Chapter 23

Biotechnology

23.0 INTRODUCTION

Definition

"The use of a natural biological system to produce a product or to achieve an end desired substances by humans."

Services of Biotechnology

(i) The genes can be removed from one organism by modern techniques, and are inserted into another organism which produce a desired substance e.g. insulin. Earlier, the insulin dependent diabetes mellitus patient received the insulin from dead animals. Today they receive human insulin. This is product of biotechnology.

(ii) Biotechnology has produced drugs and vaccines to control the human illnesses, since 1980.

(iii) Genetically engineered bacteria are used to clean up environmental pollutants, increase the fertility of the soil and kill inspect pets.

(iv) It is now possible to alter the genotype and subsequently the phenotype of plants and animals.

(v) Gene therapy is used to repair a faulty gene. However it is under clinical trials.

Some people oppose the manipulation of genes. However no bad effects of biotechnology have been observed yet. But they fear that there will be health and ecological effects of biotechnology in the future.

23.1 CLONING OF A GENE

Cloning of a gene produces many identical copies. Recombinant DNA technology is used when a very large quantity of a gene is required.

The polymerase chain reaction (PCR) creates a lesser number of copies within a laboratory test tube.

23.1.1 Recombinant DNA Technology

Definition

Recombinant DNA technology known as genetic engineering aims at the formation of recombinant DNA (which contains DNA from two different sources) is called recombinant DNA technology.

Requirements

In order to produce recombinant DNA, following are required:

1. Gene of interest, which is to be cloned.
2. Molecular scissors, to cut out the gene of interest.
3. Molecular carrier or vector, on which gene of interest could be placed.
4. Expression system where gene of interest is introduced and a specific product is made or the gene of interest along with the vector is then introduced into an expression system as a result of which a specific product is made.

Mechanism to Get a Gene

There are three possible ways to get the gene of interest.

a) To isolate it from the chromosome

b) To chemically synthesize it

c) To make it from mRNA

- Genes can be isolated from the chromosomes by cutting the chromosomes on the flanking sites of the gene using special enzymes known as **restriction endonucleases**.
- Smaller genes can be synthesized in laboratory chemically or from mRNA using reverse transcriptase. This DNA molecule is called complementary DNA (cDNA)

23.1.2 Molecular Scissors/Restriction Endonuclease

Introduction

These are natural enzymes of bacteria, which they use for their own protection against viruses.

The restriction enzyme cuts down the viral DNA, but does no harm to the bacterial chromosomes

They are called restriction enzymes because they restrict the growth of viruses.

Discovery

In 1970, ***Hamilton O. Smith***, at Johns Hopkins University, isolated the first restriction enzyme.

Example

So far more than 400 such enzymes have been isolated and out of which about 20 are frequently used in recombinant DNA technology.

EcoR1 is commonly used restriction enzyme.

Mechanism of Action

(i) Restriction enzyme cuts the double stranded DNA at very specific sites **(cleavage site)** characterized by specific sequence of four or six nucleotides arranged symmetrically in the reverse order. Such sequences are known as **palindromic sequences.**

(ii) The single stranded but complementary ends of two DNA molecules are called **sticky ends.** Because they can bind by complementary base pairing they, therefore, facilitate the insertion of foreign DNA into vector DNA.

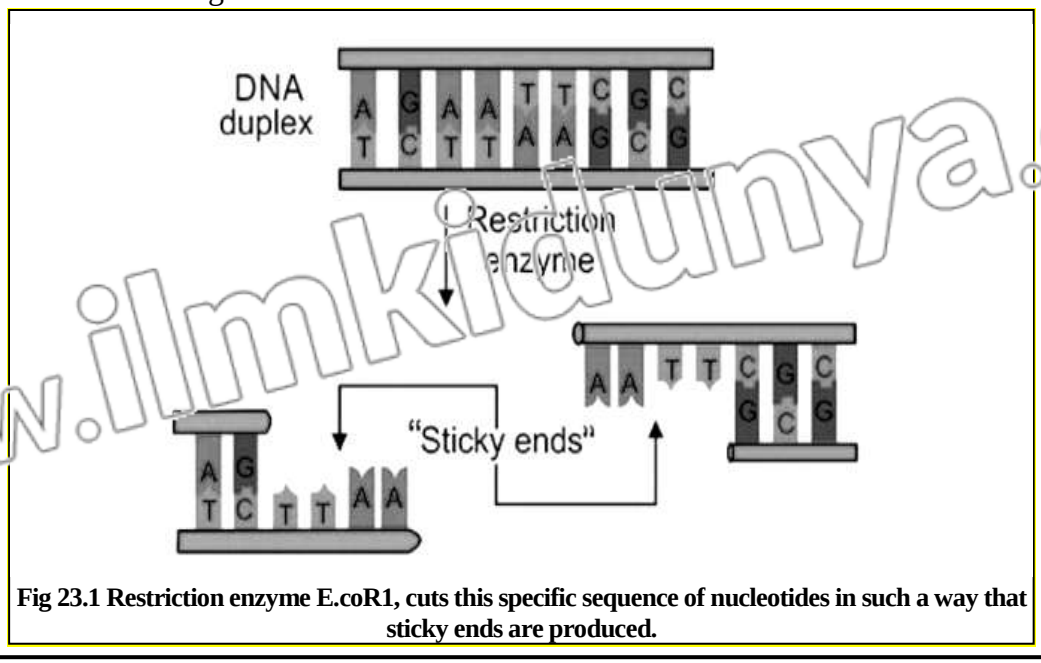


Fig 23.1 Restriction enzyme E.coR1, cuts this specific sequence of nucleotides in such a way that sticky ends are produced.

23.1.3 Molecular Carrier Vector

Introduction

To make recombinant DNA, one often begins by selecting a vector the mean by which recombinant DNA is introduced into a host cell is called vector.

Plasmid as Vector

Plasmids are most common vectors. They are natural extra chromosomal circular DNA molecules which carry genes for antibiotic resistance and fertility etc.

They were discovered by investigators studying the sex life of the intestinal bacterium *Escherichia coli*.

Examples

Some discovered plasmids are:

- ***pSC 101*** has antibiotic resistance gene for tetracycline.
- ***pBR 322*** has antibiotic resistance genes for tetracycline as well as ampicillin.

Inserting gene of interest in tetracycline resistant gene of plasmid pBR 322 would enable separating out colonies of bacteria in a medium containing ampicillin and vice versa.

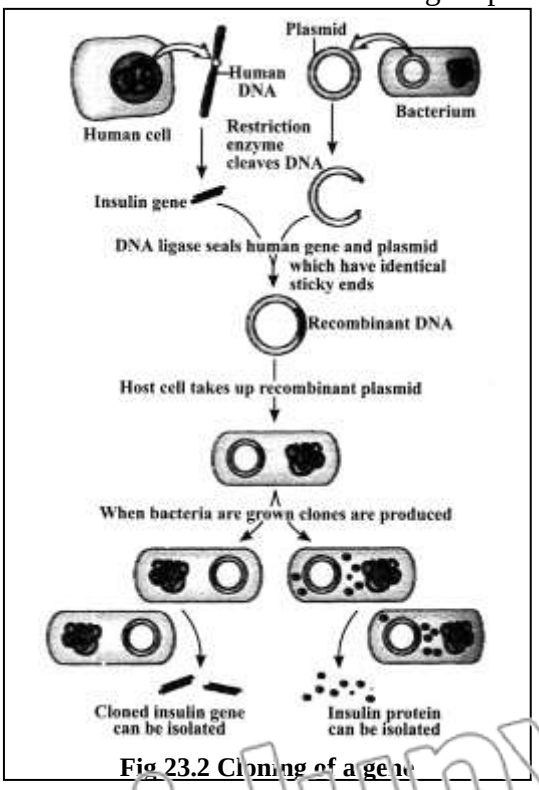


Fig 23.2 Cloning of a gene

23.1.4 Recombinant DNA

Definition

Recombinant DNA contains DNA from at least two different sources. It is also called ***chimaeric DNA***.

Preparation

Different steps involved are

i) Plasmid is cut with the same enzyme which was used for isolation of the gene of interest.

ii) The gene of interest (insulin) is then joined with sticky ends produced after cutting the plasmid with the help of another special enzyme knon as DNA ligase.

iii) DNA ligase enzyme then seals the foreign piece of DNA into the vector.

Now the two different pieces of DNA have been joined together, which is now known as ***recombinant DNA or chimaeric DNA.***

23.1.5 Expression of the Recombinant DNA

Recombinant DNA is usually expressed through cloning.

A clone can be a large number of;

- Molecules e.g. cloned genes
- Cells e.g. cloned bacteria
- Organisms that are identical to an original specimen

(1) Expression through Plasmid

Bacterial cells take up recombinant plasmid, especially, if they are treated with calcium chloride to make them more permeable. Thereafter, as the cell reproduces, a bacterial clone develops, and each new cell contains at least one plasmid. Therefore, each of the bacteria contains the gene of interest, which will express itself and will make product.

From this bacterial clone, the cloned gene can be isolated for further analysis or protein product can be separated.

(2) Expression through Bacteriophage

DNA of bacterial viruses (for example lambda phage) can also be used as a vector. After lambda phage attaches to a host bacterium, recombinant DNA is released from the virus and enters the bacterium. Here it will direct the reproduction of many more viruses. Each virus in bacteriophage clone contains a copy of the gene being cloned.

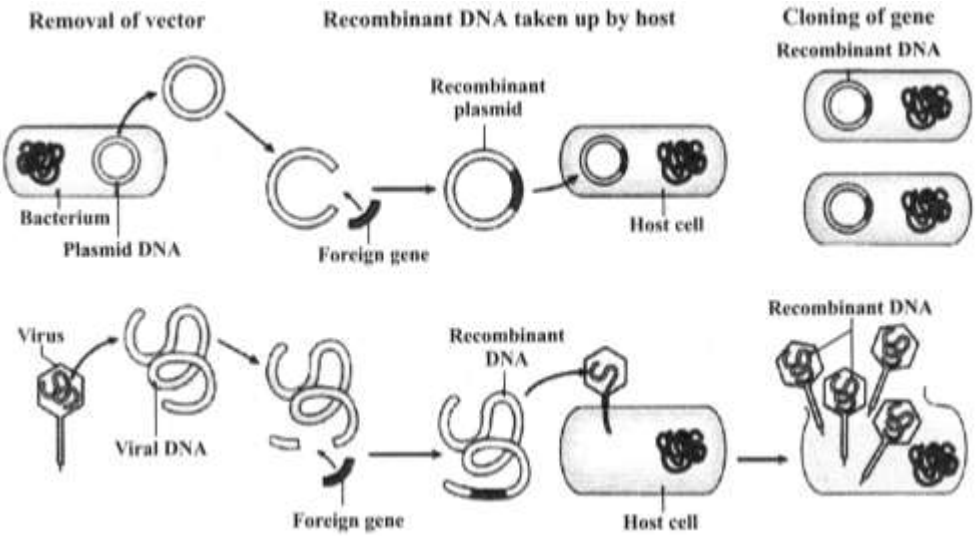


Fig 23.3 Plasmid DNA (upper part of figure) as well as viral DNA (lower part of the figure) can be used as vectors for cloning gene of interest.

QUESTIONS RELATED TO ABOVE ARTICLE

What is methodology for producing recombinant DNA to be used in gene cloning?

(Exercise Question i)

23.2 GENOMIC LIBRARY

Definition

A ***genomic library*** is a collection of bacterial or bacteriophage clones, each clone containing a particular segment of DNA from the source cell. While a ***genome*** is a full set of genes of an individual.

Development of Genomic Library

For making a genomic library, an organism's DNA is simply sliced up into pieces, and pieces are put into vectors (i.e. plasmids or viruses) that are taken up by host bacteria.

The entire collection of bacterial or bacteriophage clones that results contains all the genes of that organism.

Searching a Gene in Library

A particular ***probe*** can be used to search a genetic library for a certain gene.

A probe is

- A single stranded nucleotide sequence.
- Either radioactive or fluorescent.
- Hybridized (paired) with a certain piece of DNA.

Following steps are taken to search a particular gene in genomic library;

i) Bacterial cells, each carrying a particular DNA fragment, can be plated onto agar in a Petri dish.

ii) Probe is applied on it and it is hybridized with particular gene.

iii) After the probe hybridizes with the gene of interest, it is identified due to radioactivity or fluorescence and is isolated from the fragment.

iv) Now this particular fragment can be cloned further or even analyzed for its particular DNA sequence.

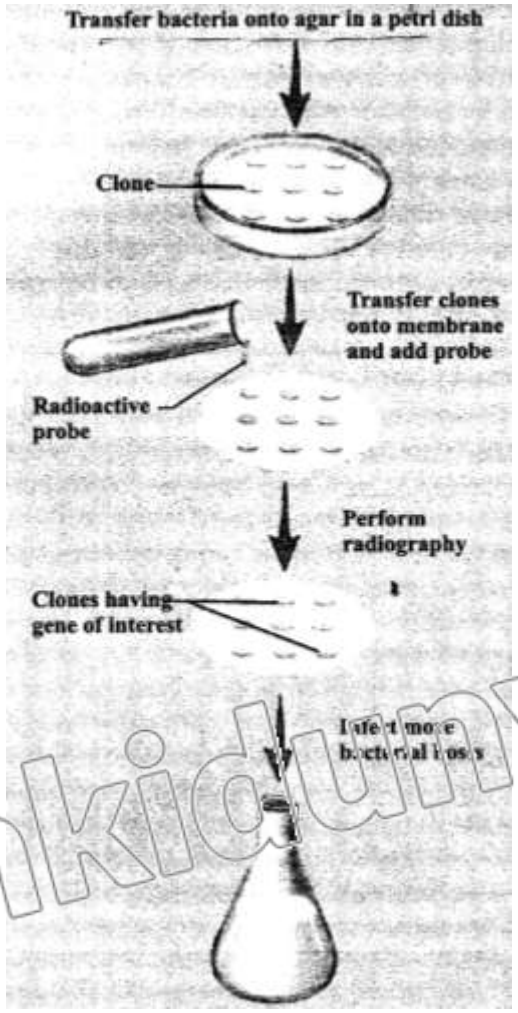


Fig 23.4 Identification of a cloned gene.

QUESTIONS RELATED TO ABOVE ARTICLE

What is a genomic library and how would you locate a gene of interest in the library?

(Exercise Question ii)

23.3 THE POLYMERASE CHAIN REACTION

Definition

It is a technique through which millions of copies of a single gene or any specific piece of DNA is produced in a short period of time by the enzyme DNA polymerase in the test tube is called polymerase chain reaction (PCR).

PCR is very specific. The targeted DNA sequence can be less than one part in a million of total DNA sample. This means that single gene or smaller piece of DNA among all human genes can be amplified using PCR.

Kary B. Mullis developed the PCR in 1983. Now a day, it is done through automatic PCR machine or ***thermocycler***.

Major Requirements of PCR

i) The Enzyme – DNA Polymerase

PCR takes its name from DNA polymerase, the enzyme that carries out DNA replication in a cell.

Different features of DNA polymerase used are;

- It is temperature insensitive (thermostable) and can withstand high temperature.
- It is extracted from the bacterium ***Thermus aquaticus***, which lives in hot springs.
- It is also called **Taq polymerase**.

ii) Primers

Before carrying out PCR, primers sequence of about 20 bases that are complementary to the bases on either side of the target DNA must be available. The primers are needed because DNA polymerase does not start the replication process; it only continues or extends the process.

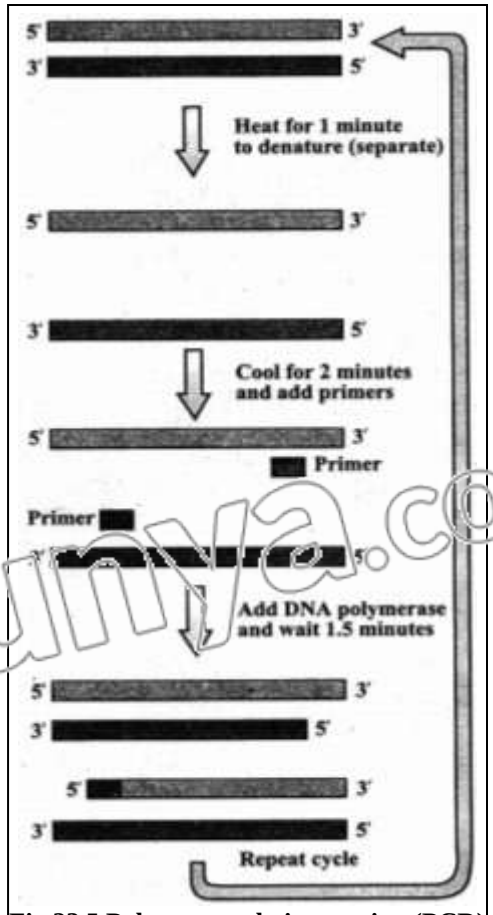


Fig 23.5 Polymerase chain reaction (PCR)

Mechanism

Different steps involved are;

i) High temperature is applied which separates double stranded DNA molecule (Denaturation).

ii) Primer is attached to the DNA (Annealing).

iii) DNA polymerase starts addition of nucleotides and leads to formation of a new strand (Extension).

iv) This process continues in same way repeatedly. This is the reason, it is also called chain reaction.

23.3.1 Analyzing DNA

The entire genome of an individual can be subjected (analyzed by) to DNA fingerprinting.

PROCESS

Different steps involved in DNA analysis are as follows;

i) Restriction Fragment Length Polymorphism

The genome is treated with restriction enzyme, which results in a unique collection of different sized fragments. These fragments vary in length and restriction enzyme separates according to this length, which is different in different individuals. This process of existing in different lengths is called restriction fragment length polymorphism (RFLPs).

ii) Gel Electrophoresis

Fragments of genome can be separated according to their lengths through a process called gel electrophoresis.

It results in formation of a number of bands that are so close together that they appear as a smear.

iii) Use of Probes

Use of probes for genetic markers produces a distinctive pattern that can be recorded on X-ray film.

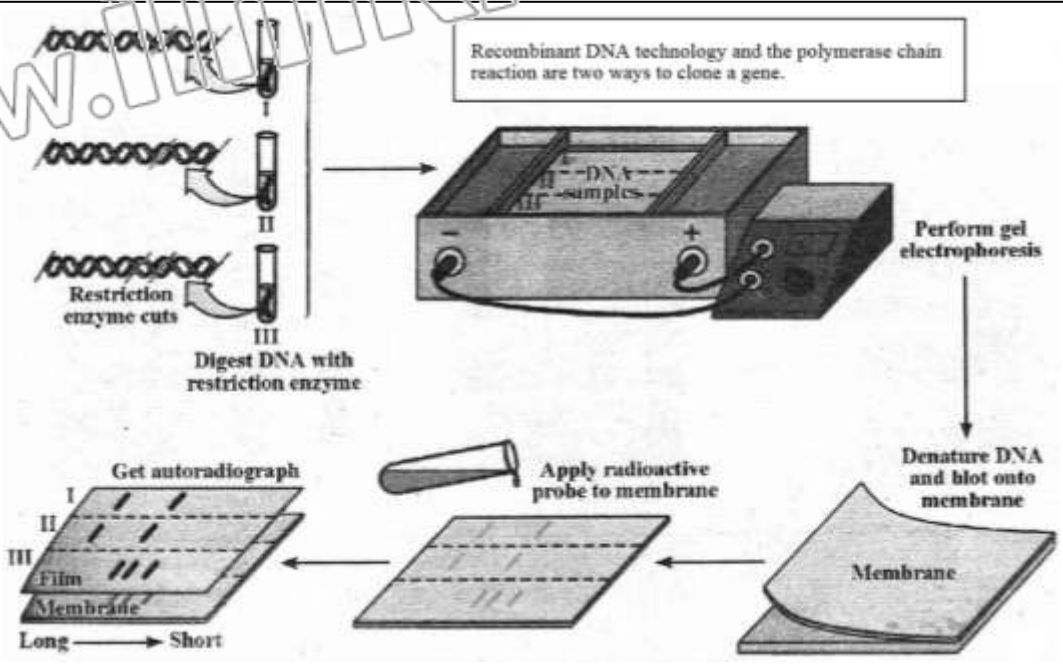


Fig 23.6 DNA fingerprinting

Three samples of DNA (I, II, III) were cut with a restriction enzyme and run on agarose gel. The gel pattern was then transferred to a membrane and DNA was denatured. The denatured DNA on the paper was hybridized with radioactive probe. Since the radioactive probes had complementary arrangement of bases to the original DNA, all DNA fragments were labeled, which appeared as black bands with autoradiogram.

IMPORTANCE OF DNA ANALYSIS

As DNA is inherited, its fingerprints resemble that of one's parents. Some important points in this relation are as follows.

i) Comparison of child's fingerprints shows similarities with that of his parents. The child has received DNA from both of his parents i.e. some bands in him are like his father and some like his mother (Fig 23.7a).

ii) The DNA from a single sperm is enough to identify a suspected rapist.

iii) DNA fingerprinting successfully identified the remains of a teenager who had been murdered eight years before because the skeletal DNA was similar to that of the parent's DNA.

iv) DNA fingerprinting can solve problems of disputed parenthood. (Fig 23.7 b)

v) DNA fingerprinting is also helpful in investigation of crimes and can be presented as forensic evidence. For example, a criminal on a deserted place assaulted a woman. She scratched his face in her defence but he murdered her and ran away. Forensic scientist recovered murderer's hair and skin cells from underneath her nails. They prepared DNA fingerprints from blood of victim, from murderer's skin and hair and from three suspects blood. Through DNA matching, it was easily found that who was murderer.

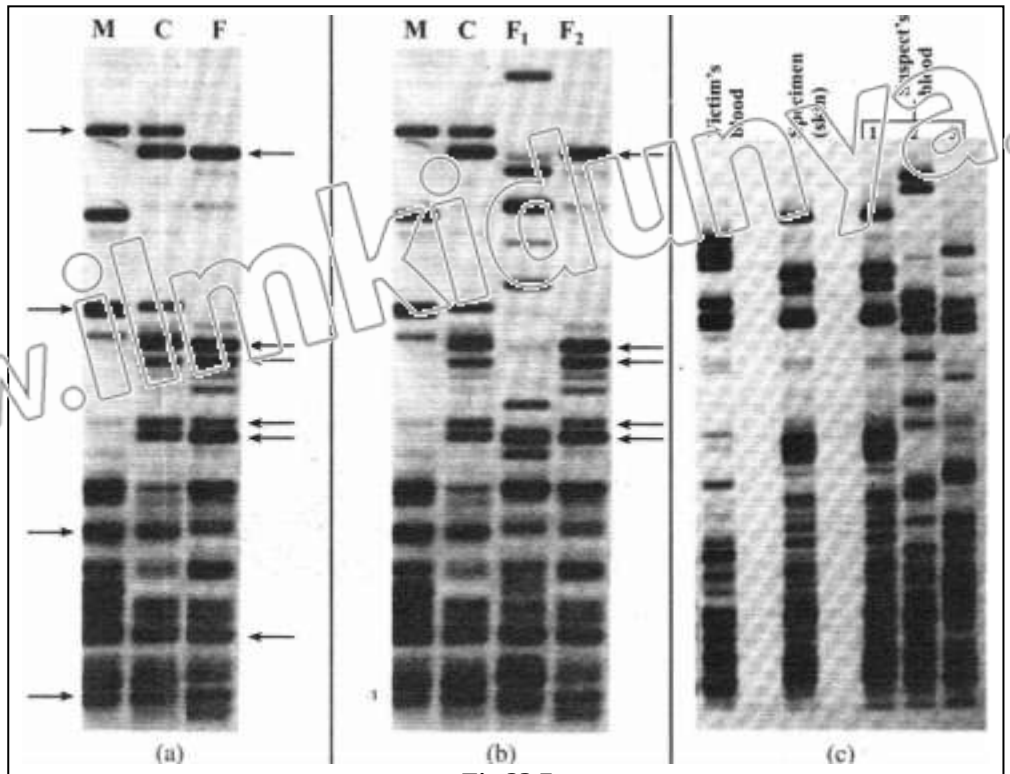


Fig.23.7

(a) Comparison of a child's DNA fingerprint (c) with his parent's DNA fingerprints (M and F). **(b) DNA fingerprints as evidence for paternity.** **(c) DNA Test - a powerful tool of forensic science.**

IMPORTANCE OF PCR AMPLIFICATION & DNA ANALYSIS

PCR amplification and DNA analysis can be used:

i) To diagnose viral infections, genetic disorders and cancer.

ii) To identify criminals in forensic laboratories.

iii) To determine the evolutionary history of human population.

It has been possible to sequence DNA taken from a 76,000 years old mummified human brain and from a 17-20 million years old plant fossil following PCR amplification.

QUESTIONS RELATED TO ABOVE ARTICLE

What is the polymerase chain reaction (PCR) and how is it carried out to produce multiple copies of a DNA segment? (Exercise Question iii)

What is DNA fingerprinting, a process that utilizes the entire genome? (Exercise Question)

23.4 GENE SEQUENCING

Methods for simple and quick determination of nucleotide sequence of any purified DNA fragment were developed in late 1970's.

Main Principles of Method

i) To generate piece of DNA of different sizes all starting from the same point and ending at different points.

ii) Separation of these different pieces of DNA on agarose gel.

iii) Reading of sequence from the gel.

Methods to Generate Piece of DNA

For generation of different sized DNA fragment, two methods are generally used.

1) ***Sanger's method*** in which dideoxyribonucleoside triphosphates are used to terminate DNA synthesis at different sites.

2) ***Maxam-Gilbert method*** in which DNA threads are chemically cut into pieces of different sizes.

Separation and Reading of Gene Sequence

The volume of DNA sequence information is now so large that powerful computers must be used to store and analyze it.

DNA sequence is now completely automated, robotic devices mix the reagents and then load, run and read the order of nucleotide bases from the gel.

Different steps involved are

i) Chain terminating nucleotides labelled with different coloured fluorescent dyes are used.

ii) All four synthesis reactions are performed in same tube and products are separated in a single lane of a gel.

iii) A detector (positioned near the bottom of the gel) reads and records the colour of fluorescent label on each band as it passes through a laser beam.

iv) A computer then reads and stores this nucleotide sequence.

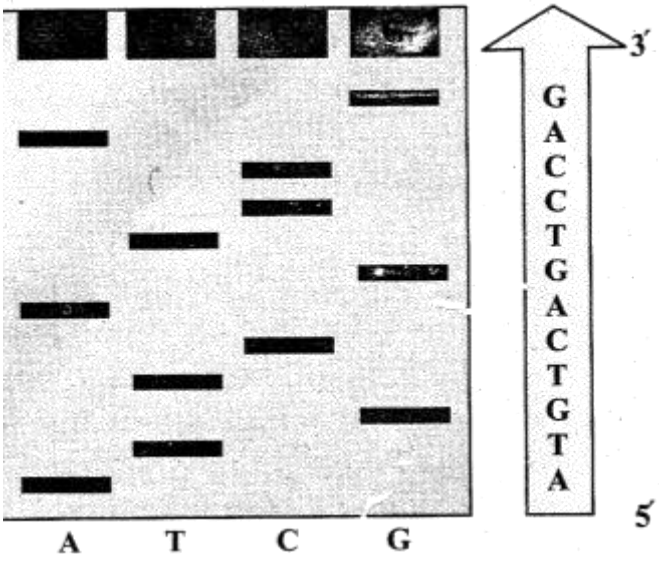


Fig 23.8 The enzymatic or dideoxy method of sequencing DNA. DNA sequence reading directly from the bottom of the gel upward, that is ATGTCAGTCCAG

Significance

Using this automation of DNA sequencing, genomes of many organisms have been sequenced e.g. plant chloroplast, animal mitochondria, bacteria, yeast, a nematode worm, *Drosophila*, model plant *Arabidopsis*, mouse, human and researchers have also deducted the complete DNA sequence of a variety of human pathogens.

23.5 THE HUMAN GENOME PROJECT

Human genome project is massive effort originally founded by the U.S. government and now by non-profit and profit gaining biochemical laboratories and pharmaceutical companies.

GOALS

There are two primary goals:

1) First goal is to construct a genetic map of human genome.

2) The second goal is to construct a base sequence map.

1) Construction of Genetic Map

- First DNA sequence in human was made of chromosome number 22, which is smallest human chromosome.
- The human genome is 25 times larger than any other genome sequenced so far.
- Aim of genetic map is to show the sequence of genes along the length of each type of chromosome.
- Some of the gene sequences are shown in diagram of X-chromosome below.
- The map for each chromosome is presently incomplete. Many scientists rely on RFLPs, which sometimes become improper in case of disease-causing gene. For example, it is known that persons with Huntington disease have a unique site where a restriction enzyme cuts DNA.

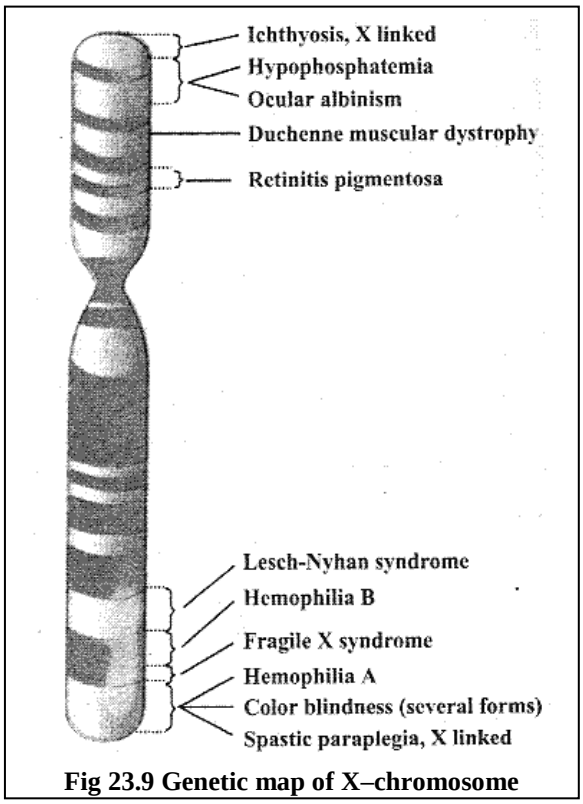


Fig 23.9 Genetic map of X–chromosome

2) Construction of Base Sequence Map

- There are three billion base pairs in the human genome and it is estimated that it could take an encyclopedia of 200 volumes, each with 1000 pages, to list all of these. Yet this goal has been reached and all the chromosomes have been sequenced.
- In this process, genome is first chopped up into small pieces, each just 1000 to 2000 base pairs long. PCR instruments copy the pieces many times and then an automatic DNA sequence determines the order of base pairs. A computer programme later strings the sequenced pieces together in the correct order. J. Craig Venter has founded a company (Celera) which has now sequenced the entire genome.

Future Goal

Knowing the base sequence of normal genes may make it possible one day to treat certain human ills by administering normal genes and/or their protein products to those who suffer from a genetic disease.

23.6 BIOTECHNOLOGY PRODUCTS

Transgenic Organisms

Organisms that have any foreign gene inserted in them are called transgenic organisms.

Examples

Examples are:

1) Transgenic bacteria
2) Transgenic plants
3) Transgenic animals

1) TRANSGENIC BACTERIA

Definition

Bacteria having foreign gene are called transgenic bacteria.

Recombinant DNA technology is used to produce bacteria that reproduce in large vats called bioreactors.

Importance

i) Use in Production of Human Proteins

Biotechnology products produced by bacteria are insulin, human growth hormone, tissue plasminogen activator, haemophilia factor VIII and hepatitis B vaccine are now in the market.

ii) Use in Promoting Plant Growth

- Bacteria that normally live on plants and encourage the formation of ice crystals have been changed from frost-plus to frost-minus bacteria.
- A bacterium that normally colonizes the roots of corn plant has now been endowed with genes that code for an insect toxin. The toxin protects the root from insects.

iii) Use in Industries

- Bacteria can be used in industries as biofilters. They prevent airborne chemical pollutants from being vented into air. They can also remove sulfur from coal before it is burned and help to clean up toxic waste dumps. They also contain suicide-genes that cause their self destruction after completion of job.
- They are also used in biosynthesis of different chemicals. For example, phenylalanine (precursor) is an organic chemical needed to make aspartame (the dipeptide sweetener) better known as Nutrasweet. Now this phenylalanine is produced by genetically engineered bacteria.
- Many major mining companies already use bacteria to obtain various metals like copper, uranium and gold from low grade sources.
- Some mining companies are testing genetically engineered organisms that have improved bioleaching capabilities.
- Bacteria are also used in cleaning up beaches after oil spills.

2) TRANSGENIC PLANTS

Definition

Plants having any foreign gene are called transgenic plants.

Mechanism of Developing Transgenic Plants

Genes are introduced into immature plant embryo or protoplast. Protoplasts are plant cells in which cell wall have been removed.

Protoplast is treated with electric current while it is suspended into a liquid containing foreign DNA. The electric current makes tiny, self-sealing holes in plasma membrane through which genetic material can enter. Then this protoplast is developed into a complete plant.

Importance

i) Development of Resistant Strains

- Transgenic forms of cotton, corn and potato have been made which are resistant to pests because they produce insect toxins.
- Soybeans have been made resistant to a common herbicide.
- Some corn and cotton plants are both pest and herbicide resistant.

ii) Production of Biodegradable Plastic

A weed called mouse-eared cress has been engineered to produce a biodegradable plastic (polyhydroxy butyrate) in cell granules.

iii) Products for Humans

Plants are being engineered to produce human hormones, clotting factors and antibodies in their seeds.

- One type of antibody made by corn can deliver radioisotopes to tumor cells.
- Antibody produced by soybean can be used as treatment for genital herpes.
- Plant made antibodies are inexpensive and have little chances of contamination.

Further Goals for Transgenic Plants

Improvements are going in

- To increase protein or starch contents.
- To modify oil or amino acid composition.
- To develop transgenic versions of wheat and rice.
- To alter stomata in order to boost carbon dioxide intake or cut down water loss.
- To increase efficiency of the enzyme RuBP.
- To introduce C4 pathway to avoid the inefficiency of carboxylase by using a different means of capturing CO_2. It will require re-engineering of plant.

3) TRANSGENIC ANIMALS

Definition

Animals having any foreign gene incorporated in them are called transgenic animals.

Methods

Two methods are important in preparing transgenic animals.

i) Microinjection of foreign gene into eggs by hand

ii) Vortex mixing method

Vortex Method

In this method, eggs are placed in an agitator with DNA and silicon-carbide needles. Needles make tiny holes through which DNA can enter the cell. When these eggs are fertilized, the resulting offsprings are transgenic animals.

Importance

i) Production of Bovine Growth Hormone

Transgenic animals have been used in production of bovine human growth hormone.

ii) Production of Large Sized Animals

This procedure has been used to produce larger fishes, cows, pigs, rabbits and sheep. Genetically engineered fishes are kept in ponds that offer no escape to the wild because there is much concern that they will upset or destroy natural ecosystem.

iii) Gene Pharming (Farming)

Gene pharming is the use of transgenic farm animals to produce pharmaceuticals.

Products through Milk of Animals

Genes that code for therapeutic and diagnostic proteins are incorporated into the animal's DNA and the proteins appear in animal's milk. These are plants to produce drugs for treatment of cystic fibrosis, cancer, blood diseases and other disorders.

Antithrombin III is used for preventing blood clot during surgery is currently being produced by a herd of goats.

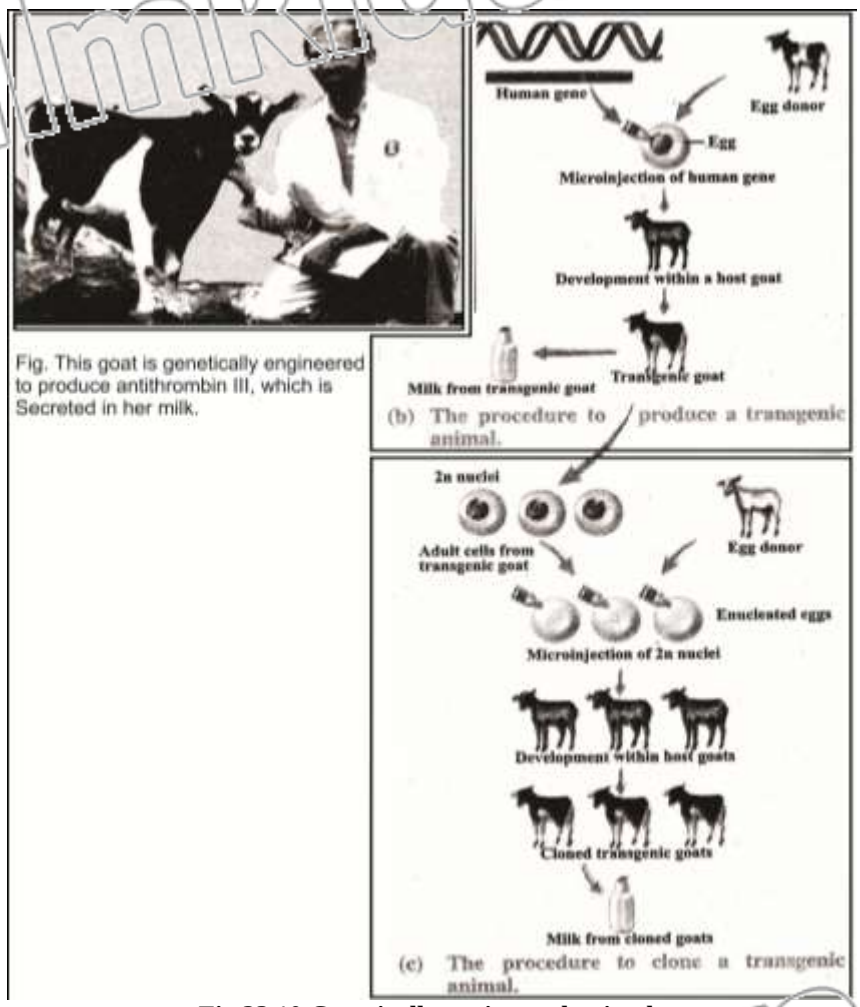


Fig. This goat is genetically engineered to produce antithrombin III, which is Secreted in her milk.

Fig 23.10 Genetically engineered animals

Products through Urine of Animals

The scientists of United States Department of Agriculture have been able to genetically engineer mice to produce human growth hormone in their urine instead of in milk.

Urine is preferable vehicle for a biotechnology product than milk because

- All the animals in a herd urinate while only female produce milk
- Animals start to urinate at birth while females do not produce milk until maturity
- It is easier to extract proteins from urine than from milk

Procedure for Producing Transgenic Mammals

Different steps involved are:

i) DNA containing the gene of interest is injected into donor eggs.

ii) In vitro fertilization is performed.

iii) Zygotes are placed in host females where they develop.

iv) After maturation of female offspring, product is secreted in the milk.

CLONING OF TRANSGENIC ANIMALS

Cloning is form of asexual reproduction and is most preferable method for getting identical copies of animals.

Old Concept about Cloning of Transgenic Animals

For many years, it was believed that adult vertebrate animals could not be cloned. Although each cell contains a copy of all the genes, yet certain genes are turned off in mature specialized cells. Different genes are expressed:

- In muscle cells, which contract.
- In nerve cells, which conduct nerve impulses.
- In glandular cells, which secrete.

Cloning of an adult vertebrate requires that all genes of an adult cell be turned on again if development is to proceed normally. It had long been thought that it is impossible.

Cloning of Transgenic Animals

In 1997, scientists at Roslin Institute in Scotland produced a cloned sheep called Dolly. Since then calves and goats have been cloned.

Now scientists have developed method to clone human, but USA government has abandoned it.

Cloning of Transgenic Mice

Different steps involved are:

i) 2n nuclei from cumulus cells (those that cling to an egg after ovulation process occurs) were taken and introduced in enucleated egg.

ii) A specially prepared chemical bath was used to stimulate the eggs to divide and begin development.

23.7 GENE THERAPY

Definition

It is the insertion of genetic material into human cells for the treatment of a disorder.

Processes Involved

Two procedures are involved

i) Removal of defective gene

ii) Insertion of healthy gene

Methods Involved

There are two main methods used for gene therapy.

i) Ex-vivo, which is most commonly used

ii) In-vivo, which is least used

Examples

i) Severe Combined Immunodeficiency Syndrome (SCID)

- Children having this disease lack an enzyme adenosine deaminase (ADA) that is involved in maturation of T and B cells. Such children are subjected to life threatening infections.
- Bone marrow stem cells are removed from the blood and infected with a retrovirus (RNA virus) that carries a normal gene for the enzyme then the cells are returned to the patient. Bone marrow stem cells are preferred for this procedure, because they divide to produce more cells with some genes.
- Patient treated with this procedure have shown considerable improvement.

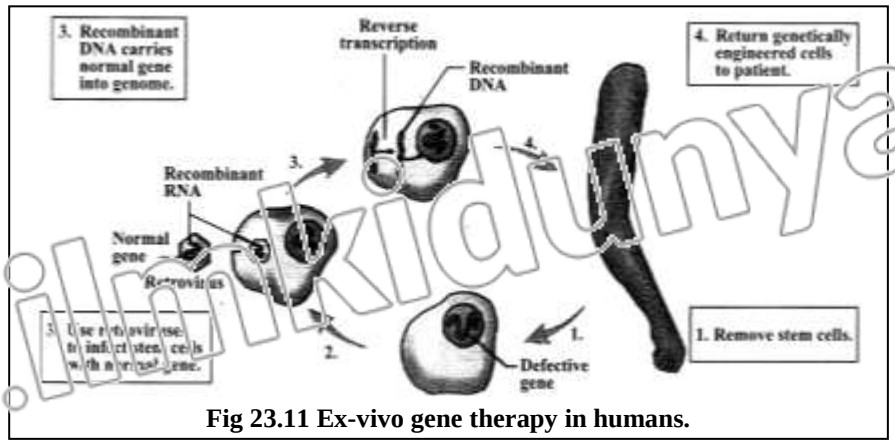


Fig 23.11 Ex-vivo gene therapy in humans.

ii) Familial Hypercholesterolemia

- It is due to deficiency of receptors in liver cells that remove cholesterol from blood.
- High levels of blood cholesterol make the patient subject to fatal heart attacks in young age.
- In a newly developed procedure, a small portion of the liver is surgically excised and infected with a retrovirus containing a normal gene for the receptor.
- Several patients have experienced a lowering of serum cholesterol levels following this procedure.

iii) Cystic Fibrosis

- Cystic fibrosis patients lack a gene that codes for trans-membrane carrier of the chloride ions.
- Patients often die due to numerous infections of the respiratory tract.
- An in-vivo method of treatment is being tried. Lipoproteins are put into a solution containing gene for cure of cystic fibrosis. After coating of this gene, we develop liposome-microscopic vesicles. This solution is sprayed into patient's nostrils.
- Due to limited gene transfer, this methodology has not as yet been successful.

iv) Cancer

Through gene therapy, researchers are trying to

- Make healthy cells more tolerant of chemotherapy
- Make tumor cells more vulnerable to chemotherapy

Once the bone marrow stem cells were protected, it was possible to increase the level of chemotherapy to kill the cancer cells.

v) Coronary Artery Angioplasty

- During coronary artery angioplasty, a balloon catheter is used to open up a closed artery. But the artery has a tendency to close up once again.
- By gene therapy, solution has been made. The balloon is coated with a plasmid that contains a gene for vascular endothelial growth factor. Expression of the gene promotes proliferation of blood vessels to bypass the obstructed area.
- Improvement has been observed in at least one patient.

vi) Others

By gene therapy, it will be possible to use in vivo therapy to cure hemophilia. Diabetes, Parkinson's disease or AIDS.

- To treat hemophilia, patients could get regular doses of cells that contain normal clotting-factor genes, or such cells could be placed in organoids (artificial organs that can be implanted in abdominal cavity).
- To cure Parkinson's disease, dopamine-producing cells could be grafted directly into the brain.

23.8 TISSUE CULTURE

Definition

"Tissue culture is the growth of a tissue in an artificial liquid culture medium."

It is also called micropropagation

Work of Gottlieb Haberlandt

German botanist Gottlieb Haberlandt in 1902 said that

- Plant cells are totipotent i.e. each cell has a full genetic potential of organism
- A single cell could become a complete plant

Work of F.C. Steward

Cornell botanist F.C. Steward in 1958 first time grew a complete carrot plant from a tiny piece of phloem.

He provided the cells with sugars, minerals and vitamins, but also added coconut milk (it was later discovered that coconut milk contains plant hormones cytokinin.

When the cultured cells began dividing, they produced a callus, which is an undifferentiated group of cells. Then the callus differentiated into shoot and roots and developed into a complete plant.

METHODS OF TISSUE CULTURING

Tissue culture techniques are used to produce millions of identical seedlings in a limited amount of space. Common methods used in this are following.

1) Meristem Culture

In this method, meristematic cells are used.

Procedure

Different steps involved are:

i) A small piece of tissue, usually mesophyll tissue from a leaf, is taken and enzymes are added to digest cell wall and convert it into protoplast.

ii) Protoplasts regenerate a new cell wall and begin to divide due to presence of auxins and cytokinins in liquid medium.

iii) Clumps of cells are manipulated to produce somatic embryos. These somatic embryos (sometimes called artificial seeds) are encapsulated in a protective hydrated gel. Somatic embryos of tomato, celery, asparagus, lilies, begonias and African violets can be produced in millions in large tanks called bioreactors.

iv) A mature plant develops from each somatic embryo. Plants generated from somatic embryo vary somewhat because of mutations that arise during the production process. These are called somaclonal variations.

Advantages

i) Identical (clonal) plants of desired traits are produced.

ii) Meristem is virus free portion of plant, so the plants produced are also virus free. The presence of plant viruses weakens plants and makes them less productive.

2) Anther Culture

It is a technique in which mature anthers are cultured in a medium containing vitamins and growth regulators.

It is useful in plants that express recessive alleles.

Procedure

Different steps involved are:

i) Haploid tube cells within pollen grain divide, producing pro-embryos consisting of as many as 20-40 cells.

ii) Pollen grains rupture releasing haploid embryos.

iii) At this level, haploid plant can be generated, or chemical agents are added that encourage chromosomal doubling

iv) After chromosomal doubling, resulting plants are diploid but homozygous for all their alleles.

3) Cell Suspension Culture

It is used to culture plant tissues to obtain different compounds.

Procedure

Different steps involved are:

i) Rapidly growing cultures are cut into small pieces and shaken in a liquid nutrient medium so that single cells or small clumps of cells break off and form a suspension.

ii) These cells produce the same chemicals as the entire plant.

Examples

Cell suspension cultures of

- *Cinchona ledgeriana* produce quinine.
- *Digitalis lanata* produce digitoxin.

Future Goal

Scientist envision that it will be possible to maintain cell suspension cultures in bioreactors for the purpose of producing chemicals used in the production of drugs, cosmetics and agricultural chemicals. If so, it will no longer be necessary to farm plants for the purpose of acquiring the chemical they produce.

23.9 GENETIC ENGINEERING OF PLANTS

HYBRIDIZATION VERSUS GENETIC ENGINEERING

- Traditionally, hybridization, the crossing of different varieties of plants or even species, was used to produce plants with desirable traits.
- Now a day's transgenic plants (having foreign gene) are produced through genetic engineering to get desired results.

PROCEDURE FOR GENERATION OF TRANSGENIC PLANTS

1) Insertion of Gene through Current

Different steps involved in this process are given below.

i) A foreign gene isolated from any type of organism is placed in the tissue culture medium.

ii) This tissue culture contains protoplasts.

iii) High voltage electric pulses are used to create pores in the plasma membrane so that DNA enters.

Example

A gene for production of firefly enzyme **(luciferase)** was inserted into tobacco protoplast and adult plant glowed when sprayed with the substrate luciferin.

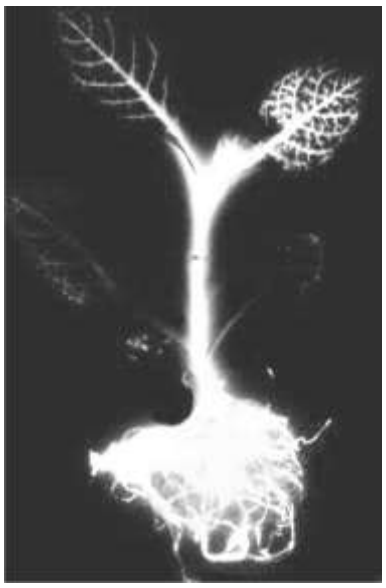


Fig 23.12 Tobacco plant containing luciferase gene glows when sprayed with luciferin.

Limitation

Corn and wheat protoplasts produce infertile plants when produced through this technique.

2) Insertion of Gene through Bacterium

This process includes following steps.

i) A plasmid is used to produce recombinant DNA. This recombinant DNA contains foreign gene.

ii) It is inserted into plasmid of bacterium **Agrobacterium**, which normally infects the plant cells.

iii) When bacterium infects the plant, recombinant DNA is introduced into plant cells.

3) Insertion through Particle Gun

This method was developed by John C. Sanford and Theodore M. Klein of Cornell University in 1987.

Many plants including corn and wheat varieties have been genetically engineered by this method.

Procedure

They constructed a device; particle gun that bombards a callus with DNA coated microscopic metal particles. Then genetically altered somatic embryos developed into adult plants.

QUESTIONS RELATED TO ABOVE ARTICLE

For what purpose have bacteria, plants and animals been genetically altered?

(Exercise Question v)

23.10 AGRICULTURAL PLANTS WITH IMPROVED TRAITS

Biotechnology has played a vital role in field of agriculture. Some of the important aspects in this context are given below.

1) Disease Resistant Varieties

- Cotton, corn, potato and soybean plants have been engineered to be resistant to either insect predation or herbicides that are judged to be environmentally safe.
- When herbicide resistant plants are planted, weeds can easily be controlled.
- In 1999, transgenic crops were planted on more than 70 million acres world wide and acreage is expected to triple in about five years.

2) Production of Salt Tolerant Plants

Irrigation leads to salinization of soil that reduced crop yield. Today crop production is limited by effects of salinization at about 50% of irrigated levels.

Recently a salt-tolerant *Arabidopsis* has been produced. It contains a gene coding for a channel protein that transport Na^+ along with H^+ across a vacuole membrane. Isolating Na^+ in a vacuole prevents it from interfering with plant metabolism.

Now scientists are trying to produce drought and cold tolerant crops.

3) Increasing Quality of Crops

- Progress has also been made to increase the food quality of crops.
- Soybeans have been developed that mainly produce the monounsaturated fatty acid oleic acid, a change that may improve human health. These altered plants also produce vernolic acid and ricinoleic acid, derivatives of oleic acid. These can be used for hardness in paints and plastics.
- Genes for these compounds were derived from Vernonia and Castor Bean seeds and were transferred into the soybean genomes.
- Scientists have aimed to produce crops that have improved agricultural or food quality traits such as those listed in table below.

Improved Agricultural Traits

Improved Traits	Plants made
Herbicide resistant	Wheat, rice, sugar beets, canola
Salt tolerant	Cereals, rice, sugarcane
Drought tolerant	Cereals, rice, sugarcane
Cold tolerant	Cereals, rice, sugarcane
Improved yield	Cereals, rice, corn, cotton
Modified wood pulp	Trees

Improved Food Quality Traits

Improved trait	Plants made
Fatty acid/ Oil content	Corn, soybeans
Protein/ Starch content	Cereals, potatoes, soybeans, rice, corn
Amino acid content	Corn, soybean
Disease protected	Wheat, corn, potatoes

4) Increasing Productivity

Genetic engineering is also expected to increase productivity. To that end;

- Stomata might be altered to boost carbon dioxide intake or cut down water loss.
- Efficiency of enzyme RuBP carboxylase, which captures CO_2 in plants could be improved.
- Japanese scientists are working on introducing the C_4 photosynthetic cycle into rice. Unlike C_3 plants, C_4 plants do well in hot dry weather.

5) Production of Human Products

i) Single gene transfers have allowed plants to produce various products such as human hormones, clotting factors and antibodies.

- Antibody made by corn can deliver radioisotopes to tumor cells.
- Antibody made by soybeans can be used as treatment for genital herpes clinical traits have begun.

ii) Scientists of Biosource Technologies in Vacaville, California have reported that they have been able to use the tobacco mosaic virus as vector to introduce a human gene into adult tobacco plants. This technology by passes the need for tissue culture completely.

iii) Tens of grams of α-galactosidase (enzyme used to treat human lysosomal storage disease) were harvested per acre of tobacco plants.

iv) Tobacco plants have been engineered to produce antigens to treat Non-Hodgkin's lymphoma in just only 30 days after being sprayed with a genetically engineered virus.

KEY POINTS

Palindromic sequences

The sequence of four or six nucleotides, which are arranged in reverse order is called Palindromic sequences. These sequences are especially cut by the end nucleases enzymes. For example ATTA, or GACGAC.

Agar

Agar is special gel like compound obtained from the algae. It is used for culturing different organism.

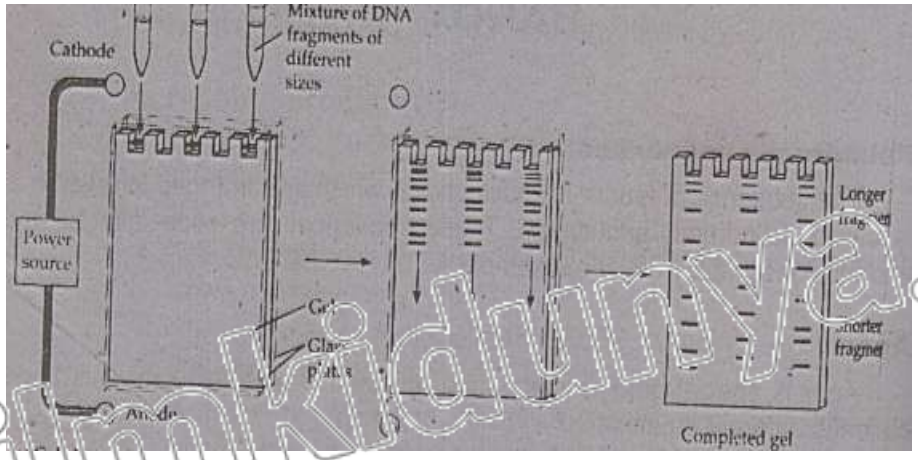
RFLP

The DNA restriction enzyme cut the piece of DNA in to small fragments. As these sequence in the restriction fragment are present at many places in the genome of an organism, even in the genome of all of the organism, so these fragments are called polymorphism (means many form). Hence termed as restriction fragment length polymorphism. Thus RFLP are fragment of DNA in the genome present in many place in the genome which can be cut selectively by a single DNA restriction enzyme.

Gel electrophoresis

It is an instrument in which gel is present between two glass plates. The fragment of DNA can easily move through this gel. Positive charge is given on one side of the gel and negative charge is given on the other side of this gel.

Gel electrophoresis separate the macromolecules on the basis of their rate of movement through a gel under the influence of electric field. The smaller fragments have greater rate of movement than the larger fragments.



Green revolution

Dr. Norman Barioug discovered high yield producing wheat varieties in 1960's. When ample inputs in the form of fertilizer, water were give to these varieties, they produce high yield. He called the discovery of these varieties as green revolution.

Per capita

The amount of total food divided by total population give per capita use of food. It gives average consumption of food by a population.

Bone marrow stem cells

There are certain cells which divide and produce other bone marrow cell. Such cells are called bone marrow stem cells.

Cystic fibrosis

It is genetic disease. In this case, a special enzyme which remove chloride ion from the cell is missing. So chloride ions accumulate in the cells. Thus osmotic pressure of these cells is increased and cell take up water from the outer mucous. Thus the mucous become thick in pancreas, lungs, digestive tract and other organs. It causes pneumonia and other infection. The effected person has to take regular antibiotics. Finally, it causes death.

Angioplasty

It is a treatment of heat disease. In this case, the coronary artery is dilated by inflation of a balloon under high pressure. This pressure of balloon ruptures the plaque in the artery.

G_4 Cycle

It is a type of dark reaction during photosynthesis. In this case, four carbon compound is produced instead of three carbon compound as in the case of Calvin cycle.

Agitator

It is a device to put something into motion by shaking or stirring.

Vegetative propagation

It is a type of asexual reproduction and refers to the process in which a new plant grows from a fragment of parent plant.

C_4 Plant

A C_4 plant is a plant that cycles CO_2 into 4C sugar compounds to enter into Calvin cycle.

EXERCISE

Q 1

Fill in the blanks.

i) The use of polymerase chain reaction (PCR) creates a _____ or copies with a laboratory test tube

ii) _____ are free living organisms in the environment that have had a foreign gene inserted into them.

iii) _____ is known sequence of DNA that are used to find complementary DNA strands; can be used diagnostically to determine the presence of particular gene.

iv) _____ is the production of many identical copies of a gene.

v) _____ are self duplicating ring of accessory DNA in the cytoplasm of bacteria.

Ans (i) Million (ii) Transgenic Organism (iii) Probe (iv) Cloning (v) Plasmids

Q 2 Encircle the correct answer from the multiple choices.

i) Which of these is true statement?
(a) Both plasmids and viruses can serve as vectors
(b) Plasmids can carry recombinant DNA but viruses cannot
(c) Vectors carry only the foreign gene into the host cell
(d) Only gene therapy uses vectors
(e) Both a and d are correct

ii) Which of these is a benefit to having insulin produced by biotechnology?
(a) It is just as effective
(b) It can be mass produced
(c) It is non allergic
(d) It is less expensive
(e) All of the above are correct

iii) Restriction fragment length polymorphism (RFLPs):
(a) Are achieved by using restriction enzymes
(b) Identify individuals genetically
(c) Are the basis for DNA finger prints
(d) Can be subjected to gel electrophoresis
(e) All of the above are correct

iv) Which of these would you not expect to be a biotechnology product?
(a) Vaccine
(b) Modified enzyme
(c) DNA probes
(d) Protein hormones
(e) Steroid hormone

v) What is the benefit of using a retrovirus as a vector in gene therapy?
(a) It is not able to enter cells
(b) It incorporates the foreign gene into the host chromosome
(c) It eliminates a lot of unnecessary steps
(d) It prevents infection by other viruses
(e) Both b and c are correct

vi) Gel electrophoresis:
(a) Cannot be used on nucleotides
(b) Measures the size of plasmids
(c) Tells whether viruses are infectious
(d) Measures the change and size of proteins and DNA fragments
(e) All of the above are correct

vii) Which of these is incorrectly matched?
(a) Protoplast – plant cell engineering
(b) RFLPs – DNA finger printing
(c) DNA polymerase - PCR
(d) DNA ligase–mapping human chromosomes

Answer keys

i	a	vi	d
ii	e	vii	d
iii	e		
iv	e		
v	e		

Q 3 Short Questions

i) How and why transgenic animals that secrete a product are often cloned?

Ans. Transgenic animals are usually cloned by tissue culture technique. This procedure is done to get large number of animals with desired results.

ii) Explain two primary goals of human genome project. What are possible benefits of project?

Ans. Two primary goals are;

- First goal is to construct a genetic map of human genome.
- The second goal is to construct a base sequence map.

We can study diseases at genetic level and can find out their solution.

iii) Explain and give examples of ex vivo and in vivo gene therapies in humans.

Ans. **Ex Vivo gene therapy:**

The gene therapy in which genes are inserted into the cell outside the body is called Ex Vivo gene therapy.

Example:

Treatment of severe combined immunodeficiency syndrome (SCID).

Treatment of hypercholesterolemia.

In vivo gene therapy:

The gene therapy in which genes are inserted in the cells within the body is called in Vivo gene therapy.

Example:

Treatment of Cystic fibrosis.

Treatment of cancer.

Treatment of hemophilia, diabetes, Parkinson's disease, or AIDS.

Q 4 Extensive Questions.

i) What is methodology for producing recombinant DNA to be used in gene cloning?

Ans (see article 23.1)

ii) What is a genomic library and how would you locate a gene of interest in the library?

Ans (see article 23.2)

iii) What is the polymerase chain reaction (PCR) and how is it carried out to produce multiple copies of a DNA segment?

Ans (see article 23.3)

iv) What is DNA fingerprinting, a process that utilizes the entire genome?

Ans (see article 23.3.1)

v) For what purpose have bacteria, plants and animals been genetically altered?

Ans (see article 23.6)