RECOMBINANT DNA

Abstract

Every country wants to enhance or improve the human life by improving health conditions, food quality, crop production, environmental conditions and many others aspects that affect human life. Science has developed several techniques for betterment of people; recombinant DNA technology is one of them. This paper describes the definition, requirements, methods, and applications of recombinant DNA technology. There are three methods to perform this technique, transformation, non-bacterial transformation and phage transformation. This technology has various applications in the field of health, clinical pharmacy, hormone production, food, agriculture, gene therapy, environmental science and pollution research.

Keywords: DNA technology, Restriction Endonucleases, RDT.

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I. INTRODUCTION

The idea of recombinant DNA (rDNA) technology has developed to enhance quality of human lives. In general, the human life is exaggerated mainly by three main priorities; food insufficiency of the food, health issues and environmental conditions. Numerous human health related problems are considered the most critical factor associated with deterioration of human life quality. Several diseases such as HIV, malaria, T.B. and dengue are threatening human life based on the global statistics from [Khan et al., 2016].

In spite of widespread hard work, presently the global food production is much lower than human necessities, and health services are even lower standard in the third-global countries. The increasing industrialization has resulted into the environmental pollution and industries release waste materials into the water bodies and pollute the that influences the life of aquatic organisms and also to, human-beings. Therefore, there is need to develop advanced technologies.

Recombinant DNA technology (RDT) is a great progress in the field of research, which helps in maintaining public health by producing new vaccines and pharmaceutical products. The therapeutic scheme is also enhanced by producing detection kits, monitoring devices, and other treatment techniques. Genetic engineering also applied environmental remediation by the conversion of wastes into bioethanol and biofuels [Khattak etal 2014 and Ullah et al., 2015] cleaning of oil spills, carbon, and other poisonous wastes, and detection of arsenic and other impurities in drinking water. The genetically engineered microorganisms are also efficiently used in bioremediation and biomining [Khan et al., 2016].

In this technique, DNA is isolated from sevesral sources, and then fragmented by restriction endonuclases followed by the joining of DNA fragments by enzyme DNA ligase to stick the preferred gene in a suitable vector. The vector with desired gene is inserted into the host cell that is allowed to grow for many generations and the resulting DNA is called rDNA (Figure 1) [Venter et al., 2007]. The first rDNA was producrd by Paul Berg, Herbert Boyer, Annie Chang, and Stanley Cohen in Stanford University and University of California San Francisco in 1973 [Khan et al., 2016]. The organism’s genome is manipulated either by inserting one or many new genes and regulatory elements or by reducing or blocking the expression of endogenous genes by recombination of elements and genes [Bazan-Peregrino et al., 2013]. A fast sheme is presented by RDT to examine the genetic expression of mutants, which were inserted into eukaryote genes via cloned insulin genes introduction inside a fragment of simian virus [Shinde et al., 2018].

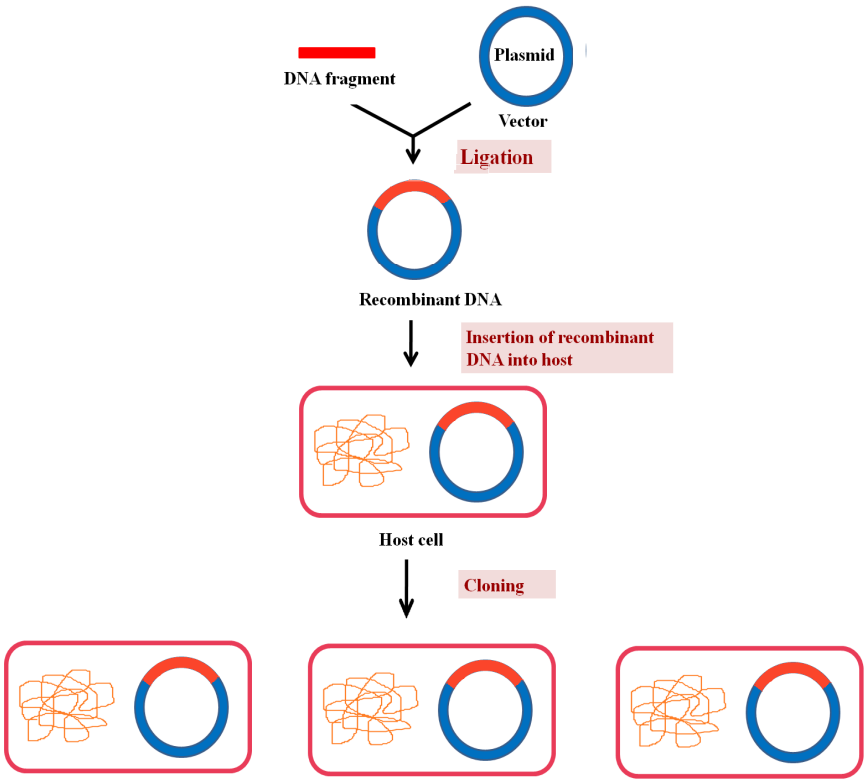

Figure 1: Representation of Recombinant DNA Formation and Gene Cloning

II. REQUIREMENTS FOR THE CONSTRUCTION OF RDNA

1. **Restriction Endonucleases:** Restriction endonucleases are deoxyribonucleases also knownn as molecular scissors which cut double-stranded DNA into smaller parts. Restriction endonucleases are classified into two groups depending upon the location of the cleavage site virtual to the identification sequence. The cleavage sites of Class I restriction endonucleases are placed in outer sideof the identification sequence and produce random sized fragments. Class II restriction endonucleases chop the DNA at sites present mostly within the identification sequence [Al-Shami et al., 2021].

   The restriction endonuclease recognizes the specific sequences called palindromic sequences at definite points and cleave the DNA at that point refers to restriction site rand lead to formation of sticky ends. The desired gene and vector are cut through the similar restriction endonuclease to produce complementary sticky ends so that ligases can easily connect the gene with vector [Shinde et al., 2018].

2. **Cloning Vectors:** Vectors are the crucial vehicle that hold the desired gene into the host cell, thus they act as very essential tool for RDT. Plasmids and bacteriophages are most widely used vectors in RDT due to their higher carrying capacity and copy number.

3. **Host Organism:** The organism into which rDNA is inserted is known host. The host cell must have compatiblity tfor rDNA. They are the eventual tool of RDT as they receive the rDNA. Several methods can be used to insert rDNA into the host including

microinjection, biolistic / gene gum, alternate cooling and heating, use of calcium ions, etc.

III. METHODS FOR THE PRODUCTION OF RDNA

1. **Transformation:** Transformation involves the selection of DNA and its insertion into appropriate vector. Then, the DNA fragment is cut into smaller parts by restriction endonucleases and joined with DNA ligase. This DNA segment has a distinctive marker that permits for future recognization of the recombinant product. The vector is introduced into a host cell and this is called transformation. Generally, *Escherichia coli* (*E. coli*) bacterium is taken as host cell. The DNA fragment should specialize to distinguish between transformed hosts from untransformed ones [Al-Shami et al., 2021].

2. **Non-Bacterial Transformation:** This process is same as the above transformation but it does not involve the use of bacteria as a vector. In this method, microinjection techniques are used for the direct insertion of DNA fragment into the nucleus of the host cell as shown in Figure 2 [Al-Shami et al., 2021].

3. **Phage Introduction:** The phage introduction is also known as transfection that is nearly alike to transformation but here, the phage (a virus which attacks bacteria) is used in place of bacteria. The phage MI3 or lambda is mostly used in this method is a lambda or MI3 phages to hold the rDNA (Figure 3) [Shinde et al., 2018].

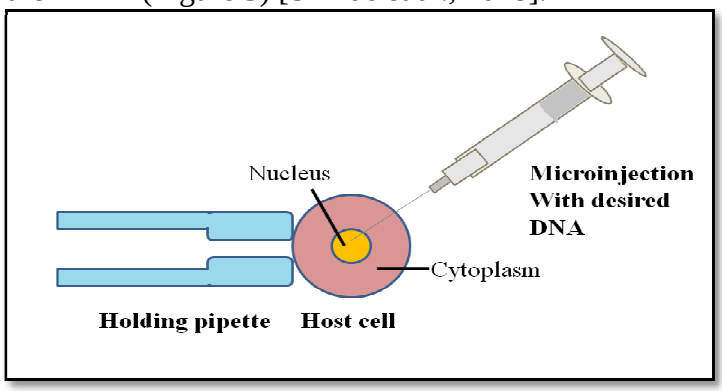

Figure 2: Microinjection Technique for Insertion of Recombinant DNA into the Host Cell

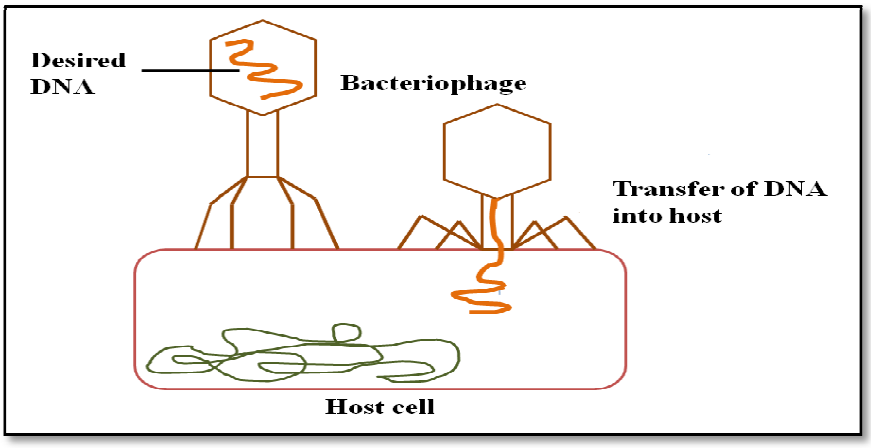

Figure 3: Phage Introduction Method for Transfer of Desired DNA into the Host Cell

4. **RDT Based Researches:** RDT shows rapid progress in the field of research and scientists have developed the engineered products and devices, which provide benefits in various fields suuch as health, agriculture, and environment. Studies show that Lispro (Humalog) is recombinant insulin which is very efficient and shows rapid action as compared to normal human insulin. Likewise, Epoetin alfa is a novel and well-identified recombinant protein that can be efficiently applied for treatment of anemia [Masson et al., 2003]. Recombinant hGH is reported to show enormous enhancement in treatment of children who unabile to produce essential quantity of hGH. FDA approved clinical testing in December 1997 for genetically modified cytokine myeloid progenitor inhibitory factor-1 (MPIF-1) which provided gratitude to this technique [Patra et al., 2000 and Macallan et al., 2008].

   Recently, clustered regularly interspaced short palindromic repeats (CRISPR) are developed with the help of RDT, has proved to solve various issues in different species. CRISPR has potential role in addition, deletion, activation, and suppression of genes in human cells, rats, mice, zebrafish, *Drosophila*, yeast, bacteria, nematodes, and plants. Mouse models are used for analysing human diseases with the help of CRISPR, which cause the rapid study of individual genes and easy analysis of gene intearactions by varying Thus the study of individual genes study becomes much faster and the genes interactions studies become easy by changing several genes in cells [Pennisi., 2013]. The CRISPR of *H. hispanica* genome is able to adapt to nonlytic viruses very effectively. The intrusive Cas3 nucleases and other Cas proteins are encoded by linked Cas operon. The strain engineering is necessary with priming CRISPR for priming the generation of crRNAs and new spacers approval. CRISPR-cas method has to incorporate new spacers into its locus for the production of adaptive immunity production [Wang et al., 2016].

   It is studied that transcription activator-like effector nucleases (TALENs) and Zinc-finger nucleases (ZFNs) are chimeric proteins consists of adaptable, sequence-specific DNA-binding modules connected to a nonspecific DNA cleavage site. TALENs and ZENs have promissing therapeutic applications [Gaj et al. 2013 and Blackburn et al., 2013]. Recombinant protein fibroblast growth factor (FGF-1) has also developed that is injected into the human myocardial tissues (biologic bypass) and helps in triggering the formation of new blood vessels in myocardial tissues and lead to higher blood supply in the heart. One product Apligraf that is approved by FDA acts as a recombinant skin replacer, used for curing leg ulcer treatment and another product DermaGraft effectively treat the diabetic ulcers [Yancopoulos et al., 2000 and Koike et al., 2004]. Insulin is successfully produced from *E. coli* by RDT and presently various animals especially cattle and pigs have been preferred as a source of insulin production, which nonetheless, elicit immune responses. The recombinant human insulin is similar to human porcine insulin and relatively occasionally generates immune responses. Moreover, it is more reasonable and can assure medical requirements more eagerly. Human growth hormone was the first protein that is expressed in tobacco plants [Ferrer-Miralles et al., 2009 and Kamionkaet al., 2011]. In addition to insulin, various RDT dependent new drugs have developed and numerous protein generation techniques have also produced. Various engineered microbial strains have produced to accomplish the formulation of drugs [Eriksson, 2016 and Urban et al., 2015].

5. **Applications:** RDT has various uses biomedical applications as well as in food processing, agriculture, production of trangenic plants and animals, enironmental field and energy, which are represented in Figure 4.

6. **Human Health:** RDT plays important role in cure of diseases and maintaining the public health. Gene therapy and genetic engineering are the techniques used as therapies for treatment of various diseases. The production of human insulin is the prominent example RDT based therapy. RDT is also used in production of antibodies their derived products. Gene therapy is applied for the cure of cardiovascular diseases by gene as it provides protection to myocardium, leads to blood vessel formation, restenosis prevention following angioplasty, repair and regeneration, avoidance of bypass graft rejection, and management of risk factor. Gene therapy also tergets various types of cancers including skin, lung, gastrointestinal, gynecological, neurological, urological, pediatric, gastrointestinal tumors, and hematological malignancies. One study shows that p53 (a tumor suppressor gene) is genetically transferred and its gene transfer is joined with chemotherapy or radiotherapy [Ginn et al., 2013 and Lam et al., 2013]. RDT show pharamceutical importance as it allows the researchers to examine the drug metabolisms to assess the efficacy and maintenance time of drugs in the human body. RDT facilitates the development of hormones involving insulin, somatotropin, somatostatin, and pendorphin. It also helps in production of vaccines, which shows better effects against many severe disease causing protozoa, bacteria, and viruses or protozoa [Shinde et al., 2018].

IV. FOOD INDUSTRIES

The RDT has employed in production of new enzymes for the improvement of the food processing and conservation. Smith, 1990 applied this technique on tomato by inserting truncated “sense” and Sheehy et al., 1988 inserted polygalacturonase gene or the “antisense”, resulted in pectin breaking during ripening, which enhanced the taste, aroma, and texture of tomato [Smith, 1990 and Sheehy et al., 1988]. Agrobacterium rhizogenes play role in transformation by raising the production of essential oil and considerably altering the allocation of monoterpene alcohols [Pellegrineschi et al., 1994]. Hence, ‘sense’ and ‘anti-sense’ methods are applied for enhancing the food quality The “antisense” procedure prohibit either the 1-amino- cyclopropane-1-carboxylate synthase or ACC oxidase that converts ACC to ethylene [Oeller et al., 1991].

The nutritional value of a food can be amplified by RDT. In transgenic oilseed plants, 12:0-acyl carrier protein thioesterase is expressed, which lead the biosynthesis of fatty acid in a support of medium-chain fatty acid [Voelker et al., 1992]. From Transgenic potatoes can provide accurately processed serum albumin in human [Sijmons et al., 1990]. “Bioreactors” are utilized for the generation of proteins, which have industrial and pharmacological importance [Dale and Belanger, 1993].

V. AGRICULTURE

Genetically modified crops provide resistance to plants, enhanced herbicides, resistance to plants, etc. [Paoletti et al., 1996]. CGN- 89546-2 was the first genetically modified crop produced in 1994, which was named as‘FlaourSanr Tomato [Bruening et al.,

2000]. It possessed properties such as extended flavor and late ripening etc. In the US 93% of soybeans and 88% of corn are genetically modified and many of them are unlabelled into processed foods [Winerip, 2003]. Various genetically customized are B.T. cotton, B.T. Brinjal, B.T. Maize etc. *Bacillus thuringenesis* is a soil dwelling bacterium used in genetically modified crops. RDT also have pharmaceutical value as U.S. Food and Drug Administration (FDA) have given the approval of many recombinant drugs in year 1997 as compared to previous years, which involves aids, anemia, aids, hereditary disorders, and cancer [Liu et al., 2013]. Numerous crops have been genetically altered to tolerate the drought or unfavourable environment by using RDT [Al-Shami et al., 2021].

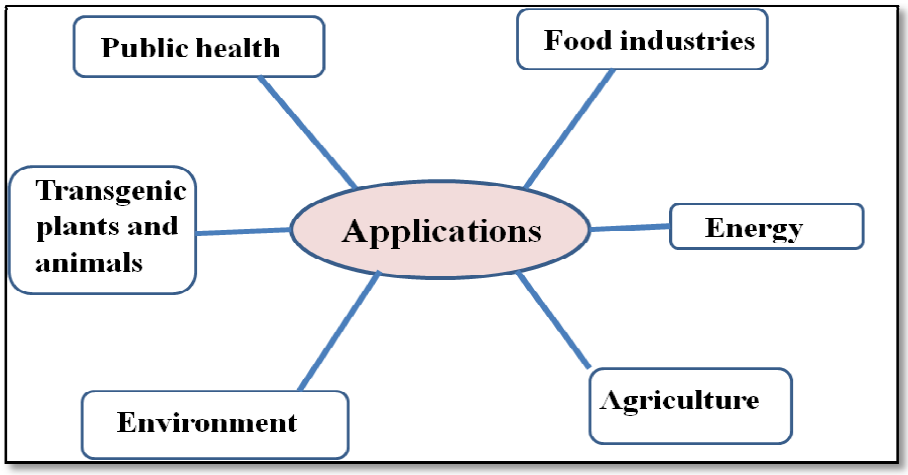

Figure 4: Applications of Recombinant DNA Technology

1. **Production of Transgenic Plants**: Genetic engineering results into the production of transgenic plants also known as genetically modified plants, which posseses qualities like resistance to insects, herbicides, and viruses or having expression of male sterility [Shinde et al., 2018].

2. **Production of Transgenic Animals:** Transgenic animals can also be produced by RDT, which enables the animal breeders to raise the collection and speed of choosy breeding in case of animals. It is helpful for the generation of superior farm animals, thus provide more marketable advantages [Shinde et al., 2018]

3. **Environmental Science and Pollution:** RDT has helped effectively to solve various environmental problems and has also decreased the related pollution problems by producing useful recombinant agents such as genetically altered microorganisms and bioplastic. The researchers of Oak Ridge National Laboratory and University of Tennessee has worked through collaboration and produced genetically modified strain *Pseudomonas fluorescens* (HK44) for the first time [Ripp et al., 2022 and Sayler et al., 1999].

4. **Energy Applications:** Various microorganisms, especifically cyanobacteria lead to production of hydrogen, which is ecofriendly source of energy. The exact generation is sustained by using the vital enzymes appropriately as these enzymes play a major role in the formation of products. However, modern techniques such as genetic engineering, variations in nutrient and growth media, united culture, cell-free techniques, and

metabolic trades [Ullah et al., 2016 and Ullah et al., 2016] have shown encouraging results to amplify the hydrogen generation in cyanobacteria and other biofuels. The commercial use of this energy source can clean the environment, so that it overcomes the limitations of traditional energy sources liberating CO_2 and other harmful chemicals [Tiwari et al., 2012].

The conductive *Geobacter sulfurreducens* biofilms are latent sources of renewable energy, bioelectronics, and bioremediation. The proteins in *G. sulfurreducens* genome encode by PilZ genes can be deleted that causes the enhanced activation of the biofilm in comparison to wild-type. The deletion of gene GSU1240 was made in CL-1ln is specific for the strain. Biofilm generation was improved along with the pili generation and exopolysaccharide. The electron acceptor CL-1 generated biofilms, which showed six-fold more conductivity than wild-type biofilms on growing with electrode. This high fold conductivity reduced the probable losses in microbial fuel cells, declining the charge transfer resistance at biofilm-anode surface and reducing the formal potential. The reduced losses lead to higher potential energy [Leang et al., 2013].

VI. CURRENT CHALLENGES AND FUTURE PROSPECTS

As microbes are most commonly used in the generation of recombinant pharmaceuticals, various difficulties come across their path confining them from generating functional proteins proficiently but these are dealt with modification in cellular systems. Genetic mutations humans result deficit in proteins generation that can be changed/treated by inclusion of external genes to seal the gaps and attain the normal intensity. The utilization of *Escherichia coli* in RDT acts as a biological scaffold that permits the workers to work in restricted manners to precisely generate the essential molecules through inexpensive processes [Vajo et al., 2001].

RDT can be employed in yeast biology by studying the manipulation of yeast genes, in test tube as well in yeast cells. Most significantly, it is now achievable to revisit to yeast by transformation with DNA and cloning of genes with the help several selectable marker systems designed for this function. RDT can cure and prevent the genetic disorders. The production of DNA vaccines is a new advancement that boosts the immunity against numerous diseases. This process involves the DNA delivery containing genes, which code for disease causing proteins. Human gene therapy is typically designed to cure cancer in medical trials. Gaucher disease, Alport syndrome, hemophilia, renal transplantation, renal fibrosis, and few other diseases are in pipeline for gene therapy [Misra et al., 2010].

VII. CONCLUSION

RDT is a significant advancement in science that enhances human life as well as plants and microbes and provides various health benefits, treatment of genetic disorders and plant diseases. It plays major role in environmental cleaning (microbial remediation and phytoremediation), provides resistance to plants against pests, salt, and drought. Thus, the technique is not only beneficial to humans but also to microbes and plants. Occasionally, the modifications in products at gene level have to face severe problems which are required to be solved. In pharmaceuticals, particularly, there are difficulities to produce better quality products as the alterations made into a gene can be rejected by the body. Regarding health

conditions, the RDT is helping in the treatment of various diseases which are unable to treat under ordinary conditions, though the immune responses obstruct attaining good results.

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