beans, beetroot, potato, eggplant., rice and also in many plants are in progress. Other versatility characters like ripening, nitrogen utilization, nutritional benefits and phosphorus utilizing property in deficient soil has been enhanced.(10,2).

 Biotechnology's uses in agriculture have generated the most debate. Genetically modified organisms (GMOs) have received calls for bans from some activists and consumer advocacy groups, as well as for labelling regulations to alert people to the rising presence of GMOs in the food supply. Bovine somatotropin (BST), a growth hormone that increases milk output in dairy cows, was approved by the FDA in 1993, marking the beginning of the introduction of GMOs into agriculture in the United States. The FDA authorised the first genetically altered whole product the following year, a tomato with a longer shelf life. Numerous agricultural GMOs have since obtained regulatory approval in the US, Europe, and other countries. These include crops that make their own insecticides and crops that can grow without a certain herbicide.(10)

 Recombinant DNA techniques may be used to create better varieties of natural enemies like parasitoids and predatory arthropods, although the technology is still in its infancy. For instance, a variety of mites are mass-reared to suppress spider mites, including the western predatory mite Metaseiulus occidentalis (Acari). However, the predatory mites are frequently wiped out by pesticides used to control other insect species. Theoretically, developing insecticide resistance genes in beneficial insects like the western predatory mite would make them resistant to chemical sprays used to eradicate insect pest species.(10,1).

3.Processing industry:

 The manufacturing of enzymes by recombinant DNA technology has several uses which are applicable in specific conditions of specified food processing industries. Important enzymes such as amylases and lipases are having major role in production of specific products in food industries. Eg: To get rid of bacterial contamination in food processing industry, lysosymes are effectively used by preventing the microbial colonization of bacteria. Hydrolized strains of exopolysaccharides from *Staphylococcus* and *E .coli* inhibits the bacterial population by an engineered gene DSP B from T7. Glucose oxidse enzyme inhibits the growth of *Salmonella infantis , S.aureus, B.cereus, L.monocytogenes, Clostridium perfringens, Camphylobacter rocolitica* and also many other food spoiling bacteria(6).

 Recently ,the scientist Mr.N.Annadurai used recombinant DNA technology to edit the DNA of bacteria which can enable the grown collagen as it is present in raw skin which gives the super strength to leather and induces flexibility. Bioleather is produced by assembling the engineered layers of collagen. Molecular biology merged with traditional industrial microbiology to promote the yield in industrial products such as biopharmaceuticals, industrial enzymes,primary and secondary metabolites and bioleathers. In addition to that, proteomics, functional genomics, novel molecules for medicine, antibiotics production, sequencing of industrial genomes are in process for the improvement of future and to introduce new industrial products.(7)

 4.Service sector:

 The most familiar service sector using recombinant DNA technology is hospital sector. This technique is used to diagnose HIV by the clinical diagnosis ELISA. With the recombinant DNA technology insulins are produced by therapeutic protein production. This technique is widely used in the production of medicines and vaccines such as hepatitis B vaccine, combination of gene therapy and drug which has high potential in recent times have been in a trial to confer chemoprotection on human during chemotherapy for glioblastoma with alkylating agents. The most frequently undergoing techniues of recombinant DNA technology is the treatment by stem cell transplantation either by in vivo or by ex vivo gene therapy. The human follicle stimulating hormone (FSH) is engineered by recombinant DNA technology in invitro process is obtained from specified gene cell line in eukaryotes a complex heterodimeric protein has been selected for the gene expression. The overlapping PCR uses the overlap in ping primers for the PCR amplification of 1-3 kb, which has long fragments has been in progress. The recombinant DNA technology is involved in various laboratory techniques to isolate and study the genetic segments of interest in research fields. It is used to study the specified modifications of higher organisms is further more helpful to educational sectors. This technology helps to understand the protein-protein interaction and in other research fields like cell biology , molecular biology, biochemistry, immunohistochemistry and many other fields.(3).

 Recombinant human antithrombin, an agent that reduces blood clotting and is used for the prevention of heart attack and stroke in high-risk patients, was the first animal-tested substance to receive approval for therapeutic use. Goats that have undergone genetic engineering emit this substance in their milk. After that, it is separated and purified from the milk, which goes through stringent safety inspections and is checked for the presence of pathogens (substances that cause disease).(14, 9, 16).

 Pharming, or the production of medications using genetically modified animals or plants. Genetically altered animals and plants are relatively cheap to produce and maintain, making pharmaceutics a useful alternative to conventional pharmaceutical development. In addition, a tiny area of injured plants or animals can produce a significant amount of pharmaceuticals such hormones, antibodies, enzymes, and vaccinations. Nevertheless, despite these benefits, pharming is still debatable because of worries regarding the manufacture and safety of pharmed agents.(16).

 Recombinant DNA techniques in forensic science assist law enforcement organisations in testing connections between biological crime sample and individuals to establish guilt or innocence. These techniques are also used to analyse familial heritability and check people for the existence or absence of genetic disorders. DNA fingerprinting using restriction fragment length polymorphisms is one method. Here, restriction digestion analysis is used to identify the distinctive repeating DNA sequences in individuals. To do this, certain bands on blots are probed with labelled nucleic acids.(8).

 Methods using recombinant DNA are widely used in biological research. They are used to build mutant alleles and test phenotypes in order to research gene function. Primers used in PCR amplification are easily modified to introduce site-specific mutations.(15).

CONCLUSION:

 Recombinant DNA technology is a significant advancement in science that has greatly facilitated human life. It has developed ways in recent years for medicinal applications such the treatment of cancer, hereditary illnesses, diabetes, and numerous plant ailments, particularly viral and fungus resistance. Recombinant DNA technology has been highly acknowledged for its contribution to environmental clean up (phytoremediation and microbial remediation) and improved plant resistance to many harmful causes (drought, pests, and salt). It made substantial improvements in plants, microbes, and humans in addition to humans. The obstacles in enhancing goods at the gene level occasionally present severe challenges that must be resolved for the benefit of the future of recombinant DNA technology.(3)

 Particularly in the pharmaceutical industry, there are significant problems with producing high-quality products since the body rejects the alteration made to a gene. Additionally, growing a product is not always a good thing because a variety of circumstances could work against its success. Recombinant technology is assisting in treating a number of diseases that cannot be treated under normal circumstances, yet the immune responses make it difficult to get satisfactory outcomes.(9)

 The methodologies for genetic engineering face a number of challenges that had to be overcome by more targeted gene augmentation in accordance with the organism's DNA. A Rec A-dependent procedure would be used to incorporate incoming single-stranded DNA into the bacterial chromosome. Sequence similarity between the bacterial chromosome and the incoming DNA is necessary for this. Plasmid reconstitution and stable maintenance might be made simple. Safety and biodiversity suffer when genetic material from one source is introduced into another. Concerns about the creation of genetically modified plants and other items are numerous. For instance, it is clear that plants that have been genetically modified can breed with wild plants, introducing their "engineered" DNA into the ecosystem and threatening our biodiversity. (17).

 Additionally, there are worries that genetic engineering could have harmful effects on health. Therefore, more in-depth research is needed in this area to address these problems and the concerns of the general public.(16).

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