Chapter 10

BIOTECHNOLOGY

Students' learning outcomes
After studying this chapter, students will be able to:

1. [B-12-J-01] Introduce genetic engineering.
2. [B-12-J-02] Explain polymerase chain reaction (PCR).
3. [B-12-J-03] Outline the Function of Restriction Enzymes.
4. [B-12-J-04] Describe plasmid as vector prokaryotes and Explain how recombinant plasmids can be formed.
5. [B-12-J-05] Define Genetically modified organism.
6. [B-12-J-06] Explain the formation of human insulin protein in bacteria.
7. [B-12-J-07] Describe how vertical food farms (soil free) work.
8. [B-12-J-08] Compare and contrast the advantages of vertical food farms with general agricultural practices prevalent in Pakistan.

The history of biotechnology is as old as the civilization history of man. Knowledge and use of biotechnology is traced back to earliest human society, when they realized to plant their own crops, breed and domesticate their own animals, for continuous supply of good food. But the term biotechnology was introduced by the end of 20th century. Biotechnology can be defined as **"use of living organisms especially microorganisms or their processes and products for the welfare of mankind"**. Use of biotechnology led human to produce yogurt and cheese from fermented milk, wine or beer from fermented juices of fruit, sugarcane or malted barley. Since early civilizations, people have been preparing the leather by tanning process which involves the use of multiple bacteria.

10.1 INTRODUCTION TO GENETIC ENGINEERING

Needs of rapidly growing human population, led the human to develop new animals with modified characteristics. Biologists imagined the possibility of growing more nutritive and naturally pest-resistant crops. Biotechnology is so vast field but this chapter touches a brief part of biotechnology, called **Genetic engineering**. Basis of genetic engineering lies on the technique called **Recombinant DNA Technology** which involves the combining DNA (genes) from different sources to create new genetic combinations. It deals with manipulation or alteration in genetic material of an organism to modify its characteristics or capabilities for human welfare. Genetic engineering of any organism can be done by adding, removing or editing specific genes within its genome. Thus the goal to give new traits to the organism, improve its existing traits or eliminate undesirable traits is achieved by genetic engineering. Human hope to develop unlimited and useful medicines and vaccines by modifying genome of microorganisms. Bacteria have been used to clean up environmental pollutants, increase the fertility of the soil and kill insect pest. Applications of genetic engineering are greatly helpful to humans in the following major fields:

Agriculture: Genetically modified (GM) crops are being produced that are resistant to pests, diseases or adverse environmental stresses.

Medicine: Research is being carried out to develop gene therapies for the treatment of genetic disorders and to find innovative and effective ways to combat fatal diseases like hepatitis and cancer. Improved biopharmaceuticals like insulin, antibiotics and vaccines are synthesized by genetic engineering.

Industry: Genetic engineering of microorganisms help in the production of biofuels, biodegradation of industrial and sewerage waste and development of environment friendly chemicals.

Genetic engineering is now one of the fastest growing areas of science, hence great genetic engineer **Craig Venter** rightly said that the 21st century is the **"Century of Biology"**. Biotechnology has been included among the six priority areas of Science and Technology by the National Commission of Science and Technology of Pakistan.

10.1.1 Recombinant DNA Technology

Recombinant DNA technology is the foundation of genetic engineering, which involves a series of steps to join DNA segments from different sources. This is an ***in-vivo*** (in living cells) method which is used for the synthesis of product of gene beside copies of desired gene (gene of interest) at industrial scale. It involves the selection and isolation of gene of interest, inserting it in a suitable vector and the transformation into suitable host by the recombinant DNA (rDNA). Components or requirements of recombinant DNA technology include:

i. Gene of interest
ii. Molecular scissors
iii. Molecular carrier or vector
iv. Molecular glue
v. Expression system

Gene of Interest

The gene of interest is the gene which is to be cloned. It can be obtained by one of the three possible ways:

a. Artificial gene synthesis is the process of synthesizing a gene ***in-vitro*** (Latin: "in glassware" means "outside the living cell") without template DNA samples with the help of DNA synthesizer machine.
b. Gene of interest can also be obtained by synthesizing it from its mRNA. Synthesis of gene from mRNA is carried out by **reverse transcriptase enzyme** which is naturally found in retroviruses. The DNA formed by this process is called complementary DNA (cDNA).
c. In most of the cases the gene of interest is directly cleaved from a chromosomal DNA by using particular DNA scissors called restriction endonucleases.

10.2 MOLECULAR SCISSORS (RESTRICTION ENDONUCLEASE)

Restriction endonucleases are enzymes that cleave the phosphodiester bonds of both strands of DNA duplex at specific sequences. They are used to cut the gene of interest during genetic recombination. Restriction endonucleases are host-defense system in bacterial cell against infecting viruses. Name of the enzyme "restriction endonuclease" is given because they are naturally found inside the bacterial cell to "restrict" the growth of the infecting viruses by digesting (chopping-up) DNA/RNA of viruses at specific sequences called restriction sites or recognition sites. Bacteria protect their own DNA by modifying their own restriction sites, generally by adding methyl (CH_3) group to the nucleotides in their recognition sequences. Methyl groups at restriction sites block the endonuclease enzymes and protect the host (Bacterial) DNA from being cleaved by its own restriction endonucleases. Thus bacterial endonucleases can recognize and cleave only viral restriction sequences, which are usually un-methylated.

In 1970, the first restriction enzyme was isolated. Many different restriction endonucleases have been isolated so far. Generally, endonucleases are *named after* the name of the genus, species and strain of the bacteria from which it was initially

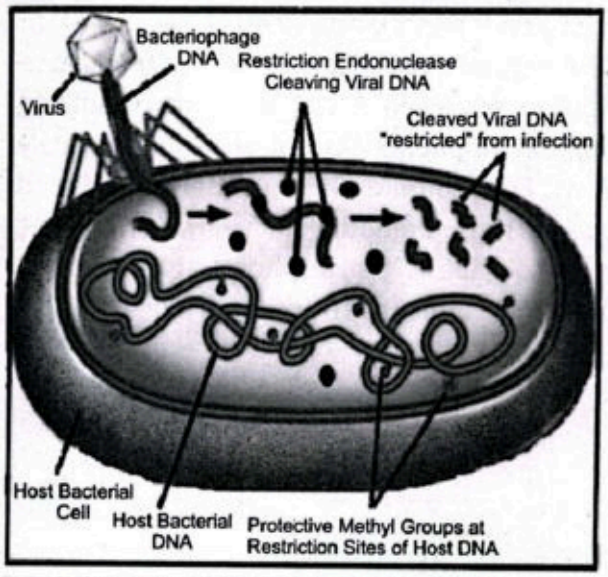

Fig. 10.1: Functioning of Restriction Endonucleases in Bacterial Cell

CRITICAL THINKING

- If restriction endonuclease enzymes are capable of destroying the DNA of bacteriophage, then why they *do not* break down the genomic DNA of host cell i.e. bacteria?
- The restriction enzymes that form sticky ends are more useful in genetic engineering. Why?

isolated. For example, *EcoRI* was isolated from *Escherichia coli*, *Hind-II* and *Hind-III* from *Haemophilus influenzae* and *XhoI* from *Xanthomonas holcicola*.

Restriction sites are recognized by endonuclease enzymes due the presence of specific palindromic sequences. A **palindromic sequence** is a four to eight base pairs in DNA in which nucleotides are arranged symmetrically in reverse order in both complementary DNA strands. Restriction enzymes either make staggered or blunt cut. A staggered cut is one in which the resulting duplex fragments show single stranded projected ends called **sticky ends**.

Sticky ends may be produced at 5' ends of the both DNA strands (by endonuclease *EcoR1*) or sticky ends may be produced at 3' end of both DNA strands (by Restriction endonuclease Kpn1). While in blunt cut the resulting duplex fragments do not show such sticky ends. For example, Blunt ends are produced by restriction endonuclease *HaeIII*, target sites for cleaving sugarphosphate backbone of DNA are indicated by green triangles.

SCIENCE TITBITS

Bacteria methylate their DNA shortly after synthesis during binary fission, by adding methyl (CH_3) groups to the nucleotides in the recognition sequences. This process is called **methylation** and is carried out by the modifying enzyme methyltransferase present in the bacterial cell.

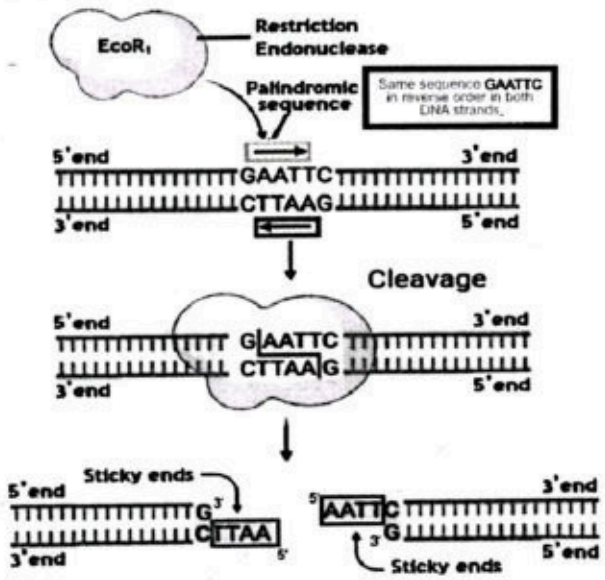

Fig. 10.2: Recognition of palindromic sequence by restriction endonuclease *EcoR1* and production of sticky ends at 5'end in both complementary DNA strands

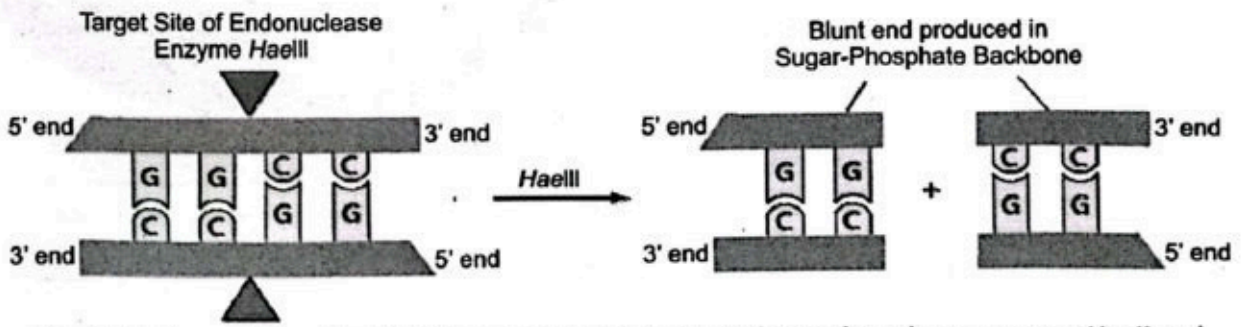

Fig. 10.3: Recognition of palindromic sequence by restriction endonuclease enzyme *HaeII* and production of blunt ends in Sugar-Phosphate backbone of double stranded DNA

10.3 MOLECULAR CARRIERS OR VECTORS

For the synthesis of recombinant DNA (rDNA), a molecular vector is needed to transfer recombinant DNA into a host cell. Vectors act as a vehicle for carrying foreign DNA (gene of interest) into a host cell for multiplication and its expression for the production of specific protein e.g. insulin. One common type of vector is a plasmid. Plasmids are small circular DNA

molecules naturally found in bacterial cells. Example of vectors other than plasmids are: lambda phage DNA, Cosmid (combination of plasmid and phage DNA), Yeast artificial chromosomes (YACs) and bacterial artificial chromosomes (BACs) etc.

Plasmids were discovered by investigators studying the sex life of the intestinal bacterium *Escherichia coli*. Plasmids are primarily found in bacterial cells but can also be found in some eukaryotes e.g. Yeast and some higher plants. They are separate from the chromosomal DNA so called extra chromosomal and can replicate independently. Plasmids carry genes for antibiotic resistance and fertility etc. One of the common plasmids discovered earlier *pSC-101* has antibiotic resistance gene for tetracycline, whereas *pBR-322* has antibiotic resistance genes for both tetracycline and ampicillin.

Plasmids are commonly used as vectors in genetic engineering due to many characteristics essentially needed as vector:

i. **Origin of Replication site:** Plasmids contain an origin of replication (Ori) site which is required for self-regulated replication of the plasmid in the host cell. Plasmids are ideal vector for producing multiple copies of a gene inserted in it.

ii. **Antibiotics resistant genes as selectable Marker:** Markers help in the selecting of cells (bacteria) that have successfully taken up the recombinant plasmids. The markers used for selection are the antibiotic resistance genes. Plasmids usually contain antibiotic resistance genes (e.g. genes for ampicillin or tetracycline resistance). When these genes are present in a bacteria it will be resistant to the specific antibiotic.

iii. **Restriction sites of different enzymes:** A vector should have a restriction site for the commonly used endonucleases. The plasmid which has at least one restriction site is required for the insertion of foreign DNA fragment. Presence of unique restriction sites provide flexibility in the choice of endonucleases for the easy insertion of foreign DNA.

iv. **Promoter regions for gene expression:** Plasmids may contain promoter sequences that enable the transcription of inserted genes in the host cell, thus facilitating the expression of the gene of interest.

v. **Small Size:** Plasmids are much smaller than chromosomal DNA, so they are easily purified as intact molecular vector and easily inserted into host cells.

vi. **High Copy Number:** Some plasmids are found in high copy numbers inside the host cell, resulting in large amounts of the cloned DNA.

10.3.1 *pBR322* as an example of Plasmids

It is the first and most common, purpose built plasmid vector, artificially developed in the laboratory by Bolivar and Rodriguez in 1977. ***pBR322*** is modified from natural bacterial plasmid. This vector has the origin of replication (ori), restriction site and also antibiotic-resistant genes. Here are the key features of the *pBR322* plasmid:

i. **Origin of replication:** It carries a fragment of the plasmid *pMB1* that acts as an origin for DNA replication to ensure the multiplication of the vector.

ii. **Size:** It is relatively small plasmid having 4,361 bps. Small size is important, as transformation efficiency is inversely proportional to size of plasmid. Transformation efficiency become very low if plasmid size is above 10 kbp, thus DNA segment up to 6 kbp can possibly be inserted in *pBR322* plasmid to form it recombinant.

iii. **Copy number:** It has relatively high copy number (~15 copies per cell)

iv. **Cloning sites:** It carries a number of unique restriction sites. Some of these are located in one of the antibiotic resistance genes (e.g., sites for *PstI*, *PvuI*, and *Sac I* are found in Ampicillin and *BamHI* and *HindIII* in Tetracycline).

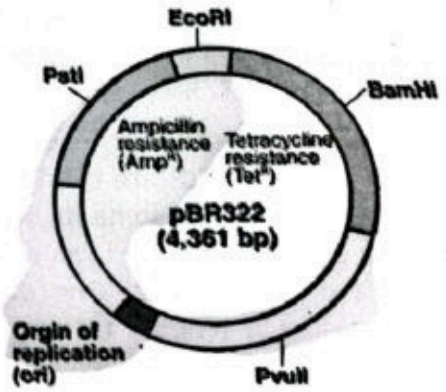

Fig. 10.4: *E. coli* cloning vector (*pBR 322* plasmid) having origin of replication (ori), restriction site and antibiotic-resistant genes

SCIENCE TITBITS

Types of Plasmids as Vectors

1. **Cloning Vectors:** These are used for the cloning of DNA fragments. They contain essential elements like Ori, selectable markers, and cloning sites.
2. **Expression Vectors:** These are designed for the expression of a gene in a host organism. They include promoters, ribosome binding sites, and termination sequences.
3. **Shuttle Vectors:** Plasmids that can replicate in multiple host species, such as in both prokaryotic and eukaryotic cells, are known as shuttle vectors. These are useful for gene transfer between different biological systems.

SCIENCE TITBITS

Applications of Plasmids

Gene Cloning: Plasmids are extensively used for cloning of genes of interest for further study and manipulation.

Protein Production: Expression vectors are used for the production of proteins by expressing inserted genes.

Gene Therapy: Plasmids are used to deliver therapeutic genes into cells of organisms with a genetic disease.

Vaccine Development: Most of the DNA vaccines are Plasmid based and are effectively used to prevent diseases.

10.3.2 Mechanism of formation of recombinant Plasmid in recombinant DNA technology

Cloning of the desired gene through recombinant DNA technology involves the formation of recombinant DNA (gene of interest + vector DNA), transformation into suitable expression system by the recombinant DNA and the identification of transformed clones.

Formation of recombinant Plasmid

The first step in the construction of a recombinant DNA, is the isolation and purification of gene of interest and relevant vectors. The gene of interest is cleaved directly from genome by specific restriction endonucleases with specific sticky ends or it may be synthesized from mRNA by reverse transcription using reverse transcriptase. Then specific vector DNA (plasmid) having same restriction site is also cleaved with same restriction enzyme by which gene of interest is cleaved so that compatible sticky ends can be produced. Next both, vector and gene of interest are incubated together in the presence of DNA ligase which connects them by forming phosphodiester linkage. When gene of interest is ligated to plasmid at the complementary sticky ends the

resultant plasmid is now called recombinant plasmid (vector).

Molecular glue (DNA Ligase) is responsible for the formation of the phosphodiester linkage between two adjacent nucleotides and thus joins two double-stranded DNA fragments; therefore it is called molecular glue. In rDNA experiments, **DNA ligase** is used to join two different DNA fragments (plasmid/vector and the foreign DNA) that are annealed by the sticky ends.

Products of recombinant DNA technology can be produced by using suitable expression system. Plasmids are usually made recombinant for the production of specific protein as a result of expression of gene of interest in natural expression system.

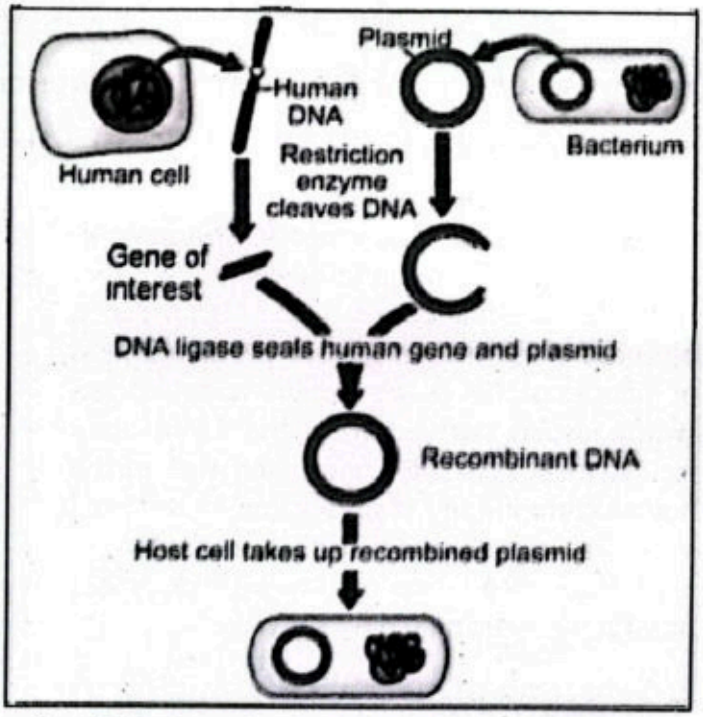

Fig. 10.5: Steps for the formation of recombinant plasmid

Expression system

Expression system may be a suitable cell or organism that can act as host for the recombinant vector for its cloning (multiplication) or expression. Therefore, the selection of suitable expression system always depends upon the type of vector which is being used while making recombinant DNA. The most important character of an ideal expression system is its short generation time and simplicity of its cellular and genetic system. So bacterial cells can act as an ideal expression system.

Transformation of expression system

Here transformation refers to the insertion of recombinant DNA into the expression system which can be performed by putting the expression system (bacterial cells that already contain no plasmids) and recombinant plasmids into the same medium. Bacterial cells take up recombinant plasmid, especially, if they are treated with calcium chloride which make them more permeable. Thereafter, as the cell reproduces, a bacterial clone forms and each new cell contains at least one plasmid. Therefore, each of the bacterial cell contains the gene of interest, which will express itself and make its product.

Identification of transformed clone

The transformed clone can be identified by adding a particular antibiotic (for which resistant gene is found in plasmid) into the medium. As the transformed clone has got resistance against the antibiotic, so it remains alive and continues to grow, whereas all the untransformed clones are killed by the antibiotic. From this transformed bacterial clone, the cloned gene can be isolated for further analysis or its protein product can be separated and used for various purposes.

10.4 FORMATION OF HUMAN INSULIN PROTEIN IN BACTERIA

Human Insulin is produced by recombinant DNA technology, which involves the following steps:

1. Identification and Isolation of the Insulin Gene

The insulin gene, which produces human insulin, is isolated from a human DNA sample. Insulin consists of two peptide chains (α and β) linked by disulfide bonds. Insulin is produced by endocrine β-cells in the islets of Langerhans of pancreas. Gene for insulin is called INS (word insulin derived from the Latin word insula, means "island") and is located on the human chromosome 11 at position 11p15.5 (on short arm (p), region 11, band 15 and sub band 5). The gene is 1.5 Kbp long having about 1,425 bp with three exons and two introns.

SCIENCE TITBITS

Insulin was discovered by Edward S. Schafer in 1916. It is the major regulator of blood sugar in the body. Fredrick Sanger and his team in 1954 first time described the complete structure of insulin. Initially, β-cells synthesize pre-pro-insulin polypeptide, having 109 amino acids. Out of 109 amino acids, 23 amino acids act as signal molecules needed for entry of the pre-pro-insulin through cell membrane. When inside the cell, it become inactive pro-insulin with 86 amino acids, that finally converted to active insulin with 51 amino acids by some proteolytic enzymes. Active insulin consists of two polypeptide chains, chain α has 21 amino acids and chain β has 30 amino acids, both linked together by disulphide bonds.

2. Selection of suitable plasmid for insertion of insulin gene

The production of human insulin by recombinant DNA technology involves plasmids that are specifically engineered to efficiently transfer insulin gene in host cells e.g. *Escherichia coli*. Two commonly used plasmids in the production of insulin are:

1. ***pBR322*** is one of the most widely used plasmids in genetic engineering. It contains antibiotic resistant genes for ampicillin and tetracycline.
2. ***pUC18/pUC19:*** These plasmids are derivatives of *pBR322* that have been modified for efficient transformation. They include a gene for ampicillin resistance.

Both above mentioned plasmids have smaller size, origin of replication, higher copy number and multiple unique restriction sites, which simplifies the insertion of the insulin gene. Human genes for chain α and β of insulin may be inserted into these plasmid, for expression in bacterial cells to synthesize insulin chains.

3. Creation of Recombinant DNA

Bacteria e.g. *E. coli* are not so advanced cells so cannot directly produce eukaryotic proteins like insulin, so two separate synthetic genes are created for the α and β chains of insulin. These genes for both chains (α and β) of insulin and separately inserted into two separate bacterial plasmid vectors i.e. *pBR322* plasmid. These genes are inserted into the plasmid by the side of β-galactosidase gene. The plasmids are engineered with a promoter sequence that helps to initiate the auto- production of insulin chains when inside the bacterial cells.

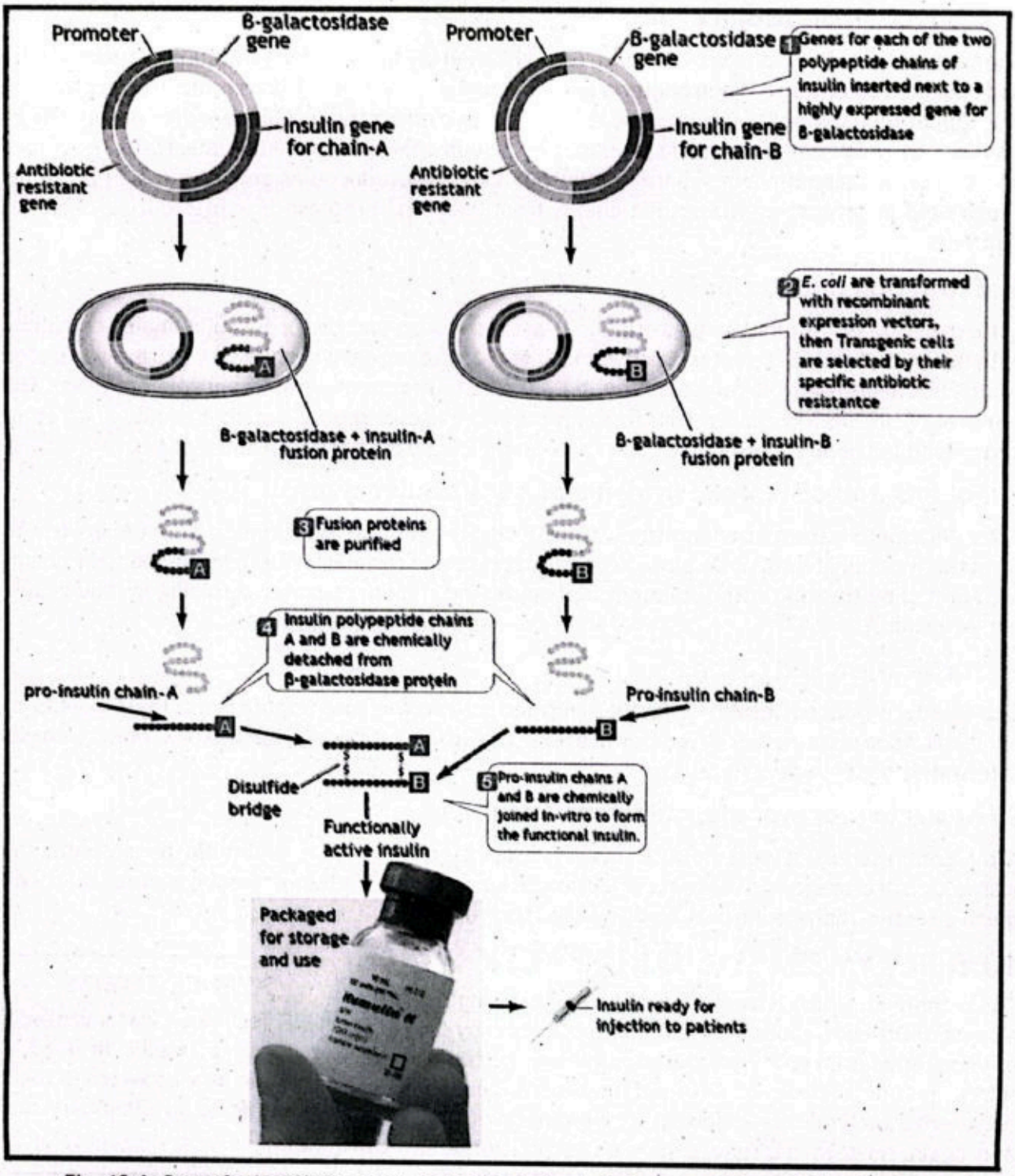

Fig. 10.6: Steps for synthesis of human insulin in bacteria by recombinant DNA technology

4. Insertion of recombinant plasmids into host Cells

The recombinant plasmids are then separately inserted into *E. coli* (or another bacterial strain) through a process called transformation. The bacteria take up the recombinant plasmids and become recombinant bacteria.

5. Production of insulin chains

The bacteria are allowed to grow in large fermentation tanks containing suitable culture medium and optimum conditions. When bacteria grow, recombinant plasmid DNA is replicated resulting in multiplication (cloning) of insulin gene. Bacteria also express the insulin genes for production of insulin. The recombinant bacteria produce pro-insulin chains i.e. fused β-galactosidase-A chain and β-galactosidase-B-chain separately. Both chains are produced as part of a fusion proteins, which help in protecting the insulin chains from bacterial protease enzymes during bacterial synthesis.

6. Extraction and Purification

For extraction of insulin, the bacterial cells are harvested and the pro-insulin chains combined with fusion proteins (β-galactosidase) are isolated. These pro-insulin chains A and B are separated from Fusion proteins, β-galactosidase by chemical treatment with cyanogen bromide. The removal of pro-insulin chains from fusion protein β-galactosidase is possible because, an extra codon is added before methionine codon at N-terminal of each gene for A and B-chain.

7. Folding and disulphide bridging of both insulin chains

After detachment from fusion proteins, A and B chains of insulin are chemically joined in-vitro to form the functional insulin. Disulphide bridges at cysteine residues of both insulin peptide chains are formed by treating with sodium disulphonate and sodium sulphite, resulting in biologically active insulin.

8. Purification and testing

The finally produced insulin is further purified to remove any impurities or by-products of bacterial gene expression. After purification, the insulin is tested for quality, effectiveness, safety and possible side effects.

9. Packaging for storage, shipment and usage

After confirmation of best quality, insulin is packaged into vials or insulin pens containing instruction of storage and usage for injection. Thus recombinant human insulin is pure, safer and much effective than insulin previously derived from animals.

10.5 POLYMERASE CHAIN REACTION (PCR)

SCIENCE TITBITS

PCR Technique was invented by Kary B. Mullis in 1983; later on he was awarded the Nobel Prize in Chemistry in 1993.

The technique, which is used to amplify (clone) a single gene or a piece of DNA into thousands to millions of copies by means of *in-vitro* replication process is called polymerase chain reaction (PCR). In this technique, DNA polymerase is compelled to polymerize (polymerase reaction) a given piece of DNA again and again, so that multiple copies are produced, thus, the technique is known as **polymerase chain reaction (PCR).**

A special DNA polymerase, the ***Taq polymerase*** is used in PCR technique, which is specialized temperature-tolerant enzyme isolated from *Thermus aquaticus*, a bacterium found in hot springs. This enzyme is stable and active at near-boiling temperatures. In order to perform PCR, template DNA (DNA to be amplified), free nucleotides (deoxyribo-nucleoside triphosphates or dNTPs), primers and *Taq polymerase* are diluted in a suitable buffer to make PCR

reaction mixture. The PCR mixture is placed in an instrument called thermocycler or PCR machine. Thermocycler regulates the temperature during various steps of PCR reaction according to the need.

Fig. 10.7: PCR machine (Thermocycler)

Mechanism of PCR Reaction

PCR cycle consists of three steps: **denaturation, primer annealing** and **extension** or **polymerization**. Each step requires a specific temperature. The time duration, temperature and sequence of the steps have to be programmed in the thermocycler.

Denaturation

Initial Denaturation

This step is performed only once before the start of cyclic steps of PCR process.

Purpose: To completely denature the DNA template and ensure all strands are separated before cycling begins. Temperature and time (typically) set at 94-95°C for 4-5 minutes.

Reason: Some DNA templates, especially GC-rich regions or complex secondary structures, require a longer initial denaturation to break hydrogen bonds fully, allowing proper primer binding.

Cyclic Denaturation

Cyclic denaturation step is performed in each cycle of the PCR process. The template DNA is heated to 94°C for one minute. At this high temperature the DNA undergoes complete denaturation and the double-stranded DNA (dsDNA) becomes single-stranded DNA (ssDNA). Each single ssDNA can act as the template for the in vitro DNA synthesis.

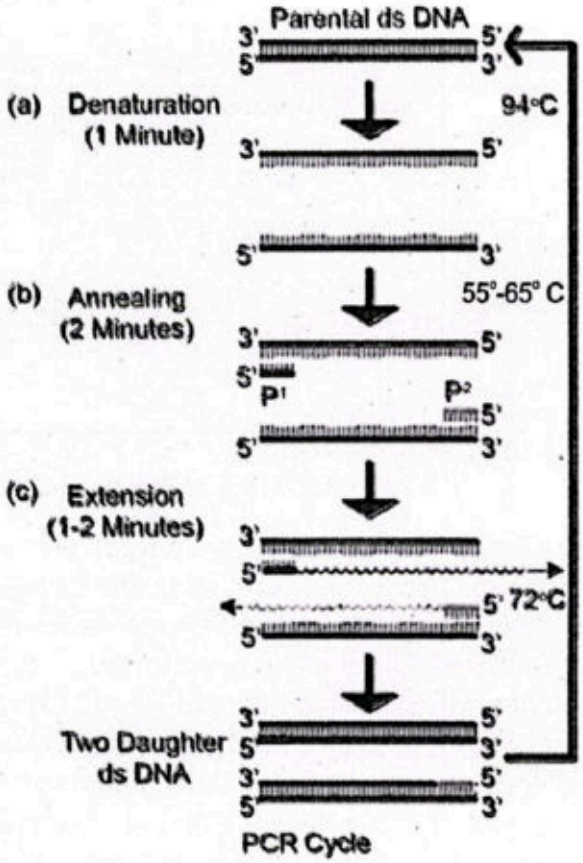

Fig. 10.8: Mechanism of PCR reaction P1 is forward primer and P2 is reverse primer

Primer annealing

The next step is the primer annealing. In this step the two primers, the forward primers and the revese primers, anneal or hybridize to the single-stranded template DNA at its complementary regions. Annealing is usually carried out at a lower temperature depending on the length and sequence of the primers. In standard cases temperature is between 55°-65°C depending upon size of the primer and the length of the gene. Approximate time required for this step is 2 minutes.

Extension or Polymerization

Cyclic Extension

Cyclic Extension is performed in each cycle of the PCR process. This is final step in each cycle of primer extension or polymerization in which the Taq polymerase synthesizes new DNA strands to the 3' ends of primers using dNTPs. The optimum temperature for carrying out the primer extension reaction or polymerization of dNTPs is standardized at 72°C. This step takes just one minute to be completed in each cycle.

Final Extension

On the completion of cyclic steps of PCR process final extension is performed only once.

Purpose: For complete synthesis of all PCR products by Taq DNA polymerase. Temperature and time is usually 72°C for 5-7 minutes.

Reason: The final extension ensures that any incomplete remaining DNA strands during cyclic extension, are fully extended resulting in complete double-stranded products.

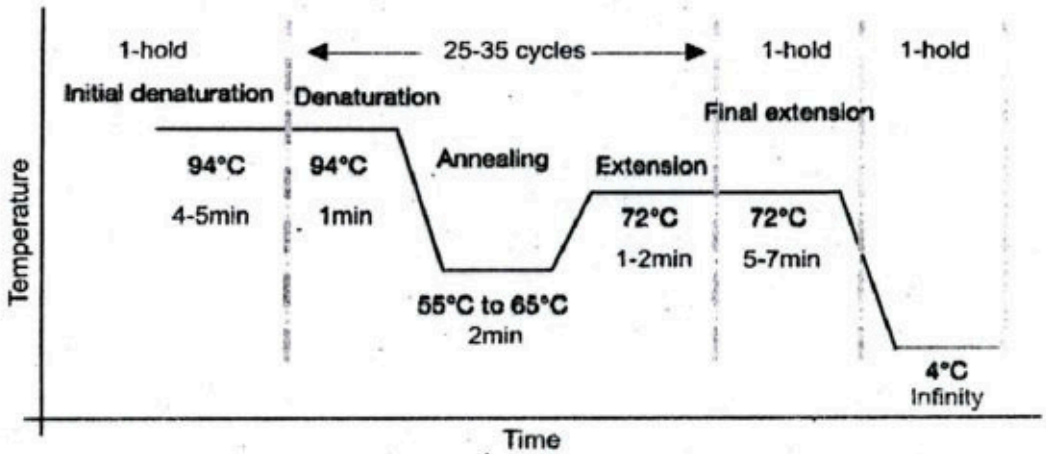

Fig. 10.9: Steps of PCR mechanism as set in thermocycler (PCR machine)

At the end of first cycle, one target dsDNA molecule is converted into two daughter dsDNA molecules. The second cycle immediately starts with the denaturation by heating at 94°C, so that all the newly synthesized dsDNA molecules are also denatured to single strands, which again act as templates. It will again be followed by the primer annealing and extension and thus the cycle of denaturation, primer annealing, and extension continues resulting in the amplification of the selected DNA sequence at an exponential rate i.e., the number of existing DNA molecules become doubled after each cycle. Both non cyclic steps i.e. initial denaturation and final extension contribute to the efficiency and accuracy of PCR, leading to better yields and more reliable results.

Storage: At the end of the extension, temperature is set at 4°C for longer time to safely store the PCR products (i.e. replicated DNA copies) until it is not shifted to refrigerator.

SCIENCE TITBITS

- Why heat is used in PCR technique to denature the target DNA instead of using DNA helicase and DNA gyrase enzymes?
- Why human DNA polymerase cannot be used in PCR technique? Why already synthesized primers are used in PCR technique instead of using primase enzyme?

Science, Technology and Society Connections

The application of polymerase chain reaction.

PCR has application in almost all areas of molecular biology, genetics, and in clinical areas.

1. PCR is an efficient diagnostic technique used for the detection of specific genotypes of infectious agents.
2. Reactions for DNA sequencing are also simplified by introducing the PCR method.
3. DNA fingerprinting is also made simple by PCR.
4. The genetic mutations responsible for certain genetic diseases and cancers can be detected using PCR tools. Early detection of genetic disease is even possible in embryonic conditions or even in sex cells—sperm and egg.

10.6 GENETICALLY MODIFIED ORGANISMS (GMOs)

"Genetically Modified Organisms (GMOs)" are the organisms (e.g. Bacteria) whose genetic material (DNA) has been altered in a way that does not occur naturally through fertilization or natural recombination. Techniques of genetic engineering are used to insert foreign genes, remove existing genes or modify specific genes in naturally free living organisms to convert them into genetically modified organisms with desirable traits. GMOs have improved nutritional content, enhanced growth rates and are resistant to pests, diseases and environmental conditions. Genetically modified organisms are being widely used in agriculture, animal husbandry, medicine and research. Many transgenic organisms such as animals, plants, and bacteria have been produced.

SCIENCE TITBITS

Both strains of *Pseudomonas syringae* occur naturally, but recombinant DNA technology has allowed for the synthetic removal or alteration of specific genes, enabling the creation of the ice- minus strain. Modifying *P. syringae* may have unexpected consequences for climate. A study has shown that its ice nucleating proteins may play an important part in causing ice crystals to form in clouds. If humans increase the frequency of bacteria lacking these proteins then it could potentially affect rainfall.

10.6.1 Transgenic Bacteria

Unlike other organisms bacteria can be easily transformed due to their simple genetics. The first example of this occurred in 1978 when a version of the human insulin gene was inserted into the bacterium *Escherichia coli* to produce synthetic "human" insulin. The transgenic bacteria are not only being used to produce different human proteins, they are also being used in improvement of plant growth, removal of environmental pollutants and extraction of metals from low grade ores.

10.6.2 Transgenic Plants

The first field trials of genetically engineered plants occurred in France and USA in 1986, when tobacco plants were engineered to be resistant to herbicides. In most cases the aim of developing transgenic plant is to introduce a new trait to the plant which does not occur naturally in this species. Examples include resistance to certain pests, diseases or environmental conditions or the production of a certain nutrient or pharmaceutical agent.

10.6.3 Transgenic Animals

A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. Genetic engineering has also been used to improve the traits of farm animals. In addition these animals are also used to produce drugs. Such transgenic animals which also produce human proteins or drugs are called **transpharmer animals.**

SCIENCE TITBITS

The first genes available for genetic engineering of crop plants for pest resistance were known as Bt genes from a bacterium ***Bacillus thuringiensis***. These are specific to particular group of insect pests and are not harmful to other useful insects like butter flies and silk worms. Transgenic crops with *Bt* genes (e.g. cotton, rice, maize, potato, tomato, brinjal, cauliflower, cabbage, etc.) have been developed. This has proved to be an effective way of controlling the insect pests and has reduced the pesticide use.

SCIENCE TITBITS

Concerns about the Genetically Modified Organisms (GMOS)

The main areas of consideration for safety aspects in biotechnology are the following:

a. How to dispose-off spent microbial biomass and purify the effluents from biotechnological processes?
b. The toxicity of the allergy associated with microbial production.
c. How to deal with the increase in the number of antibiotic resistant pathogenic microorganisms?
d. How to evaluate the pathogenicity of the genetically engineered microorganisms to infect humans, plants and animals?
e. How to prevent contamination, infection or mutation of the processed strains?

Science, Technology and Society Connections

Global Transgenic Crops

Since 1996, the cultivation of transgenic crops has expanded rapidly. In 2005, transgenic crops were planted globally on 90 million hectares, which increased to 190.4 million hectares by 2019. Major GM crops include herbicide tolerance and insect resistance soybean, maize, cotton, and canola. Leading producers are the United States, Brazil, Argentina and Canada.

Transgenic Crops in Pakistan

Pakistan commercially adopted Bt cotton in 2010, which is now grown over 2.3 million hectares (95% of the cotton area). New triple gene cotton varieties, resistant to pests and herbicides, have been introduced in 2023. GM maize production increased sharply, from 1 million metric tons in 2013 to 10.5 million metric tons in 2023. Recently, GM varieties of sugarcane has also been approved for cultivation. (Discourse: 2024, PIDE, Islamabad)

SCIENCE TITBITS

Most of the countries of the world are signatories to the Biological Weapons Conventions of 1972. As a signatory, it is a voluntary pledge by a nation "never to produce microbial or other biological agents or toxins, whatever may be their method of production, for use in wars. However, many people have expressed their concerns about the possible use of genetic manipulations for military purposes in the near future.

Science, Technology and Society Connections

· Justify the need of genetic counselling.

Genetic counselling is a service that provides information and advice about genetic conditions. Counselling is conducted by healthcare professionals who have been specially trained in the science of human genetics (a genetic counsellor or a clinical geneticist). The counsellor will discuss the risks, benefits and limitations of genetic testing with you. They will also explain how the information found as a result of genetic testing could have implications for both you and your family.

·Investigate careers that require an understanding of biotechnology and genetic engineering.

Many careers require an understanding of biotechnology and genetic engineering. Such as: Healthcare professionals, Teachers of biological sciences, biomedical engineers, crime lab analyst, crime scene investigator, environmental impact analyst, forensic scientist, genetic engineer, molecular biologist etc.

· Describe briefly the accomplishments of the renowned genetic engineers working in private and public sector institutions in her or his province.

It is advised to the administration that they should arrange study tour of student to public and privet sector institutions of genetic engineering so that students can meet with renowned genetic engineers and can directly ask about their accomplishments.

· Suggest measure she/he would take to solve related problems by using knowledge gained in this chapter.

Students should evaluate themselves to know what they have learnt in this chapter and should apply this knowledge to solve related problems under the guidance of their class teacher.

· Describe the role of genetic screening.

Genetic screening includes all those diagnostics tests which are used to determine whether a person or a new born baby is at risk of genetic diseases or not. Generally there are two types of genetic screening, screening of children and adults, and screening of unborn children. Genetic screening of children and adults has two purposes: first it can confirm whether the person has a mutated gene of certain disease or characteristics. The second purpose is to test adults to see if their children will be at risk of certain disease. Knowing that one or both parents carries a dominant allele for a genetic disease might affect the decision of parents about having birth of affected children. Now a days, genetic screening is used for approval of marriage licenses in some countries, like Denmark.

10.7 WORKING OF VERTICAL (SOIL-FREE) FOOD FARMS

Vertical farming is the soil free agriculture farming by growing plants in vertically stacked layers or towers, allowing food production in urban areas or places with limited land. Vertical farming is a common practice in densely populated areas and can be perfectly done in small spaces including greenhouses, warehouses or empty spaces in offices and houses.

Vertical farming or soil free farming is typically growing plants without the use of soil. As soil is heavy that may weigh down the vertical stacking system for growing plants so, farmers use air and water to create a suitable artificial environment needed for plant growth. On the basis of technique and plant growing medium soil free farms are called hydroponics, aeroponics or aquaponics.

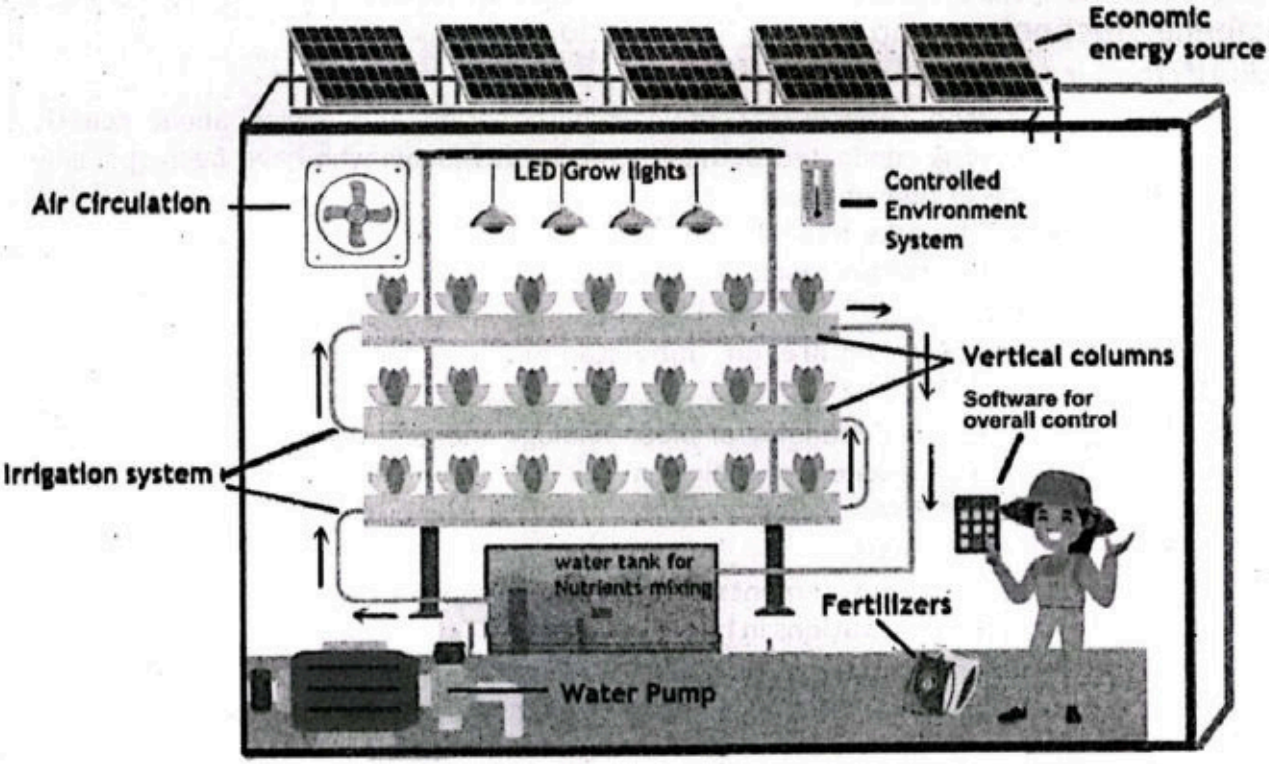

Fig. 10.10: Working of vertical (Soil-free) food farms

1. Hydroponics

Plants are grown with their roots submerged in nutrient rich water solution instead of soil. Water is efficiently used and set to flow through channels providing nutrients directly to the plants and then recycled to conserve the resources up to 90% less than traditional farming.

Fig. 10.11: Harvesting of fruits from vertical (Soil-free) food farms

2. Aeroponics

In aeroponics, plant roots are suspended in the air, instead of being submerged in water. Nutrient rich water is sprayed directly onto the roots after specific intervals for efficient absorption of moisture and nutrients by roots. Aeroponics are economical to hydroponics for even less water usage, better oxygenation for the roots and higher crop yields.

3. Aquaponics

Sometimes fish farming in combination with hydroponics: is carried out and called aquaponics. Water from fish tanks is circulated

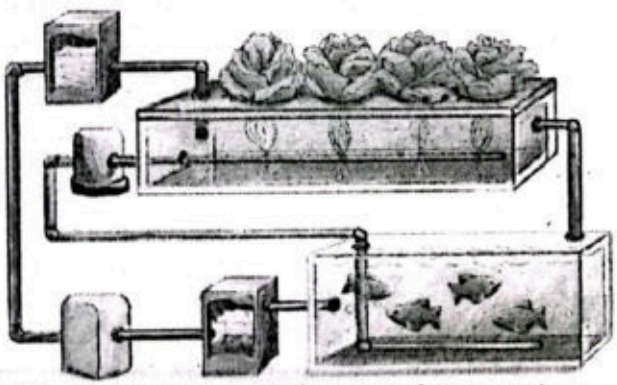

Fig. 10.12: Schematic diagram of Aquaponics: Vertical (Soil-free) food farming with fish farming

Fig. 10.13: well established large scale vertical (Soil-free) food farm

through plant beds where plants absorb the nutrients. Fish excreta provides natural nutrients to the plants and in return plants filter the water for the fish. This creates a self-sustaining ecosystem and reduces the need for synthetic fertilizers.

Vertical farming uses controlled environment agriculture (CEA) technology to maintain an optimal growing environment around the year. CEA includes vertically placed LED grow lights with inbuilt timers to ensure light spectrum necessary for photosynthesis in plants, temperature regulators to ensure the optimal farm climate and various sensors for the continual monitoring of growth. Thus soil free farming provide faster growth and year round production with limited resources. Vertical farming is also sustainable, compared to traditional farming because it uses less water and land, releases less CO_2 and produces higher crop yields per square foot.

Now a days, vertical farms are equipped with automated systems of sensors and AI support, to monitor plant growth, adjust climatic conditions, water and nutrient supply and optimized production.

10.8: COMPARISON OF VERTICAL FOOD FARMS WITH GENERAL AGRICULTURAL PRACTICES IN PAKISTAN

Agriculture is the major contributor to Pakistan's economy. Agriculture provide employment to a large population especially in rural areas and act as of major labour force in Pakistan. It provides raw materials for tèxtile, sugar, food and dairy industry of Pakistan. Main aspects of agriculture in Pakistan are:

i. Pakistan has 47% agricultural land, which is higher than the global average of 38%. About 82% of

cultivated land is irrigated through well planned canal system and 18% is arid land.

ii. The major crops in Pakistan are wheat, rice, sugarcane and potatoes as staple crops and cotton and maize as main cash crops. Pakistan has two main crop seasons i.e. Kharif and Rabi.

iii. Livestock is complementary to agriculture and is a major contributor to economy. Pakistan is the 5th largest in dairy products and 4th largest exporter of leather item in world.

iv. Organic farming is also common by using recycled remainder of crop and animal manure, using no or minimum pesticides and relying on crop rotation, thus minimizing the environmental impact.

Vertical food farms and traditional agricultural practices as prevalent in Pakistan have some advantages and challenges.

Table 10.1 Comparison between vertical food farming and general agricultural practices

Vertical food farming	General agricultural practices
1. Space Utilization	
Advantage: Utilize less horizontal space by stacking plants in vertical layers. This is ideal for urban environments where land is limited or expensive. In big cities, vertical farming is ideal due to insufficient land.	Disadvantage: Requires large horizontal fields for crop cultivation, making it less feasible in urban or densely populated areas. Crops like wheat, rice and sugarcane need vast fields, as grown in rural areas of Punjab and Sindh.
2. Water Usage	
Advantage: Use 90-95% less water compared to traditional farming. Hydroponic systems recycle water and aeroponics uses water spray, minimizing water wastage. This is crucially relevant in regions facing severe water shortages, like Thar and Baluchistan.	Disadvantage: Traditional farming methods consume large amounts of water, especially rice and sugarcane which consume huge water. Pakistan frequently faces drought and wastage of water due to floods in traditional irrigation systems.
3. Yield and Productivity	
Advantage: Crops grow faster due to optimized nutrient delivery systems. It is possible to achieve higher yields per square foot by using controlled environments (temperature, light, humidity and fertilizers) for stacked plants. Leafy greens vegetables, herbs and small fruits like strawberry are grown throughout the year with higher production.	Disadvantage: Mainly depends on seasonal cycles, weather conditions and unpredictable factors like floods and droughts. Annual productivity per unit of land, remains inconsistent and lower as a crop failures due to possible adverse climatic conditions. Wheat production is limited to winter seasons and monsoons may damage cotton and rice crops.
4. Labour and Automation	
Advantage: May be easily managed by automatic and AI systems to monitor and control plant health, watering times and light durations. Thus need for manual labour is	Disadvantage: Mainly depends upon manual labour and mostly uses outdated tools and techniques. Less use of modern and efficient machinery lead to low productivity.

minimal. Perhaps, high cost for automation may be a barrier for making it common especially in rural areas of Pakistan.	
5. Crop Varieties	
Disadvantage: It is suited for only short-cycle crops like leafy green vegetables, herbs and small fruit plants like strawberries. Hence, large scale growing staple crops (wheat, rice, maize) in Pakistan may not be easily grown in vertical systems due to land usage challenges.	Advantage: It can produce a wide range of crops, including grains (wheat, rice, maize), fruits (mangoes, apples, oranges) and vegetables like potatoes. Traditional agriculture methods provide food security.
6. Pesticide and Chemical Usage	
Advantage: There are rare chances of insects and pest in controlled environments so it has minimal need for pesticides and herbicides, resulting in pesticide and toxin free food products. Thus, it may improve food quality and safety by avoiding pesticide contamination in agricultural zones.	Disadvantage: Frequent and extensive use of pesticides and fertilizers is needed pests in traditional agriculture farming to avoid loss of food by insects and other. Improper handling of toxins may harm to human health and lead to environmental degradation.
7. Environmental Impact	
Advantage: As they are usually located in cities, so negative impacts on environment is rare. It is sustainable because of less usage of water and land sources, minimal need of transportation, negligible fossil fuel emissions and no soil erosion since. However, demand of energy usage for LED grow lights and climate control may be managed by using renewable energy like solar electricity.	Disadvantage: This agriculture practice is greatly at risk to be affected by environmental hazards like soil degradation, salinity, floods and drought. Another issue is deforestation which is due to desire for expanding agricultural land. Extreme climate changes have often deteriorated the traditional agriculture and resulted in loss of agriculture land.
8. Accessibility and Investment	
Disadvantage: Vertical farming require higher initial investment to establish infrastructure for lighting, climate control and automation. Thus it is less accessible to small farmers in Pakistan. However, it may be ideal for agriculture farming business in urban areas.	Advantage: Lower initial investment in many cases, particularly for small-scale farmers who rely on simple tools and natural resources. Disadvantage: Yields are generally lower, and dependence on natural weather cycles makes it unpredictable.

STEAM ACTIVITY 10.1

Make a model of DNA probe and restriction enzymes.

EXERCISE

Section I: Multiple Choice Questions
Select the correct answer:

1. Which tool of recombinant DNA technology is incorrectly paired with its use?
   A. Restriction enzyme – RFLP production
   B. DNA ligase – cuts DNA
   C. Reverse transcriptase – makes cDNA
   D. Electrophoresis – separates DNA

2. Which of the following is a key difference between vertical farming and traditional agriculture?
   A. Vertical farming uses more horizontal space, suitable for rural areas.
   B. Vertical farming is not ideal for crops like wheat, rice, and sugarcane.
   C. Vertical farming is ideal for urban areas; traditional farming needs large fields.
   D. Traditional farming grows crops in vertical layers; vertical farming requires vast fields.

3. A paleontologist finds tissue from a 400-year-old extinct bird and wants to compare its DNA with living birds. Which method would best increase the bird DNA for testing?
   A. a RFLP analysis
   B. polymerase chain reaction (PCR)
   C. electroporation
   D. Southern hybridization

4. Which of the following sequences in double-stranded DNA is mostly likely to be recognized as a cutting site for a restriction enzyme?

A.	B.	C.	D.
AAGG	AGTC	GGCC	ACCA
TTCC	TCAG	CCGG	TGGT

5. A plasmid
   A. is used as DNA vector
   B. is a type of bacteriophage
   C. is a type of cDNA
   D. is a retrovirus

6. DNA molecules with complementary sticky ends associate by
   A. covalent bond
   B. hydrogen bond
   C. ionic bond
   D. disulphide bond

7. Which technique rapidly replicates specific DNA fragments without cloning?
   A. gel electrophoresis
   B. cDNA libraries
   C. genetic probe
   D. polymerase chain reaction

8. The PCR technique uses
   A. heat resistant DNA polymerase
   B. reverse transcriptase
   C. DNA ligase
   D. restriction enzymes

9. If eukaryotic DNA contains five cleavage sites for a particular restriction enzyme, how many fragments will be produced upon complete digestion of the DNA with that enzyme:
   A. 2
   B. 4
   C. 6
   D. None

10. The dideoxynucleotides (ddATP, ddTTP, ddGTP and ddCTP) are important in DNA sequencing because they:
    A. cause premature termination of growing DNA strand
    B. are used as prime
    C. cause the DNA fragments that contain them to migrate more slowly through the gel
    D. are not affected by high temperatures

Section II: Short Answer Questions

1. What is the role of restriction endonucleases in gene cloning?
2. What are molecular carriers?
3. What is the role of restriction DNA ligases in gene technology?
4. What are the concerns about genetically modified organisms?
5. Define following terms:
   (i) Biotechnology (ii) genetic engineering (iii) gene cloning (iv) palindromic sequence (v) recombinant DNA technology (vi) DNA ligase (vii) polymerase chain reaction (viii) primers(ix) Taq polymerase (x) genome (xi) transgenic organisms
6. Write the difference between:
   (a) biotechnology and genetic engineering (b) staggered and blunt cut restriction enzymes (c) genome and chromosome(d) trangenic organisms and hybrid organisms (e) biotechnology and nanotechnology
7. Shortly suggest, which type of farming is better from vertical farming and traditional agriculture farming in Pakistan.
8. Explain the role of restriction endonucleases and DNA ligases in gene cloning.
9. Describe the selection and isolation of the gene of interest.
10. Explain the properties and the role of vectors in recombinant DNA technology.
11. State the steps for the integration of DNA insert into the vector.
12. Briefly state the technique applied for the selection of the vectors that take up the DNA insert.
13. Describe the steps involved in gene amplification through polymerase chain reaction.
14. State the objectives of the production of transgenic bacteria, transgenic plants and transgenic animals.
15. Explain the scope and importance of biotechnology in promoting human welfare.
16. List the hazards of using genetically modified organisms.

Section III: Extensive Answer Questions

1. Describe the components of recombinant DNA technology under the following heads:
   (a) Gene of interest (b) Molecular scissors (c) Molecular carriers (d) Molecular glue (e) Expression system
2. Describe the mechanism or procedure of recombinant DNA technology.
3. What is polymerase chain reaction? Discuss its components and mechanism?
4. Describe vertical agriculture farming. Explain hydroponics, aeroponics and aquaponics.
5. Discuss the scope and importance of biotechnology in promoting human welfare.
6. What are the ethical, legal and social implications of using biotechnology?
7. Compare vertical farming and traditional agriculture farming in Pakistan to clear advantages and disadvantages of both.