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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. Nonetheless, 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. Genetically modified plants, particularly in agriculture, have increased resistance to hazardous agents, improved product yield, and demonstrated increased adaptation for better survival. Recombinant medications are also currently being used with confidence and gaining commercial authorization quickly. Recombinant DNA technology, gene therapy, and genetic modification techniques are also frequently utilised in bioremediation and the treatment of serious illnesses. This review article primarily focuses on the significance of recombinant DNA technology and its potential applications in daily life because of the technology's great advancement and a wide variety of applications.

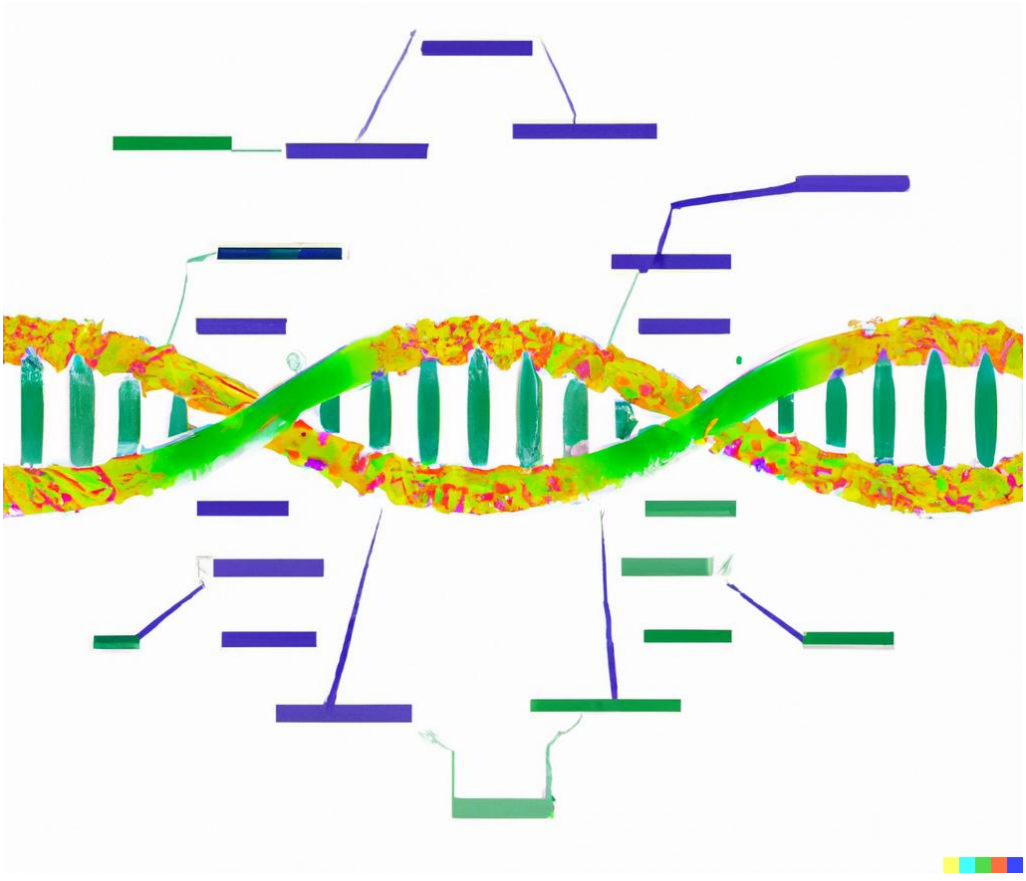
KEYWORDS: DNA technology, desirable features, gene therapy, pharmaceuticals.

INTRODUCTION

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 food needs are growing quickly as a result of the world population's rapid growth. People demand affordable, wholesome food. There are many

deaths worldwide due to various human health conditions. According to http://GlobalIssues.org/, non-communicable and communicable diseases like cancer, diabetes, AIDS/HIV, tuberculosis, malaria, and several others cause the deaths of almost 36 million people worldwide every 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. Industrial waste is permitted to directly into the water supply, which harms aquatic life and indirectly on people. Environmental contamination has increased due to the rapid expansion of industrialisation. Thus, these problems must be solved using contemporary technologies. 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 pollutant degradation through conventional techniques, respectively. For instance, genetic engineering only distributes a tiny block of targeted genes to the recipient, as opposed to conventional breeding, which transfers a huge number of both specified and nonspecific genes to the recipient. diverse methods, including biolistic and Agrobacterium-mediated transformation, on the target.[1] Homologous recombination-dependent gene targeting or nuclease-mediated site-specific genome editing are the two methods used to modify plant genomes. It is also possible to apply oligonucleotide-directed mutagenesis and site-specific genome integration mediated by recombinases.[2] By creating novel vaccinations and medications, recombinant DNA technology is significantly enhancing health conditions. By creating new therapy modalities, monitoring tools, and diagnostic kits, treatment techniques are also improved. 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 like turning wastes into biofuels and bioethanol[4–7], cleaning up oil spills, carbon, and other toxic wastes, and identifying toxins like arsenic in drinking water. The bacteria that have been genetically altered are also useful for bioremediation and biomining. Recombinant DNA technology's introduction transformed biological research and sparked several significant changes. By changing microbes, animals, and plants to generate medicinally valuable compounds, it provided the new potential for innovators to produce a wide range of therapeutic goods with immediate effect in medical genetics and biomedicine.[8,9] 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 anaemia, AIDS, cancer (Kaposi's sarcoma, leukaemia, colorectal, kidney, and ovarian cancers), hereditary disorders (cystic fibrosis, familial Site-specific integration and carefully regulated gene expression are essential advanced methods[10] because plants have a multigene transfer. Plant biotechnology faces several significant hurdles, including the precise control of transgenic expression, the efficiency of endogenous genes in novel environments, and the transcriptional regulation of these genes.[11] Several things endanger human life, including food shortages that create 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.



Principal of genetic engineering

The fundamentals of recombinant DNA technology are straightforward. shows the cleavage of chromosomal DNA from one organism and plasmid DNA from Escherichia coli by a restriction enzyme, followed by their mixing and ligation by DNA ligase. Escherichia coli is subsequently given the recombinant DNA molecule so that it can reproduce foreign chromosomal DNA. The genes in the donor segment are said to be cloned, and the DNA molecule that carries them is known as the vector. It is connected to a plasmid that contains an origin of replication. Restriction endonucleases are used to insert the gene into the DNA carrier molecule. These enzymes are found in bacteria and work naturally to block invasive DNA molecules. Specifically, Escherichia coli, Haemophilus influenzae, Bacillus globigii, Providencia stuarti, and Serratia marcescens provide the Eco RI, Hind III, Bgl H, Pst I, and Sma I restriction endonucleases. These enzymes cleave DNA molecules in palindromic order at certain base sequences. Type I restriction endonucleases can only cut within the recognition sites of a given sequence after recognising it. Genetic engineering uses type II restriction endonucleases. Two single-strand breaks are created by these enzymes, one in each strand. These breaks may create complementary, cohesive, or sticky ends that can overlap for two to four bases at the symmetry centre (flush or blunt ends) or the same relative point in each strand. DNA ligase, a sealing or ligating enzyme, may reunite DNA sliced into fragments. The phase T4-encoded ligase is the most widely utilised one that is commercially accessible.[11,12,13,14]

Application of genetic engineering in medicine

1. Gene Therapy: Gene therapy uses genetic engineering to introduce or replace dysfunctional genes into the body to cure hereditary illnesses. A new gene is inserted into a patient's cells during gene therapy to replace a damaged one. Cancer and conditions like cystic fibrosis have both been treated using this method
2. Manufacture of Medicinal Proteins: Therapeutic proteins like insulin, growth hormone, and clotting factors, which are used to treat a variety of disorders, can be produced by genetic engineering.
3. Vaccine Development: By introducing genes that code for particular antigens into a virus or bacteria, genetic engineering can be used to create novel vaccinations. This method has been used to create vaccinations against illnesses like the human papillomavirus and hepatitis B. (HPV).

4. Pharmacogenomics: Genetic engineering can be used to investigate how a person's genes influence how they react to medications. With this data, personalised medicine can be created, and patients who are more likely to have negative drug reactions can be identified.
5. Genome Editing: With genetic engineering, disease-causing genetic mutations can be fixed in an individual's genome. CRISPR/Cas9 genome editing technologies have shown promise in the treatment of illnesses like sickle cell anaemia and Huntington's disease.[15,16]

Gene Therapy

In the course of gene therapy, a patient's cells are given a new gene to replace a damaged one. This can aid in the treatment of hereditary diseases like haemophilia, sickle cell disease, and cystic fibrosis. Delivering a functional copy of a damaged gene to the patient's cells through gene therapy can treat the disease's underlying cause. Somatic cell gene therapy and germline gene therapy are the two main varieties of gene therapy.

A functioning gene is inserted into a patient's bodily cells as part of somatic cell gene therapy. This kind of gene therapy is used to cure conditions like cystic fibrosis and haemophilia that are brought on by abnormalities in just one gene. A viral vector, a modified virus that can insert the gene into the patient's cells, is used to deliver the functional gene to the patient's cells.

A functioning gene is inserted into a patient's reproductive cells as part of germline gene therapy. This particular form of gene therapy is particularly contentious since it has the potential to permanently alter the patient's genetic composition and pass those changes on to subsequent generations. Although it has been used on animals, germline gene therapy is not currently licenced for use in humans.

Gene therapy has been used successfully in several human applications. For instance, gene therapy was used to successfully cure a young girl with severe combined immunodeficiency (SCID) in 1990. To restore the patient's immune system to normal operation, the treatment entailed inserting a functional copy of the ADA gene into the patient's bone marrow cells. However, there are also potentially harmful side effects of gene therapy, such as an immunological reaction to the viral vector or the incorrect placement of the functional gene in

the patient's genome. Gene therapy is therefore still an experimental procedure and is only applied in a small number of circumstances.[17,18]

Production of Therapeutic Proteins

A gene that codes for a particular protein must be inserted into a host cell, such as yeast or bacteria, to produce therapeutic proteins using genetic engineering. The protein is then produced by the host cell and can be medicated after being purified. This method has completely changed how therapeutic proteins are produced and increased access to numerous life-saving medications.

The creation of insulin to treat diabetes is one instance of how therapeutic proteins are produced using genetic engineering. Diabetes affects the body's ability to create adequate insulin, a hormone that controls blood sugar levels. Diabetics cannot make enough insulin on their own. Before the development of genetic engineering, animals with a pancreas, such as pigs and cows, were used to produce insulin. This procedure took a long time and produced a meagre amount of insulin, which was frequently of variable quality.

Genetic engineering was used by researchers in the 1980s to create a method of producing human insulin. Scientists gave bacteria or yeast, which can make enormous amounts of the protein, the gene that codes for human insulin. The resulting insulin can be manufactured more quickly and cheaply than insulin derived from animals, and it is identical to human insulin.

Growth hormones, clotting factors, and erythropoietin are just a few of the therapeutic proteins that have been produced by genetic engineering. These proteins are used to treat several illnesses, including anaemia, haemophilia, and growth hormone insufficiency.

The ability to generate therapeutic proteins in vast numbers in a reliable and regulated manner is one benefit of employing genetic engineering to do so. This has decreased the cost of treatment for many people and increased access to numerous life-saving medications. Genetic engineering-based therapeutic protein synthesis is not without its difficulties, though, including the possibility of protein contamination and the requirement for meticulous quality control to guarantee the end product's efficacy and safety.[19,20]

Vaccine Development

The production of vaccines, a vital tool in halting the spread of infectious illnesses, is greatly aided by genetic engineering. The immune system is normally stimulated by weaker or inactivated versions of the pathogen, but genetic engineering has made it possible to create newer, more potent vaccination kinds.

Using recombinant DNA technology to create protein subunit vaccines is one instance of genetic engineering being used in the production of vaccinations. These vaccines are created by introducing a pathogen's gene that codes for a particular protein into a host cell, like a yeast or bacterial one. The protein is subsequently produced in huge quantities by the host cell and can be isolated for use as a vaccine. Vaccines against illnesses like hepatitis B, the human papillomavirus (HPV), and COVID-19 have been created using this method.

The use of viral vector vaccines is another illustration of genetic engineering in the creation of vaccinations. These vaccines carry genetic material from the disease into the body using a modified virus as a vector. Once a pathogen has entered the body, its genetic information tells cells to make a specific protein that causes an immunological reaction. For the development of vaccinations against diseases like Ebola and COVID-19, this method was employed.

DNA vaccines, which require injecting a small fragment of DNA that codes for a particular pathogen protein into the body, have also been made possible by genetic engineering. The DNA tells cells to create the protein once it has entered the body, causing an immunological reaction. For viruses like the COVID-19 virus and the Zika virus, DNA vaccines have been created.

The ability to quickly create new vaccines in response to newly developing infectious illnesses is one benefit of using genetic engineering in vaccine production. For instance, genetic engineering was crucial in the rapid creation of multiple novel vaccinations during the COVID-19 epidemic. To assure the safety and efficacy of the finished product, thorough testing and quality control are required, which are some of the difficulties related to the use of genetic engineering in vaccine development.[21,22,23]

Pharmacogenomics

Pharmacogenomics is the study of how a person's genetic makeup influences how they react to medications. Increasing drug efficacy and lowering the likelihood of negative drug

reactions, entails using genetic information to customise pharmacological therapy to a person's unique genetic profile.

Cancer treatment is one instance of how pharmacogenomics is used in medicine. Other individuals may not respond to the therapy at all, and many cancer medications have terrible side effects that can be fatal. Doctors can determine which patients are most likely to benefit from a specific treatment by looking at their genetic makeup, and they can then adjust the amount to reduce the likelihood of side effects. This strategy has been used to increase the potency of medications like the breast cancer treatment medicine tamoxifen.

Cardiovascular disease treatment is another application of pharmacogenomics. When a person has a specific genetic variation, the blood clot-preventing medication clopidogrel is less effective. Doctors can identify patients who are less likely to respond to clopidogrel and recommend an alternative medication by looking at the genetic profile of the patient.

Pharmacogenomics has also been used to pinpoint genetic elements that influence the toxicity of medications. For instance, a severe allergic reaction to the HIV medication abacavir has been linked to the genetic mutation HLA-B*5701. Doctors can prevent this potentially fatal side effect by screening individuals for this genetic mutation before giving abacavir.

The ability for more individualised and accurate medication therapy, which can result in better treatment outcomes and a lower risk of adverse drug reactions, is one benefit of employing pharmacogenomics in medicine. However, using pharmacogenomics also comes with significant difficulties, such as the requirement for extensive genetic testing and the interpretation of complicated genetic data.[24,25]

Genome Editing

A potent technology in genetic engineering called genome editing enables precise changes in an organism's DNA. By making precise cuts in the DNA sequence at a given site, this approach uses designed nucleases like CRISPR-Cas9 to deliver the desired genetic alteration.

Many medical uses for genome editing exist. The treatment of genetic illnesses is one of the most promising. Instead of only treating the disease's symptoms, it may be feasible to deliver a permanent cure by employing genome editing to fix the underlying genetic abnormality that causes a disease. For instance, CRISPR-Cas9 genome editing was used to treat the first patient with sickle cell disease in the US in 2019, with encouraging outcomes.

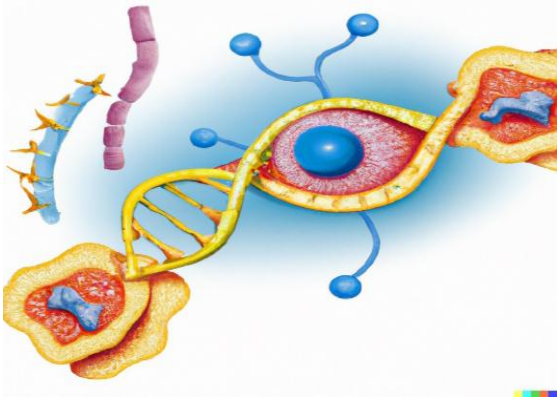
Animal models of human diseases can also be created via genome editing, which can aid researchers in understanding the pathophysiology of the disease and testing new treatments. For instance, scientists have utilised genome editing to develop animal models of Huntington's disease and cystic fibrosis.

In the area of regenerative medicine, genome editing has the potential to be used in more medical procedures. It is feasible to instruct stem cells to differentiate into particular cell types that can be employed to repair or replace damaged or ill tissue by employing genome editing to change the DNA of stem cells. For instance, scientists have utilised genome editing to transform human stem cells into insulin-producing cells to treat diabetes.

Yet, genome editing also raises ethical and security issues, particularly when it comes to changing the human germline (i.e., the DNA that is passed on to future generations). The possibility of producing so-called "designer babies" with desired qualities raises worries, as does the possibility of unforeseen off-target impacts or undesirable genetic modifications. As a result, the use of genome editing in human research is subject to stringent laws.[26,27,28,29]

Human insulin produced through recombinant DNA technology

Synthetic insulin or recombinant DNA insulin are other names for human insulin made using recombinant DNA technology. This kind of insulin is made by introducing the human insulin gene into a bacterial or yeast host cell, which results in the production of insulin that is the same as that made by the human pancreas in its natural state.



Insulin was previously extracted from the pancreases of slaughtered animals, particularly pigs and cows, before the invention of recombinant DNA technology. Unfortunately, this method had several disadvantages, including the possibility of allergic reactions to animal proteins and the danger of spreading diseases from animals to people.

Since its introduction in the 1970s, recombinant DNA insulin has quickly replaced traditional insulin manufacturing due to its efficiency and safety. There are various steps in the production process

1. **Extraction of the Human Insulin Gene:** Using restriction enzymes, which make particular site-specific DNA cuts, the Human Insulin Gene is extracted from Human DNA.
2. **Insertion of the Gene into a Host Cell:** Using a plasmid, a tiny, circular bit of DNA that can replicate independently of the DNA of the host cell, the isolated gene is introduced into a bacterial or yeast host cell.
3. **Insulin Gene Expression:** After the gene is introduced into the host cell, it is translated from mRNA into insulin protein. Then, using a variety of procedures, the insulin protein is isolated from the host cell.

Recombinant DNA insulin has several advantages over animal insulin, including

1. **Purity:** Recombinant DNA insulin is pure and free of any potentially allergenic animal proteins.
2. **Consistency:** As recombinant DNA insulin is made in a controlled environment, its potency and purity are guaranteed to be the same in every batch.
3. **Safety:** There is no chance that recombinant DNA insulin may expose humans to animal-to-human disease transmission.
4. **Availability:** The most widely utilised type of insulin worldwide is recombinant DNA insulin, which is abundantly available.
5. The manufacture of insulin has been transformed by recombinant DNA technology, and insulin therapy for diabetics is now much safer and more effective.[30,31]

RESULT

Recombinant DNA technology has transformed several disciplines of research, including health, agriculture, and biotechnology. Scientists can edit and modify DNA to generate new species with desired properties or to produce useful items on a massive scale via genetic engineering. Recombinant DNA technology has enormous promise, with applications ranging from the discovery of life-saving medications to the development of genetically modified crops resistant to pests or environmental conditions. However, in addition to its potential benefits, the use of recombinant DNA technology raises ethical, social, and environmental concerns. Despite the problems, recombinant DNA technology continues to play an important

role in creating the future of science, and its impact on society and the environment will be a topic of discussion and investigation in the coming years.

CONCLUSIONS

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 as 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 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.

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 products since the body rejects the alteration made to a gene. However, 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 several diseases that cannot be treated under normal circumstances, yet the immune responses make it difficult to get satisfactory outcomes.

The methodologies for genetic engineering face several challenges that had to be overcome by more targeted gene augmentation following the organism's DNA. A RecA-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, plants that have been genetically modified can breed with wild plants, introducing their "designed" DNA into the ecosystem and threatening our biodiversity. However, there are worries that genetic engineering could have harmful effects on health. Therefore, a more in-depth research is needed in this area to address these problems and the concerns of the general public.

ACKNOWLEDGEMENT

Indeed, advances in recombinant DNA technology have resulted in substantial advances in a variety of disciplines, including agriculture, health, gene therapy, and bioremediation. These breakthroughs have the potential to solve pressing concerns such as food security, human health, genetic diseases, and environmental sustainability. It is critical to recognise the enormous contributions of recombinant DNA technology in these fields while simultaneously keeping in mind the ethical, social, and environmental implications of its use. Implementing recombinant DNA technology responsibly and sustainably can lead to greater improvements and benefits for society as a whole.

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