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Basic Approach of Recombinant DNA Technology and its Applicability in Various Sectors

Dr. NamrataMittra
Department of Biosciences
Integral University,
Dasauli, Kursi road, Lucknow, 226026, India
Email: mnamrata@iul.ac.in

Abstract- Recombinant DNA (r-DNA) technology or genetic engineering is offering new strategies and opportunities to combat severe health and environment issues. Recombinant DNA is constructed by inserting the DNA of interest with the help of the restriction enzymes and ligated into the vector by DNA ligases. Restricted DNA fragments and the vector are cut with the same restriction enzyme to make it compatible to each other which ligates them together by the DNA ligases. Depending upon the ends generated by the restriction enzymes either sticky or blunt ended fragments, DNA ligases act upon them accordingly. The vector replicates independently and swiftly once introduced into host cell. The vector is selected depending on the size of the DNA insert i.e. smaller to larger. The recombinant colonies or plaques are screened from the non-recombinants by blue-white screening or antibiotic resistance strategy. The rDNA technology is involved in the therapeutics of severe human diseases such as Hepatitis B, human growth hormone, insulin etc. The rDNA technology is not limited to therapeutics of human diseases but to the production of beneficial industrial products and insect resistant crops. It is moving towards to resolve environmental issues by degradation of the environmental pollutants which is major area of concern. To maintain a balance between the health and environment, the rDNA technology has step forward in resolving the severe issues related to it. The review explores the basic strategies involved in the rDNA technology and its effectiveness to the world in sustaining balance between human health and environment.

Keywords-recombinant DNA technology, restriction enzyme, DNA ligase, plasmid, bacteriophage, cosmids, bacterial artificial chromosomes, yeast artificial chromosomes, recombinant colonies, r-DNA applications.

I. INTRODUCTION

Recombinant DNA (rDNA)technologyis the process which involves the joining of two different DNA molecules from different organisms inserting into the suitable vector and then introducing into the host cell to produce new genetic recombination that are beneficial in science, industry, human health (Khan et al; 2016,Ashwini et al; 2016).

The first recombinant DNA was discovered in 1973 in a living organism by Herbert Boyer and Stanley Cohen. They both used the Escherichia coli restriction enzymes to introduce the foreign DNA into the vector. The different key steps involved in the rDNA technology involve the following: isolation of the foreign DNA or the genetic material from the organism, the restriction enzyme digestion of the foreign DNA by the use of restriction enzymes, amplification of the digested DNA fragments by the polymerase chain reaction (PCR), ligation of the restricted DNA fragments into the vector by DNA ligases, insertion of the recombinant vector (containing the DNA of interest) into the host cell, selection of the recombinants from the non-recombinants (Ashwini et al; 2016).

First of all the DNA of interest is being isolated by the suitable extraction procedure. The isolated DNA is cut by the restriction enzyme through restriction enzyme digestion process. Restriction enzymes are specific restriction endonucleases that cut the DNA at specific sequences or the recognition site (Roberts; 2005).

The vector is also being cut by the suitable restriction enzyme so as to incorporate the restricted foreign DNA fragments. After the restriction enzyme digestion, different fragments of the DNA are obtained which are then ligated into the suitable cloning vector. The cloning vector is being selected on the basis of the size of the DNA of interest to be inserted by the suitable DNA ligases. Depending on the size of the foreign DNA to be cloned, vector is being choose, if the foreign DNA is small then the plasmid vector is being used generally.

For large DNA inserts, bacterial artificial chromosome (BAC) or yeast artificial chromosome (YAC) is being used. The vector should composed of unique restriction site, origin of replication to replicate independently, antibiotic resistant genes to enable the survival of the host cell containing recombinant vector and a tag gene to

screen the cells that contain the foreign DNA. The DNA ligase helps in joining and ligating the DNA fragments to the vector. The recombinant vector containing the DNA of interest is introduced into the host cell by various techniques described previously (Ashwini et al; 2016).

Furthermore, screening of recombinants is being done by various techniques like blue white screening which is used to identify recombinant bacteria. The review reveals the basic approach in the rDNA technology such as the major enzymes involved and the different type of vectors involved in the rDNA technology, screening of the recombinant bacteria and applications of the rDNA technology in human health and disease therapeutics.

1. Major Enzymes Used in rDNA Technology:

1.1 Restriction Enzymes: Restriction enzymes are the restriction endonucleases that cleave the sugar phosphate backbone between the deoxyribose sugar and the phosphate group within or close to the recognition site (Roberts; 2005). The restriction enzymes are also recognized as "scissors" of molecular cloning. The restriction enzymes are named as "restriction" because of their ability to restrict the growth of the bacteriophages. Most of the restriction enzymes are isolated from the bacterial species. There is a restriction-modification system which protects the bacteria own DNA from being restricted (Roberts et al; 2010).

The enzyme methyl transferase methylated the bacteria own DNA and prevents its own restriction. Till date wide variety of the restriction enzymes have been isolated and they cut the DNA into fragments specifically. Due to the different combinations of the restriction enzymes, DNA can be cut or manipulated accordingly. The nomenclature or naming of the restriction enzymes is on the basis of the genus and species names of the organism from where they are isolated i.e. Eco R1 (Escherichia coli).

There are three types of the restriction enzymes generally: Type I restriction enzymes recognize the specific sequences of the DNA and cleave at 1000 base pairs (bp) away from the recognition site. The type I restriction enzyme is composed of the single large submit having both modification (methylation) and restriction activities. It basically requires adenosine triphosphate (ATP) for its activity or to cut the DNA. The type I restriction enzymes recognize long sequences ranging from 8-16bp (Murray; 2010 and Loenen et al; 2014). The type I enzymes are Eco k and Eco B. The type II restriction enzymes are very specific as they cut the DNA within or too close to the recognition site (Pingoud et al; 2005).

They do not require ATP to cut the DNA. They are generally used in the rDNA technology due to their specificity. It is composed of separate proteins for modification and restriction purpose. Out of these two one methylates the DNA and protects it from restriction and other recognizes and cuts the DNA. Type II restriction enzymes recognize the sequences ranging from 4-9bp long. They cut the DNA and result in either sticky or blunt ends as shown in the figure 1 of the article. When type II restriction enzymes cut the DNA straight across the axis of symmetry between the same two bases as on the complementary strand generates sticky ends (Hung et al; 1984).

There are two types of sticky ends generating 5' overhangs or 3' overhangs. If the cut is near the 5'end, it generates 5' overhangs or vice-versa. Example of 5'overhang is Bam HI (Bacillus amyloliquefaciens H) and 3' overhang is Kpn I (Klebsiella pneumonia).When type II restriction enzyme cut the DNA strand in the middle of the recognition sequence then it generates blunt ends, for example Sma I (Serraticamarcescens).

Finally, Type III restriction enzymes cut the DNA about 25bp away from the recognition site and require ATP to cut the DNA, for example Eco P1 and Eco P5 (Rao et al; 2014).The type III restriction enzymes recognize the short sequences ranging 5-7bp.

1.2 DNA Ligase:DNA ligase is an enzyme that facilitates the joining of the two DNA strands by forming a phosphodiester bond between the phosphate group of one strand and deoxyribose group of the other strand and result in the unbroken DNA molecule (Ashwini et al; 2016). In rDNA technology, DNA ligase joins the restricted DNA fragments into the suitable vector. Ligation is being done according to the DNA fragments generated by the restriction endonucleases either sticky ends or blunt ends. In the blunt end ligation, the DNA fragments are joined directly by creating single base overhangs for example 'A tailing' with ThermusAquaticus (Taq) polymerase and dATP.

In the sticky ends, overhangs are being produced either 5' or 3', thus the overlapping region of the complementary single stranded DNA hydrogen bond to each other and the sugar phosphate backbone are connected by the DNA ligases. In this way sticky end or blunt end ligation is made possible by the DNA ligases. The two most commonly used DNA ligases in the rDNA are E. coli and T4 (bacteriophage) DNA ligases. The E. coli DNA ligase joins the cohesive/sticky end DNA fragments and requires nicotinamide adenine dinucleotide (NAD) as a cofactor. The T4 Ligase joins both sticky as well as blunt ended DNA fragments and requires adenosine triphosphate (ATP) as a cofactor.

1.2 Vectors Used in the rDNA Technology:

Vector is a vehicle or plasmid which carries the DNA of interest or the foreign DNA and then introduced into the host cell where they have the capability of autonomous replication i.e. they replicate independently and possess own origin of the replication. There are different types of

vectors that are being constructed till date depending upon the insertion capability to insert DNA fragments of different size. The rDNA technology uses the plasmid vectors in human health for drug delivery to insert the desired drug in the body for example insulin, human growth hormone. Here are the few commonly used vectors in the rDNA technology.

2.1 Plasmids: Plasmids are the small circular; double stranded DNA molecules apart from the chromosomal DNA possess the capability of the independent replication. Plasmids play an important role in the rDNA technology or molecular cloning or gene therapy for the insertion of the therapeutic genes in the human body to fight against the infectious diseases. They have the capability to target the dysfunctional cells and trigger the therapeutic or functional genes in them. Plasmids compose of antibiotic resistance genes for the bacteria to survive against natural antibiotics. The size of the plasmid varies depending on the type of plasmid. Its smaller size is more beneficial to be used in the therapeutic purposes through rDNA technology. The size of the insert is dependent on the size of the cloning vector.

Plasmids carry upto 8-12 kbp of the DNA inserts. Plasmids produce virulence factors that enable a bacterium to colonize a host and ability to degrade the toxic compounds. Plasmids can be conjugative or non-conjugative. The conjugative plasmids have the capability to transfer from one bacterium to another (Smillie et al; 2010 and Chiang et al; 1988). The non-conjugative do not possess this capability but can be transferred through conjugation by the help of non-conjugative plasmids.

On the basis of the functionality, plasmids are classified into the following groups: Fertility plasmids containing transfer (tra) genes and are capable of conjugation, col E1 plasmids code for bacteriocins which are the proteins that kill other bacteria, degradative plasmids that avoid the digestion of unusual substances, resistance plasmids provide resistance against the antibiotics and virulence plasmids which turn the bacterium in to the pathogen (Gil et al; 1991).

2.2 Bacteriophage: Bacteriophages also known as lambda phage is the viruses that infect and replicate in the E. coli bacteria (Chauthaiwale et al; 1992, Cue et al; 2001 and Stemberg et al; 1990). It is a temperate phage and resides in to the host genome by lysogeny. They act as a vector and have own origin of replication to replicate independently inside the bacterial cell. They enter the genetic material of the bacteria and the bacteria treats the viral genetic material as if its own and more virus particles are manufactured.

Lambda phage is a virus particle that is composed of head known as capsid, tail and tail fibers. The phage head is composed of double stranded circular DNA and the cossite which serve as a packaging initiation site. The phage binds the bacterium (host cell) and transfers its phage DNA into bacterial cytoplasm ejected by tail (Arber et al; 1963). After the phage DNA enters the bacterial cell, the phage particles are multiplied and released into the environment through lysis of the bacterial cell along with all the cell contents. Bacteriophage lambda is more efficient in carrying the DNA inserts and in replication in to the host cell. The lambda libraries are easy to amplify and the lambda plaques are easy to screen as more plaques can be screened per plate compared to the plasmid colonies. The capability of the lambda phage to carry DNA insert is about 20-25kbp.

Replacement and insertional vectors are the two categories of Bacteriophage lambda (Cue et al; 2001 and Stemberg et al; 1990).The replacement vectors is composed of the pair of the restriction sites to excise the central stuffer (non-essential) fragments which can be replaced by the desired DNA fragment/ sequence with compatible ends. The replacement vectors are too useful in the genomic library construction and can carry DNA insert upto 25kilo bp. The commonly used replacement vectors are EMBL and Charon. The insertional vectors are involved in cloning of the small DNA fragments such as cDNA. The small DNA fragments can be introduced without the removal of the stuffer fragments. The most commonly used insertionallambda vectors are lambda gt10 or gt11.

2.3 Cosmids: The cosmids are hydrid of bacteriophage lambda + plasmid and often used in genetic engineering or rDNA technology as a suitable cloning vector. Cosmids are basically utilized in the construction of the genomic libraries due to large insert capability of about 50kbp (Cohen et al; 2010). The main advantage ofcosmid cloning is that the vector clones large DNA fragments in contract to transformation which involves the cloning of the smaller DNA fragments.

The cosmids can be packed in vitro into phage capsids with a lambda phage as a vector (Lund et al; 1982). They possess the characteristics such as origin of replication, antibiotic resistance genes, cos sites for packaging of DNA. The insert capacity depends on the size of the vector. It generally involves the similar procedure like isolation of gene of interest, packaging into the cosmid vector, insertion of the cosmid carrying the gene of interest into the host cell such as E.coliby transduction via in vitro packaging. Furthermore, the screening of the cosmid colony is made possible by suitable methods. The widely used cosmid vector in the rDNA technology is SuperCos 1.

2.4 Bacterial/Yeast artificial chromosome (BAC/YAC): Bacterial artificial chromosome are the cloning vectors with large insert capacity of about 100-300 kbp that are transformed and cloned in bacteria such as E.coli (Moralli et al; 2020, Nilmalgoda et al; 2003 and

Baga et al; 2005). The bacteria take up the recombinant BAC and amplify the BAC DNA which is further isolated and used in DNA sequencing. The BAC is used in the genome sequencing projects such as human genome sequencing. The sequenced parts are analyzed in silico resulting in entire genome of the organism. They compose of a unique restriction enzyme site, rep E for plasmid replication and copy number, antibiotic resistance gene, based on fertility (F) plasmid for cloning in bacteria, T7 and Sp6 phage promoters for transcription of the inserted genes. The BAC is being used as a therapeutic in genetic and infectious diseases. The BAC is used in various neurological disorders such as Alzheimer's disease and are widely used because of their capability to carry larger DNA fragments with an ease and stability as compared to the other cloning vectors of small insert capability.

Yeast artificial chromosome (YAC) is the genetically engineered chromosome derived from yeast (Sacharomycescerevisiae) DNA which is further ligated into the bacterial plasmid (Moralli et al; 2020). The YAC can carry large DNA inserts upto 1000kbp. The components that genetically engineered from yeast are centromere, telomere and autonomously replicating sequence. The unique feature of YAC is that they can express eukaryotic proteins that require posttranslational modification but due to the stability issues the BAC was preferred over the YAC.

3. Transfer of recombinant vector into the bacterial host cell:

There are different techniques involved in the transfer of the recombinant DNA (composed of DNA of interest) into the bacteria through transformation, transfection and electroporation. The vectors have the capability to carry the foreign DNA to the bacterial cells. Once, bacteria take the recombinant DNA, it replicates further. This technique is called transformation and is chemically induced by treating the bacterial cell with calcium chloride to take up the foreign DNA by plasmid vector.Transfectiontakes place with the help of the bacteriophages which carries gene from one host cell to the other. The electroporation is a technique which creates pores in the cell membrane of bacteria by inducing high electric current to take up the foreign DNA.

4. Screening of the recombinant colonies/plaques:

The most widely used technique for the screening of the recombinants containing DNA of interest from the non-recombinants is blue-white screening and the antibiotic resistance screening (Bell et al; 1999). The foreign DNA is being inserted in the vector at that sequence (lac z gene) which encodes an enzyme β-galactosidase resulting in the blue colored colony in a culture medium. This enzyme is present in the host E.coli which cleaves the lactose into glucose and galactose. If the foreign DNA is inserted in the β-galactosidase encoding sequence, it disables the function of the enzyme resulting in the white colony that is composed of the transformed DNA. If the foreign DNA is not inserted in the β-galactosidase encoding sequence it will result in the blue colonyindicating non recombinants due to the functional β-galactosidaseenzyme.The vector is composed of the antibiotic resistance gene which make them resistant to the action of the antibiotics such as streptomycin, tetracycline etc. The recombinant colonies/plaques remain resistant from the action of the antibiotics, hence screened from the non-recombinants.

5. Applications of rDNA technology:

5.1 Human health and medicines: People suffering from the hemophilia are unable to produce sufficient quantities of factor VIII to induce blood coagulation. Such patients suffering from this disease were in requirement of factor VIII by processing large quantities of human blood from various blood donors that caused severe blood borne diseases and infections like hepatitis B and human immunodeficiency virus (HIV). The recombinant DNA technology produced the recombinant blood clotting factor VIII which do not contain plasma and albumin, therefore cannot spread blood borne diseases or infections.This recombinant factor VIII is genetically engineered which do not come from human plasma.

The insulin from the animal source such as pigs and cattle's were used to treat insulin dependent diabetic patients who resulted into the onset of severe infections. To combat the infections, recombinant insulin was produced to treat the insulin dependent patients (Vajo et al; 2001 and Johnson et al; 1983). The human insulin gene was inserted into the E. coli or saccharomyces cerevisiae (yeast) to produce the insulin that can be used for humans.

The patients whose pituitary glands are producing insufficient amount of the human growth hormone (HGH) were suffering from the abnormal growth and development. The human growth hormone is responsible for inducing growth and development. Previously, the human growth hormone was obtained from the cadavers and the patients treated with this HGH were suffering from the Creutzfeldt - Jakob disease, a fatal degenerative brain disorder. To elucidate the problem, the recombinant HGH was produced (Lal et al; 2019).

The recombinant hepatitis B vaccine was produced to treat the patients suffering from the hepatitis B infection. The recombinant hepatitis B vaccine composed of hepatitis B surface antigen produced in the yeast cells. Furthermore, to diagnose the HIV infection, the recombinant DNA technology was used. The antibody test used the recombinant HIV protein to test the presence of the antibodies that the patient's body has developed in response to the HIV infection. Thus, recombinant DNA technology resolved many of the health issues in humans. The majority of the recombinant protein drugs are the human proteins to compensate the in vivo defects caused due to the malfunctioning of the functional proteins. These

drugs possess greater safety and good approach of production.

5.2 Agriculture:
To protect the plant from the harm of insects and herbicides, the recombinant DNA technology came into action and developed herbicide as well as insect resistant crops (Chen et al; 2019 and Paoletti et al; 1996). The re-engineered plants had greater disease resistance to produce viral proteins. The plants were made resistant to the insects with the resistant genes isolated from the bacterium which were being inserted into them to make plants resistant against the pests and insects like caterpillars.Various insect and herbicide resistant crops were developed by the rDNA technology to increase the crop productivity and yield (Whitelaw et al; 2005 and Fayerman et al; 1986).

The first agricultural product developed by the rDNA technology was the rot resistant tomato. The antisense molecule was produced by the re-engineered plants which inhibits the enzyme that encourages rotting in tomatoes. There are varieties of the agricultural crops that incorporate a recombinant gene that provide resistance to the herbicide such as glyphosate. The herbicide resistant crops are maize, cotton, corn, alfalfa and soy. To make plants resistant to insecticide, the rDNA technology was used to make transgenic crops where they expressed a recombinant form of the bacterial protein. Previously, the bacterium named Bacillus thuringeiensis produces a natural protein Bt toxin. The bacterium was used in plants to provide insect resistance in agriculture for years.

5.3 Industry: The recombinant DNA technology has been used for the production of various products that are beneficial to human (Katz et al; 2018, Khan et al; 2016). These products are composed of the primary metabolites which are directly involved in growth and development. The primary metabolites primarily maintain the physiological function in an organism and balance the basic metabolism. The primary metabolites are amino acids, vitamins, organic acids, lactic acid and carotenoids etc (Schneider et al; 2011).

The different strains of the bacterium used for the production of the amino acids are Brevibacterium, Serratia and Corynebacterium. The variety of large scale production of amino acids such as L-Lysine, L-alanine, L-threonine, L-proline, aromatic amino acids were initiated by the rDNA technology. The vitamins were produced in large quantities such as riboflavin, biotin and vitamin C by the rDNA technology. Furthermore, a wide variety of organic acids were produced such as lactic acid, acetic acid, succinic acid etc. The secondary metabolites were also produced by the rDNA technology. The secondary metabolites are produced by the modification of the primary metabolites. The secondary metabolites are not involved in growth and development but involved in defense mechanisms by serving as antibiotics. B-lactams, cephalosporin and Penicillin G are the antibacterial provide defense against bacterial infection.

The production of the chymosin was initiated by the recombinant DNA technology (Mohanty et al; 1999). Chymosin is a proteolytic enzyme produced from the stomach of calf. The enzyme is responsible to coagulate the milk which is important for the milk digestion in the young animals. Later on, curd is obtained as the milk clots. The curd is then processed to form cheese. By this technique, a larger quantity of cheese was not possible to produce. The recombinant DNA technology came into the frontline to produce the recombinant cheese (Mohanty et al; 1999).

The fungi mainly produce the recombinant cheese through the rDNA technology. The bacteria can also be used to produce the recombinant cheese. The technique used in the production of cheese by the rDNA technology is same as described in the article. The basic approach is to obtain a tissue from the calf stomach to isolate the DNA from the cells. As the DNA gets isolated, it is being amplified by the PCR to amplify the chymosin gene to increase the number of gene fragments. Then these fragments are ligated in to the suitable vector most commonly is the plasmid vector. Once, the plasmid composed of the gene of interest is inserted into the host cell i.e. targeted microbes. Then the microbes start producing the chymosin due to the self-replicating ability of the recombinant plasmid (containing the gene of interest) and finally, the product can be screened and purified.

The most widely used fungi for the chymosin production by the rDNA technology are Aspergillusniger and Escherichia coli. The metabolic pathways and cell genetics of these fungi are very well known and this is the reason they are widely used in the rDNA technology in the production of various products, amino acids, proteins etc. These products are highly beneficial for humans and can be obtained in the larger quantities. Due to the sacrifice of large number of the claves in the production of cheese, the rDNA technology came forward to fulfill the desire in the production of the beneficial products which not only stopped the requirement of the animal sacrifice but also initiated the production on a large scale to fulfill the need of the world (Stryjewska et al; 2013).

5.4 Environmental Concerns: Pollutants are the molecules, particles or the elements that pollute the environment and cause deleterious health effects. There are different types of the pollutants that may pollute air, water or soil. Pollutants are generally the by-product of human. The excessive use of chemicals by the human in day to day life has made the environment polluted and have disturbed the environmental health and balance (Khan et al; 2016). A variety of methods have been incorporated to reduce the rate of pollution. There are

some natural methods to reduce the effect of pollutants which are low cost and safe too. Although there are three types of methods physical, chemical and biological to reduce the pollution, yet none of them found effective completely to resolve the issue.

The physical method was labor intended, chemical method involved the use of hazardous chemical which was not safe to be applied for, later the biological involved the use of micro-organisms to degrade the pollutant which was termed as bioremediation involved the process of the biodegradation. There are few xenobiotic compounds that are found in the environment and act as a pollutant.

The rate of pollution is more as compared to resolving methods that are being engaged. To solve this issue, rDNA technology opened the hands again to resolve the elevating rate of pollution in the environment by degrading the xenobiotic compounds effectively. The rDNA technology evolved the micro-oraganism ability to degrade the environmental pollutants such as xenobiotics by detecting the dagradative genes and transforming them into the appropriate host cell via suitable plasmid vector possessing all the suitable conditions.

Hence, genetically modified organisms were used to the bioremediation process to restore the environmental health and safety by degradation of the large number of the complex compounds. Thus, the rDNA technology was involved in the bioremediation of the environmental pollutant effectively (Khan et al; 2016). The recombinant DNA technology play a wide variety of role in combating deleterious human diseases, elevating agricultural productivity, industrial benefits as well as resolving environmental issues as depicted in figure 2.

II. DISCUSSION

Recombinant DNA technology is a well suited technology for the therapeutics of various health disorders. It is one of the emerging tools in the healthcare and industry to cure severe genetic and infectious diseases (Khan et al; 2016 and Stryjewska et al; 2013). It has been a blessing to the mankind in resolving diseases which are life threatening such as hemophilia, diabetes,hepatitis B, HIV and various infectious diseases (Vajo et al; 2001, Johnson et al; 1983).

A variety of enzymes are required to make the recombinant DNA technology possible. Restriction enzymes are tools of rDNA technology in cutting the DNA into smaller fragments so to fit them in the desired vector as per the insert capability. The restriction enzymes cut the vector as well so that restricted DNA fragments can fit in to the vector in a similar fashion as the key fits into a lock. The same restriction enzyme is used to cut the vector and DNA of interest to obtain complementary sticky ends to make easier for DNA ligases to work upon them by joining the DNA of interest into the vector accordingly (Sambrook et al; 2001 and Roberts; 2005). Depending on the size of the DNA to be inserted, the vector is selected accordingly. To carry small size of DNA, the plasmid vector like pBR322, pBR323 fits in to it. If the size of insert is 40-45Kbp then bacteriophage lambda is best suited otherwise BAC/YAC is best for 100 Kbp/1000Kbp respectively.The most commonly used vectors are plasmids and bacteriophage lambda vectors due to their high copy number speciality (Chiang et al; 1988 and Cue et al; 2001).

The locking of the restricted DNA fragments into the vector is made possible by the DNA ligases. DNA ligases joins the DNA fragments into the vector to make a recombinant vector which means a vector composed of DNA of interest. As soon the recombinant vector gets ready, it is introduced in to the host (bacterial) cell by transformation or by other techniques. The bacteria replicate to produce multiple copies.

Finally, the recombinant colonies undergo a screening strategy which differentiates well between the recombinant and non-recombinants. The vector is composed of a selectable marker gene like antibiotic resistance gene which show resistance to certain antibiotics such as tetracycline, ampicillin etc. A wide variety of screening techniques such as blue-white screening or antibiotic resistance test can be initiated to screen the recombinant colonies/plaques from the non-recombinant colonies/plaques (Bell et al; 1999).

III. CONCLUSION

This review article clearly defines the basic approach to rDNA technology and its applicability in the real world such as healthcare, diseases, agriculture, industry and environmental concern. The article states about the basic approach of rDNA technology from cutting the DNA of interest to the creation of the recombinant vector followed by the screening of recombinants as well. The entire approach can be very well summarized in the figure 3of this article to understand the basics of the rDNA technology.

The rDNA technology or gene cloning is a blessing to the living world especially a “lifeline to human health”. It can be stated or concluded as a “two sides of the same coin” where on one side humans are possessing a unhealthy lifestyle and moving towards disease onset reaching towards severity in many of the cases, on the other side of the coin “rDNA” technology is acting as a life saver.

The rDNA technology have been utilized in the production of vaccines such as insulin, Hepatitis B, HIV, human growth hormone, antibiotics, cytokines and gene therapy as well. Besides, its applicability in healthcare it is moving towards environment too covering major environmental issues.

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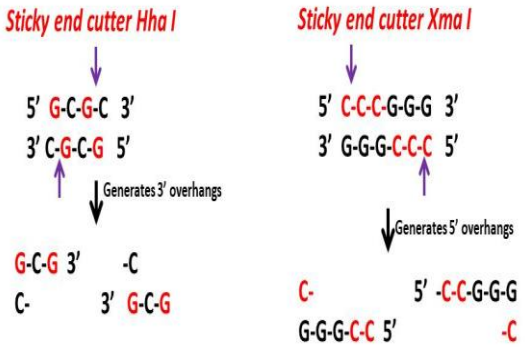
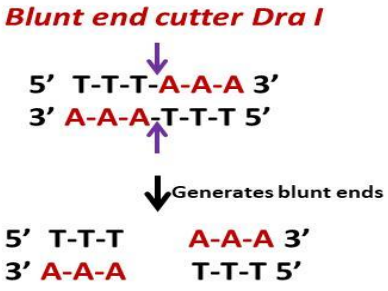
IV. FIGURE LEGENDS



(a)



(b)

Fig 1.A diagram depicting the restriction enzyme to generate sticky and blunt ended DNA fragment.

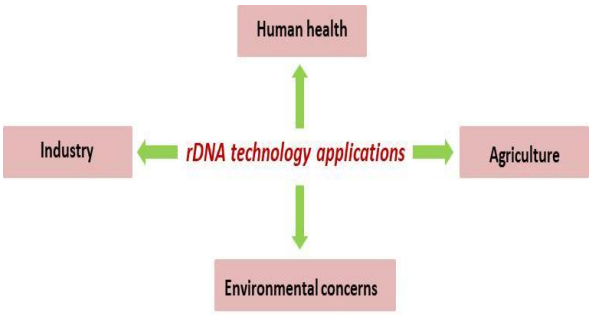


Fig 2.The flowchart reveals the applicability of the r-DNA technology in various sectors.

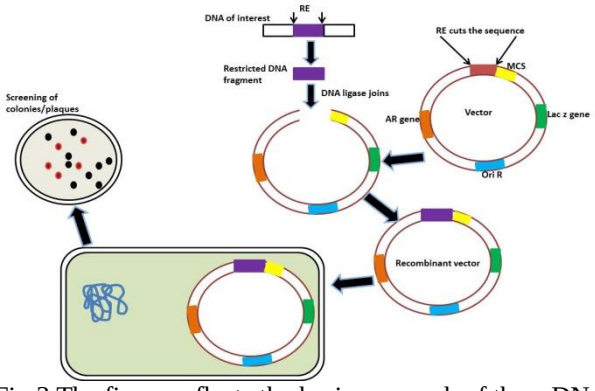


Fig 3.The figure reflects the basic approach of the r-DNA technology.

V. ACKNOWLEDGEMENT

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REFERENCES

[1] Ashwini M, Murugan SB, Balamurugan S, Sathishkumar R (2016) [Advances in Molecular Cloning].MolBiol (Mosk).; 50(1):3-9. Russian. doi: 10.7868/S0026898416010134. PMID: 27028806.

[2] Baga MR, Fowler BD, Chibbar NR (2005) Construction and characterization of a BAC library of a coldtoleranthexaploid wheat cultivar. Crop Science vol. 45, no. 4, pp. 1571–1577.

[3] Bell A, Feng X, Reder TA (1999) Efficient and Rapid Procedure for Blue-White Screening of Recombinant Bacterial Clones. BioTechniques 27:1126-1128

[4] Chauthaiwale VM, Therwath A, Deshpande VV (1992).Bacteriophage lambda as a cloning vector. MicrobiolRev; 56(4):577-91. doi: 10.1128/mr.56.4.577-591.1992. Erratum in:Microbiol Rev 1993 (1):290-1. PMID: 1480110; PMCID: PMC372889.

[5] Chen K, Wang Y, Zhang R, Zhang H, Gao C. CRISPR/Cas (2019) Genome Editing and Precision Plant Breeding in Agriculture. Annu Rev Plant Biol; 70: 667-697. doi: 10.1146/annurev-arplant-050718-100049. PMID: 30835493.

[6] Chiang CS and Bremer H (1988) Stability of pBR322-derived plasmids. Plasmid 20: 207–220.

[7] Cohen JI (2010) The varicella-zoster virus genome.Curr Top MicrobiolImmunol; 342:1-14. doi: 10.1007/82_2010_10. PMID: 20225013; PMCID: PMC3413377.

[8] Collins J, Hohn B (1978) "Cosmids: a type of plasmid gene-cloning vector that is packageable in vitro in bacteriophage lambda heads". Proceedings of the National Academy of Sciences of the United States of America75 (9): 4242–6

[9] Cue D and Feiss M (2001) Bacteriophage lambda DNA packaging: DNA site requirements for termination and processivity. J. Mol. Biol 311:233–240.

[10] Fayerman, J (1986) New Developments in Gene Cloning in Antibiotic Producing Microorganisms. Nat Biotechnol **4,** 786–789

[11] Gil D and Bouche PJ (1991) ColE1-type vectors with fully repressible replication. Gene 105:17–22.
[12] Hung CH, Wensink CP (1984) Different restriction enzyme-generated sticky DNA ends can be joined in vitro, Nucleic Acids Research Volume 12, Issue 4, Pages 1863–1874
[13] Johnson IS (1983) Human insulin from recombinant DNA technology. Science 219(4585):632–637.
[14] Katz L, Chen YY, Gonzalez R, Peterson TC, Zhao H, Baltz RH (2018) Synthetic biology advances and applications in the biotechnology industry: a perspective. J IndMicrobiolBiotechnol. (7):449-461. doi: 10.1007/s10295-018-2056-y. Epub 2018 Jun 18. PMID: 29915997.
[15] Khan S, Ullah MW, Siddique R, Nabi G, Manan S, Yousaf M, Hou H (2016) Role of Recombinant DNA Technology to Improve Life.Int J Genomics; 2016:2405954. doi: 10.1155/2016/2405954. Epub 2016 Dec 8. PMID: 28053975; PMCID: PMC5178364.
[16] Lal RA, Hoffman AR (2019) Perspectives on long-acting growth hormone therapy in children and adults. Arch EndocrinolMetab; 63(6):601-607. doi: 10.20945/2359-3997000000190. PMID: 31939485; PMCID: PMC7237337.
[17] Loenen WA, Dryden DT, Raleigh EA, Wilson GG (2014) Type I restriction enzymes and their relatives. Nucleic Acids Res; 42(1):20-44. doi: 10.1093/nar/gkt847. Epub 2013 Sep 24. PMID: 24068554; PMCID: PMC3874165.
[18] Lund T, Grosveld GF and Flavell AR (1982) 'Isolation of transforming DNA by cosmid rescue', Proc. Natl. Acad. Sci. USA 79, 520–4.
[19] Mohanty KA, Mukhoupadhyay KU, Grover S, Batish KV (1999) Bovine chymosin: production by rDNA technology and application in cheese manufacture. BiotechnolAdv 17(2-3):205-17.
[20] Moralli D, Monaco ZL (2020) Gene expressing human artificial chromosome vectors: Advantages and challenges for gene therapy. Exp Cell Res.; 390(1):111931. doi: 10.1016/j.yexcr. Epub 2020 Feb 29. PMID: 32126238; PMCID: PMC7166075.
[21] Murray NE (2000) Type I restriction systems: sophisticated molecular machines (a legacy of Bertani and Weigle) Microbiol. Mol. Biol. Rev 64:412–434
[22] Nilmalgoda DS, Cloutier S, Walichnowski ZA (2003) Construction and characterization of a bacterial artificial chromosome (BAC) library of hexaploid wheat (Triticumaestivum L.) and validation of genome coverage using locus-specific primers, Genome vol. 46, no. 5, pp. 870–878.
[23] Paoletti MG, Pimentel D (1996) Genetic engineering in agriculture and the environment-assessing risks and benefits. BioScience 46(9):665– 673.
[24] Pingoud A, Fuxreiter M, Pingoud V, Wende W (2005) Type II restriction endonucleases: structure and mechanism. Cell Mol. Life Sci 62:685–707
[25] Rao DN, Dryden DT and Bheemanaik S (2014) Type III restriction-modification enzymes: a historical perspective. Nucleic Acids Res 42, 45–55
[26] Roberts RJ (2005) how restriction enzymes became the workhorses of molecular biology. Proc. Natl. Acad. Sci. U.S.A 102, 5905–5908.
[27] Roberts RJ, Vincze T, Posfai J and Macelis D (2010) REBASE—a database for DNA restriction and modification: enzymes, genes and genomes. Nucleic Acids Res 38, D234–D236.
[28] Schneider J, Niermann K, Wendisch FV (2011) Production of the amino acids l-glutamate, l-lysine, l-ornithine and l-arginine from arabinose by recombinant Corynebacteriumglutamicum. Journal of BiotechnologyVolume 154, Issues 2–3 Pages 191-198
[29] Smillie C, Garcillán-Barcia MP, Francia MV, Rocha EP, de la Cruz F (2010) Mobility of plasmids". Microbiology and Molecular Biology Reviews74 (3): 434–52.
[30] Sternberg N (1990) Bacteriophage P1 cloning system for the isolation, amplification, and recovery of DNA fragments as large as 100 kilobase pairs. Proceedings of the National Academy of Sciences of the United States of America 87: 103–107.
[31] Stryjewska A, Kiepura K, Librowski T, Lochyński S (2013) Biotechnology and genetic engineering in the new drug development. Part I. DNA technology and recombinant proteins. Pharmacol Rep; 65(5):1075-85. doi: 10.1016/s1734-1140(13)71466-x. PMID: 24399704.
[32] Vajo Z, Fawcett J, Duckworth C.W (2001) Recombinant DNA Technology in the Treatment of Diabetes: Insulin Analogs. Endocrine Reviews Volume 22, Issue 5, Pages 706–717
[33] Whitelaw CB, Sang HM (2005) Disease-resistant genetically modified animals. Rev Sci Tech 24(1):275–283.