International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 2381-2389 www.ijprajournal.com ISSN: 2249-7781

Role of Recombinant DNA Technology

K. Malleswari*[1], D.Rama Brahma Reddy*[2], Mallela Bhavani[3], Manda Swetha[4], Mudamanchu Renuka[5]

[1*]*Associate Professor, Nalanda Institute of Pharmaceutical Science Siddharth Nagar, Kantepudi (V), Sattenapalli (M), Guntur (Dist)-522438.*

[*2]*Principal & Professor, Nalanda Institute Of Pharmaceutical Science Siddharth Nagar, Kantepudi (V),Sattenapalli (M), Guntur (Dist)-522438.*

[3 4 5]*Students of Nalanda Institute Of Pharmaceutical Science Siddharth Nagar, Kantepudi (V), Sattenapalli (M), Guntur (Dist)-522438.*

Submitted: 15-08-2023 Accepted: 25-08-2023

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

KEY WORDS:
Biological pathway, genetic material, gene manipulation recombinant DNA

I. INTRODUCTION:

Human life is greatly affected by three factors: deficiency of food, health problems, and environmental issues. Food and health are basic human requirements beside a clean and safe environment. With increasing world's population at a greater rate, human requirements for food are rapidly increasing. Humans require safe-food at reasonable price. Several human related health issues across the globe cause large number of deaths. Approximately 36 million people die each year from noncommunicable and communicable diseases, such as cardiovascular diseases, cancer, diabetes, AIDS/HIV, tuberculosis, malaria, and several others according Despite extensive efforts being made, the current world food production is much lower than human requirements, and health facilities are even below standard in the third-world countries. Rapid increase in industrialization has soared up the environmental pollution and industrial wastes are directly allowed to mix with water, which has affected aquatic marines and, indirectly, human-beings. Therefore, the, "Role of genetic engineering in agriculture,"[se issues urge to be addressed modern technologies Unlike tradition approaches to overcome agriculture, health, and environmental issues through breeding, traditional medicines, and pollutants degradation through conventional techniques respectively, the genetic engineering utilizes modern tools and approaches, such as molecular cloning and transformation, which are less time consuming and yield more reliable products.For example, compared to conventional breeding that transfers a large number of both specific and nonspecific genes to the recipient, genetic engineering only transfers a small block of desired genes to the target through various approaches, such as biolistic and Agrobacterium -medicated transformation .The alteration into plant genomeis brought either by homologous recombination dependent gene modification .Recombinant DNA technology is playing a vital role in improving health conditions by developing new vaccines and pharmaceutical.The treatment strategies are also improved by developingdiagnostic kits, monitoring devices, and new therapeutic approaches. Synthesis of genetically modified bacteria

DEFINITION:

Deliberate alteration in the genetic material of any organism by direct modification of

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 2381-2389 www.ijprajournal.com ISSN: 2249-7781

the nucleic acid is referred to gene manipulation or gene cloning or genetic engineering and is achieved by numerous techniques collectively termed as recombinant DNA (rDNA) technology (fig:1). It comprises practices for analysing or joining fragments of DNA from one or more organisms. Involving the rDNA molecule introduction into a cell for its replication or incorporation into the target cell.

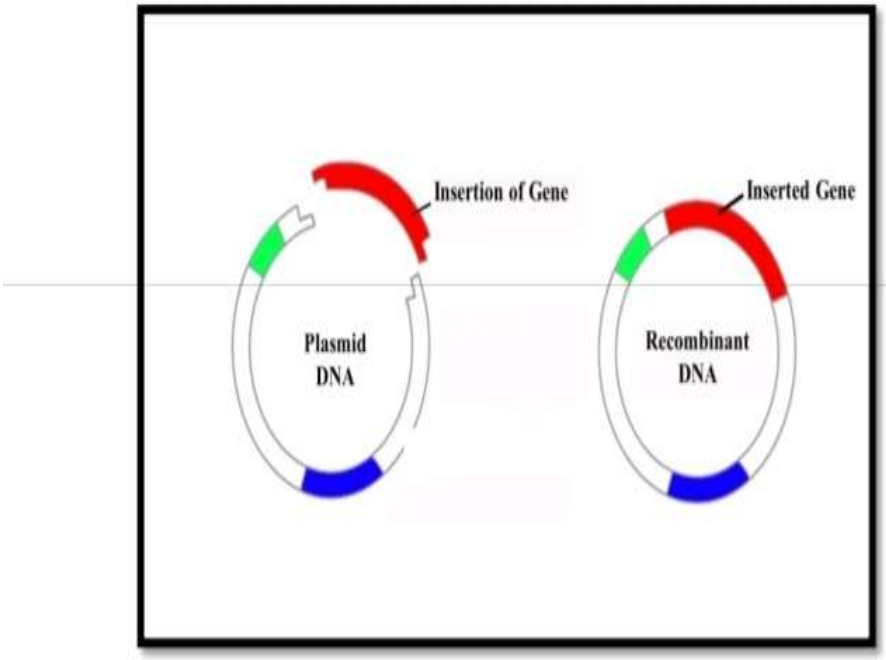


Figure 1: Basic Method of Recombinant DNA Production

HISTORY:

For many years, through selective breeding of plants and animals, human beings have transformed living organism's genetic structure. the thoughtful alteration of the genetic material by unswervingly modifying its nucleic acids is known as genetic engineering or gene manipulation. These alterations involve countless techniques collectively designated as rDNA technology.In the late 1960s, Werner Arber, Daniel Nathans and Hamilton O. Smith discovered restriction endonucleases in microorganisms. It is considered as the key breakthrough in the beginning of rDNA technology.Herbert Boyer, in 1969, reported that the restriction enzyme ECORI (isolated from Escherichia coli).Cuts DNA between the G and A nucleotides in the sequence GAATTC Rapid advancement was continuously achieved in rDNA technology following this discovery. The detection of reverse transcriptase from retroviruses by H. Temin and D. Baltimore, the first recombinant DNA molecule by D. Jackson, R. Symons and P. BergThe development of a recombinant plasmid by S.N. Cohen and H. Boyer and the uncovering of specific DNA fragments by E.M. Southern are some of the noteworthy landmarks of this technology.Some more accomplishments in this path include the DNA sequencing method by F. Sanger, G. Brownlee and B. Barrell; the first gene cloning and construction of rDNA to produce insulin by J. Baxter; first genetically modified crop (Debnath and Sadhukhan; Barman et al., which was an antibiotic-resistant tobacco plant; first recombinant vaccine (Hepatitis B); first mammalian clone, attained through nuclear transplantation from a non-reproductive cell of an adult animal (Dolly).

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 2381-2389 www.ijprajournal.com ISSN: 2249-7781

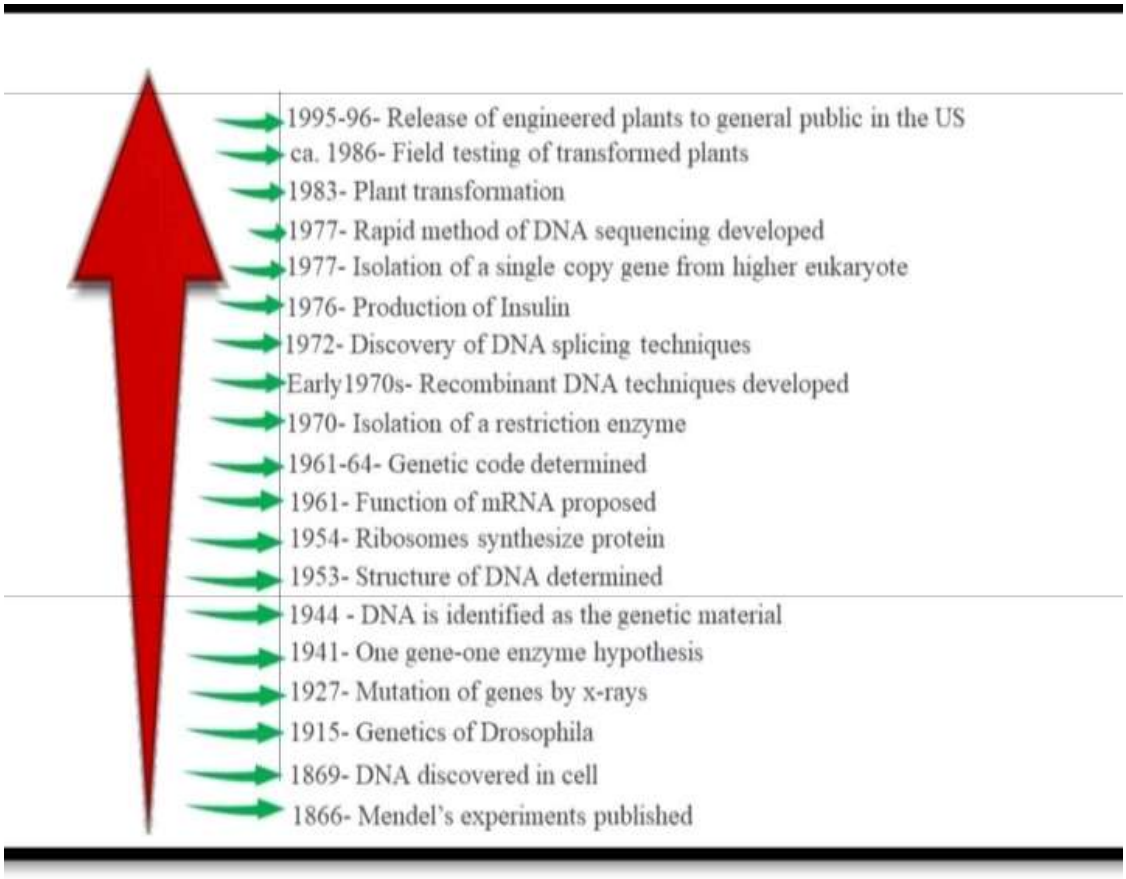


Fig:2 History of RDNA technology.

Recombinant DNA Technology:

Recombinant DNA technology comprises altering genetic material outside an organism to obtain enhanced and desired characteristics in living organisms or as their products.This technology involves the insertion of DNA fragments from a variety of sources, having a desirable gene sequence via appropriate vector . Manipulation in organism's genome is carried out either copy of the incorporated DNA fragment in culture, and finally clones containing a relevant DNA fragment are selected and harvested. The first recombinant DNA (rDNA) molecules were generated in 1973 by Paul Berg, Herbert Boyer, Annie Chang, and Stanley Cohen of Stanford University and University of California San Francisco. In 1975, during "The Asilomar Conference" regulation and safe use of rDNA technology was discussed.Paradoxically to the view of scientists at the time of Asilomar, the recombinant DNAmethods to foster agriculture and drug developments took longer than anticipated because of unexpected difficulties and barriers to achieve the satisfactory results. However, since the mid-1980s, the number of products like hormones, vaccines, therapeutic agents, and diagnostic tools has been developed continually to improve health . A quick approach is offered by recombinant DNA technology to scrutinize the genetic expression of the mutations that were introduced into eukaryote genes through cloned insulin genes insertion inside a simian virus fragment . In a similar way, tumour growth was inhibited by adenoviral vector that encodes endostatin human secretory form through antiangiogenic effects. Antiangiogenic effect can be enhanced by dl1520 through rescuing replication of Ad-Endo . Targeted gene disruption has been used to produce antitumor derivatives in other hosts which were structurally similar for the production pathway . Besides, longer acting therapeutic proteins have been developed through recombinant DNA technologies; for example, sequences containing additional glycosylation site are one of the most followed approaches. A new chimeric gene has been developed through this technique which contains the FSH β- subunit coding

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 2381-2389 www.ijprajournal.com ISSN: 2249-7781

sequences and the C-terminal peptide of the HCG β-subunit coding sequences. . Researchers have also developed vectors and combined vectors for gene therapy and genetic modification approaches. Presently, viral vectors have received immense consideration in clinical settings, some of which have also been commercialized. In principle, viruses are modified approaches. Presently, viral vectors have received immense consideration in clinical settings, some of which have also been commercialized. In principle, viruses are modified to be safe for clinical purposes. They have several application including treatments of several diseases including cancer either through vivo or gene therapy (ex vivo) vaccination and protein transduction approaches.[1].

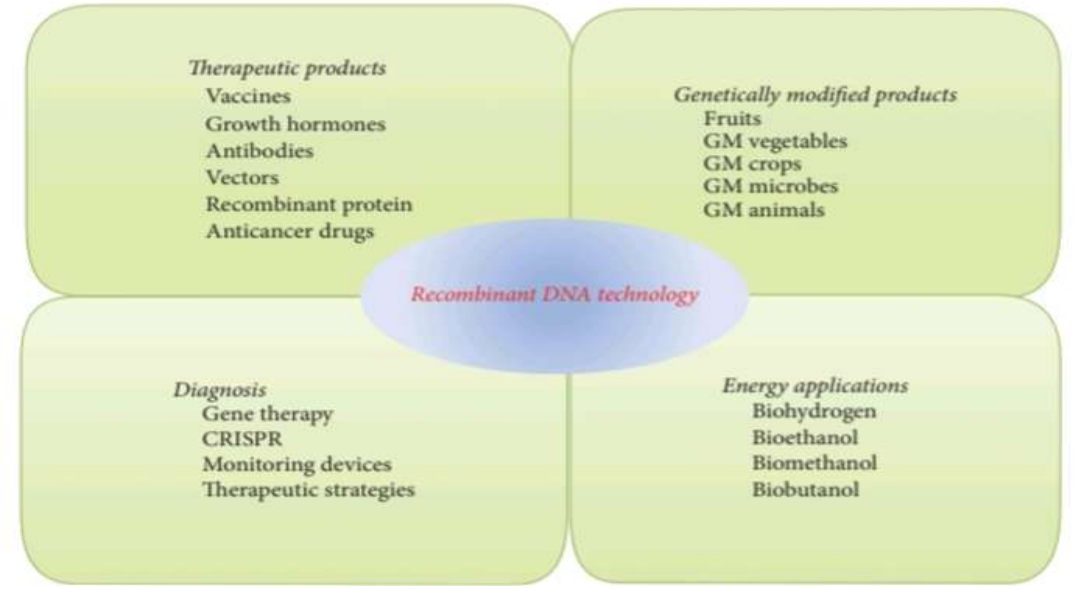


Fig:3 Illustration of various applications of recombinant DNA technology.

Advantages of Recombinant technology:

- Provide substantial quantity
- No need for natural or organic factor
- Unlimited utilizations
- Tailor made product that you can control
- Cheap

Disadvantages:

- commercialized and became big source of income for businessmen
- natural immune system of the body [2].

Current Research progress:

RDNA technology is a fast-growing field and researchers around the globe are developing new approaches, devices, and engineered products for application in different sectors including agriculture, health, and environment. For example, Lispro (Humalog), in comparison with regular human insulin, is a welleffective and fast acting recombinant insulin . Similarly, Epoetin is a novel and well-recognized recombinant protein that can be effectively used in curing of anaemia Recombinant HGH was found with a great improvement in treating children lacking the ability to produce HGH in a required quantity. Clinical testing approval by the FDA in December 1997 for a recombinant version of the cytokine myeloid progenitor inhibitory factor1 (MPIF-1) was an achievement to give recognition to this technology. With its help anticancer drug's side effects can be mitigated whereas it has the ability to mimic the division of immunologically important cells The following section summarizes the most recent developments of recombinant DNA technology. Clustered regularly interspaced short palindromic repeats (CRISPR), a more recent development of recombinant DNA technology.[3]

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 2381-2389 www.ijprajournal.com ISSN: 2249-7781

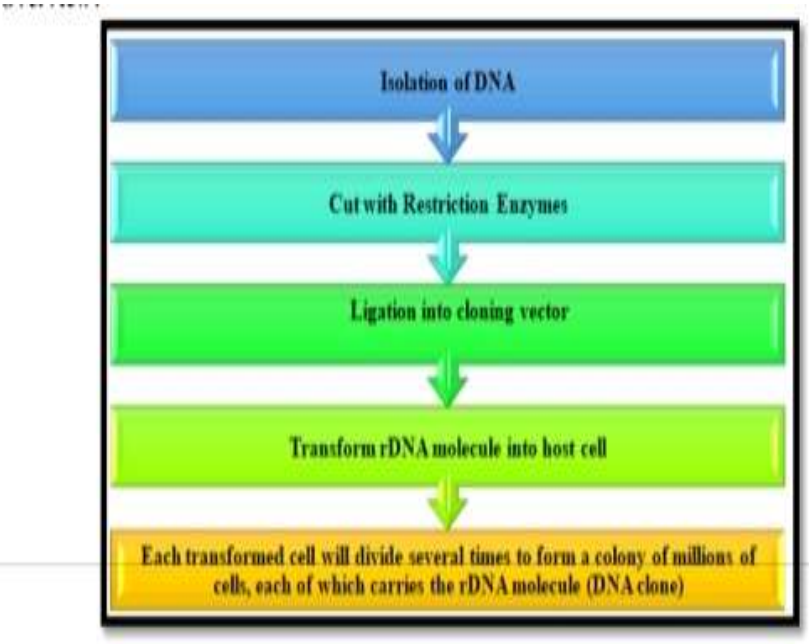


Fig:4 Current research process.

APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY:

Recombinant DNA technology (RDT) has many applications that have made it feasible to produce unique proper enzymes for particular food processing circumstances. Due to their specialized functions and applications in the food processing industry, several significant enzymes, like amylases, lipases etc., are available for specific productions.

Another considerable accomplishment made possible with RDT is the production of microbial strains. By selective engineering, numerous strains of microorganisms have been created that can form enzymes, specifically proteases. Some strains of the fungal pathogen have undergone modifications to lessen their capacity to generate lethal compounds Lysozymes are the most efficient tools for eliminating harmful microorganisms in the food industry.

It is regarded as one of the most crucial enzymes in the food business for eliminating various zoonotic bacteria. Recombinant proteins utilized as medications were recently derived from the initial plant, and many more are now ready to be employed for additional manufacturing of similar medically significant proteins. In order to be used as enzymes in industries, a wide range ofrecombinant proteins have been stated in numerous plants. Additionally, novel polymeric proteins are used in both the medicinal and industrial fields . Food and Agriculture. Recombinant DNA technology has major uses which made the manufacturing of novel enzymes possible which are suitable in conditions for specified foodprocessing. Several important enzymes including lipases and amylases are available for the specific productions because of their particular roles and applications in food industries. Microbial strains production is another huge achievement that became possible with the help of recombinant DNA technology. Health and Diseases. Recombinant DNA technology has wide spectrum of applications in treating diseases and improving health conditions. The following sections describe the important breakthroughs of recombinant DNA technology for the improvement of human health.Environment. Genetic engineering has wide applications in solving the environmental issues. The release of genetically engineered microbes, for example, Pseudomonas fluorescens strain designated HK44, for bioremediation purposes in the field was first practiced by University of Tennessee and Oak Ridge National Laboratory by working in collaboration . The engineered strain contained naphthalene catabolic

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 2381-2389 www.ijprajournal.com ISSN: 2249-7781

plasmid pUTK21 and a transposon-based bioluminescence-producing lux gene fused within a promoter that resulted in improved naphthalene degradation and a coincident bioluminescent response. HK44 serves as a reporter for naphthalene bioavailability and biodegradation whereas its bioluminescence ability makes it able to be used as an online tool for in situ monitoring of bioremediation processes . The production of bioluminescent signal is detectable using opticfibre.[4]

Current Challenges and Future Prospects:

The fact that microbial cells are mostly used in the production of recombinant pharmaceutical indicates that several obstacles come into their way restricting them from producing functional proteins efficiently but these are handled with alterations in the cellular systems. Common obstacles which must be dealt with are posttranslational modifications, cell stress responses activation, and instability of proteolytic activities, low solubility, and resistance in expressing new genes. Mutations occurring in humans at genetic levels cause deficiencies in proteins production, which can be altered/treated by incorporation of external genes to fill the gaps and reach the normal levels. The use of Escherichia coli in recombinant DNA technology acts as a biological framework that allows the producers to work in controlled ways to technically produce the required molecules through affordable process.[5].

Creating the clone:

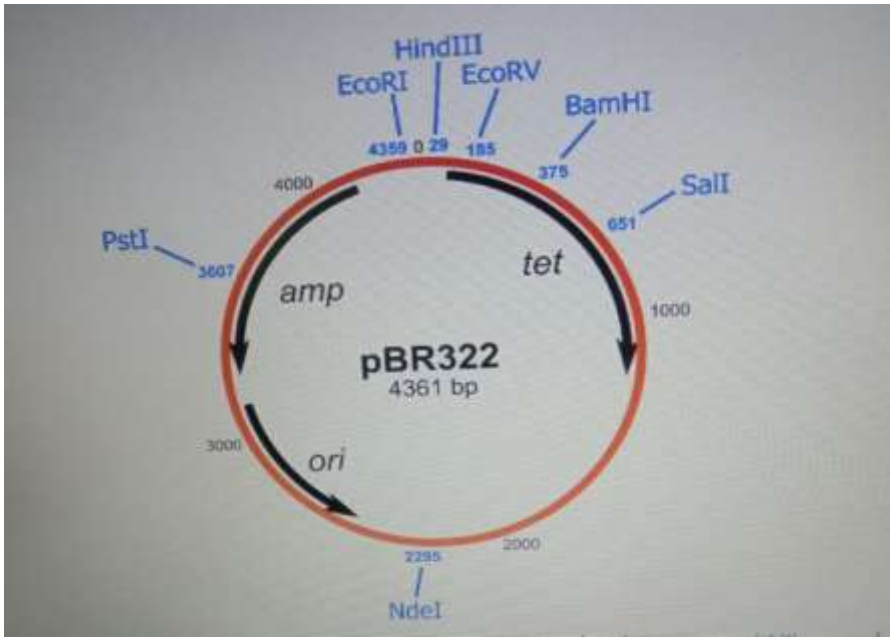
In general, cloning means the creation of a perfect replica. Typically, the word is used to describe the creation of a genetically identical copy. In biology, the re-creation of a whole organism is referred to as "reproductive cloning." Long before attempts were made to clone an entire organism, researchers learned how to copy short stretches of DNA—a process that is referred to as molecular cloning.Molecular cloning allows for the creation of multiple copies of genes, the expression of genes, and study the of specific genes. To get the DNA fragment into a bacterial cell in a form that will be copied or expressed, the fragment is first inserted into a cloning vector.[6]

Cloning vector:

A Cloning vector small piece of DNA that can be stably maintained in an organism, and into which a foreign DNA fragment can be inserted for cloning purposes.The cloning vector may be DNA taken from a virus, the cell of a higher organism, or the plasmid of a bacterium. The vector contains features that allow for the convenient insertion or removal of a DNA fragment to or from the vector, for example by treating the vector and the foreign DNA with a restriction enzyme that cuts the DNA. DNA fragments thus generated contain either blunt ends or overhangs known as sticky ends, and vector DNA and foreign DNA with compatible ends can then be joined together by molecular ligation. After a DNA fragment has been cloned into a cloning vector, it may be further subcloned into another vector designed for more specific use.There are many types of cloning vectors, but the most commonly used ones are genetically engineered plasmids. Cloning is generally first performed using Escherichia coli, and cloning vectors in E. coli include plasmids, bacteriophages (such as phage λ), Cosmides, and bacterial artificial chromosomes (BACs). [7].

Some DNA, however, cannot be stably maintained in E. coli, for example very large DNA fragments. For these studies, other organisms such as yeast may be used.Cloning vectors in yeast include yeast artificial chromosomes (YACs). The common bacterial cloning plasmid, pRB322, is shown the cloning vector may be DNA taken from a virus, the cell of a higher organism, or the plasmid of a bacterium. The vector contains features that allow for the convenient insertion or removal of a DNA fragment to or from the vector, for example by treating the vector and the foreign DNA with a restriction enzyme that cuts the DNA. DNA fragments thus generated contain either blunt ends or overhangs known as sticky ends, and vector DNA and foreign DNA with compatible ends can then be joined together by molecular ligation. After a DNA fragment has been cloned into a cloning vector, it may be further.[8]
subcloned into another vector designed for more species use

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 2381-2389 www.ijprajournal.com ISSN: 2249-7781



Restriction enzymes (also called restriction endonucleases) recognize specific DNA sequences and predictably cut them; they are naturally produced by bacteria as a defence mechanism against foreign DNA.

As the name implies, restriction endonucleases (or restriction enzymes) are "restricted" in their ability to cut or digest DNA. The restriction that is useful to biochemists is usually a palindromic DNA sequence. Palindromic sequences are the same sequence forwards and backward. Some examples of palindromes: RACE CAR, CIVIC, A MAN A PLAN A CANAL PANAMA. DNA has two complementary strands. Therefore, the reverse complement of one strand is identical to the other.

Like with a palindromic word, the DNA palindromic sequence reads the same forward and backward. In most cases, the sequence reads the same forward on one strand and backward on the complementary strand. Restriction enzymes often cut DNA into a staggered pattern. When a staggered cut is made in a sequence, the overhangs are complementary.[9].

MAIN STEPS INVOLVED IN MOLECULAR CLONING USING RECOMBINANT TECHNOLOGY:

1. DNA from donor is isolated and purified.
2. Restriction Enzymes (endonuclease)generate fragments of purified DNA by cutting the DNA at recognition site. There are well over a hundred restriction enzymes, each cutting in a very precise way a specific base sequence of the DNA molecule not exceeding 4- 6 bp.
3. Fragments are inserted, pasted or spliced into plasmid.
4. Plasmid transferred to host cell.
5. As host cell replicates, recombinant molecules are passed on to progeny known as 'clone.
6. Cloned DNA can be recovered &analysed from the host cell after processing.

CUTTING AND LIGATION OF FRAGMENTED DNA FROM DONOR AND FROM THE PLASMID STEPS IN DETAILS:

This "sticky ends" from two different DNA molecules can hybridize together; then the nicks are sealed using ligase. The result is recombinant DNA. When this recombinant vector is inserted into E. coli, the cell processes the instructions and by translation & transcription, it assembles the amino acids forming the protein product of interest. More importantly, the new instructions are passed along the next generation of E. coil cells forming recombinant .[15]

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 2381-2389 www.ijprajournal.com ISSN: 2249-7781

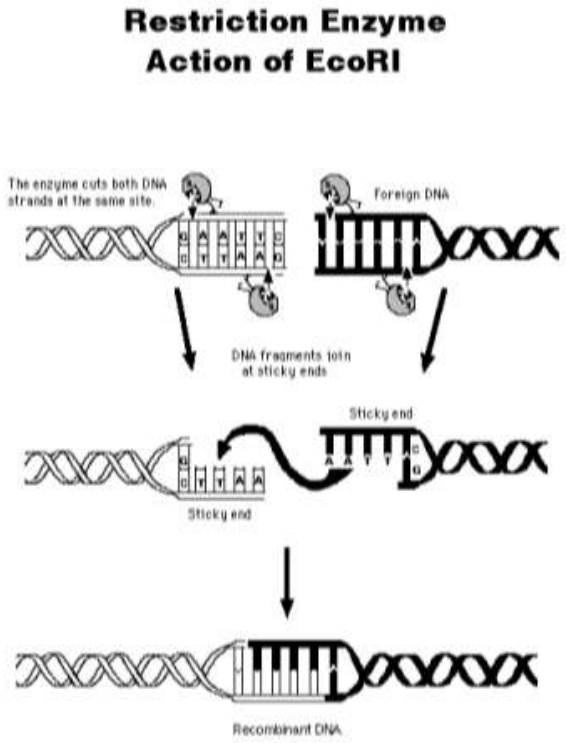


Fig: 6 Restriction enzyme action of ECORI

II. CONCLUSION :

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

III. ACKNOWLEDGEMENT:

We would like to thank Mrs.K.Malleswari,our associate professor and our principal,D.Rama Brahma Reddy for their support and their guidance in completing our project on the topic(Role of Recombinant DNA Technology).It was a great learning experience. I would like to take this opportunity to express my gratitude to all of my group members M.Bhavani, M.Swetha, M.Renuka.The project would not have been successful without their cooperation and inputs.

REFERENCE:

[1]. B. C. J. M. Fauser, B. M. J. L. Manner's, P. Devore, A. Leader, I. Biome, and D. T. Baird, "Advances in recombinant DNA technology j Human Reproduction Update,2009: 15, 3, 309– 321.

[2]. E. A. Masson, J. E. Patmore, P. D. Brash et al., "Pregnancy outcome in Type 1 diabetes mellitus treated with insulin lispro (Humalog),j," Diabetic Medicine,2003: 20, 46–50.

[3]. Z. S. Eclampsia-Beer, R. I. Merker, M. D. Ditto, and M. J. DiNovi, "Food-processing enzymes from recombinant microorganisms—a review," Regulatory Toxicology and Pharmacology,2006: 45, 2, 144–158.

[4]. Z.-X. Lian, Z.-S. Ma, J. Wei, and H. Liu, "Preparation and characterization of immobilized lysozyme and evaluation of its application in edible coatings," Process Biochemistry,2012: 47, 2, 201–208.

[5]. N. Ferrer-Miralles, J. Domingo-Esp 'in, J. Corchero, E. Vazquez, and A. Villaverde, "Microbial factories for recombinant

pharmaceuticals," Microbial Cell factories 2009:8,260-268.

[6]. J. M. DeJong, Y. Liu, A. P. Bollon et al., "Genetic engineering of Taxol biosynthetic genes in Saccharomyces cerevisiae," Biotechnology and Bioengineering, 2006: 43, 2, 212–224.

[7]. Nigro,O,culley,A& steward. Standards in genomic creative clone j of biotechnology 2012:6,3, 415-426.

[8]. Nigro,O. steward. Standards in genomic cloning vector j of biotechnology 2012:6,3, 415-426.

[9]. Tiwari, A., & Pandey, A. Cyanobacterial hydrogen production–a step towards clean environment. International journal of hydrogen energy, (2012):37(1), 139-150.

[10]. Barman, M., & Kundu, S. Molecular markers and a new vista in plant breeding International Journal of Current Microbiology anAppliedSciences,2019:8(12), 1921-1929.

[11]. M., & Singh, Green super rice and asagriculture," Plant Archives,2021; 15;1–6.

[12]. Berk and S. L. Zipursky, Molecular Cell Biology, WH Freeman, New York, NY, USA, 2000:4,7,2-6.

[13]. Z. S. Olesko-Beer, R. I. Merker, M. D. Ditto, and M. J. DiNovi, "Food-processing enzymes from recombinant microorganisms—a review," Regulatory Toxicology and Pharmacology,2006: 45:2, 144–158.

[14]. N. Ferrer-Miralles, J. Domingo-Esp 'in, J. Corchero, E. Vazquez, and A. Villaverde, "Microbial factories for recombinant pharma John Davies group at UFT Canada 2008:2:10-1

[15]. Barman M., Kumar, S.S., Kumar, A., & Parveen, R. Cacogenics as New Horizon in Crop Improvement. Research Journal of Biotechnology,2021:16(1), 200-208.