GENETIC ENGINEERING AND ITS USE

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Abstract

Genetic engineering is the set of the techniques using for manipulation of DNA. Basically this technique involves gene cloning. For cloning of DNA, four essential components, restriction enzymes, DNA library for gene isolation, vector and analysis of cloned DNA, are needed. There are different applications of genetic engineering. Medicine and agriculture are the areas in which rapid progress have been made. A large number of vaccines and other pharmaceutical drugs and transgenic plants and animal are being produced by genetic engineer and some of them are being released to the markets for use.

Keywords: genetic engineering, gene cloning, restriction enzymes, vectors, gene isolation, analysis of cloned DNA

Genetik Mühendisliği ve Kullanımı

Özet

Genetik mühendisliği DNA manipulasyonu için kullanılan teknikler setidir. Temel olarak genetik mühendisliği gen klonlamayı içerir. DNA klonlanması için dört zorunlu komponente, restriksiyon enzimleri, gen isolasyonu için DNA kütüphanesi, vektör ve klonlanmış DNA'nın analizi, ihtiyaç duyulur. Genetik mühendisliğinin birçok farklı uygulamaları vardır. Eczacılık ve ziraat en fazla ilerlemelerin yapıldığı alanlardır. Genetik mühendisleri tarafından çok sayıda aşı ve diğer eczacılıkla ilgili ilaçlar ve transgenik bitki ve hayvanlar üretilmekte ve bunlardan bazıları kullamı için pazarlara sürülmektedir.

Anahtar Kelimeler: Genetik Mühendisliği, Gen Klonlama, Restriksiyon Enzimleri, Vektör, Gen İzolasyonu, Klonlanmış DNA Analizi

1. Introduction

The definition of biotechnology is that 'the application of biological organisms, systems or processes to manufacturing and service industries' (Trevan *et al.*, 1997). Biotechnology has opened a new way in science for scientists after developing such techniques like PCR and recombinant DNA technology called new biotechnology (Gubta, 1996).

Genetic engineering originated in the 1970s as the result of development of several techniques. The first, DNA sequencing, allows the sequence of bases in any stretch of DNA to be determined. The second technique is making recombinant DNA in the test-tube using enzymes isolated from microorganisms to cut and join pieces of DNA together. Hence by cutting and joining bits of viruses, plasmids and mobile genetic elements together, appropriate vectors are made for transferring genes from a donor species to a recipient species that do not naturally interbreed with it. The third technique is the chemical synthesis of DNA of any desired base sequence. A fourth technique, the Polymerase Chain Reaction (PCR), discovered in 1988, allows specific gene sequence(s) in a

mixture to be rapidly replicated many tens or hundreds of thousand times, and is extensively used in forensic DNA finger-printing (Ho, 1997).

The aim of this review is to discuss the basic understanding of the major techniques used in genetic engineering.

2. Genetic engineering

Techniques for isolating, modifying, multiplying and recombining genes from different organisms are genetic engineering. Manipulating DNA has allowed breakage of DNA at two desired places to isolate a specific DNA segment and then insert it in another DNA molecule at a desired position (Fig.1) The product obtained via these techniques is called recombinant DNA and the technique often called genetic engineering or referred as recombinant DNA (rDNA) technology (Gupta, 1996; Lewin, 1997).

Using this technique we can isolate and clone single copy of a gene or a DNA fragment into an indefinite number of copies, all identical. Geneticists can transfer any genes between species belonging to different kingdoms. For example a fish gene can be transferred into potato, human genes can be transferred to sheep, pig or the bacterium, *Esherichia coli that* inhabit the gut of all mammals. This is achieved due to vectors like plasmids and phages reproduce in a host (e.g. *E. coli*) in their usual manner even after insertion of foreign DNA, so that the inserted DNA will also replicate faithful with the parent DNA (Ho, 1997).

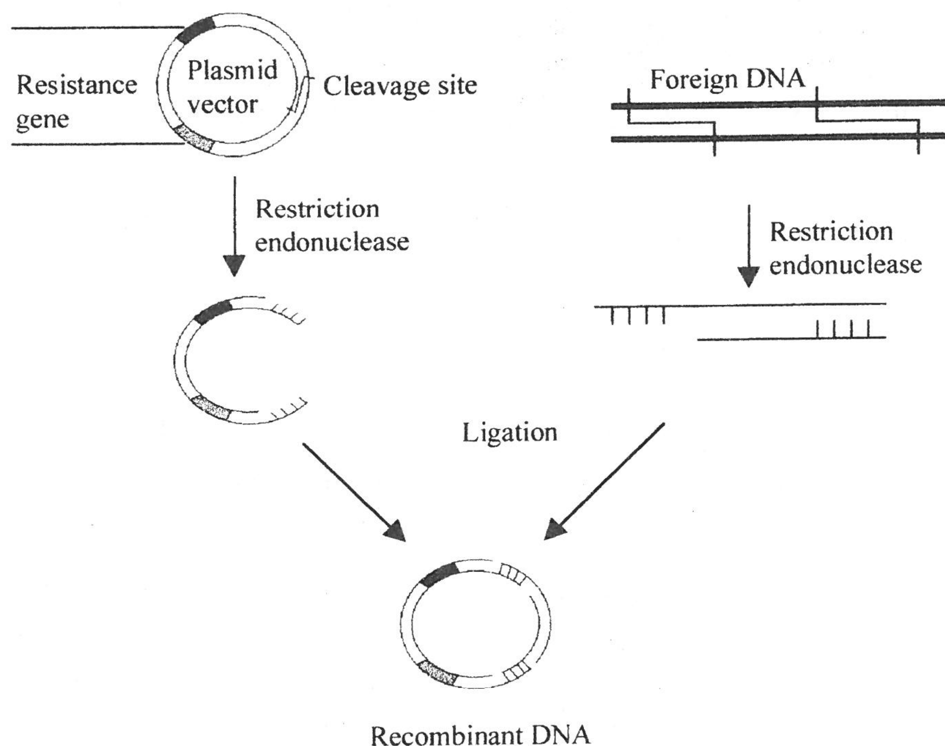

Figure 1. Recombinant DNA technology (Old and Primrose, 1996).

2. Gene cloning

Gene cloning is essentially the insertion of a specific piece of foreign DNA into a cell, in such a way that the inserted DNA is replicated and handed onto daughter cells during cell division (Trevan *et al.,* 1997). This involves the construction of novel DNA molecules by joining sequences from different sources. Cloning a fragment of DNA allows indefinite amounts to be produced from even a single original molecule (Lewin, 1997). There are varieties of different procedure for cloning DNA into either plasmid or viral vectors but the basic scheme of events is broadly the same. The basic procedures involve series of steps (Trevan *et al.,* 1997):

1-DNA fragments are generated by using enzymes so called restriction endonuclease that recognize and cut DNA molecule at specific nucleotide sequences.

2-The fragments produced by digestion with restriction enzymes are joined to other DNA molecules that serve as vectors.

3-The recombinant DNA molecule, consisting a vector carrying an inserted DNA segment, is transferred to a host.

4-As host cells replicate, the recombinant DNA is passed on to all progeny, creating a population of identical cells, all carrying the cloned sequence.

5-The cloned DNA segments can be recovered from the host cell, purified, and analysed.

6-Potentially, the cloned DNA can be transcribed its mRNA translated, and the gene product isolated and studied.

2.1. Restriction Endonucleases

At the heart of gene cloning lie the restriction endonucleases (Trevan *et al.,* 1997). Cloning of DNA becomes a reality after identification and manipulation of restriction endonucleases in the 1960s and early 1970s (Turner *et al.,* 1998). Restriction enzymes, which naturally occur in bacteria as a weapon against the introduction of foreign DNA into the cell, restrict or prevent viral infection by degrading the invading nucleic acid (Trevan *et.al.,* 1997).

Restriction enzymes are named for the organism in which they were discovered, using a system of letters and numbers (Table. 1). The name of enzyme is always written in italics. This letter codes denotes the origin of enzymes. For example *Eco*RI comes from *Esherichia coli* and *Hae*III comes from *Haemophilus aegyptus.* In certain cases more than one enzyme are isolated from single origin, which is denoted by Roman numerals (I, II, III) to distinguish the enzymes. e.g. *Hind* I, *Hind* II, *Hind* III etc. (Old and Primrose, 1996).

There are three types of restriction endonucleases (Old and Primprose, 1996). Type II enzymes are most important for cloning purposes. (Table. 1). Restriction enzymes recognize short sequences of double stranded DNA as targets for cleavage (Stryer, 1995). Different enzyme recognize different, but specific sequences, each ranging in length from 4 to 8 base and hydrolyse a phosphodiester bond in each strand in this region. The recognized sequence is palindromic and the cleavage sites are symmetrical positioned e.g.

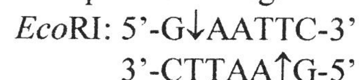

Table. 1. Some restriction enzymes using in gene cloning (Old and Primrose, 1996).

Microbial Orijin	Enzyme	Recognition Site
Escherichia coli	*Eco*RI	5'- G↓AATTC -3'
Hemophilis influenze	*Hind*III	5'- A↓AGCTT -3'
Hemophilus aegypius	*Hae*III	5'- GG↓CC -3'
Providencia ituartri	*Pst*I	5'- CTGCA↓G -3'
Seriatia marcescens	*Sma*I	5'- CCC↓GGG -3'
Xanthomonas malvacearum	*Xma*I	5'- C↓CCGGG -3'
Bacillus amyloliquefaciens H	*Bam*HI	5'- G↓GATCC -3'

2.1.1. Restriction digestion

Restriction digestion of plasmid or genomic DNA is carried out using restriction enzyme and buffer solution. DNA is incubated with appropriate enzyme and buffer at the optimum temperature (usually 37°C) in volume of perhaps 20µl. (Turner *et al.,* 1998).

2.1.2. Gel electrophoresis

Restriction fragments can be separated by electrophoresis and visualized when a DNA molecule is cut by a restriction enzymes. Agarose gel will separate larger DNA fragments but polyacrylamide gel are needed to separate small DNA fragments (Hames *et al.,* 1998; Stryer, 1995)

Agarose which is a polysaccharide derived from seaweed is used for electrophoresis (Sambrook *et al.,* 1989). When an electric field applied to an agarose gel in the presence of a buffer solution, which will conduct electricity, DNA fragments move through the gel towards the positive electrode (DNA is highly negatively charged) at the rate that is dependent on its size and shape. Small linear fragments move more quickly than large ones. Hence, this process of electrophoresis may be used to separate mixture of DNA fragments on the basis of size. The DNA is stained by the inclusion of ethidium bromide that binds to DNA, in the gel. The DNA shows up as an orange band on illumination by UV light (Turner *et al.,* 1998).

2.1.3. Ligation of restriction fragments

If DNAs from different sources share the same palindromic recognition sites, both will contain complementary single-stranded tails when treated with a restriction endonuclease. If the cut fragments are placed together under proper conditions, the DNA fragments from these two sources can form recombinant molecules by hydrogen bonding of the sticky ends (Fig. 2). The enzyme DNA ligase can be used to covalently link the phosphate-sugar backbones of two fragments, producing a recombinant DNA molecule (Hames *et al.,* 1998).

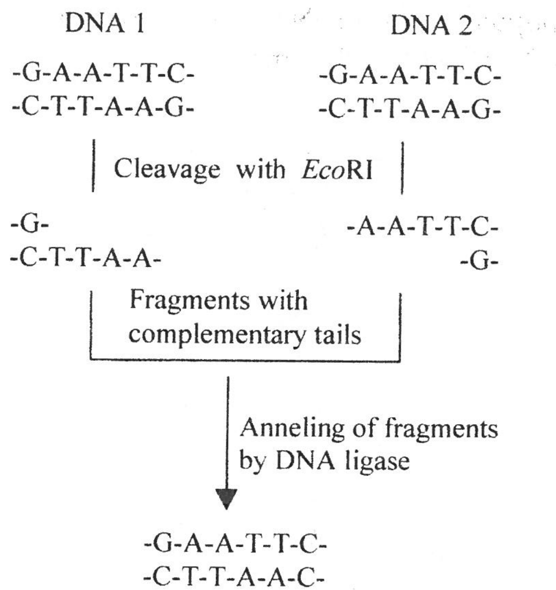

Figure 2. Ligation of DNA molecules (Stryer, 1995)

3. Cloning vectors

DNA cloning can be achieved with the help of another DNA molecule. This other DNA molecule is called as a vector, which could be a plasmid, a bacteriophage, a derived cosmid or phagemid, transposon or even virus. All vectors have common characteristics. They are typically small, well-characterized molecules of DNA. They contain at least one replication origin and can be replicated within the appropriate host, even when they contain foreign DNA. They code for a phenotypic trait as a selectable marker that can be used detect their presence. Another feature of any cloning vector is that it should possess at a site at which foreign DNA can be inserted without disrupting any essential function. Sometimes vectors are modified by inserting a DNA segment, which has restriction sites for enzymes to create unique site(s). This inserted DNA is called a polylinker (Gupta, 1996). Alternative vector systems are used for cloning different sized DNA fragments for different purposes. Generally, plasmid vectors are used for sub-cloning experiments because they are small and easy to handle. Plasmid vectors carry markers allowing the selection of transformed cells, which form colonies on selective media. The vector pBR322 was one of the first genetically engineered plasmids to be used in recombinant DNA (Fig.3).

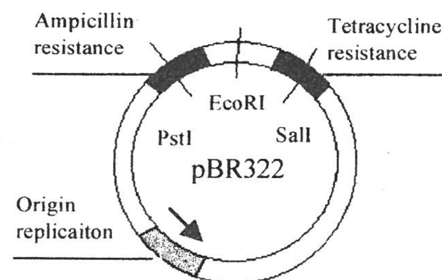

Figure. 3. Genetic map of pBR322 (Stryer, 1995)

Bacteriophage λ vectors are used for DNA library construction because they have a greater capacity than plasmids, they are more stable in long-term storage, and plaques are easier to screen than colonies (Paterson, 1996; Twyman, 1998). There are different type of vectors, which exploit features of both phage and plasmids. One of them is cosmid which is a plasmid carrying a λ *cos* site, allowing them to be packaged into phage heads. The basic cosmid is very small, so these vectors can accommodate large donor DNA fragments and are used for genomic library construction. Another one is phagemids which are the plasmids carrying the bacteriophage M13 (or similar) origin of replication, which allows the plasmid to replicate as a single-stranded DNA phage. These vectors are used to produce single-stranded DNA for applications such as

sequencing, *in vitro* mutagenesis and probe synthesis. The other one is the phasmids, which are composite λ-plasmid vectors, basically lambda insertion vectors containing an entire plasmid. Such vector, e.g. λ ZAP, are extremely versatile, allowing cDNA libraries to be constructed in phage vectors but excised as plasmids for easy downstream manipulations, without sub-cloning (Twyman, 1998). There are also some cloning vectors which they have very large amount of DNA cloning capacities like yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC) and their derivations (Paterson, 1996).

4. Isolation of DNA

The techniques used in genetic engineering allow any DNA sequence to be inserted into a vector and cloned to facilitate further analysis and manipulation. It may be possible isolate the desired donor DNA fragment directly, and insert it into a vector for cloning where the source DNA is not complex. If the source of a particular target sequence is complex, it is necessary to construct a DNA library, a representative collection of all DNA fragments from a particular source cloned in vectors (Old and Primrose, 1996; Twyman, 1998). DNA libraries are sets of DNA clones (a clone a genetically distinct individual or set of identical individuals), each of which has been derived from the insertion of a different fragment into a vector followed by propagation in the host (Turner *et al.*, 1998). There are two major types of library; genomic libraries, prepared from total genomic DNA and cDNA libraries, prepared by reserve transcription of a population of mRNA molecules (Fig. 4) (Darnell *et al.*; Twyman, 1998).

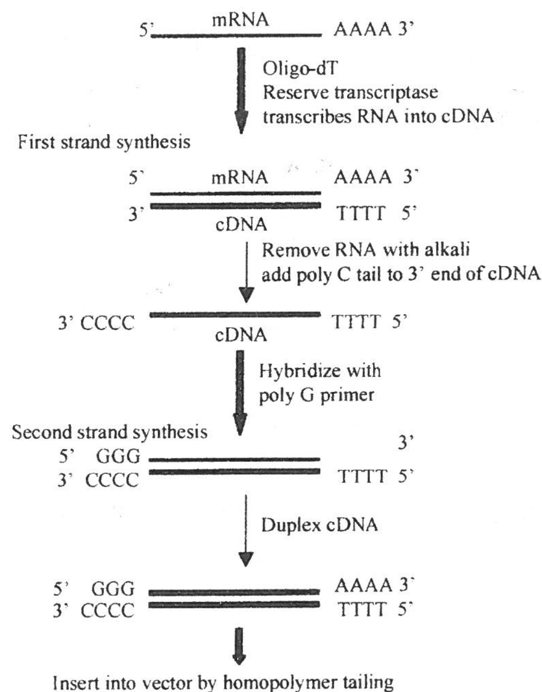

Figure 4. Preparation of a cDN (Darnell *et al.* 1990)

4.1. Screening Library

Identification of one particular clone containing the gene of interest from among the very large number of others in the gene library is called screening. Colony and plaque hybridisation methods can be used for screening of gene libraries. After the initial step both screening methods are essentially the same. If the library is available in the form of bacteria, bacterial colonies must be Lysol first to release their DNA, and this usually is done by growing a replica of the colonies on the dish directly on the membrane surface (replica plating) (Turner *et al*, 1998).

5. The analysis of cloned DNA

The identification and recovery of specific sequences is a powerful tool for the analysis of gene structure and function. Different techniques are used for characterisation of cloned DNA.

5.1. Restriction Mapping

After isolation of a novel clone, the first stage of analysis is the creation of a restriction map. A restriction map is a compilation number, order, and distance between restriction enzyme cutting sites along a cloned segment of DNA. Any double-stranded DNA can be cut by a variety of restriction enzymes that have different recognition sequences (Fig.5).

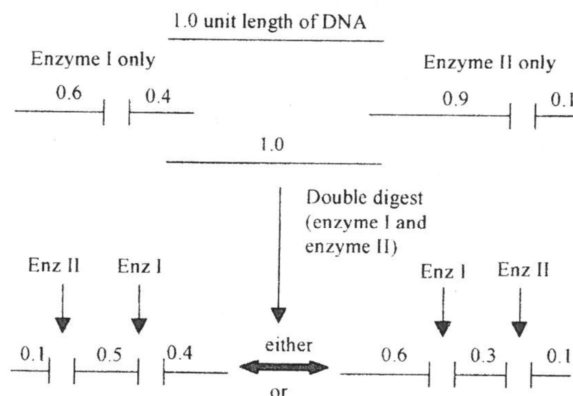

Figure 5. Mapping of the cleavage sites of two restriction enzymes (Darnell *et al.*, 1990).

It is possible to deduce where on the DNA molecule each restriction enzyme cuts by separating the restriction fragments and measuring their sizes by gel electrophoresis. A restriction map of the DNA molecule can be drawn showing the location of these cut sites. Two DNA molecules can be compared by looking at their restriction maps without determining the nucleotide sequence of each DNA. Restriction map shows the size of the donor DNA, and identifies sites which may be used for subcloning. (Twyman, 1998; Hames *et al.*, 1998).

5.2. Southern, Northern and Western Blots

A mixture of DNA, RNA, or protein can be separated by electrophoresis and separated bands can be confirmed via hybridisation of labelled probe to these bands (Gupta, 1996). Southern blots (for DNA), Northern blots (for RNA), Western blots (for protein) are carried out. The former is named after its inventor and the latters was extrapolated from the former (Turner *et al.*, 1998) The nucleic acid molecules are separated by agarose gel electrophoresis and transferred to nitro-cellulose membrane and then hybridised with a labelled nucleic acid probe. Washing removes nonhybridised probe, and the membrane is then treated to reveal the bands produced. (Figure. 6). Specific RNA species are detected on Northern blots, whereas the bands on Southern blots could be genes in genomic DNA or parts of cloned genes (Turner *et al.*, 1998). Western blotting is used for detection of particular proteins (Gupta, 1996).

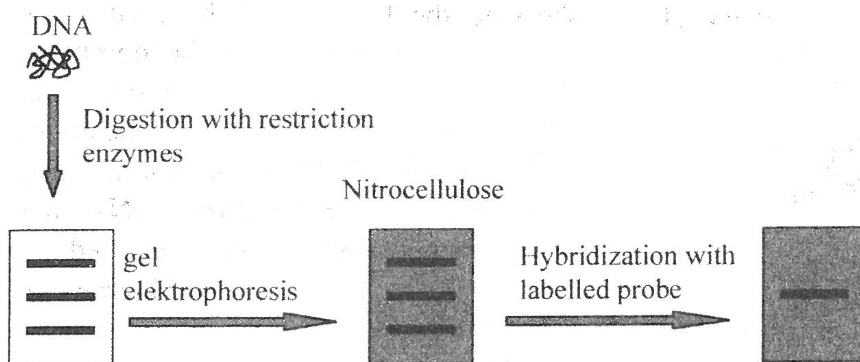

Figure 6. DNA hybridisation (Darnell *et. al.*, 1990).

5.3. DNA Sequencing

After cloning a gene or a DNA fragments, DNA can be sequenced. Two different methods are used for determination of DNA. One method of DNA sequencing devised by Alan Maxam and Walter Gilbert. The second method, which is commonly used, was developed by Fredrick Sanger and his colleagues (Fig. 7) (Twyman, 1998; Gubta, 1996).

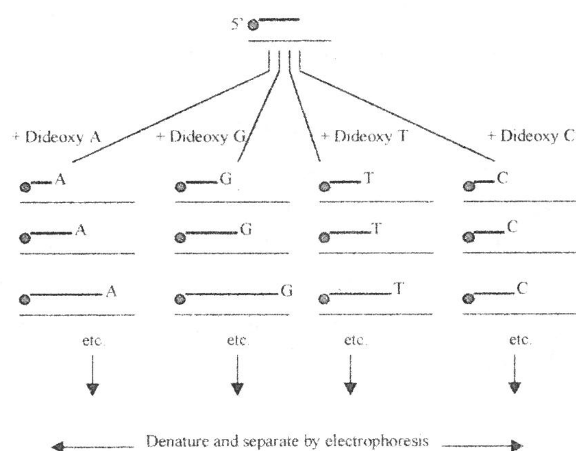

Figure 7. DNA sequencing by the Sanger (dideoxy) method (Darnell *et al.*, 1990).

Maxam and Gilbert sequencing involves the chemical degradation of a restriction fragment with reagents that modify defined bases. The Sanger sequencing method involves DNA synthesis, and each reaction includes a small amount of one of the four 2', 3'-dideoxynucleoside triphosphates (ddNTPs). This technique is often termed the dideoxy method or the chain terminator (Twyman, 1998). In this method, four incubation mixtures are set up, each containing the DNA template, a specific DNA primer, *E. coli* polymerase I and all four deoxyribonucleoside triphosphates (dNTPs), one of which is radioactively labelled. In addition, each mixture contains a different dideoxynucleoside triphosphate analog, ddATP, ddTTP, ddGTP, and ddCTP. In this reaction The DNA polymerase begins copying template molecules by extending the bound primer. The incorporation of the analog blocks further growth of the new chain because it lacks of 3'-hydroxyl terminus needed to form the next phosphodiester bond. Hence, fragment of various length are produced in which the dideoxy analog is the 3' end. Four such sets of chain-terminated fragments are then electrophoresed and the base sequence of the new DNA is read from

autoradiogram of the four lanes (Stryer, 1995).

5.4. PCR Analysis

The polymerase chain reaction (PCR) is a technique for amplifying DNA sequences in vitro (Twyman, 1998). Polymerase chain reaction involves synthesizing multiple copies of a gene, or a region of DNA from oligonucleotide primers which bind to opposite strands. The reaction requires the target DNA, the two primers, all four deoxyribonucleoside triphosphates and thermostable DNA polymerase such as *Taq DNA polymerase*. A PCR cycle consists of three steps, denaturation of the DNA, primer annealing and elongation. This cycle is repeated for a set number of times depending on the degree of amplification request. In this method, the reaction mixture is heated to 95° C for a short time period to denature the target DNA into single strands. The mixture is then rapidly cooled to a defined temperature which allows the two primers to bind to the sequences on each of the two strands flanking the target DNA (primer annealing). The temperature of the mixture is raised to 72° C and kept at this temperature for a pre-set period of time allow DNA polymerase to elongate each primer by copying the single stranded templates. DNA segment with the terminus consisting of the 5' end of the primer becomes a template for the next round, resulting in exponential amplification of original target DNA. Thus, in 20-30 cycles, it becomes possible to amplify the original sequence by a million-fold (Fig. 8) (Hames *et al.*, 1998).

There are many applications of PCR in molecular biology. Especially molecular marker techniques based PCR as well as RFLP (Restriction fragment length polymorphism) that are shown to be genetically linked to a trait of interest can be used for gene cloning, medical diagnostics, and for trait introgression in plant and animal breeding program (Gubta, 1996).

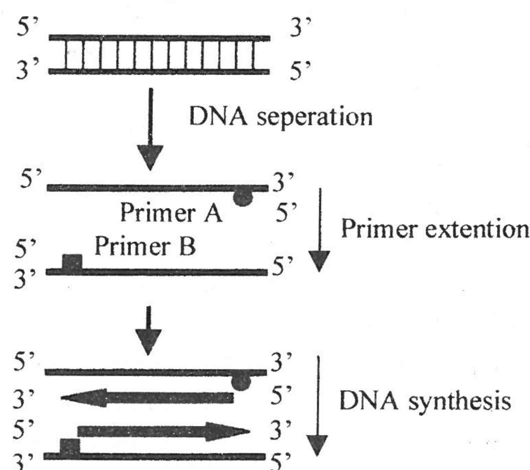

Figure 8. The Polymerase chain reaction (PCR) (Oldand Primrose, 1997).

6. Application of genetic engineering

The applications of recombinant DNA technology are almost without limit. Once a particular DNA clone has been isolated, it may be exploited in a great number of ways (Twyman , 1998). These include the biotechnological production of proteins, the generation of modified organisms, especially for improved food production, the development of test kits for medical diagnosis, the application of the polymerase chain reaction (PCR) and cloning in forensic science and studies of evolution, and the attempts to correct genetic disorders by gene therapy, etc. (Turner *et al.*, 1998).

The techniques mentioned here has also the potential for the study of the function of the genes by in vitro

manipulation of sequences (in vitro mutagenesis) that are then reintroduced into the original organisms from which they came, allowing dissection of gene function. Proteins of interest that are impossible to isolate from the original organism can be produced in bulk in bacteria cells. Characterisation of gene and genome structure, and of gene expression, gene mapping, functional analysis of gene and their products and gene transfer etc. can be studied (Twyman, 1998).

There is a big potential of genetic engineering in pharmaceutical. Many proteins that are normally produced in very small amounts are known to be missing or defective in various disorders (Turner *et al.*, 1998). The best-known example is a genetically engineered polypeptide used for the treatment of human is insulin. This hormone is essential for the correct regulation of blood sugars levels. There is a huge market for insulin in the world. Another group of polypeptides is the interferons that are produced naturally by the human body in response to viral infections. However, these compounds could be produced from human tissue. Other medical applications of genetically engineering are the production of vaccines. This involves cloning and expressing the genes for the coat proteins in bacteria, and using the bacterial product as a vaccine (Trevan *et al*, 1988). Insulin and interferons synthesised by bacteria have already been released for sale (Gubta, 1996).

A great variety of medical conditions arise from mutation. PCR primers have been used for medical diagnosis. By using sequence information to design PCR primers and probes, many tests have been developed to screen patients for clinically important mutations. Checking for the presence of mutations in a gene can confirm a diagnosis that is based on other clinical presentations. A large number of vaccines (including Hepatitis B), DNA probes and monoclonal antibodies (including ELISA tests) for diagnosis of various diseases, human growth hormone and other pharmaceutical drugs for treatment of diseases are being released or are in the process of being released. DNA fingerprinting techniques are used for identification of criminals like murderers and rapists though the study of DNA or antibodies from blood and semen stains, urine, tears, saliva, or hair roots, etc. (Gupta, 1996).

It can also be used to show pedigree in animals bred commercially and to disorder mating habits in wild animal disorders. Attempts have been made to treat some genetic disorders by delivering a normal copy of the defective gene to patients. This is known as gene therapy. The gene can be administered on its own or cloned into a defective virus used as a vector that can replicate but not cause infection. Gene therapy is in its infancy, but has great potential. (Turner *et al.*, 1998).

Another area that much progress is being done of genetic engineering is agriculture. Genetically modified organisms are created when cloned genes are introduced into germ cells. In eukaryotes, if the introduced genes are derived from another organism, the resulting transgenic plants or animals can be propagated by normal breeding. Several types of transgenic plant and animal have been created and tested for safety in the production of foodstuffs. (Turner *et al.*, 1998).

Transgenic animals have already produced in mice, pigs, goats, chicken, cow, etc., (Gubta, 1996). Transgenic sheep have been produced with the intention of producing valuable proteins

in their milk. Purification of the protein from milk is easier than cultured cells or blood (Turner *et al.*, 1998) Transgenic plants which is carrying desirable traits like disease resistance (Jongedijk *et al.*, 992), insect (Perlak *et al.*, 1990) and herbicide resistance (Gasser and Frayley, 1992), improved food quality (Townsed *et al.*, 1992), improved storage proteins by transformation (Sheeh *et al.*, 1988) have already been created.

On the other hand, It is possible to use of genetic engineering for solving some environmental problems like pollution control depletion of natural resources for non-renewable energy, restoration of degraded lands, biodiversity conservation. For instance, microbes are being developed to be used as bio-pesticides, biofertilizers, biosensors, etc., and for recovery of metal, cleaning of spilled oils and for a variety of other purposes (Gubta, 1996).

7. Result

Genetic engineering has created a new commercial environment with enormous applications. Different companies related to genetic engineering in the world have many commercial projects for helping to improve life quality due to engineered organisms. But unexpected toxins and allergens can be seen via genetically engineered foods. Rules and laws have been framed to safeguard against the risks, because of the fears about the implication of genetic engineering thinking that harmful organisms can be created via this technology.

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