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A REVIEW ON: RECOMBINANT DNA TECHNOLOGY

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ABSTRACT
Recombinant DNA (rDNA) technology, also known as genetic engineering, involves manipulating DNA to create artificial DNA by combining or inserting DNA strands. It has significantly impacted various fields, including agriculture, public health, gene therapy, environmental science, and pharmaceutical development. The technology uses methods such as transformation, non-bacterial transformation, and phage introduction. It has enabled innovations in producing therapeutic products, developing vaccines, and improving health conditions by modifying microorganisms, animals, and plants. This review highlights the importance of recombinant DNA technology in advancing medicine, biomedicine, and other applications**.**

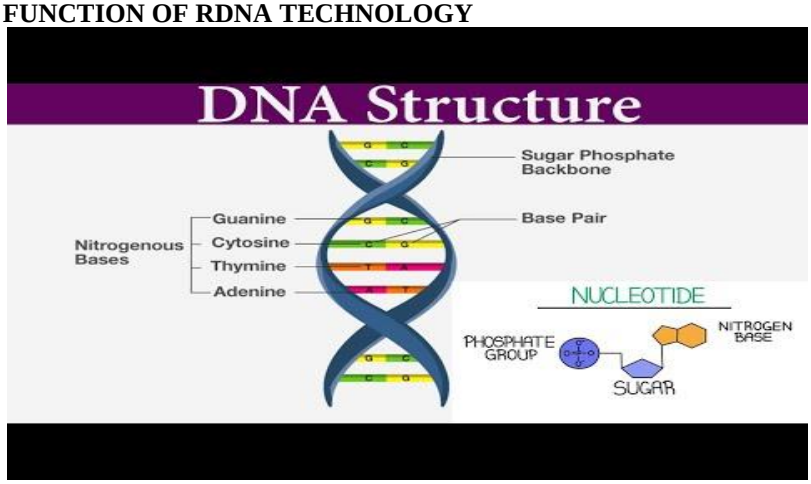
KEYWORDS: RDNA, Restriction Enzymes, Transgenic Plants, Chimeric DNA.

INTRODUCTION

Human life is significantly impacted by food scarcity, health problems, and environmental issues. With the world's population growing rapidly, the demand for safe and affordable food is increasing. Health problems, such as cardiovascular diseases, cancer, and infectious diseases, lead to millions of deaths annually. Environmental pollution, caused by industrialization, affects water quality and aquatic life, indirectly impacting human health. Genetic engineering, using advanced technologies like molecular cloning and transformation, offers solutions to these problems. Unlike traditional approaches, genetic engineering targets specific genes to improve food production, health treatments, and environmental management. For example, genetically modified bacteria produce synthetic human insulin, and engineered microbes are used for bioremediation and waste management. Recombinant DNA technology has revolutionized medicine, leading to the development of life-saving drugs and vaccines.

While genetic engineering has great potential, challenges remain in areas like gene expression regulation in plants. However, it continues to be a critical tool in addressing global issues related to food, health, and the environment.

STRUCTURE AND FUNCTION OF RDNA TECHNOLOGY



DNA (Deoxyribonucleic Acid) is a molecule found in the nucleus of cells, responsible for storing and transmitting genetic information that controls the development and function of organisms. It consists of

three components: a phosphate group, a sugar (deoxyribose), and nitrogenous bases (adenine, thymine, guanine, and cytosine). These bases pair specifically: adenine with thymine, and guanine with cytosine, forming the "double helix" structure, discovered by James Watson and Francis Crick in 1953.

DNA's sequence of bases forms genes, which are used to synthesize proteins through processes involving RNA and ribosomes. While DNA itself doesn't directly create organisms, it provides the instructions for making proteins, which in turn form the organism. Changes in DNA sequence can alter protein formation, potentially resulting in different or inactive proteins.

Recombinant DNA Technology

Recombinant DNA (rDNA) technology is a powerful tool in molecular biology used to manipulate genetic material for various applications, especially in healthcare. It involves combining DNA from different sources to create new genetic sequences that would not naturally occur. This process, first developed in 1973 by Herbert Boyer and Stanley Cohen, is used to produce valuable products like hormones, vaccines, and diagnostic kits. A key example is the production of human insulin and erythropoietin using genetically modified bacteria. The process typically involves inserting DNA fragments with desirable genes into vectors, often bacterial plasmids, which then replicate the recombinant DNA. The DNA sequences can come from any species, allowing for cross-species genetic combinations, such as plant DNA in bacteria or human DNA in fungi. Recombinant DNA can lead to the production of recombinant proteins, which are produced when the DNA is expressed in a host organism.

While the technology has faced initial concerns about its safety and ethical implications, it has since become widely used, particularly from the 1980s onward, to create a variety of therapeutic products and vaccines.

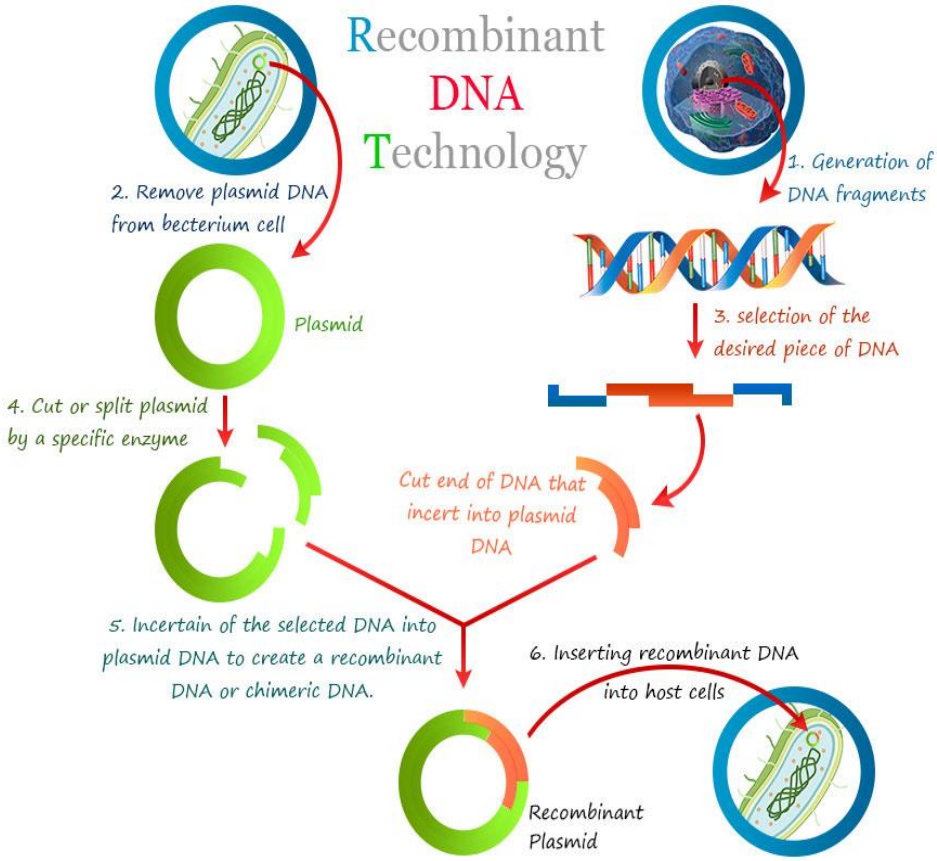


FIG: Recombinant DNA Technology.

METHODS INVOLVED IN RDNA TEHNOLOGY

There are three different methods by which recombinant DNA is made.

1. Transformation
2. Non bacterial transformation
3. Phage introduction

Transformation is a process where DNA is inserted into a vector, usually by cutting the DNA with restriction enzymes and ligating it into the vector using DNA ligase. The vector contains a selectable marker, typically an antibiotic resistance gene, allowing identification of recombinant molecules. The vector is then introduced into a host cell, like E. coli, in a process known as transformation. Host cells can be distinguished from untransformed ones using the selectable marker.

Non-bacterial transformation is a similar process but

doesn't involve bacteria as the host. DNA is directly injected into the host cell's nucleus via microinjection, which uses microprojectiles coated with DNA.

Phage introduction, or transfection, uses viruses (phages) instead of bacteria to carry recombinant DNA. Phages like lambda or MI3 are used to create recombinant plaques, which can be selected based on differences from non-recombinants.

STEPS INVOLVED IN RECOMBINANT DNA TECHNOLOGY

The process of recombinant DNA technology involves several key steps:

Selection and isolation of DNA insert

⬇

Selection of suitable cloning vector

⬇

Introduction of DNA-insert into vector to form rec DNA molecule

⬇

rDNA molecule is introduced into a suitable host.

⬇

Selection of transformed host cells.

⬇

Expression and multiplication of DNA-insert in the host.

1. **Selection and Isolation of DNA Insert**: The desired DNA segment is identified and isolated enzymatically. This segment, known as the DNA insert or cloned DNA, is the target for cloning.
2. **Selection of Suitable Cloning Vector**: A self-replicating DNA molecule, such as a plasmid or bacteriophage, is chosen to carry the DNA insert.
3. **Introduction of DNA Insert into Vector**: The isolated DNA insert is cleaved with restriction enzymes and ligated into the cloning vector to form a recombinant DNA (rDNA) molecule.
4. **Introduction of rDNA into Host**: The recombinant DNA molecule is introduced into suitable host cells (commonly E. coli, but yeast and fungi can also be used) through a process called transformation.
5. **Selection of Transformed Host Cells:** The host cells that have successfully taken up the recombinant DNA are separated from those that haven't, using selection markers.
6. **Expression and Multiplication**: The recombinant DNA is expressed in the host cells to ensure it produces the desired product. The transformed cells are then multiplied to generate enough copies of the desired DNA or protein.

This process allows for the cloning and expression of specific genes in host organisms for research or industrial applications.

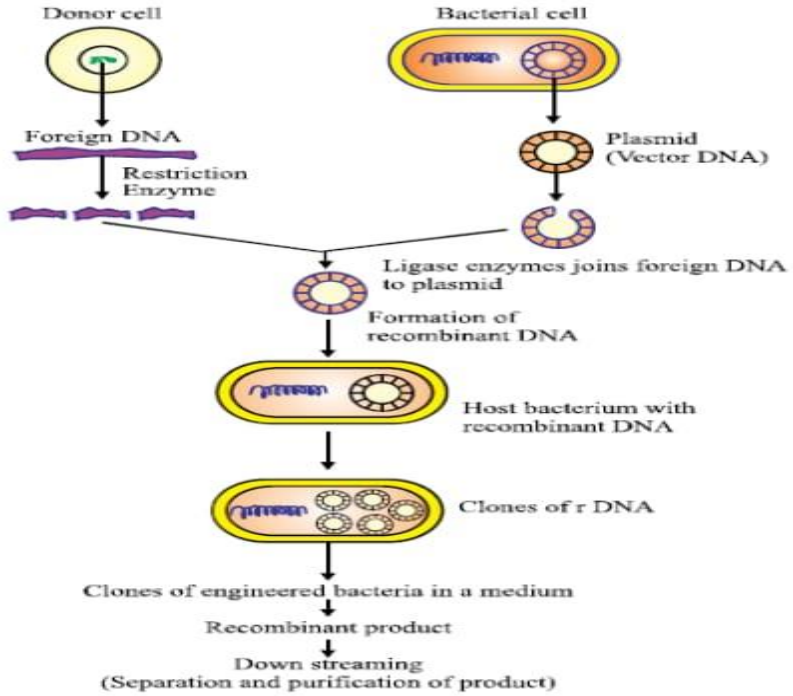


Fig: Steps Involved In Rdna Technology.

IMPORTANCE OF RDNA TECHNOLOGY

Recombinant DNA (rDNA) technology is becoming increasingly important, with significant applications in various fields. As genetic diseases rise and agricultural

land decreases, rDNA holds promise in areas such as:
Better Crops: Developing crops with resistance to drought and heat.
Recombinant Vaccines: Producing vaccines like the Hepatitis B vaccine.
Genetic Disease Treatment: Offering potential cures for genetic disorders like sickle cell anemia and cystic fibrosis.
Clotting Factor Production: Producing clotting factors for hemophilia treatment.
Insulin Production: Producing insulin for diabetes management.
Recombinant Pharmaceuticals: Creating pharmaceutical products through genetic engineering.
Insect-resistant Plants: Engineering plants to produce their own insecticides.
Gene Therapy: Enabling both germline and somatic gene therapies for treating genetic diseases.
As these advancements unfold, rDNA will continue to revolutionize medicine, agriculture, and biotechnology.

BIOLOGICAL TOOLS OF RDNA TECHNOLOGY

Recombinant DNA technology involves several key tools:
1. Enzymes
2. Foreign DNA
3. Vehicle/Vector
4. Host Cell
5. Culture Media, Buffers, Reagents

Enzymes

Restriction enzymes (endonucleases and exonucleases) are used to cut DNA at specific sites. Endonucleases cut within the DNA, while exonucleases remove nucleotides from the ends. Restriction enzymes recognize palindromic sequences, creating sticky ends that allow ligases to bind the desired gene to a vector.

Vectors: Vectors (such as plasmids and bacteriophages) carry the desired gene into the host organism. They consist of an origin of replication, a selectable marker (resistance genes), and cloning sites for the insertion of foreign DNA.

Host Organism: The host organism receives the recombinant DNA through methods like microinjection, gene guns, or the use of calcium ions. It then expresses the inserted gene, completing the process of recombinant DNA technology.

In summary, enzymes cut and bind DNA, vectors carry the gene, and host organisms facilitate the expression of the recombinant DNA.

APPLICATIONS OF RDNA TECHNOLOGY

Recombinant DNA (rDNA) technology has a wide range of applications across various fields:
1. Transgenic Plants and Animals: Genetically modified plants and animals have been created for improved traits such as pest resistance or enhanced production, including transgenic animals for pharmaceuticals.
2. Production of Hormones and Vaccines: Bacteria like E. coli are used to produce human hormones (e.g., insulin) and vaccines against diseases like polio and hepatitis. Recombinant DNA also aids in the production of interferons with antiviral properties.
3. Antibiotics and Commercial Chemicals: rDNA technology enhances antibiotic production and the manufacturing of chemicals like alcohol, organic acids, and vitamins.
4. Enzyme Engineering and Disease Diagnosis: It enables enzyme modifications and plays a crucial role in disease prevention and diagnosis through tools like monoclonal antibodies.
5. Gene Therapy: Gene therapy involves introducing specific genes into patients to treat genetic disorders, representing a major advancement in medicine.
6. Forensic Science: rDNA techniques, such as DNA fingerprinting, help identify individuals and solve cases of parentage or crime.
7. Biofuel Production: Genetic engineering optimizes organisms for biofuel production, improving yields and efficiency in bioenergy generation.
8. Environmental Protection: rDNA technology aids in waste treatment, bioremediation, and pollution reduction, contributing to environmental sustainability.
9. Food and Agriculture: It enhances food processing and preservation by producing specialized enzymes and genetically modified crops that withstand adverse conditions.
10. Health and Disease Treatment: The technology is pivotal in producing therapeutic products, including hormones and vaccines, and has widespread applications in treating diseases.

TECHNIQUES USED IN RDNA TECHNOLOGY

Gel electrophoresis

It is a technique used to separate molecules, such as DNA, RNA, or proteins, based on their size. The molecules are placed in a gel matrix, typically made of agarose or polyacrylamide, and an electric field is applied. Molecules with a net negative charge, like DNA and RNA, move toward the positive electrode, with smaller molecules migrating faster through the gel's pores, while larger molecules move more slowly and remain near the well.

DNA, RNA, and protein molecules are measured by different units: DNA in base-pairs (bp), RNA in nucleotides and proteins in Daltons (Da). The distance traveled by molecules is roughly proportional to the log of their inverse molecular weight. Gels are typically stained to visualize the separated molecules. DNA and RNA are commonly stained with ethidium bromide (EtBr), which fluoresces under UV light, while proteins are stained with Coomassie Blue or Silver Stain.

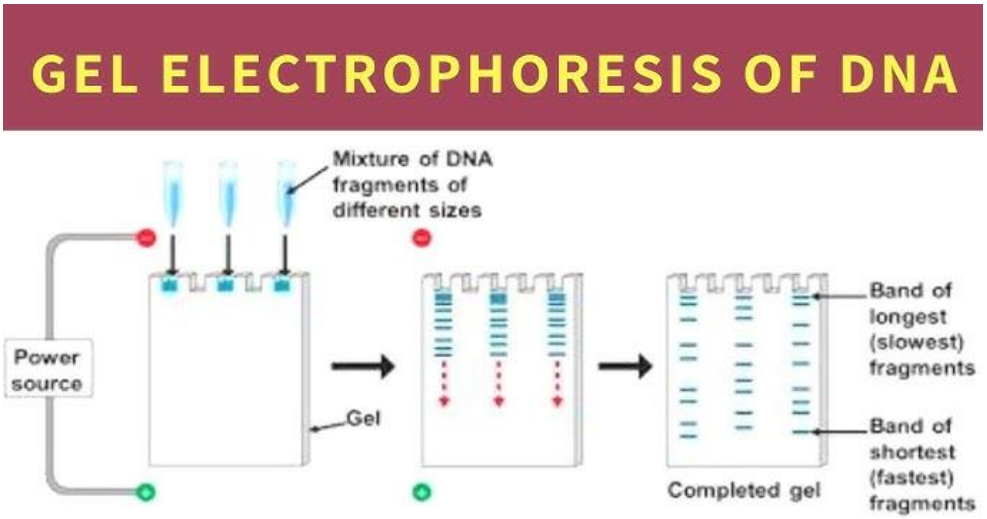


FIG: Gel Electrophoresis.

Restriction endonucleases

They are enzymes that cut DNA at specific sites, typically 4-6 base pairs long, by recognizing palindromic sequences (the same forwards and backwards). These enzymes are found in bacteria, which use them to defend against foreign DNA by cutting it at these specific sequences. When two DNA molecules are cut by the same restriction enzyme, they produce "sticky ends" that can hybridize and be joined using ligase to form recombinant DNA.

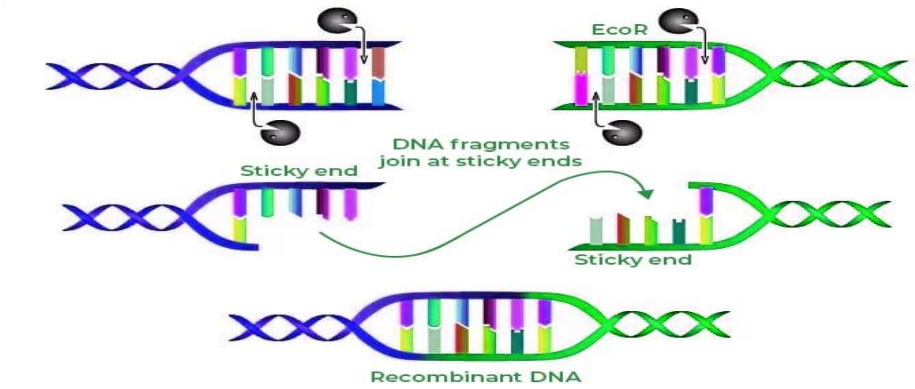


FIG: RESTRICTION ENDONUCLEASES.

This recombinant DNA can then be inserted into E. coli for gene cloning, allowing the production of proteins like insulin. In addition, a radioactive probe can be created by determining a short segment of a protein's amino acid sequence, back-translating it into possible DNA sequences, and synthesizing complementary DNA oligos. These radiolabeled oligos are applied to blotted clones and will hybridize with clones containing the desired DNA sequence encoding the target protein.

Reverse transcription

Reverse transcription or cDNA cloning, is used to clone genes that contain introns. To avoid the problem of introns when expressing a gene in bacteria, mRNA is first isolated from tissues that produce the desired protein. Using the enzyme reverse transcriptase, the mRNA is reverse transcribed into cDNA, which is free of introns. This cDNA is then ligated with "sticky ends" and inserted into a phage or plasmid vector for cloning and expression.

Polymerase Chain Reaction (PCR)

It is a method used to amplify specific DNA sequences. It uses a heat-stable DNA polymerase, typically Taq Polymerase from the thermophilic bacterium Thermus aquaticus, or the even more heat-resistant Vent Polymerase from a hyper thermophilic microbe.

In PCR, the DNA to be amplified, along with primers (short DNA sequences specific to the region of interest), four nucleotide bases, and the polymerase, are placed in a thermal cycler. The temperature is raised to denature the DNA, then lowered to allow primers to hybridize to the DNA strands. The polymerase extends new DNA strands, and this cycle is repeated multiple times. With each cycle, the amount of DNA doubles, leading to exponential amplification of the target sequence, reaching millions of copies in a few hours.

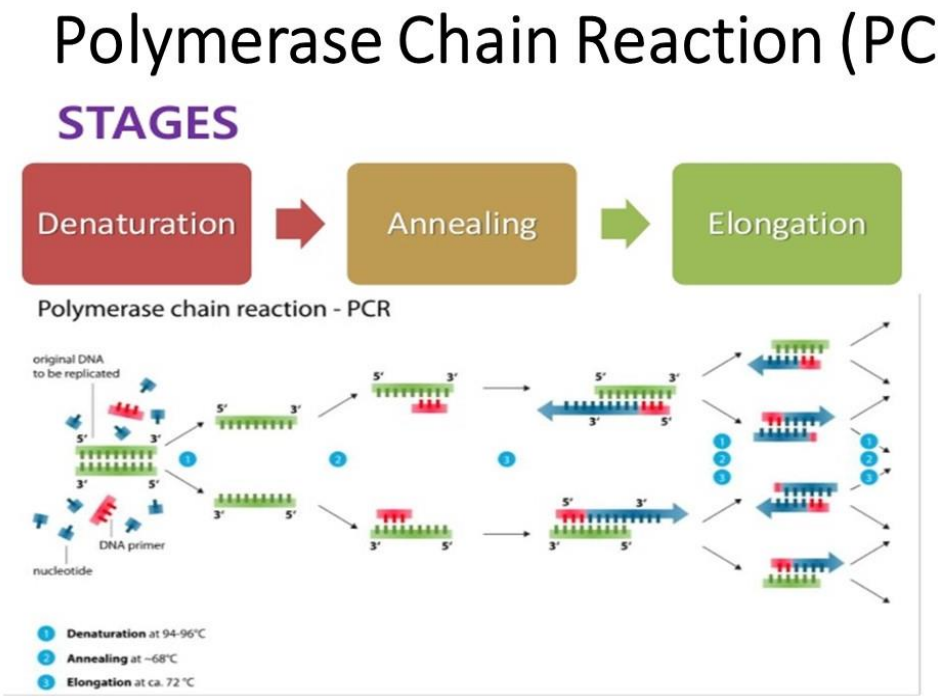


FIG: POLYMERASE CHAIN REACTION.

CONCLUSION

Recombinant DNA technology has significantly impacted various fields, improving human life by enabling advancements in biomedical applications such as cancer treatment, genetic diseases, and diabetes. It has also enhanced plant resistance to viral, fungal, and environmental stressors like drought, pests, and salt. The technology has been pivotal in improving plants, microorganisms, and pharmaceuticals, though it faces challenges like gene integration issues and immune responses that hinder effectiveness in some treatments.

In agriculture, recombinant DNA technology allows for the development of genetically engineered plants, but concerns exist about their potential to cross-breed with wild plants and affect biodiversity. Additionally, there are health concerns about the safety of genetically engineered products. These issues highlight the need for further research to resolve these challenges and ensure the safe application of recombinant DNA technology.

Despite these challenges, recombinant DNA technology has made significant strides, particularly in the development of human insulin, which has saved millions of lives. It continues to play a crucial role in treating diseases, improving health conditions, and advancing food and agricultural practices. Further research and refinement are essential for overcoming current difficulties and maximizing its potential in various sectors.

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