**Futuristic Trends in the field of Recombinant DNA Technology to improve Human Life**

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**Abstract**

Recombinant DNA technology has significantly advanced human life by enabling the production of essential proteins for health problems and dietary purposes. This technology has multidisciplinary applications, such as improving health, enhancing food resources, and resisting adverse environmental effects. Genetically modified plants in agriculture have enhanced resistance to harmful agents, increased product yield, and better survival. Recombinant pharmaceuticals are now widely used, and techniques like gene therapy and genetic modifications are used for bioremediation and treating serious diseases. The importance of recombinant DNA technology in daily life is evident due to its wide range of applications and potential applications.

**Keywords:** Health problems, Dietary purpose, Essential proteins, DNA technology, Recombinant, Application, Human life, Treating and Therapy.

**1. Introduction**

Human life is significantly impacted by three factors: food deficiency, health problems, and environmental issues. Food and health are essential for human survival, and the increasing world population demands safe, affordable food. Health issues, such as cardiovascular diseases, cancer, diabetes, AIDS/HIV, tuberculosis, and malaria, cause significant deaths annually. Despite efforts, global food production is lower than human needs, and health facilities are below standard in third-world countries. Industrialization has led to environmental pollution, allowing industrial waste to mix with water, affecting aquatic marines and humans indirectly. Addressing these issues requires modern technologies to improve living conditions and reduce the impact of these issues on our planet [1].

Modern techniques and methods like molecular cloning and transformation are used in genetic engineering to address difficulties with agriculture, human health, and the environment. This method takes less time and produces more trustworthy results. Genetic engineering, in contrast to conventional breeding, transforms the target by biolistic and *Agrobacterium*-mediated transformation to add a tiny block of desired genes. Homologous recombination-dependent gene targeting, nuclease-mediated site-specific genome modification, recombinase-mediated site-specific genome integration, and oligonucleotide-directed mutagenesis are all methods that may be used to modify plant genomes [2].

By creating novel vaccinations and medications, recombinant DNA technology is significantly enhancing health conditions. Additionally, the therapy approaches are enhanced. Through creating novel therapy strategies, monitoring tools, and diagnostic kits. One of the most prominent instances 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 [3]. Similar to this, genetic engineering techniques have been used to address environmental problems including turning wastes into biofuels and bioethanol [4–7], cleaning up oil spills, carbon, and other hazardous wastes, and identifying toxins like arsenic in drinking water. The bacteria that have been genetically altered are also useful for bioremediation and biomining.

The development of recombinant DNA technology transformed biology and profoundly altered medical genetics and biomedicine. It made it possible to create therapeutic items by altering microbial, animal, and plant life to produce beneficial compounds for treating illnesses. The majority of biotechnology medicines are recombinant, and they are essential in the fight against fatal human illnesses. In 1997, the FDA authorised more recombinant medications for anaemia, AIDS, malignancies, hereditary conditions, diabetic foot ulcers, diphtheria, genital warts, Hepatitis B, Hepatitis C, human growth hormone insufficiency, and multiple sclerosis than in all prior years combined. For overcome these difficulties, advanced techniques like site-specific integration and controlled gene expression are essential [8-10]. Challenges in plant biotechnology include tanscriptional regulation of endogenous genes, effective new locations, and precise transgene expression control, requiring further development for successful application [11].

Numerous things endanger human life, including food shortages that induce starvation, various deadly illnesses, environmental issues brought on by rapid industrialisation and urbanisation, and many more. 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 now being used.

**2. Recombinant DNA Technology**

In recombinant DNA technology, genetic material outside of an organism is changed to produce improved and desirable features of living things or the things they produce. This method entails inserting DNA fragments with acceptable gene sequences from a number of sources using the right vector [12]. 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 [13]. Utilising restriction endonucleases for specific target sequence DNA sites, enzymes are used to cleave DNA into various pieces, which are then joined together by DNA ligase activity to fix the desired gene in the vector. The host organism is then given the vector, which is subsequently grown to make many copies of the inserted DNA fragment in culture. Clones that contain the appropriate DNA fragment are then chosen and collected [11]. Paul Berg, Herbert Boyer, Annie Chang, and Stanley Cohen of Stanford University and University of California San Francisco produced the first recombinant DNA (rDNA) molecules in 1973. Regulation and safe application of rDNA technology were considered in 1975 at "The Asilomar Conference". Contrary to the expectations of scientists at the time of Asilomar, the recombinant DNA techniques to support agricultural and pharmaceutical innovations took longer than predicted due to unforeseen challenges and impediments to achieving the desired outcomes. To enhance health, however, a growing variety of products—including hormones, vaccinations, therapeutic agents, and diagnostic tools—have been created since the middle of the 1980s [13].

Recombinant DNA technology provides a rapid method to examine the genetic expression of the changes that were introduced into eukaryote genes by the insertion of cloned insulin genes within a simian virus fragment [3]. Similar to this, the antiangiogenic properties of an adenoviral vector that encodes endostain human secretory form prevented the formation of tumours. Dl1520 can increase the antiangiogenic impact by saving Ad-Endo replication [14]. In other hosts, targeted gene disruption has been employed to generate anticancer compounds that had structural similarities with the routes for synthesis [15].

Recombinant DNA techniques have been used to create therapeutic proteins with longer half-lives that contain glycosylation site sequences. Researchers have created a novel chimeric gene that combines HCG and FSH subunit coding sequences. For gene therapy and genetic alteration, researchers have also created vectors and vector combinations. Viral vectors are currently commonly employed in therapeutic settings and are available for purchase [16].

With applications in the treatment of serious illnesses including cancer, immunisation, and protein transduction, viruses are modified for medical use. Clinical-grade viral vectors are now easier to get because to improvements in manufacturing technology. Retroviral vectors are becoming less used because of their negative side effects, although they efficiently transmit genes. When administered directly into tissues, especially muscles, the most basic nonviral gene delivery technique, employing "naked" DNA, results in large gene expression levels with little adverse effects [17-19].

In order to electroporate recombinant DNA into E. coli and create a 15,000 clone library with an average insert size of 130–150 kb, a P1 vector was created. It is possible to map and analyse complicated genomes with this PAC cloning technique. pWSK29, pWKS30, pWSK129, and pWKS130 are examples of low copy number vectors that may be utilised for run-off transcription, complementation analysis, and unidirectional deletions. The technique of recombinant DNA has several uses, including genome mapping, analysis, and gene editing. Figure 1 provides an overview of a wide variety of recombinant DNA technology uses. [20-21].

Recombinant DNA Technology

Genetically modified products Fruits GM vegetables GM crops GM microbes GM animals

Therapeutic products Vaccines Growth hormones Antibodies Vectors Recombinant protein Anticancer drugs

Energy applications Biohydrogen Bioethanol Biomethanol Biobutanol

Diagnosis Gene therapy CRISPR Monitoring devices Therapeutic strategies

**Figure 1: Illustration of various applications of recombinant DNA technology.**

**3. Current Research Progress**

Recombinant DNA technology is a rapidly expanding subject, and scientists from all over the world are creating novel techniques, tools, and modified goods for use in a variety of fields, including agriculture, health, and the environment. For instance, Lispro (Humalog), a well-effective and quick-acting recombinant insulin, is superior to conventional human insulin [3]. Similar to this, epoetin alfa is a brand-new, well recognised recombinant protein that may be utilised to successfully treat anaemia [22]. Recombinant hGH has proven to be quite effective in treating kids who are unable to manufacture enough hGH on their own. An accomplishment to acknowledge this technique was the FDA's approval of clinical trials for a recombinant form of the cytokine myeloid progenitor inhibitory factor-1 (MPIF-1). Since it can imitate the division of immunologically significant cells, it can reduce the negative effects of anticancer medications [23, 24]. The most recent advancements in the field of recombinant DNA technology are outlined in the section that follows.

CRISPR, a more recent advancement in recombinant DNA technology, has provided answers to a number of issues in several animals. This technique may be used to specifically target and eliminate human cell gene copies. The approach was shown to be effective by activating, suppressing, adding, and deleting genes in human cells, mice, rats, zebrafish, bacteria, fruit flies, yeast, nematodes, and crops. With CRISPR, mouse models may be handled for the research of human illnesses, with individual gene studies becoming much faster and the study of gene connections becoming simple by altering several genes in cells [25].

The *H. hispanica* genome's CRISPR system can adapt to nonlytic viruses quite well. The interfering Cas3 nucleases and other Cas proteins are encoded by the related Cas operon. In order to prime CRISPR for the synthesis of new spacers and priming crRNAs, a strain must be engineered. In order to generate adaptive immunity, the CRISPR-cas system has to incorporate additional spacers into its locus [26]. Cleavage of foreign DNA and RNA is a regulated process that is sequence-specific. By incorporating a photo-spacer into the CRISPR system, the host system is able to preserve information on the genetic material of the intrusive [27].

DNA endonucleases that utilise RNA molecules to recognise particular targets include Cas9t (gene editing tool) [28]. For genome editing procedures, a Class 2 CRISPRCas system with a single protein effector can be used. Dead Cas9 is crucial for the initiation of transcription, the localisation of fluorescent protein labels, the recruitment of histone modifying enzymes, and transcriptional repression [29]. CRISPR-induced mutations are used to target the genes implicated in the isolation process for homozygous gene knockouts. Analysing important genes in this way enables the study of "potential antifungal targets" [30]. To create strains that are resistant to various types of disruptive viruses, natural CRISPR-cas immunity has been used [31].

The CRISPR-Cas system, which consists of a CRISPR array with short repeating elements and spacers, is the only adaptive immune system seen in prokaryotes. An AT-rich leader sequence precedes the array, and cas genes that encode Cas proteins flank it. New spacers are promoted in Escherichia coli by the cas1 and cas2 catalases through complex formation. Since the target sequence is chosen intentionally, interference and acquisition necessitate the photo-spacer adjacent motif (PAM). After CRISPR array transcription, memory of the invader's sequence starts to form, and the mechanism resists self-targeting during the last phases of immunisation [32-34]. The CRISPR loci in Sulfolobus species include several spacers, which largely resemble conjugative plasmids. Active viral DNA replication has an impact on spacer acquisition, and DNA breaks at replication forks function as a catalyst. In complex biological systems, the CRISPR-Cas system has a special function that improves immune stability and stability [35].

Chimeric nucleases, such as zinc-finger nucleases (ZFNs) and transcription activatorlike effector nucleases (TALENs), are made up of programmable, sequence-specific DNA-binding modules connected to a nonspecific DNA cleavage domain. ZFNs and TALENs have more specialised and focused therapeutic potential [25, 36, 37]. The recombinant protein fibroblast growth factor (FGF-1), which encourages the creation of new blood vessels in the myocardium, has also been created. When it is injected (through biological bypass) into a human myocardial, the heart's blood flow is boosted. Diabetic ulcers can be successfully treated using DermaGraft and Apligraf, two FDA-approved products that act as recombinant skin replacers and are intended for the treatment of leg ulcers [38–40].

Recombinant DNA technology allowed for the effective creation of insulin from E. coli, and as a result, numerous species, including cattle and pigs, are now being used as sources for producing the hormone. This selection, however, has resulted in immunological reactions. Recombinant human insulin seldom triggers immunogenic reactions and is equivalent to human porcine insulin. Additionally, it is more reasonably priced and better suited to meet medical demands. The first protein produced in tobacco plants was human growth hormone [41, 42]. In addition to insulin, a number of novel medications associated with recombinant DNA technology have improved during the research process, and several protein manufacturing methods have been created. In order to formulate medications, several modified microbial strains have been created [41, 43, 44].

Serious problems with recombinant DNA techniques and the biology of the cells that act to make medically significant chemicals exist in the development of molecular medicine that is specifically based on proteins. There is a pressing need to increase the amount and quality of medications based on a molecular phenomena in order to solve these challenges. In recombinant DNA technologies, cell factories are thought to be crucial, but these need to be investigated in more complexity and depth since the demands cannot be met by traditional factories [42].

Similarly, an oncolytic adenovirus that mimics breast cancer was created using endothelial growth factor and Notch signalling.A selective agent for the expression of the antagonist. This further functions as an anticancer drug by disrupting tumour angiogenesis. This results in a substantial alteration in the overall number of blood vessels as well as the perfused vessels, indicating greater effectiveness against the tumour and vascular effects [13].

Recombinant DNA technology has been used to attempt to alter the influenza virus genome in order to create vaccinations. Engineering of vectors for the expression of foreign genes is the basis of the modifications. In actuality, a foreign gene, often chloramphenicol acetyltransferase gene, was used to substitute the influenza virus's NS gene.After transfection with pure influenza A virus and helper virus, the previously recombined RNA is produced and packed into viral particles. It has been established that the influenza A virus's 5 and 3 terminal nucleotides are enough to create signals for RNA transcription, RNA replication, and RNA packaging into influenza viruses [15].

The aforementioned new manufacturing techniques improve the pipelines for the creation of numerous medications and vaccines, among other things. High-quality protein production is influenced by a cell's physiology and the environment it is given. If a cell experiences stress, the expression of proteins is delayed, which in certain situations may also favour the synthesis. Therefore, more advancements are needed for better and safer manufacturing at the genetic and metabolic levels. Considered to be the most practical hosts for the production of molecular drugs are microorganisms. These cells have less resistive barriers that enable the assimilation of foreign genes, and expression can be readily regulated.

Microbial systems offer less complex machinery than plant and mammalian cells to be used as hosts, which eventually improves the performance and quality of synthesis of proteins. The utilisation 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 medications and the requirement for quality can be met with improved outcomes (Table 1) [41, 45, 46].

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Method  | Mechanism | Overhang (bp) | Scar (bp) | Comments | Examples of applications |
| BioBricks  | Type IIP restriction endonuclease | 8 | 8 | Sequentially assembles small numbers of sequences | Construction of a functional gene expressing enhanced cyan fluorescent protein |
| BglBricks  | Type IIP restriction endonuclease | 6 | 6  | Uses a highly efficient and commonly used restriction endonuclease, the recognition sequences of which are not blocked by the most common DNA methylases | Construction of constitutively active gene-expression devices and chimeric, multidomain protein fusions |
| Pairwise selection  | Type IIS restriction endonuclease | 65  | 4 | Requires attachment tags at each end of fragments to act as promoters for antibiotic resistance markers; rapid, as a liquid culture system is used | Assembly of a 91 kb fragment from 1-2 kb fragments |
| GoldenGate  | Type IIS restriction endonuclease | 4  | 0 | One-step assembly of 2-3 fragments |  |
| Overlapping PCR  | Overlap  | 0 | 0 | Uses overlapping primers for the PCR amplification of 1–3 kb-long fragments | Usually used for 1–3 kb-long fragments, for example, for gene cassette construction |
| CPEC  | Overlap | 20–75  | 0 | Uses a single polymerase for the assembly of multiple inserts into any vector in a one-step reaction in vitro | One-step assembly of four 0.17–3.2 kb-long PCR fragments |
| Gateway  | Overlap | 20 | 0 | Uses a specific recombinase for small-scale | assembly One-step assembly of three 0.8–2.3 kb-long fragments |

**4. Applications of Recombinant DNA Technology**

**4.1. Food and agricultural.**

 Recombinant DNA technology has several applications that have made it feasible to produce unique enzymes that are suited for certain food processing settings. Due to their specialised functions and uses in the food industry, several significant enzymes, such as lipases and amylases, are accessible for the specific manufactures.

Another significant accomplishment made possible with the use of recombinant DNA technology is the generation of microbial strains. Numerous microbial strains have been created that can manufacture enzymes, specifically proteases, by targeted engineering. A few fungus strains have undergone modifications to lessen their capacity to produce hazardous substances [47]. Lysozymes are the most efficient tools for eliminating microorganisms in the food industry. They avoid colonisation of microscopic creatures. Fruits, vegetables, cheese, and meat may all be preserved with it since it extends the shelf life of these foods. Lysozyme immobilised in polyvinyl alcohol films and cellulose can be used to suppress food-spoileding germs. Fish skin gelatin gels that have been impregnated with lysozyme have longer shelf lives and are less likely to deteriorate [48–50]. It is possible to hydrolyze the exopolysaccharides of Staphylococcus and E. coli by using the T7-engineered DspB. The bacterial population declines as a result of DspB's ability [50].

The combined activity of serine proteases and amylases can be used to eliminate biofilms associated with the food industry [51]. Yersinia enterocolitica, Campylobacter jejuni, L. monocytogenes, S. aureus, Salmonella infantis, Clostridium perfringens, B. cereus, and certain other food-rotting microbes can all be suppressed by glucose oxidase. It is regarded as one of the most crucial enzymes in the food business for eliminating a variety of foodborne pathogens [50].

Recombinant proteins that are employed as pharmaceuticals were recently developed by the first plant, and many more are on the way to being used for additional synthesis of comparable medically significant proteins [52]. Various plant species have been employed to produce a wide variety of recombinant proteins for usage as enzymes in various sectors.

The nutrition-related proteins found in milk and novel polymeric proteins utilised in businesses and the medical area are two of the most commonly employed proteins in research [52]. The idea of oral vaccination using edible plants has gained appeal with the development of HBV vaccine manufacturing in plants. Several therapeutic protein products, including casein and lysozyme for enhancing child health and protein polymers for tissue replacement and surgery, have been produced by plants. Additionally, tobacco plants can be genetically modified to make human collagen.

One of the key objectives being thought about in the realm of recombinant DNA technology is the production of high yielding molecular proteins [52]. The identification of a rice variety with protein kinase called as PSTOL1 (phosphorus starvation tolerance1), which aids in boosting root development in the early stages and tolerates phosphorus deprivation, was made possible by traditional breeding and quantitative trade locus (QTL) analysis [53]. Overexpression of this enzyme permits roots in phosphorus-deficient soil to absorb nutrients in sufficient amounts, thus increasing grain output [54]. The chloroplast genome sequences play a crucial role in the phylogeny and evolution of plants. It is believed that Rpl22 was transported from the chloroplast to the nuclear genome.

A peptide found in this gene aids in the transfer of proteins from the cytosol to the chloroplast. Except for ycf1 and ycf2, it has been seen that a number of crucial genes removed from the chloroplast transfer into the nucleus to prevent abnormalities in photosynthesis and other critical functions. Since nuclear transgenic plants struggle with decreased expression and transgene emigration through pollen, trans-genesis into chloroplast is regarded as stable. Transgenes have been integrated into the chloroplast genome in about ten thousand copies [55–57]. Although not under cellular control, heterologous regulatory sequences are necessary for transgene expression. Success has been discovered in the engineering of T7gene10 against salt stress, although with a decreased expression rate into nongreen tissues. The inner chloroplast membrane develops in layers as a result of the introduction of the -tmt gene into the chloroplast genome. The insertion of lycopene -cyclase genes into the tomato plastid genome increases the conversion of lycopene to provitamin A [57, 58].

Gene expression profiles can be used to identify genes unique to an organ or tissue. The primary tools for gene expression profiling are full-length cDNAs. To analyse the transcriptome of rice cultivated in the field, a 44 K Agilent Oligonucleotide microarray is employed. Transcriptomic data and weather data can be used to predict gene expression variation and transcriptome dynamics. These procedures and forecasts can assist increase crop yield and crop resilience to microbial or environmental challenges.

The WRKY45 gene in rice, which is triggered by the plant activator benzothiadiazole, which stimulates the plant's innate immune system, can increase resistance to bacterial and fungal diseases.The qSW5 gene can be inserted to produce grains of a bigger size. By suppressing the development of the abscission layer, qSH1 results in the loss of seed shattering. The black colour of rice, which makes it resistant to disease assault, is caused by the kala4 gene [59, 60]. In order to facilitate the gene-by-gene introduction of well-known features, genetic modification is required. It enables access to a wider variety of an organism's genes.

Numerous plants, including potatoes, beans, eggplant, sugar beetroot, squash, and others, are being created with beneficial traits, such as tolerance to the herbicide glyphosate, insect resistance, drought resistance, disease resistance, and salt tolerance.

**4.2. Health and Diseases.**

 Recombinant DNA technology has a wide range of uses for curing illnesses and enhancing physical well-being. The significant advances in recombinant DNA technology for the enhancement of human health are described in the sections below: Enhancements to nutritional flexibility, ripening, and nitrogen uptake have also been made [61].

**(4.2.1). Gene Therapy**

A cutting-edge method with therapeutic promise in healthcare is gene therapy.A more sure path towards healing the most lethal genetic disorders was offered by the first successful report in the area of gene therapy to treat a genetic condition [62, 63]. The main immunodeficiency adenosine deaminase-deficiency (ADA-SCID) responds well to therapy using this approach. When this technique was first developed, it was difficult to maintain patients on PEGylated ADA (PEG-ADA) during gene therapy, and it was difficult to target gene transfer to T-lymphocytes, which led to poor outcomes [64, 65]. Later, however, targeting haematopoietic stem cells (HSCs) with an enhanced gene transfer methodology and a myeloablative conditioning regimen led to effective outcomes [66].

It is conceivable for X-linked disease and adrenoleukodystrophy (X-ALD) to develop when certain genes are expressed by lentiviral vectors based on HIV-1 [67]. The expression of the XALD protein shows that real HSCs' genes were properly corrected. For the first time, lentiviral vectors were successfully used to cure hereditary human illness [68]. By increasing the expression of a particular protein, immunotherapy was used to treat metastatic melanoma in 2006. This achievement in the health sciences opens up new avenues for the development of immunotherapy as a severe life-threatening illness treatment [69].

In two patients, a retrovirus encoding a T-cell receptor was used to produce blood levels of cells tailored for tumour identification that led to the regression of metastatic melanoma lesions up to a year after infusion. Later, patients with metastatic synovial cell cancer were treated using this approach [70]. For the therapy of chronic lymphocytic leukaemia, autologous T-cells were genetically altered to express chimeric antigen receptors (CAR) with specificity for the B-cell antigen CD19. Despite the repair of just a few number of progenitors, genetically edited cells undergo selective expansion for disorders like SCID-X1 and ADASCID as a result of in vivo selection provided by the disease pathogenesis. The possibility for combining gene and pharmacological treatment has recently been emphasised in a trial designed to give human HSCs chemoprotection during chemotherapy with alkylating drugs for glioblastoma [71].

A focused approach with the potential to be therapeutically beneficial is gene transfer to a few cells at anatomically distinct places. For incurable autosomal recessive dystrophies such congenital blindness and Leber congenital amaurosis (LCA), it shown amazing outcomes. In April 2006, a Swiss-German phase I/II gene therapy clinical study that was intended to treat chronic granulomatous illness was successful [72]. Two-thirds of the patients who received retrovirally transduced mobilised CD34+ cells extracted from peripheral blood exhibited obvious improvement from the therapy. After the therapy, methylation of the viral promoter caused the transgene to be silenced, which worsened the illness and ultimately resulted in the patient's death [73].

Gene therapy has been used to treat a wide range of cancers, including haematological malignancies, paediatric tumours, lung, gynaecological, cutaneous, urological, neurological, and gastrointestinal tumours. Different approaches have been tried to treat many forms of cancer, including the insertion of tumour suppressor genes into immunotherapy, oncolytic virotherapy, and gene guided enzyme prodrug treatment. Cancer treatment strategies heavily rely on the tumour suppressor gene p53, which is frequently inherited. P53 gene transfer is used in conjunction with radiation or chemotherapy in some of the techniques. Vaccination with tumour cells created to produce immunostimulatory molecules, vaccination with recombinant viral vectors expressing tumour antigens, and vaccination with host cells engineered to express tumour antigens are the three most significant approaches now being used [19].

Ad5/35-EGFP, a novel fibre chimeric oncolytic adenovirus vector, provides an effective new anticancer agent for the more effective treatment of hepatocellular carcinoma. The correct assaying of these vectors demonstrated their importance for improving transduction, and more viral offspring were generated in HCC. While maintaining the normal cells' cytotoxicity resistance, a greater level of transgenic expression was induced and an improved antitumor impact was seen on in vitro HCC cells. Using this technique also prevented the formation of tumours [74]. In recent years, cancer gene therapy has evolved and shown an increase in effectiveness [75].

Gene therapy for the treatment of cardiovascular disorders is a significant medical research method. Gene therapy will open up new possibilities for therapeutic angiogenesis, myocardial protection, regeneration, and repair, prevention of restenosis after angioplasty, avoidance of bypass graft failure, and risk factor management in the field of cardiovascular medicine. Wiskott-Aldrich Syndrome (inherited immunodeficiency) is brought on by a mutation in the gene that codes for WASP, a protein that controls the cytoskeleton. When matched donors are not available for stem cell transplantation, the condition is treated by injecting autologous HSPCs that have undergone ex vivo gene therapy [76].

Through immunotherapy based on the adoptive transfer of gene-engineered T-cells, metastatic cancer can be retreated. This therapy primarily focuses on accurate targeting of antigens produced by tumours and the accompanying vasculature, as well as the effective use of gene engineering to retarget T-cells before their transfer into the patient [77]. T-cell survival and migration are suppressed by the microenvironment of cancer cells, which frequently renders them nearly "invisible" to the immune system. Genetic engineering of T-cells is the answer to these problems. Cancer patients' T cells can be altered by recombining the genes that recognise cancer-specific antigens, provide immunity against immunosuppression, extend longevity, and promote migration to tumours [78].

Anaplastic lymphoma kinase (ALK) inhibitor sensitivity is conferred by fusion between the genes echinoderm microtubule-associated protein like 4 (EML4) and anaplastic lymphoma kinase (ALK), which is generated by an inversion on the short arm of chromosome. Specific chromosomal rearrangements are brought about when the CRISPR/Cas9 system is delivered through a vial to somatic cells of mature animals [79]. One of the main oncogenic pathways in many malignancies is wnt signalling. An appealing therapeutic strategy for treating cancer is to target the Wnt system, and LGK974 potently suppresses Wnt signalling, has good effectiveness in mouse tumour models, and is well tolerated. High levels of LGK974 response are observed in head and neck cancer cell lines with loss-of-function mutations in the Notch signalling pathway [80].

A codonoptimized gene was created and cloned into a recombinant modified vaccinia virus Ankara (MVA) based on the coding sequence of the influenza virus hemagglutinin gene. The ferret MVA-H7-Sh2 viral vector vaccine was found to be immunogenic because mock-vaccinated unprotected animals developed interstitial pneumonia and lost weight, whereas MVAH7-Sh2 immunisation shielded the animals from severe illness [81]. One of the most effective and significant treatments for head and neck cancer is viral gene therapy. Viral treatment first targeted the p53 gene function because it targets tumor-associated genes. Oncolytic viruses can kill cancer cells by replicating the virus and equipping themselves with therapeutic transgenes [82].

Cells that have the high density lipoprotein gene ABCA1 mutation can develop into macrophages. Gene knockouts improve the capacity of cells to develop into macrophages and precisely target the targeted pathogens in embryonic stem cells. Studying protein coding alterations and regulatory variations involved in altering mRNA transcription and stability in macrophages will be made easier thanks to the allele substitutions in this situation [83].

Production of antibodies and their derivatives is covered in 4.2.2. Recently, many antibodies and their derivatives have been developed and expressed in plant systems.Most crucially, seven antibodies and antibody derivatives have achieved the required stages in a good manner. It is possible to create chimeric secretory IgA/G, also known as CaroRx, from transgenic tobacco plants. This antibody can identify the Streptococcus variants, an oral pathogen that causes tooth decay. Antigen carcinoembryonic, which is still regarded as an affectively characterised marker in malignancies of the epithelia, may be functionally recognised by the monoclonal antibody T84.66 [84, 85].

An anti-HSV and anti-RSV full-length humanised IgG1 that is functional. In transgenic soybean and Chinese Hamster Ovary (CHO) cells, glycoprotein B has been produced as the herpes simplex virus (HSV)-2 recognising agent. When applied topically, antibodies from both sources have been demonstrated to stop the transmission of HSV-2 through the vagina in mice; if this were also true in people, it would be regarded as an effective and affordable method of preventing infections that are spread through sexual contact [86–88]. scFv antibody 38C13 is based on the malignant B lymphocyte idiotype in the well-studied mouse lymphoma cell line 38C13.

When mice were given the antibody, it caused the creation of anti-idiotype antibodies that could identify 38C13 cells, helping to protect the animals from the deadly challenge of being injected with lymphoma cells [89, 90]. Through this technique, distinct markers recognising enzymes could be created, most significantly the surface markers of malignant B-cells to serve as a successful treatment for non-Hodgkin lymphoma-like disorders in humans [61]. Human chorionic gonadotropin recognition is specific for a monoclonal antibody known as PIPP. Transgenesis and agroinfiltration in transiently transformed tobacco allowed for the generation of full-length monoclonal antibodies, as well as scFv and diabody derivatives [91].

In cells cultivated by LEYDIG, stimulated hCG can suppress the generation of testosterone, and in mice, who are used to measure hCG activity, uterine weight increase can be slowed. Antibodies can be used in the diagnosis and treatment of tumours [61].

**4.2.3: Research on Drug Metabolism.**

 For optimum therapeutic effectiveness and effects, it is essential to examine the complex system of enzymes involved in drug metabolism. Recent advances in recombinant DNA techniques have contributed to its function through heterologous expression, in which the genetic code for the enzyme is produced in vitro or in vivo by the transfer of a gene [92, 93].

**4.2.4. Production of Recombinant Hormones and Vaccines.**

Recombinant vaccines are more effective and specific than traditional vaccinations in comparison. Nasal transfer is a quick and effective way to fight mucosal diseases while also being painless and fearless way to transmit adenovirus vectors encoding pathogen antigens.

By expressing a transgene in the airway, this serves as a pharmacological vaccination that can create an anti-influenza state [74].

Through the use of recombinant DNA technology, human follicle-stimulating hormone (FSH) may now be produced in vitro. FSH is a very complicated heterodimeric protein that has been expressed in a particular cell line from eukaryotes. A success of recombinant DNA technology is the stimulation of follicular development in assisted reproductive therapy. Through the use of r-FSH, many patients are being treated. The most intriguing development was the effective recombination of r-FSH and luteinizing hormone (LH) to improve ovulation and pregnancy [94, 95].

 **4.2.5. 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 of gene therapy. These medications might 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 other genes. In A. rhizogenes vector systems, it is primarily carried with modified genes to improve properties for particular uses. The cultures developed into a useful tool for researching the biochemical characteristics and gene expression pattern of metabolic pathways. Turned cultures can clarify the intermediates and important enzymes involved in the manufacture of secondary metabolites [96, 97].

**4.2.6. Medically Important Compounds in Berries**

The rolC gene has contributed to an improvement in strawberries' nutritional value. This gene elevates the amount of sugar andantioxidant performance. Anthocyanins must be glycosylated using the enzymes transferase and glycosyl-transferase. For enhancing the component of interest by genetic modification, several nutrition-related genes for various strawberry components, including proanthocyanidin, l-ascorbate, flavonoid, polyphenols, and flavonoid, are relevant.

In the case of raspberries, the anthocyanin components are controlled by the bHLH and FRUITE4 genes, whereas ERubLRSQ072H02 is connected to flavonol. These genes have the ability to boost production and raise quality through precise transformation. All of the aforementioned substances have therapeutic benefits [98].

**4.3. Environment**

 Numerous environmental problems can be solved through genetic engineering. the distribution of genetically. The University of Tennessee and Oak Ridge National Laboratory were the first to use engineered microorganisms, such as the Pseudomonas fluorescens strain designated HK44, for bioremediation in the field [99, 100]. The altered strain had a transposon-based bioluminescence-producing lux gene linked within a promoter that led to increased naphthalene breakdown and a concomitant bioluminescent response [101, 102] as well as a naphthalene catabolic plasmid called pUTK21 [101]. In addition to acting as a reporter for naphthalene bioavailability and biodegradation, HK44 may also be employed as an online tool for in situ monitoring of bioremediation processes due to its bioluminescence signalling capabilities [102]. Fibre optics and photon counting modules can be used to identify the presence of a bioluminescent signal [101].

**4.4. Development of Plant Resistance and Phytoremediation.**

For the detection and absorption of pollutants in drinking water and other samples, genetic engineering has been widely applied. For instance, the insertion of the AtPHR1 gene into the garden plants Verbena, Torenia, and Petunia altered their capacity to absorb Pi. Effective phytoremediation in contaminated aquatic settings may be facilitated by AtPHR1 transgenic plants with improved Pi absorption capacity [103]. The binary vector pBinPLUS was modified to incorporate a segment of the AtPHR1 gene and an improved cauliflower mosaic. The 35S viral promoter. Agrobacterium tumefaciens was utilised to convert Petunia and Verbena [104] using the plasmid known as pSPB1898 [105]. Although AtPHR1 is successful in other plant species as Torenia, Petunia, and Verbena [103], overexpression of AtPHR1 may prevent posttranscriptional alteration of the endogenous AtPHR1 counterpart.

Plant metabolism identifies its value in using them to remove toxins from the environment. Some of the compounds are not easily digested or broken down.

TNT only undergoes a partial digestion process, during which time the nitrogen interacts with the oxygen to produce lethal superoxide. In order to solve this problem, monodehydroascorbate reductase gene is knocked out, increasing plant tolerance to TNT. Enhancing the plant responses to hazardous metals by knockout engineering and fine-tuning enzyme activity. A method of improving tolerance to heavy metals through enzymatic activity attenuation was discovered by phytochelatin synthase, an enzyme that produces heavy metal-binding peptides [106].

Recombinant DNA technology has demonstrated its efficacy in eliminating particles of arsenic, which are regarded as severe pollutants in soil. Arabidopsis expressed PvACR3, a crucial arsenite [As(III)] antiporter that exhibited increased arsenic tolerance. Unlike wild-type seeds, which often die in the presence of greater than usual concentrations of arsenate [As(V)], seeds of plants genetically altered with PvACR3 may germinate and flourish under these conditions. As reductase is an enzyme found in A. thaliana that reduces arsenic (As). Phytochelatins limit the migration of arsenic in phloem companion cells and root cells. The transporters for cadmium (Cd) absorption and retention are OsNramp5 and OsHMA3 [107].

Brassino-steroid (BR) has a role in controlling physiological and developmental processes in plants. Its action is initiated by activating a cascade of phosphorylation or dephosphorylation [108]. Biosorption, phytostabilization, hyperaccumulation, dendroremediation, biostimulation, mycoremediation, cyanoremediation, and genoremediation are recent biotechnology techniques for bioremediation that primarily depend on boosting or blocking certain gene activity. The difficulties in implementing the effective method, however, must be disregarded [109].

**4.3.2. Energy Applications**

 A number of microbes, particularly cyanobacteria, facilitate the creation of hydrogen, an ecofriendly energy source. By appropriately utilising the necessary enzymes, which are crucial to the synthesis of the product, the desired production is maintained. However, cutting-edge techniques like metabolic engineering, cell-free technology, combination culture, genetic engineering, and changes to the nutritional and growth conditions have all been successful in boosting the hydrogen generation in cyanobacteria and other biofuels [3, 4]. while this energy source is commercialised, the environment will remain clean, which is impossible while utilising current energy sources that release CO2 and other dangerous pollutants [113].

Additionally, it is possible to modify cyanobacteria such that they can convert CO2 into reduced fuel components. This will render carbon-based energy sources environmentally safe. This strategy has proved effective for a wide variety of common compounds, primarily energy carriers like short- and medium-chain alcohols [114].

Geobacter sulfurreducens' conductive biofilms are potential sources for bioelectronics, bioremediation, and renewable energy. The G. sulfurreducens genome's PilZ gene deletion rendered the biofilm more active than it would have been under wild-type conditions. For the strain where the gene GSU1240 was deleted, CL-1ln is given. Along with pili and exopolysaccharide synthesis, biofilm generation was improved. When biofilms were cultivated with electrode, the electron acceptor CL-1 generated biofilms that were six times more conductive than wild-type biofilms. This high fold conductivity reduced the potential losses in microbial fuel cells by lowering the formal potential and reducing the charge transfer resistance at the biofilmanode surface. Lower losses led to an increase in potential energy [115].

**5. Current Obstacles and Prospects**

The predominant usage of microbial cells in manufacturing. Understanding recombinant pharmaceuticals shows that a number of barriers prevent them from manufacturing functional proteins effectively, but they are overcome with changes to the biological systems. Posttranslational changes, activated cell stress responses, instability of proteolytic activities, limited solubility, and resistance to the expression of additional genes are typical challenges that must be overcome. Human genetic mutations lead to shortages in the synthesis 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 operate in regulated ways to technically create the needed molecules through cost-effective methods [41, 116].

By enabling the investigation and modification of yeast genes both in the test tube and in living yeast cells, recombinant DNA research holds considerable potential for improving our understanding of yeast biology. Most notably, it is now feasible to get back to yeast through DNA transformation and gene cloning utilising a number of specially created selectable marker systems. Because of these developments in technology, it is now possible to manipulate and analyse yeast genetic material at the molecular level as well as the traditional genetic level. Recombinant DNA technology has been most successful in solving biological issues whose core difficulty is the structure and organisation of individual genes [117, 118].

Recombinant DNA technology is now undergoing rapid growth, which has drastically altered study areas and offered new, exciting avenues for investigating biosynthetic processes through genetic modification. Actinomycetes are utilised in the creation of pharmaceuticals, such as certain helpful chemicals in the health sciences and the modification of biosynthetic pathways for the synthesis of innovative medications.These have been heavily taken into account when building recombinant medications since they produce a significant portion of biosynthetic chemicals. Their compounds have demonstrated high level effectiveness against numerous types of bacteria and other harmful germs, making them more relevant in clinical studies. Additionally, these substances have demonstrated immunosuppressive and anticancer properties [119].

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 illnesses. The DNA supplied during this procedure contains genes that produce harmful proteins. In clinical trials, human gene therapy is mostly used to treat cancer. High transfection effectiveness in relation to creating gene delivery systems has been the major focus of research. It is still being researched whether transfection might be used for cancer gene therapy with minimum side effects, such as in cases of brain, breast, lung, and prostate cancer. Additionally, gene therapy is being considered for renal transplantation, Gaucher disease, haemophilia, Alport syndrome, renal fibrosis, and certain other illnesses [120].

**6. Recommendations**

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 cleanup (phytoremediation and microbial remediation) and improved plant resistance to many harmful causes (drought, pests, and salt).

It made substantial advancements in plants, microbes, and people 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.

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

The methodologies for genetic engineering face a number of challenges that have to be resolved by more targeted gene augmentation in accordance with the organism's genome. Single-stranded DNA would be incorporated into the bacterial chromosome by a RecA-dependent mechanism. 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. There are various issues with the creation of products and plants that have been genetically modified.

For instance, it is clear that plants that have been genetically modified may mate with wild plants, introducing their "engineered" DNA into the ecosystem and threatening our biodiversity. Additionally, there are worries that genetic engineering might have harmful effects on health. Therefore, more in-depth study is needed in this area to address these problems and the concerns of the general public.

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