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Applications and Protocols of Recombinant DNA Technology in Medical Science

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Abstract: Recombinant deoxyribonucleic acid (DNA) is an artificial form of DNA that cannot be found in natural organisms. It is made in the laboratory by joining together genes taken from different sources. This is done by selecting and cutting out a gene at a specific point on a strand of DNA using restriction enzymes which act as molecular scissors. The gene is chosen on the basis of its ability to code for or alter different traits in another organism. It is inserted into a circular piece of bacterial DNA, called a plasmid, or a bacterial virus, called a phage and then put into a host organism, such as the *Escherichia coli*, for replication by its cell machinery. Recombinant DNA technology has enabled scientists to provide cheap, pure and readily available medicines for a variety of illnesses. By using restriction enzymes, plasmids and ligases, scientists can cut and paste the genes for almost any protein into bacteria and express usable proteins. These proteins include human growth hormone, insulin and many others used to treat dwarfism, diabetes, stroke and heart attacks, as well as cancer. In our case, still it is in its infant stage when it is compared with the neighboring countries and a close look at the current situation reveals a number of constraints and gaps that contributed to the underdevelopment of biotechnology. Hence, the government should give emphasis to the role of biotechnology especially rDNA in rural development and must enhance the development and application of this technology.

Key words: Gene · Phage · Plasmid · Recombinant DNA · Restriction Enzyme

INTRODUCTION

The scientific possibility of recombinant DNA (rDNA) technology began with the work of the Austrian monk Gregor Mendel in the 1860s. He studied selective breeding and trait inheritance in pea plants and kept highly detailed documentation of his research. The significance of his genetic studies was not noticed by the scientific community immediately. In fact, his work was not recognized until 1900, when it was rediscovered and reaffirmed. The idea of rDNA was first proposed by Peter Lobban, a graduate student of Prof. Dale Kaiser in the Biochemistry Department at Stanford University Medical School [1]. The first publications describing the successful production and intracellular replication of recombinant DNA appeared in 1972 and 1973 [2].

The cornerstone of most molecular biology technologies is the gene. To facilitate the study of genes, they can be isolated and amplified. One method of isolation and amplification of a gene of interest is to clone the gene by inserting it into another DNA molecule that serves as a vehicle or vector that can be replicated in living cells. When these two DNAs of different origin are combined, the result is arDNA molecule. Although genetic processes such as crossing-over technically produce rDNA, the term is generally reserved for DNA molecules produced by joining segments derived from different biological sources. The rDNA molecule is placed in a host cell, either prokaryotic or eukaryotic. The host cell then replicates (Producing a clone) and the vector with its foreign piece of DNA also replicates. The foreign DNA thus becomes amplified in number and following its amplification can be purified for further analysis [3].

Recombinant DNA is a form of artificial DNA that is made through the combination or insertion of one or more DNA strands, therefore combining DNA sequences as per your requirement, within different species i.e. DNA sequences that would not normally occur together [4]. Recombinant DNA technology is the technique of genetic engineering in which recombinant DNA is prepared by cutting the DNA into small fragments and joining different

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fragments together taken from different organisms. This technique makes it possible to take any gene from any species and place this gene in any other organism or species. It is similar to cloning because when the foreign gene is incorporated in an organism like bacteria then multiple copies are made through cloning to use the gene in different application [5].

Genetic engineering has applications in medicine, research, industry and agriculture and can be used on a wide range of plants, animals and microorganisms. In medicine, genetic engineering has been used to mass produce insulin, human growth hormones, follistim (For treating infertility), human albumin, monoclonal antibodies, anti-hemophilic factors, vaccinesand many other drugs [6].

Therefore, the objectives of this seminar paper are:

- To illustrate the range of techniques whichare used in recombinant DNA technology.
- To highlight the applications of recombinant DNA in medicine.

Basic Techniques of Recombinant DNATechnology: The organism under study, which will be used to donate DNA for the analysis, is called donor organism. The basic procedure is to extract and cut up DNA from a donor genome into fragments containing from one to several genes and allow these fragments to insert themselves individually into opened-up small autonomously replicating DNA molecules such as bacterial plasmids. These small circular molecules act as carriers, or vectors, for the DNA fragments. The vectormolecules with their inserts are called rDNA because they consist of novel combinations of DNA from the donor genome (Which can be from any organism) with vector DNA from a completely different source (Generally a bacterial plasmidor a virus). The rDNA mixture is then used to transform bacterial cellsand it is common for single recombinant vector molecules to find their way into individual bacterial cells. Bacterial cells are plated and allowed to grow into colonies. An individual transformed cell with a single recombinant vector will divide into acolony with millions of cells, all carrying the recombinant vector. Therefore an individual colony contains a very large population of identical DNA inserts and this population is called a DNA clone. A great deal of the analysis of the cloned DNA fragment can be performed at the stage when it is in the bacterial host. Later, however, it is often desirable to reintroduce the cloned DNA back into cells of the original donor organism to carry out specific manipulations of genome structure and function [7].

Isolation of the Gene of Interest and Use of Restriction Endonuclease Enzyme: Every gene manipulation experiment requires a source of nucleic acid, in the form of either DNA or RNA. It is therefore important that reliable methods are available for isolating these components from cells. The three basic requirements are: opening the cells in the sample to expose the nucleic acids for further processing, separation of the nucleic acids from other cell components and recovery of the nucleic acid in purified form. A variety of techniques may be used, ranging from simple procedures with few steps up to more complex purifications involving several different stages [8].

DNA is extracted from the organism under study and is cut into small fragments of a size suitable for cloning. Most often this is achieved by cleaving the DNA with a restriction enzyme. Restriction enzymes are extracted from several different species and strains of bacteria, in which they act as defense mechanisms against viruses. They can be thought of as “Molecular scissors, ” cutting the DNA at specific target sequences. The most useful restriction enzymes make staggered cuts; that is, they leave a single-stranded overhang at the site of cleavage. These overhangs are very useful in cloning because the unpaired nucleotides will pair with other overhangs made using the same restriction enzyme. So, if the donor DNA and the vector DNA are both cut with the same enzyme, there is a strong possibility that the donor fragments and the cut vector will splice together because of the complementary overhangs [9].

Bacteria have a variety of restriction endonucleases that cleave DNA at more than a hundred distinct recognition sites, each of which consists of a specific sequence of four to eight base pairs. Since restriction endonucleases digest DNA at specific sequences, they can be used to cleave a DNA molecule at unique sites. For example, the restriction endonuclease EcoRI recognizes the six-base-pair sequence GAATTC. This sequence is present at five sites in DNA of the bacteriophage λ, so EcoRI digests λ DNA into six fragments ranging from 3.6 to 21.2 kilo bases long [10]. Restriction enzymes (Endonucleases) 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 [4].

Table 1: Recognition sites of representative restriction endonucleases

Enzyme	Source	Recognition site
BamHI	Bacillus amyloliquefaciens H	GGATCC
EcoRI	*Escherichia coli* RY13	GAATTC
HaeIII	Haemophilusaegyptius	GGCC
HindIII	Haemophilusinfluenzae Rd	AAGCTT
HpaI	Haemophilusparainfluenzae	GTTAAC
HpaII	Haemophilusparainfluenzae	CCGG
MboI	Moraxella bovis	GATC
NotI	Nocardiaotitidiscaviarum	GCGGCCGC
SfiI	Streptomyces fimbriatus	GGCCNNNNNGGCC
TaqI	Thermusaquaticus	TCGA
HInfI	Haemophilusinfluenzae	GANTC
Sau3A	Staphylococcus aureus	GATC

Source: (http://www.ncbi.nlm.nih.gov/books/ NBK9950/table/A450/?report=objectonly)

Separation of the Segment of DNA by Gel Electrophoresis: Gel electrophoresis is the process by which genetic materials and proteins can be separated by charge and size and is one of the most indispensable tools in a biological laboratory. From one gel, a wide range of information can be extracted, such as how large a protein is or if a gene was successfully inserted into a fragment of DNA. For this reason, gel electrophoresis has become a staple in all DNAand protein analysis. Gel electrophoresis consists of a few basic steps: preparing agel, loading samples and then finally switching on the electricity. The final product is a visual schematic of what each segment of DNA is like in terms of size and charge [11].

The technique of gel electrophoresis is vital to the genetic engineer, as it represents the main way by which nucleic acid fragments may be visualized directly. The method relies on the fact that nucleic acids are polyanionic at neutral pH; that is, they carry multiple negative charges because of the phosphate groups on the phosphodiester backbone of the nucleic acid strands. This means that the molecules will migrate towards the positive electrode when placed in an electric field. As the negative charges are distributed evenly along the DNA molecule, the charge/mass ratio is constant; thus, mobility depends on fragment length. The technique is carried out using a gel matrix, which separates the nucleic acid molecules according to size. The type of matrix used for electrophoresis has important consequences for the degree of separation achieved, which is dependent on the porosity of the matrix. Two gel types are commonly used: agarose and polyacrylamide. Agarose gels are usually run using the submerged agarose gel electrophoresis (SAGE) technique. Polyacrylamide-based gel electrophoresis (PAGE) is sometimes used to separate small nucleic acid molecules; in applications such as DNA sequencing as the pore size is smaller than that achieved with agarose. Electrophoresis is carried out by placing the nucleic acid samples in the gel and applying a potential difference across it. This potential difference is maintained until a marker dye (Usually bromophenol blue, added to the sample prior to loading) reaches the end of the gel. The nucleic acids in the gel are usually visualized by staining with the intercalating dye ethidium bromide and examining under UV light. Nucleic acids show up as orange bands [8].

Principle of gel electrophoresis First, a slab of gel material is cast. Gels are usually cast from agarose or polyacrylamide. These gels are solidand consist of a matrix of long thin molecules forming sub-microscopic pores. The size of the pores can be controlled by varying the chemical composition of the gel. The gel is cast soaked with buffer. The gel is then set up for electrophoresis in a tank holding buffer and having electrodes to apply an electric field. The pH and other buffer conditions are arranged so that the molecules being separated carry a net negative charge so that they will be moved by the electric field from left to right. As they move through the gel, the larger molecules will be held up as they try to pass through the pores of the gel, while the smaller molecules will be impeded less and move faster. This results in a separation by size, with the larger molecules nearer the well and the smaller molecules farther away. Note that this separates on the basis of size, not necessarily molecular weight [12].

Protocols: Separation of Nuclear and Organelle DNA Using Cesium Chloride Gradient: This protocol describes steps after obtaining high molecular weight nucleic acids most typically through phenol chloroform preparations. First, measure the volume of solution of

total DNA that will be used for the cesium chloride gradients. For every ml of solution, add 1 g of finely ground cesium chloride (Using a mortar and pestle) to the solution slowly, keeping it warm by placing the tube in one's hands. Agitate the tube gently. Then, while the cesium chloride is dissolving, add 15 ml of ethidium bromide (10 mg/ml stock) to a Beckman 13 ml quick seal tube. After that, add the DNA/cesium chloride solution to the quick seal tube till the volume reaches the lower part of the neck of the seal. Then balance tubesand seal. Then, centrifuge at 40, 000 rpm for 40-48 hrs. Once centrifugation is complete, carefully remove tubes without disturbing the gradient and place in a rack. Then, use a small, hand-held UV lamp to visualize the bands. There should be at least 4 bands in the tubes. The lowest density band (And therefore the highest one) is the mitochondrial DNA; the next one is the plasmid DNA. The third lower band is sheared rDNA and the bottom band is the nuclear DNA. Then, using a large gauge needle to puncture a hole next to the fraction that is to be isolated. If all 3 fractions are to be isolated, then start with the topmost band (Mitochondrial) and work down the layers. Then, slowly draw up the band until everything is collected and place in separate collection tubes. Then, extract DNA with 100% iso-butanol two to three times to remove the ethidium bromide by mixing an equal volume of iso-butanol and inverting the tubes several times. The aqueous phase (Top) containing the DNA should lose the pink color of ethidium bromide with each wash. Extract one extra time after all ethidium bromide appears to be removed from aqueous phase. After collecting all 3 types of DNAs, the solutions must be dialyzed in tris-EDTA (TE) buffer solution (Usually several changes of 1-2 L overnight) at 4°C. This will remove all the cesium chloride. After dialysis, the DNA can be used for several techniques (PCR, restriction, digest, Southern and Northern blot analysis and genomic libraries). This solution must be kept at 4°C (Freezing the DNA will shear the longer strands) [13].

DNA Sequencing: The ability to determine the sequence of bases in DNA is a central part of modern molecular biology and provides what might be considered the ultimate structural information. Rapid methods for sequence analysis were developed in the late 1970s and the technique is now used in laboratories worldwide. In recent years the basic techniques have been revolutionized by automation, which has improved the efficiency of sequencing to the point where genome sequencing is possible [8].

Principles of DNA Sequencing: The determination of a DNA sequence requires that the bases are identified in a sequential technique that enables the percussive identification of each base in turn. There are three main requirements for this to be achieved: rDNA fragments need to be prepared in a form suitable for sequencing. The technique used must achieve the aim of presenting each base in turn in a form suitable for identification. The detection method must permit rapid and accurate identification of the bases. Automated sequencing procedures tend to use fluorescent labels and a continuous electrophoresis to separate the fragments, which are identified as they pass a detector. There are two main methods for sequencing DNA. In one method, developed by Allan Maxam and Walter Gilbert, chemicals are used to cleave the DNA at certain positions, generating a set of fragments that differ by one nucleotide. The same result is achieved in a different way in the second method, developed by Fred Sanger and Alan Coulson, which involves enzymaticsynthesis of DNA strands that terminate in a modified nucleotide. Analysis of fragments is similar for both methods and involves gel electrophoresis and autoradiography (Assuming that a radioactive label has been used). The enzymatic method or chain termination method has now almost completely replaced the chemical method as the technique of choice, although there are some situations where chemical sequencing can provide useful data to confirm information generated by the enzymatic method [8].

The chemical method of DNA sequencing consists in determining the nucleotide sequence of a terminally labeled DNA molecule by breaking it at adenosine, guanine, cytosine and thymine with chemical agents. Partial cleavage at each base produces a nested set of radioactive fragments extending from the labeled end to each of the positions of the base. The autoradiograph of a gel produced from four different chemical cleavages, shows a pattern of bands from which the sequences are read directly [14]. The chain termination method depends on DNA replication and termination of replication at specific sequences. For that, Sanger's technique is based on an enzymatic synthesis from a single-stranded DNA template with chain termination on DNA polymerase, using dideoxynucleotides (ddNTPs). The principle of this method relies on the dideoxynucleotide lacking a 3'OH group, which is required for extension of the sugar phosphate backbone. Thus, DNA polymerases cannot extend the template copy chain beyond the incorporated ddNTP. Both methods rely on four-lane high-resolution

polyacrylamide gel electrophoresis to separate the labeled fragment and allow the base sequence to be read in a staggered ladder-like fashion. Sanger sequencing was technically easier and faster, becoming the main basis of DNA sequencing, being modified and automated to aid large scale sequence procedure [15].

Automatic Sequencing: An automatic sequencing is an improvement of Sanger sequencing, through the use of different fluorescent dyes incorporated into DNA extension products primers or terminator. The use of different fluorophores in the four bases specific extension reactions means that all reactions can be loaded in a single lane. For each base one color are used, emitting a different wavelength when excited. Throughout electrophoresis, the fluorescence signs are detected and recorded [16].

Amplification by PCR: The Polymerase chain reaction (PCR) is elegantly simple in theory. When a DNA duplex is heated, the strands separate or melt. If the single stranded sequences can be copied by a DNA polymerase, the original DNA sequence is effectively duplicated. If the process is repeated many times, there is an exponential increase in the number of copies of the starting sequence. The length of the fragment is defined by the 5 ends of the primers, which helps to ensure that a homogeneous population of DNA molecules is produced. Thus, after relatively few cycles, the target sequence becomes greatly amplified, which generates enough of the sequence for identification and further processing [8].

PCR is an in vitro method for amplifying a selected nucleic acid sequence. To target the amplification to a specific DNA segment, two primers bearing the complementary sequences that are unique to the target gene are used. These two primers hybridize to opposite strands of the target DNA, thus enabling DNA polymerase to extend the sequence between them. Each cycle produces a complementary DNA strand to the target gene. Consequently, the product of each cycle is doubled, generating an exponential increase in the overall number of copies synthesized [12].

Cloning of DNA in the Host Cell Using Vectors: In every case, the recombinant DNA must be taken up by the cell in a form which it can be replicated and expressed. This is achieved by incorporating the DNA in a vector. A number of viruses, both bacterial and of mammalian cells can serve as vectors. Cloning vector is a DNA molecule that carries foreign DNA into a host cell, replicates inside a bacterial or yeast cell and produces many copies of itself and the foreign DNA. The molecular analysis of DNA has been made possible by the cloning of DNA. The two molecules that are required for cloning are the DNA to be cloned and a cloning vector. Vectors are specialized plasmids, phages or hybrids which have been developed to make the construction of recombinant libraries and the identification of individual recombinant clones simpler. Three features of all cloning vectors are: Sequences that permit the propagation of itself in bacteria or in yeast for YACs, a cloning site to insert foreign DNA; the most versatile vectors contain a site that can be cut by many restriction enzymes and a method of selecting for bacteria or yeast for YACs containing a vector with foreign DNA; usually accomplished by selectable markers for drug resistance [12].

The term ‘Clone’ means, exact copy of the parent. A duplicate or look like carrying the same genetic signature or genetic map. Cloning is the best application of recombinant DNA technology and could be applied to something as simple as DNA fragment or larger, sophisticated mammalian specie such as humans. Molecular cloning is carried out in-vitro where a specific fragment of DNA is isolated from an organism ‘donor’ and introduced into a ‘Plasmid’ that replicates in a ‘Host’ cell making multiple copies of that DNA fragment. Plasmids are double stranded DNA that are usually circular and mostly found inside certain bacterial specie e.g. *E. coli*. However, most plasmids are now commercially available, ready to be used, providing specific fragment insertion sites. Plasmids in genetic engineering are also known as ‘vectors’. Vectors also include viruses known as bacteriophage that use bacteria as their host to replicate. Hence, a bacteriophage can be used to transfect and create several copies of the DNA fragment of interest by replicating several times in a bacterium [4].

Microinjection and Gene Transfer: Microinjection is a proven and relatively simple method for introducing DNA into worms [17] Moreover, microinjection is a very effective approach to RNA interferenceand can be used to deliver synthetic mRNAs or other molecules directly to cells [18-20]. Microinjection is an effective method for creating transgenic animals, for RNAi of selected genesand for introducing various types of molecules directly to cells. For DNA transformation, the easiest approach is to inject DNAs into the distal arm of the gonad [17].

Microinjection directly into oocyte nuclei can induce chromosomal integration of transgenes, but this technique is relatively difficult to do [21]. The probability of formation of stably transformed mammalian embryos as a result of direct gene microinjection may depend on the stage of development of the egg or embryo at the time of injection. The optimal time for gene microinjection may coincide with the fertilization process [22].

It is based on the injection of a foreign DNA construct into a fertilized oocyte. The construct integrates randomly into the host oocyte genome, subsequently the zygote continues embryonic developmentand the embryo is transferred to a foster mother and eventually develops to a transgenic animal. However, this method has strong limitations: on average, less than 1 percent of embryos injected and 10 percent of animals born are transgenic, genes can only be added, not replaced or deletedand multiple copies of the transgene are inserted at random, hindering the correct regulation of gene expression and possibly interfering with endogenous gene function [23]. This requires large amounts of oocytes to be injected, as the overall efficiencyof the process is very low and basically a trial and-error process, whose outcome can only be influenced to a small extent [24].

Applications of Recombinant DNA Technology in Medicine: Recombinant DNA technology had made it possible to treat different diseases by inserting new genes in place of damaged and diseased genes in the human body. It has brought many revolutionary changes in the field of medicine and introduced such methods of treating diseases and delivering the drug which were just imaginary. Recombinant DNA technology has allowed the scientists to develop human insulin which is a hormone made up of protein by usingthe bacteria as ahost cell and it is also available in the market. It is believed that the drugs produced through microbes are safer than the drugs produced traditionally like vaccine which is also a biological substance and it is prepared from the suspension of weak or dead pathogenic cells. This technology enables the scientists to develop vaccines by cloning the gene used for protective antigen protein. Viral vaccines are most commonly developed through this technology for example, Herpes, Influenza, Hepatitis and Foot and Mouth Disease. In recent years, scientists have developed many growth hormones using recombinant DNA technology. Human growth hormones, it is a polypeptide hormone. It is responsible for growth, reproduction of the cells and regeneration in humans as well as animals. It is secreted by somatotrophic cells present in the pituitary glands and the disease dwarfism is treated with this hormone [25].

When a foreign object enters the body, immune system of the body releases a specific protein called as antibody. Hybridoma technology has made it possible to produce monoclonal antibodies. In this technique, the lymphocytes or B cells are joined with myeloma cells; the resulting substance is called as hybridoma. This hybridoma produces unlimited antibodies called monoclonal antibody in the culture. These antibodies are used to produce vaccines against different viral infections; a glycoprotein which has the ability to block the multiplication or division of viruses in the cells or in the nearby cells is called as interferon. Interferon can be used to treat cancer like hairy cell leukemia. Recombinant DNA technology produces this protein using *E. coli*. Interferon alpha is used to treat lymphoma and myelogenous leukemiaand alsoantibiotics which are chemical substances that are used against bacterial infections such as penicillin is produced using recombinant DNA technology [5].

The chemical structure of everyone's DNA is the same. The basic difference between two individual's DNA is the order of base pairs. Using these sequences, every person could be identified solely by the sequence of their base pairs. Scientists usually use a small numbers of sequences of DNA that are known to vary among individuals. In medicine, DNA fingerprinting has application in genetic counseling, proof of parentageand identification of criminals in thefts etc. Since a person inherits his or her VNTRs (Variable numbers of tandem repeats), which are, dispersed islands throughout the genome and are made up of a variable numbers of end to end duplications of identical or almost identical sequences of 280 each. VNTRs are polymorphic due to difference in numbers of repeat units at a given locus or position in a chromosome from his or her parents. Thus, analysis of VNTR patterns can be used to establish paternity and maternity. DNA can be isolated from blood, hair, skin cells etc and can be compared with that of a suspected criminal for a particular VNTR pattern. Novel disease diagnostic approaches have been developed by biotechnology which are efficient, specific precise and rapid. Among these use of probes is the most important. Probes are small (15-30 bases long) nucleotide (DNA/RNA) sequences used to detect the presence of

complementary sequences in nucleic acid samples. Use of probes for disease diagnosis is advantageous over conventional diagnostic tools like in the following ways: High specificity, rapid and much simpler, no culturing is required, can detect infections even in a very latent stage where antibodies are yet not generated, probe can be easily prepared and a single species-species probe can identify all the serotypes of pathogen [26].

Advantages and Disadvantages of Recombinant DNA Technology: Recombinant DNA technology provides substantial quantity, no need for natural or organic factors, tailor made product that you can control, unlimited utilizations, cheap, resistant to natural inhibitors. Whereas the disadvantages of recombinant technology are commercialized and became big source of income for businessmen, effects natural immune system of the body, can destroy natural ecosystem that relies on organic cycle, prone to cause mutation that could have harmful effects, major international concern: manufacturing of biological weapons such as botulism and anthrax to target humans with specific genotype and concerns of creating super-human race [4].

Genetic engineering promises to increase the quality of human life in the near future if done in the proper way. But if handled carelessly rDNA practice may come up with dangerous outcomes. People continue to worry about the danger of recombinant DNA work. There is immediate and obvious concern that cancer or toxin gene will escape from the laboratory. In other words, recombinant DNA technology could accidentally create a bacterium or plasmid that contained toxin or tumor gene. The modified bacterium or plasmid could then accidentally infect people. The most hazardous experiments which deal with manipulation of tumor virus and toxin genes requires extreme care including negative pressure, air blocks to laboratory and experiments must be done in the laminar flow hoods, with filtered or incinerated exhausted air [27].

Moreover, critics persist in offering a number philosophical argument against transgenic animals. Transgenic animal violet species integrity. Patenting of live reflects domionistic and materialistic attitude towards living being that preclude proper regard for their inherent nature, says John Hoyt, president of human society of USA. Similarly in some people, cloning of animal raise numerous ethical questions such as, Is it man preview to select the genetic composition of animals and propagate these animals? But most consider unacceptable [28].

Status of Biotechnology in Ethiopia: Ethiopia is one of the major genetic centers of origin with huge biodiversity of flora and fauna. A few examples of the legacy of Ethiopia to the rest of the world as a source of important genes include the yellow dwarf virus (BYDV) resistance gene found in barley [29] mlo-11 and related genes for powdery mildew resistance gene [30].

Around 65 projects are underway in the country and most of them are in areas of tissue culture, bio-fertilizers, molecular markers, embryo transfer , immunology, vaccine and diagnostic kit development and epidemiology, mainly for crops such as coffee, grass pea, teff and forest trees [31, 32]. The institutes involved in biotechnology in Ethiopia include the Addis Ababa University (AAU), Ethiopian Institute of Agriculture Research (EIAR), Federal Research Centers (FRCs), Institute of biodiversity conservation and research (IBCRI), National Veterinary Institute (NVI)and National Health and Nutritional Institute [32]. Biotechnology research and development in Ethiopia is at its infant stage compared to the neighboring countries [33].

The lack of guidelines, lack of proper assessments of the level of biotechnological capacity available in some institutions and research centers, lack of public's appreciation of opportunities provided by agro-biotechnology and financial constraints suggest that the government need to move steadfastly to address the situations. Ethiopian, being a party to the Cartagena protocol and one of the eligible countries, has prepared its Nation Bio-Safety Framework (NBF) through financial assistance from Global Environment Fund. This NBF comprises of policy, legal administrative and technical instrument that have been development, handling and use of Living Modified Organisms (LMOs), also referred to as Genetically Modified Organisms (GMOs) and their products that emanate from modern biotechnology and may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risk to human health and finally a big step taken by the Ethiopian government is approval of bio-safety law by the parliament on the 7th of July 2009 which expected to encourage genetic engineering research as well marketing of its products in the country in the responsible way. Genetic engineering offers several benefits when used responsibly by addressing the environmental and food safety concerns with rigorous bio safety regulations. Until recently, guidelines with genetic engineering

research and exploitation of GMOs do not exist in the country. This situation discourage Ethiopian scientists from initiating genetic engineering projects and participating in similar network activities at regional and international level and consequently significantly hampering the research and capacity building process in modern biotechnology research and development in the country [34].

CONCLUSION AND RECOMMENDATIONSR

Recombinant DNA technology allows us to manipulate every DNA of living organisms and to make conscious change in that DNA. There are various means of manipulating DNA and means of transforming DNA to a recipient cell like transformation. Moreover, one can do with the DNA that has been transferred to a recipient cell. The transferred DNA may be from the same species or from a different species than the recipient. Such successfully transferred DNA is said to be cloned. The purpose of doing recombinant DNA technology are many and various among them; to repair a genetic defect, to enhance an effect already natural to that organism like to increase its growth rate, growth hormone, to increase an already existing immunity such as vaccine, to enable microorganisms to do something it would not normally do such as to produce human insulin for diabetics, or a sheep to produce a human blood clotting protein in her milk; to make monoclonal antibodies, interferon's, antibiotics and for effective diagnosis of infectious disease and also to do DNA fingerprints and forensics. Recombinant DNA technology comes up undesirable outcome unless handled carefully and there are some fundamental ethical objections to the production of and use of genetically modified animals and products. But in our country's case, even though it has conducted research on various fields like plant tissue culture, use of molecular markers, diagnostics and characterization of crop disease, animal production and healthand bio-fertilizer and bio-pesticides, but still it is in its infant stage when it is compared with the neighboring countries and a close look at the current situation reveals a number of constraints and gaps that contributed to the underdevelopment of biotechnology.

Therefore based on the above conclusion, the following recommendations are forwarded in order to strengthen the national biotechnology capacity in the country and realize its benefits:

- The government should give emphasis to the role of biotechnology especially rDNA in rural development and must enhance the development and application of this technology.
- Research and academic institutions of the country should also use recombinant technologies for the development of disease diagnostic kits, vaccines and therapeutic agents.
- Universities offering biotechnology course should upgrade their laboratory in terms of manpower and facilities also it must be handled carefully not to release some undesirable products to the environment.
- There should be sufficient financial resources and institutional linkage within the country as well as collaborations with foreign academic and industrial entrepreneurs in the field of biotechnology.
- The wise utilization of the country's biodiversity by molecular characterization and introduction of marker assisted breeding and isolation of potentially useful genes should be promoted.

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