Class: 12th

Subject: Biology

Chapter 23: BIOTECHNOLOGY

🩸 Important MCQs:

1. The rediscovery of Mendel's work took place in:

(a) 1800

(b) 1856

(c) 1900 ✅

(d) 1953

2. The production of human insulin through biotechnology began in:

(a) 1960s

(b) 1970s

(c) 1980s ✅

(d) 1990s

3. The branch of biology that deals with manipulation of genes is called:

(a) Cytology

(b) Genetic Engineering ✅

(c) Microbiology

(d) Embryology

4. The technique used to produce a large number of copies of a gene is:

(a) Cloning ✅

(b) Transcription

(c) Translation

(d) Transformation

5. The polymerase chain reaction (PCR) helps in:

(a) Producing proteins

(b) Making copies of DNA in a test tube ✅

(c) Breaking DNA strands

(d) Isolating RNA

6. The enzyme used to cut DNA molecules at specific sites is called:

(a) DNA ligase

(b) Restriction endonuclease ✅

(c) DNA polymerase

(d) Reverse transcriptase

7. Restriction enzymes were first isolated by:

(a) Gregor Mendel

(b) James Watson

(c) Hamilton O. Smith ✅

(d) Charles Darwin

8. Restriction enzymes recognize and cut DNA at:

(a) Random sequences

(b) Palindromic sequences ✅

(c) Start codons

(d) Stop codons

9. The ends of DNA fragments produced by restriction enzymes are called:

(a) Hard ends

(b) Sticky ends ✅

(c) Smooth ends

(d) Soft ends

10. The enzyme that joins the gene of interest to the plasmid is:

(a) DNA polymerase

(b) DNA ligase ✅

(c) RNA polymerase

(d) Endonuclease

11. In recombinant DNA technology, the vector used to carry the gene is often a:

(a) Ribosome

(b) Chromosome

(c) Plasmid ✅

(d) Nucleus

12. Plasmids are:

(a) Circular extra-chromosomal DNA ✅

(b) Linear DNA molecules

(c) RNA fragments

(d) Chromosomal segments

13. The plasmid PBR 322 contains genes for resistance to:

(a) Streptomycin only

(b) Tetracycline and ampicillin ✅

(c) Chloramphenicol only

(d) Penicillin only

14. Complementary DNA (cDNA) is synthesized from mRNA using the enzyme:

(a) DNA ligase

(b) RNA polymerase

(c) Reverse transcriptase ✅

(d) Restriction endonuclease

15. Recombinant DNA formed by joining DNA from two sources is also called:

(a) Complementary DNA

(b) Chimaeric DNA ✅

(c) Messenger DNA

(d) Genomic DNA

16. A clone is a group of:

(a) Different genes

(b) Identical molecules, cells, or organisms ✅

(c) Mutated organisms

(d) Similar species

17. Calcium chloride treatment in bacteria helps to:

(a) Kill the bacteria

(b) Make cells more permeable for plasmid uptake ✅

(c) Stop protein synthesis

(d) Destroy DNA

18. Which of the following can be used as a vector besides plasmids?

(a) Ribosomes

(b) Lambda phage ✅

(c) tRNA

(d) Mitochondria

19. A genomic library is:

(a) A collection of mRNA molecules

(b) A collection of bacterial or viral clones containing DNA fragments ✅

(c) A set of ribosomes in a cell

(d) A DNA repair mechanism

20. The total set of genes in an individual is called:

(a) Chromosome

(b) Genome ✅

(c) Gene pool

(d) Nucleus

21. The tool used to find a specific gene in a genetic library is called:

(a) Ligase

(b) Probe ✅

(c) Vector

(d) Polymerase

22. A probe can be detected because it is:

(a) Colorless

(b) Radioactive or fluorescent ✅

(c) Acidic

(d) Transparent

23. The Polymerase Chain Reaction (PCR) was invented by:

(a) Paul Berg

(b) Kary B. Mullis ✅

(c) Francis Crick

(d) Rosalind Franklin

24. PCR is used for:

(a) Cutting DNA

(b) Making millions of copies of DNA ✅

(c) Repairing DNA

(d) Sequencing RNA

25. The enzyme used in PCR that is heat-stable is:

(a) DNA ligase

(b) RNA polymerase

(c) Taq polymerase ✅

(d) Restriction enzyme

26. Taq polymerase was isolated from:

(a) Escherichia coli

(b) Thermus aquaticus ✅

(c) Bacillus subtilis

(d) Lactobacillus casei

27. The machine used for PCR is called:

(a) Centrifuge

(b) Thermocycler ✅

(c) Spectrophotometer

(d) Electrophoresis chamber

28. DNA fingerprinting is based on differences in:

(a) Protein size

(b) Restriction Fragment Length Polymorphism (RFLP) ✅

(c) Amino acid sequence

(d) Ribosomal RNA

29. DNA fragments are separated by:

(a) PCR

(b) Gel electrophoresis ✅

(c) Centrifugation

(d) Chromatography

30. DNA fingerprinting can be used to:

(a) Cure diseases

(b) Identify criminals and determine parentage ✅

(c) Produce vaccines

(d) Synthesize proteins

31. The Sanger method of DNA sequencing uses:

(a) Radioactive probes

(b) Dideoxynucleotides to stop DNA synthesis ✅

(c) Restriction enzymes

(d) RNA primers

32. The Maxam-Gilbert method involves:

(a) Using fluorescent dyes

(b) Chemical cleavage of DNA at different sites ✅

(c) Enzyme digestion

(d) Protein modification

33. Automated DNA sequencing uses:

(a) Manual reading of gels

(b) Colored fluorescent dyes and laser detection ✅

(c) X-ray films

(d) Chemical labels only

34. The Human Genome Project began as an initiative of:

(a) The United Nations

(b) The U.S. government ✅

(c) World Health Organization

(d) European Union

35. The main goals of the Human Genome Project were to:

(a) Clone human organs

(b) Map and sequence all human genes ✅

(c) Produce artificial DNA

(d) Eliminate genetic diseases

36. Organisms that have a foreign gene inserted into them are called:

(a) Clones

(b) Transgenic organisms ✅

(c) Recombinant organisms

(d) Hybrid species

37. The large tanks used to culture genetically modified bacteria are called:

(a) Incubators

(b) Bioreactors ✅

(c) Thermocyclers

(d) Biochambers

38. Insulin and human growth hormone are produced by:

(a) Transgenic animals

(b) Transgenic bacteria ✅

(c) Transgenic plants

(d) Stem cells

39. Frost-minus bacteria are genetically engineered to:

(a) Cause ice crystal formation

(b) Prevent frost damage in plants ✅

(c) Increase photosynthesis

(d) Produce antibiotics

40. Bacteria used for cleaning oil spills and toxic wastes are called:

(a) Pathogenic bacteria

(b) Bio-remediating bacteria ✅

(c) Nitrogen-fixing bacteria

(d) Sulphur bacteria

41. Suicide genes are inserted into bacteria to:

(a) Make them reproduce faster

(b) Cause them to self-destruct after completing their task ✅

(c) Increase their lifespan

(d) Enhance resistance to antibiotics

42. The amino acid used in the production of the sweetener aspartame (NutraSweet) is:

(a) Lysine

(b) Phenylalanine ✅

(c) Glycine

(d) Tryptophan

43. The use of bacteria to extract metals from low-grade ores is called:

(a) Biodegradation

(b) Bioleaching ✅

(c) Bioluminescence

(d) Biomining

44. Transgenic plants are developed by introducing foreign genes into:

(a) Mature leaves

(b) Immature embryos or protoplasts ✅

(c) Flower petals

(d) Fruit tissues

45. In transgenic plants, an electric current is used to:

(a) Kill harmful microorganisms

(b) Create tiny holes in the plasma membrane for DNA entry ✅

(c) Activate chlorophyll

(d) Increase respiration rate

46. Transgenic crops like cotton and corn have been made resistant to:

(a) Viruses only

(b) Pests and herbicides ✅

(c) Fungal infections

(d) Drought stress

47. The process of using transgenic farm animals to produce pharmaceuticals is known as:

(a) Gene cloning

(b) Gene pharming ✅

(c) Genetic mapping

(d) DNA fingerprinting

48. Antithrombin III, used to prevent blood clots, is produced by transgenic:

(a) Cows

(b) Goats ✅

(c) Sheep

(d) Pigs

49. The first cloned sheep produced by scientists was named:

(a) Mary

(b) Dolly ✅

(c) Lucy

(d) Molly

50. The main purpose of gene therapy is to:

(a) Clone humans

(b) Insert normal genes to cure genetic diseases ✅

(c) Create designer babies

(d) Increase cell mutation rate

51. The growth of plant tissues in an artificial medium is called:

(a) Hybridization

(b) Tissue culture ✅

(c) Grafting

(d) Vegetative propagation

52. The concept of totipotency was first proposed by:

(a) F.C. Steward

(b) Gottlieb Haberlandt ✅

(c) John Sanford

(d) Theodore Klein

53. F.C. Steward first grew a complete carrot plant from a piece of:

(a) Root

(b) Phloem tissue ✅

(c) Leaf

(d) Flower

54. Coconut milk promotes plant growth because it contains:

(a) Auxin

(b) Cytokinin ✅

(c) Gibberellin

(d) Ethylene

55. The undifferentiated mass of plant cells formed during tissue culture is called:

(a) Protoplast

(b) Callus ✅

(c) Embryo

(d) Meristem

56. The commercial production of thousands of identical seedlings is called:

(a) Polyploidy

(b) Micropropagation ✅

(c) Cross-breeding

(d) Hybridization

57. Micropropagation through shoot tips is achieved by:

(a) Meristem culture ✅

(b) Anther culture

(c) Root culture

(d) Embryo culture

58. Plants produced through meristem culture are:

(a) Mutated plants

(b) Clonal and virus-free ✅

(c) Heterozygous

(d) Polyploid

59. Naked plant cells without cell walls are known as:

(a) Meristems

(b) Protoplasts ✅

(c) Callus cells

(d) Somatic embryos

60. Somatic embryos encapsulated in hydrated gel are known as:

(a) True seeds

(b) Artificial seeds ✅

(c) Clonal seeds

(d) Hybrid seeds

61. Plants produced by anther culture are useful for expressing:

(a) Dominant alleles

(b) Recessive alleles ✅

(c) Hybrid traits

(d) Polyploid traits

62. Cell suspension culture is mainly used to:

(a) Produce drugs and useful chemicals ✅

(b) Produce hybrid seeds

(c) Grow ornamental plants

(d) Develop insect resistance

63. The plant Digitalis lanata in suspension culture produces:

(a) Quinine

(b) Digitoxin ✅

(c) Morphine

(d) Vinblastine

64. The bacterium Agrobacterium is used in plant genetic engineering because:

(a) It produces auxins

(b) It can transfer plasmid DNA into plant cells ✅

(c) It fixes nitrogen

(d) It kills pests

65. The device used to introduce DNA-coated metal particles into plant cells is called:

(a) Gene gun (Particle gun) ✅

(b) Plasmid injector

(c) Micro pipette

(d) DNA ligase

66. Scientists who developed the particle gun method were:

(a) F.C. Steward and Haberlandt

(b) John Sanford and Theodore Klein ✅

(c) Watson and Crick

(d) Smith and Mullis

67. Salt-tolerant Arabidopsis plants were produced by introducing a gene for:

(a) Na^+ channel protein ✅

(b) RuBP carboxylase

(c) Luciferase

(d) Herbicide resistance

68. The enzyme RuBP carboxylase is involved in:

(a) Protein synthesis

(b) Carbon dioxide fixation ✅

(c) Nitrogen metabolism

(d) DNA replication

69. Transgenic soybeans have been engineered to produce high amounts of:

(a) Saturated fatty acids

(b) Oleic acid ✅

(c) Linoleic acid

(d) Palmitic acid

🩸Q3: Exercise Short Questions

1. How and why transgenic animals that secrete a product are often cloned?

👉 **Answer:**

Transgenic animals that secrete a useful product, such as a human hormone or protein in their milk, are often cloned to produce identical offspring carrying the same inserted gene.

✅ **Reason:**

Cloning ensures that all animals:

- Have the same genetic makeup
- Express the desired gene efficiently
- Produce the same quantity and quality of the product

Example:

A cloned sheep or goat may produce human insulin or growth hormone in its milk, which can then be purified for medical use.

2. Explain two primary goals of the Human Genome Project. What are possible benefits of the project?

👉 **Answer:**

The Human Genome Project (HGP) was launched to map and understand all the genes of human beings.

✅ **Two Primary Goals:**

1. To identify and sequence all human genes (approx. 30,000–40,000 genes)

2. To determine the complete sequence of human DNA (about 3 billion base pairs)

✅ Possible Benefits:

- Detection of genetic disorders before symptoms appear
- Development of gene therapies for inherited diseases
- Improved drug design and personalized medicine
- Understanding human evolution and genetic diversity

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

👉 Answer:

Gene therapy is a technique in which a normal gene is inserted into human cells to correct genetic disorders.

(a) Ex Vivo Gene Therapy:

- The defective cells are removed from the patient's body.
- A normal gene is inserted in the lab using a vector (like a virus).
- The modified cells are then returned to the patient.

Example: Treatment of SCID (Severe Combined Immunodeficiency) by inserting the normal ADA gene into patient's white blood cells.

(b) In Vivo Gene Therapy:

- The gene is directly inserted into the patient's body, where it targets the defective cells.

Example: Introducing a corrected gene into lung cells of cystic fibrosis patients using a viral vector.

🩸 Important Short Questions:

1. What is the aim of recombinant DNA technology?

Answer:

The aim of recombinant DNA technology is to create DNA molecules that contain genes from two different sources to produce useful biological products such as insulin or vaccines.

2. What is the difference between recombinant DNA technology and PCR?

Answer:

Recombinant DNA technology produces large quantities of a gene by inserting it into a vector and cloning it, while PCR (Polymerase Chain Reaction) makes multiple copies of DNA in a test tube without using a living organism.

3. What are the main components required for producing recombinant DNA?

Answer:

Four components are required:

1. Gene of interest
2. Restriction enzyme (molecular scissors)
3. Vector (molecular carrier)
4. Expression system for producing the desired product

4. What are restriction endonucleases and who discovered them?

Answer:

Restriction endonucleases are enzymes in bacteria that cut DNA at specific sequences. They were first isolated by Hamilton O. Smith in 1970 at Johns Hopkins University.

5. What is meant by palindromic sequence in DNA?

Answer:

A palindromic sequence is a specific DNA sequence that reads the same forward and backward on complementary strands, allowing restriction enzymes to recognize and cut it.

6. What are sticky ends and why are they important?

Answer:

Sticky ends are single-stranded overhangs created when restriction enzymes cut DNA. They are important because they allow foreign DNA to attach to a vector by complementary base pairing.

7. What is a plasmid and how is it used as a vector?

Answer:

A plasmid is a small, circular DNA molecule found in bacteria that replicates independently. It is used as a vector to carry the foreign gene into a host cell for cloning.

8. What are antibiotic resistance genes and how are they helpful in genetic engineering?

Answer:

Plasmids often carry antibiotic resistance genes (e.g., for tetracycline or ampicillin). These help scientists identify bacteria that have successfully received the recombinant DNA by growing them on selective media.

9. What enzyme is used to join foreign DNA with vector DNA?

Answer:

The enzyme DNA ligase is used to seal the foreign DNA into the vector DNA, forming recombinant DNA.

10. What is recombinant or chimaeric DNA?

Answer:

Recombinant DNA (or chimaeric DNA) is the combined DNA molecule formed by joining DNA from two different sources, such as a human gene and a bacterial plasmid.

11. What is meant by a clone?

Answer:

A clone is a group of identical molecules, cells, or organisms derived from a single parent and having the same genetic makeup.

12. How can bacterial cells be made permeable to take up recombinant plasmid?

Answer:

Bacterial cells are treated with calcium chloride ($CaCl_2$) to make their cell walls more permeable for uptake of recombinant plasmids.

13. What is a genomic library?

Answer:

A genomic library is a collection of bacterial or bacteriophage clones, each containing a specific DNA segment of an organism's genome.

14. What is the function of a probe in a genomic library?

Answer:

A probe is a single-stranded DNA or RNA sequence used to identify a specific gene by hybridizing with its complementary sequence.

15. Who developed the Polymerase Chain Reaction (PCR) and when?

Answer:

PCR was developed by Kary B. Mullis in 1983 to amplify specific DNA sequences rapidly in a test tube.

16. What is the function of primers in PCR?

Answer:

Primers are short sequences of DNA that bind to the target DNA and provide a starting point for DNA polymerase to begin replication.

17. What is Taq polymerase and from where is it obtained?

Answer:

Taq polymerase is a thermostable enzyme obtained from the bacterium Thermus aquaticus, used in PCR because it withstands high temperatures.

18. What is DNA fingerprinting?

Answer:

DNA fingerprinting is a technique used to identify individuals by analyzing unique patterns in their DNA fragments produced by restriction enzymes.

19. What is RFLP?

Answer:

RFLP (Restriction Fragment Length Polymorphism) refers to differences in DNA fragment lengths between individuals due to variations in restriction enzyme cutting sites.

20. Give two applications of PCR technology.

Answer:

1. Diagnosing genetic disorders, viral infections, and cancers.

2. Identifying criminals in forensic laboratories.

21. What is gene sequencing?

Answer:

Gene sequencing is the process of determining the exact order of nucleotide bases (A, T, G, C) in a DNA molecule.

22. Name the two common methods of DNA sequencing.

Answer:

1. Sanger's method (dideoxy method)

2. Maxam-Gilbert method

23. What is the Human Genome Project (HGP)?

Answer:

The Human Genome Project is an international research program aimed at mapping and sequencing all the genes present in human chromosomes.

24. What are the two primary goals of the Human Genome Project?

Answer:

1. To construct a genetic map of all human chromosomes.

2. To determine the base sequence of the entire human genome.

25. What is the significance of RFLPs in the Human Genome Project?

Answer:

RFLPs help scientists locate and identify disease-causing genes, as certain RFLPs are inherited along with defective genes like those causing Huntington's disease.

26. What are transgenic organisms?

Answer:

Transgenic organisms are those that have a foreign gene inserted into their DNA using recombinant DNA technology.

27. Name some biotechnology products produced by transgenic bacteria.

Answer:

Insulin, human growth hormone, tissue plasminogen activator, haemophilia factor VIII, and hepatitis B vaccine.

28. What are bioreactors and what is their purpose?

Answer:

Bioreactors are large vats where genetically engineered bacteria are grown to produce biotechnology products in large quantities.

29. How are transgenic bacteria useful in agriculture?

Answer:

Transgenic bacteria protect plants from frost, insects, and promote soil fertility, for example, frost-minus and insect-resistant bacteria.

30. How can bacteria help in environmental cleanup?

Answer:

Genetically modified bacteria are used to clean oil spills, remove sulfur from coal, and degrade toxic wastes.

31. What are suicide genes in bacteria and why are they used?

Answer:

Suicide genes cause engineered bacteria to self-destruct after completing their job, preventing ecological harm.

32. Give one industrial use of genetically engineered bacteria.

Answer:

They are used to produce phenylalanine, a key ingredient in the artificial sweetener aspartame (Nutrasweet).

33. What are transgenic plants and how are they produced?

Answer:

Plants with foreign genes introduced into their DNA; produced by inserting genes into plant embryos or protoplasts using electric current.

34. Give examples of pest- and herbicide-resistant transgenic crops.

Answer:

Pest-resistant corn, cotton, and potato; herbicide-resistant soybeans.

35. What are future goals of genetic engineering in crops?

Answer:

To increase crop yield, improve protein/starch content, and enhance photosynthetic efficiency.

36. What is the C4 cycle and how is it related to genetic engineering in rice?

Answer:

The C4 cycle is a more efficient photosynthetic process; scientists are trying to introduce it into rice to improve carbon dioxide fixation.

37. Give examples of pharmaceutical products produced by transgenic plants.

Answer:

Human hormones, antibodies, and clotting factors are produced in seeds of corn and soybeans.

38. What is gene pharming?

Answer:

Gene pharming is the use of transgenic animals to produce therapeutic and diagnostic proteins in their milk or urine.

39. Give one example of a biotechnology product obtained from transgenic animals.

Answer:

Antithrombin III, a drug used to prevent blood clots during surgery, is produced by transgenic goats.

40. What was the first cloned mammal and by whom?

Answer:

The first cloned mammal was a sheep named Dolly, produced by scientists at the Roslin Institute, Scotland, in 1997.

41. What is tissue culture?

Answer:

Tissue culture is the growth of plant tissues or cells in an artificial nutrient medium under sterile conditions.

42. Who proposed the concept of totipotency in plants and when?

Answer:

German botanist Gottlieb Haberlandt proposed in 1902 that plant cells are totipotent — each cell can develop into a complete plant.

43. Who successfully grew a complete carrot plant from a single cell and when?

Answer:

F. C. Steward grew a complete carrot plant from a single cell in 1958.

44. What is a callus?

Answer:

A callus is an undifferentiated mass of plant cells formed during tissue culture before they develop into shoots and roots.

45. What is micropropagation?

Answer:

Micropropagation is a commercial method of producing thousands or millions of identical plants (clones) through tissue culture.

46. What is meristem culture and its advantage?

Answer:

Meristem culture involves growing shoot tips in nutrient medium; it produces virus-free clonal plants.

47. What are protoplasts and how are they obtained?

Answer:

Protoplasts are plant cells without cell walls, obtained by digesting the walls with enzymes.

48. What are somatic embryos?

Answer:

Somatic embryos are embryos formed from body (somatic) cells rather than from gametes during tissue culture.

49. What are artificial seeds?

Answer:

Somatic embryos encapsulated in a protective gel are called artificial seeds; they can be stored or transported easily.

50. What is somaclonal variation?

Answer:

Somaclonal variation refers to genetic variations that appear in plants regenerated from tissue culture due to mutations during cell division.

51. What is another culture and what is its significance?

Answer:

Anther culture is a technique where anthers are cultured to produce haploid plants; useful for obtaining plants expressing recessive traits.

52. What is cell suspension culture?

Answer:

It is a technique in which plant cells are grown in a liquid medium forming a suspension, used to produce valuable chemicals like quinine and digitoxin.

53. What is the role of Agrobacterium tumefaciens in genetic engineering of plants?

Answer:

Its plasmid acts as a vector to transfer foreign genes into plant cells, creating transgenic plants.

54. What is a particle gun and who developed it?

Answer:

A particle gun bombards plant cells with DNA-coated metal particles to insert genes; developed by John C. Sanford and Theodore M. Klein in 1987.

55. What is the significance of producing salt-tolerant transgenic plants?

Answer:

Salt-tolerant plants can grow in saline soils, improving crop yield and helping solve food shortages caused by soil salinization.

🩸 Q.4. Extensive questions.

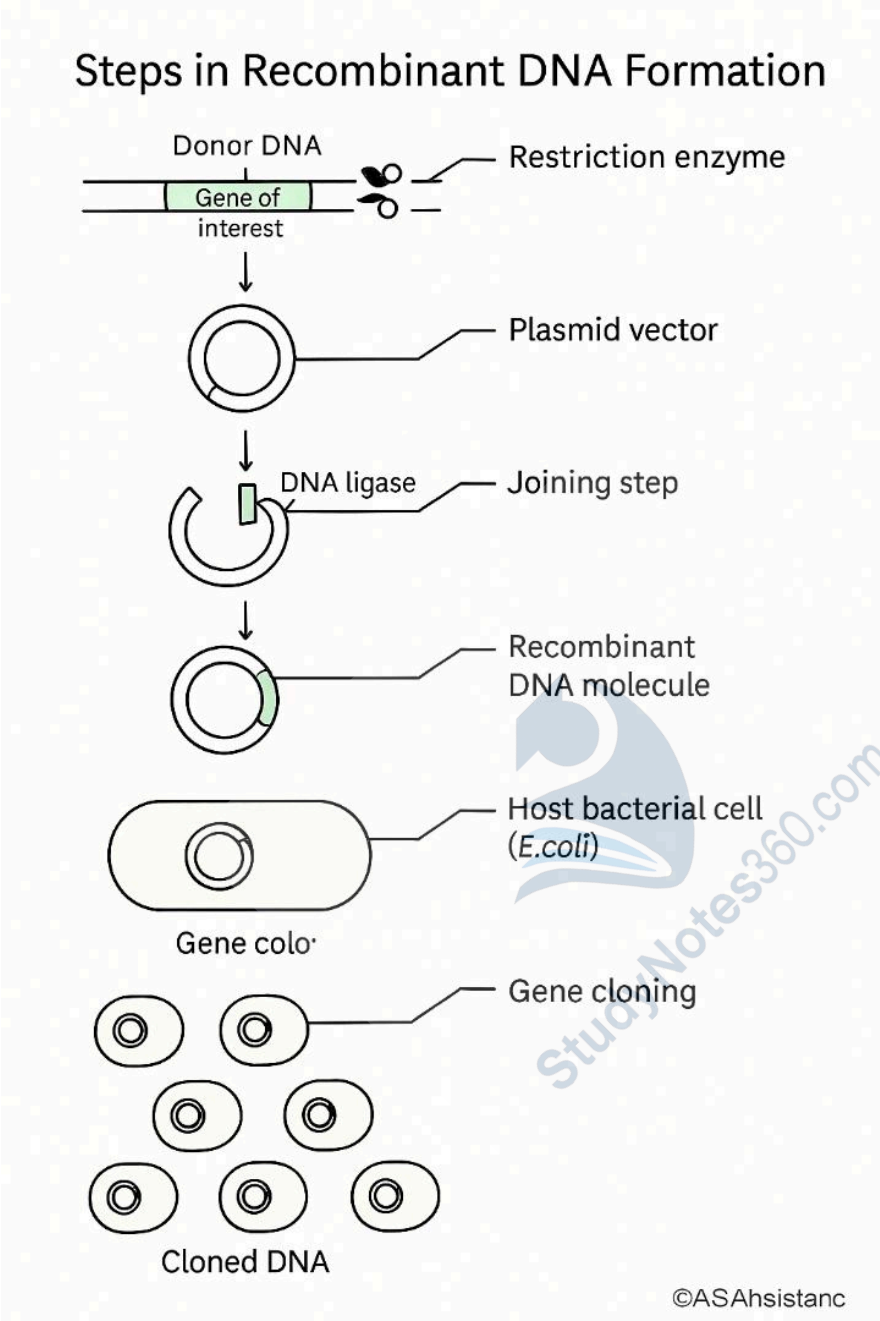
🌟 Q1: What is the methodology for producing recombinant DNA to be used in gene cloning?

❖ **Introduction:**

- Recombinant DNA technology, also called genetic engineering, allows scientists to combine DNA from different organisms.
- It helps in producing valuable biological products such as hormones, vaccines, and enzymes.

🔹 **Definition:**

Recombinant DNA (rDNA) is a hybrid DNA molecule formed by combining genes from two different sources to obtain a desired product.



- **Steps in the Methodology of Producing Recombinant DNA:**

Step 1: Isolation of Gene of Interest

The desired gene is obtained by:

1. Cutting it from the donor chromosome using restriction enzymes, or

2. Synthesizing it artificially in the lab, or

3. Producing it from mRNA using reverse transcriptase enzyme (cDNA).

Step 2: Cutting DNA with Restriction Enzymes

- Both the donor DNA and vector DNA (usually a plasmid) are cut with the same restriction enzyme (e.g., EcoRI).
- These enzymes produce sticky ends that can join easily.

Step 3: Insertion of Gene into Vector

- The donor gene is inserted into the plasmid vector.
- The enzyme DNA ligase joins the foreign gene with the plasmid — forming recombinant DNA.

Step 4: Introduction into Host Cell

- The recombinant plasmid is introduced into a host organism such as E. coli through transformation.
- The host cell then replicates, producing multiple copies of the recombinant DNA.

Step 5: Selection and Expression of Recombinant Gene

- Only transformed cells are selected using antibiotic resistance markers.
- The recombinant gene expresses itself, producing the desired protein or product (e.g., human insulin).

🔹 Applications of Recombinant DNA:

1. Medicine: Production of insulin, vaccines, interferons.

2. Agriculture: Development of pest-resistant, high-yield crops.

3. Industry: Production of enzymes and amino acids.

4. Environment: Creation of pollution-degrading microorganisms.

✦ Summary:

- Recombinant DNA technology involves isolating a gene, cutting it with restriction enzymes, joining it with a vector, and introducing it into a host cell for replication and expression.
- This technique helps in producing medicines, improving crops, and advancing biotechnology.

🌟 Q2: What is a genomic library, and how would you locate a gene of interest in the library?

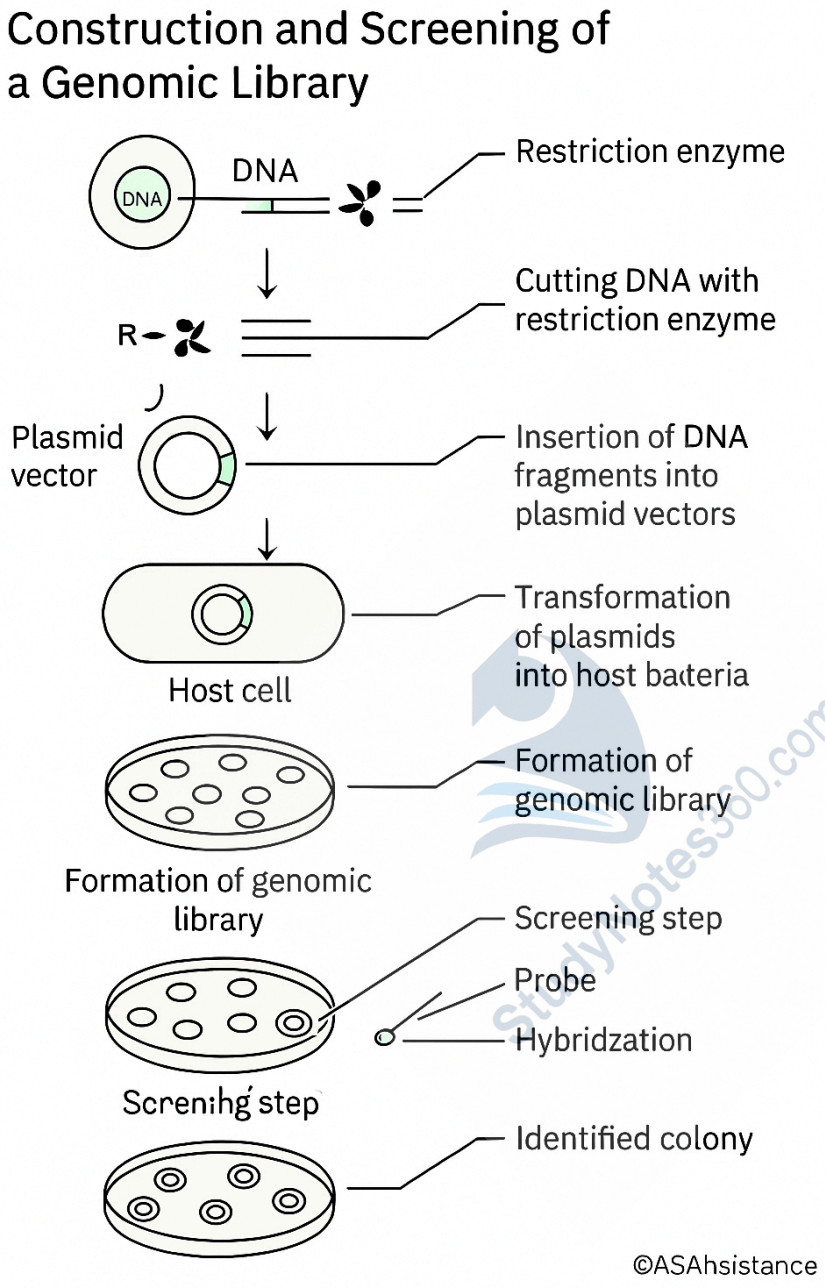
❖ Introduction:

- In recombinant DNA technology, a genomic library serves as a valuable resource for storing and retrieving genes.
- It contains all the genetic material (DNA fragments) of an organism, stored in a collection of vectors.
- This allows scientists to isolate, identify, and study specific genes of interest.

🔹 Definition:

A genomic library is a complete set of DNA fragments that represent the entire genome of an organism, cloned into vectors (like plasmids, bacteriophages, or cosmids) and stored in host cells.

🧬 Each clone in the library contains a different DNA fragment, and together, all the clones make up the entire genome.



- **Steps in Construction of a Genomic Library:**

Step 1: Isolation of Total DNA

- The genomic DNA of the organism is extracted and purified from its cells.

Step 2: Fragmentation of DNA

- The large DNA molecule is cut into smaller fragments using restriction enzymes.
- These enzymes produce overlapping DNA pieces that cover the entire genome.

Step 3: Insertion into Vectors

- Each DNA fragment is inserted into a suitable cloning vector (like a plasmid or bacteriophage).
- This produces recombinant vectors, each carrying a unique segment of the genome.

Step 4: Introduction into Host Cells

- The recombinant vectors are introduced into bacterial host cells (e.g., E. coli) through transformation.
- Each bacterium now carries one DNA fragment.

Step 5: Storage and Maintenance

- All these transformed cells collectively form the genomic library.

- Each cell line is stored and can be grown whenever a specific gene needs to be studied.

🔹 Locating a Gene of Interest in the Genomic Library:

To find a specific gene within the large collection of DNA fragments, scientists use DNA hybridization techniques.

Step 1: Preparation of a Gene Probe

A gene probe is a short, single-stranded DNA or RNA molecule that is:

- Complementary to the desired gene sequence
- Radioactively or fluorescently labeled for detection

Step 2: Screening the Library

- Colonies containing recombinant DNA are grown on agar plates.
- The DNA is transferred onto a nitrocellulose or nylon membrane.

Step 3: Hybridization Process

- The membrane is exposed to the labeled gene probe.
- The probe binds (hybridizes) with any DNA fragment containing the complementary sequence.

Step 4: Detection

- The radioactive or fluorescent signal identifies the colony containing the gene of interest.
- The identified colony is then isolated and cultured to extract or clone the desired gene.

🔹 Applications of Genomic Library:

1. Gene isolation and characterization.

2. Study of gene function and regulation.

3. Production of recombinant proteins.

4. Identification of disease-causing genes.

5. Comparative genomic studies among species.

✦ Summary:

- A genomic library is a collection of cloned DNA fragments representing the whole genome of an organism.
- The gene of interest can be located using a labeled DNA probe that hybridizes with its complementary sequence.

- This technology is essential for gene cloning, genome mapping, and genetic engineering research.

🌟 Q3: What is the Polymerase Chain Reaction (PCR), and how is it carried out to produce multiple copies of a DNA segment?

❖ Introduction:

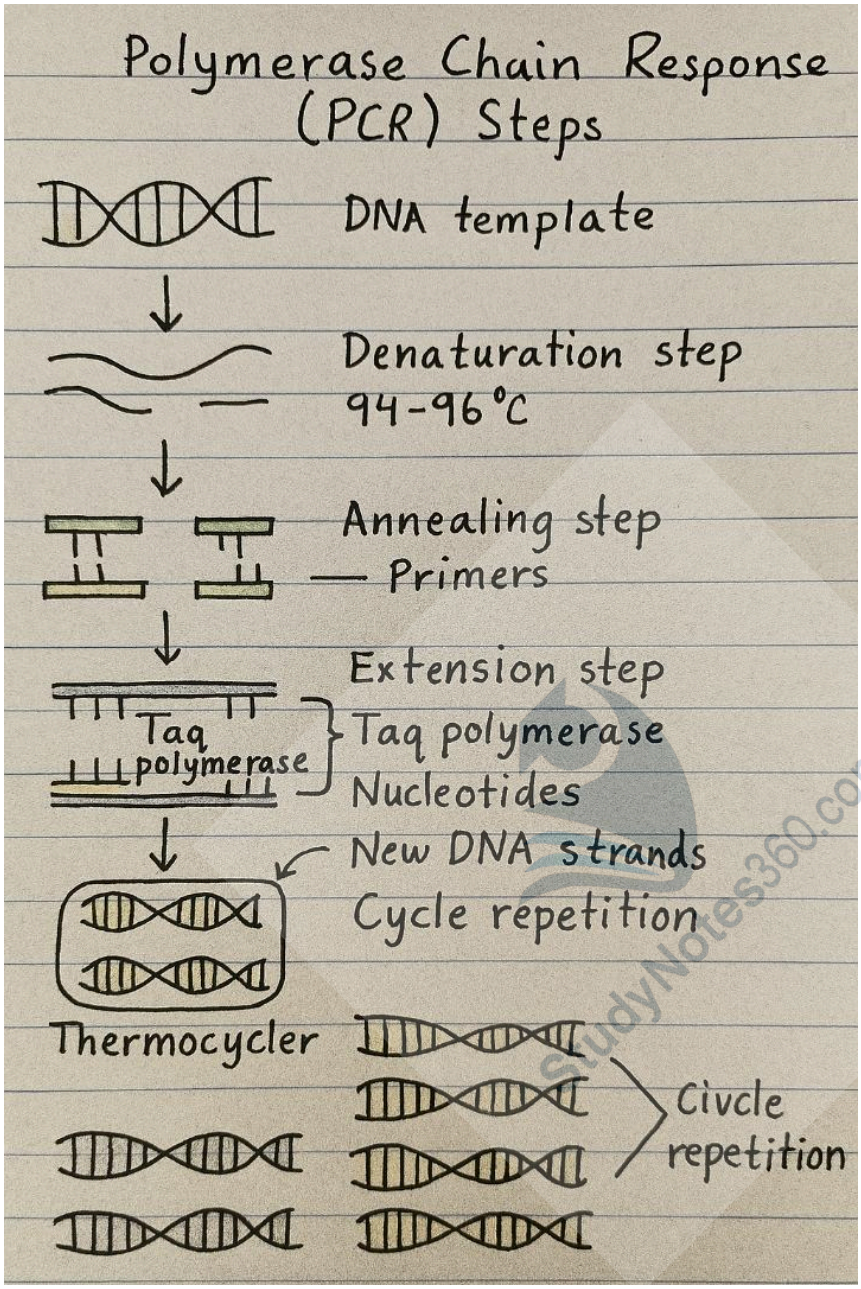
- The Polymerase Chain Reaction (PCR) is one of the most powerful techniques in molecular biology.
- It was developed by Kary Mullis in 1985, and it allows scientists to amplify (make millions of copies) of a specific DNA segment within a few hours.

🔹 Definition:

PCR is a laboratory technique used to make multiple copies of a specific DNA sequence in vitro (outside the living cell), using a DNA polymerase enzyme.

🔹 Purpose of PCR:

- To obtain a large quantity of DNA from a small initial sample.
- Used in gene cloning, medical diagnostics, forensic science, and DNA fingerprinting.



- **Steps Involved in PCR Process:**

Step 1: Denaturation (Separation of DNA Strands)

- The double-stranded DNA is heated to about 94–96°C.

- This causes the hydrogen bonds between the two DNA strands to break, resulting in two single-stranded DNA molecules.

Step 2: Annealing (Binding of Primers)

- The reaction mixture is cooled to about 50–65°C.
- Primers (short single-stranded DNA sequences) attach to the complementary bases on each single strand.
- These primers define the starting and ending points for DNA synthesis.

Step 3: Extension (Synthesis of New DNA Strands)

- The temperature is raised to about 72°C, which is the optimum temperature for Taq DNA polymerase (enzyme obtained from Thermus aquaticus bacterium).
- The enzyme adds nucleotides to the primers and synthesizes new complementary DNA strands.

Step 4: Repetition of the Cycle

- The above three steps (denaturation, annealing, and extension) are repeated 30–40 times in an automatic machine called a thermocycler.
- Each cycle doubles the amount of DNA, leading to millions of copies within hours.

🔹 Key Components Required:

1. Template DNA – the DNA to be copied

2. Primers – short sequences marking the target region

3. Taq DNA Polymerase – heat-stable enzyme for DNA synthesis

4. Free Nucleotides (dNTPs) – building blocks of new DNA strands

5. Buffer Solution – maintains optimal conditions for the enzyme

🔹 **Applications of PCR:**

Medical Diagnosis: Detecting infections such as HIV, hepatitis, COVID-19.

- **Forensic Science:** Identifying criminals through DNA fingerprinting.
- **Genetic Research:** Cloning genes and studying mutations.
- **Evolutionary Studies:** Comparing ancient and modern DNA samples.

✦ **Summary:**

- PCR is a revolutionary technique that mimics DNA replication in a test tube.
- It involves denaturation, annealing, and extension steps, repeated in cycles to amplify DNA exponentially.
- It has countless applications in medicine, research, and forensic science.

🌟 Q4: What is DNA Fingerprinting, a process that utilizes the entire genome?

❖ **Introduction:**

- Every individual (except identical twins) has a unique DNA sequence.
- This uniqueness in DNA makes it possible to identify individuals with great accuracy.
- The technique that makes use of this uniqueness for identification is called DNA Fingerprinting or DNA Profiling.
- It was developed by Sir Alec Jeffreys in 1984 at the University of Leicester (England).

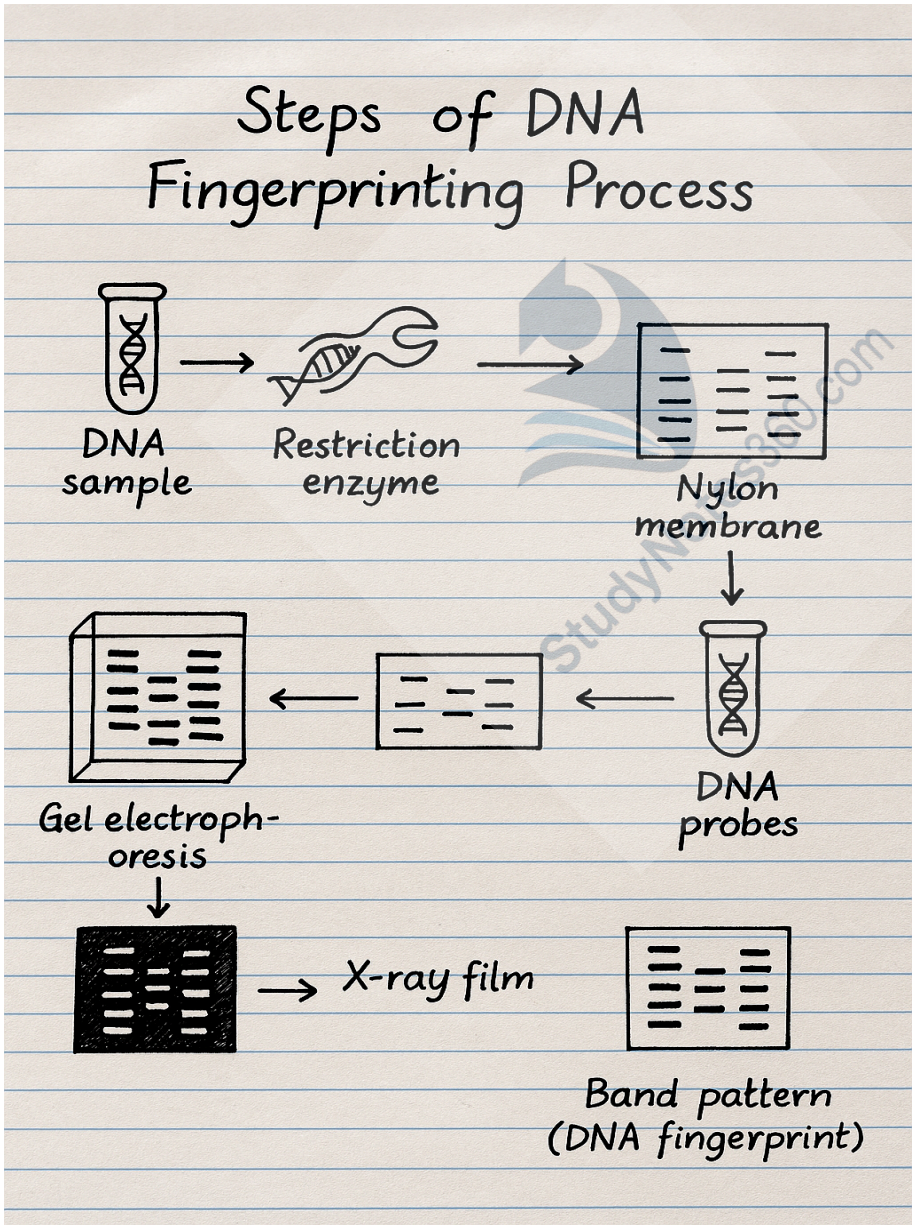
🔹 **Definition:**

DNA Fingerprinting is a technique used to identify and compare an individual's unique pattern of DNA sequences using samples from blood, hair, skin, or other tissues.

🔹 **Basic Principle:**

The process is based on variations in specific non-coding regions of DNA known as VNTRs (Variable Number Tandem Repeats) or STRs (Short Tandem Repeats).

These sequences are different in every person, forming a unique “DNA pattern” or “fingerprint.”



🔹 Steps Involved in DNA Fingerprinting:

Step 1: Isolation of DNA

- DNA is extracted from cells (blood, hair root, semen, saliva, etc.).
- Care is taken to ensure the DNA sample is pure and undamaged.

Step 2: Cutting DNA with Restriction Enzymes

- The isolated DNA is cut into fragments using restriction endonucleases (molecular scissors).
- These enzymes cut at specific sequences, producing fragments of various lengths.

Step 3: Separation of DNA Fragments (Gel Electrophoresis)

- The DNA fragments are placed on an agarose gel and an electric current is passed through it.
- The negatively charged DNA moves toward the positive electrode.
- Smaller fragments move faster and farther, thus separating according to size.

Step 4: Transfer to Nylon Membrane (Southern Blotting)

- The separated DNA fragments are transferred from the gel onto a nylon or nitrocellulose membrane for analysis.

Step 5: Hybridization with DNA Probes

- The membrane is exposed to radioactively or fluorescently labeled DNA probes that bind to specific DNA sequences.
- Only the fragments complementary to these probes become visible.

Step 6: Detection (Autoradiography)

- The membrane is placed against an X-ray film, and a pattern of dark bands appears.
- These banding patterns represent the DNA fingerprint of the individual.

Step 7: Comparison of DNA Patterns

- The DNA pattern from the sample is compared with other samples (e.g., from a crime scene, parent, or suspect).
- If the banding patterns match, it confirms a genetic relationship or identity.

🔹 Applications of DNA Fingerprinting:

1. Forensic Science: Identification of criminals through evidence (blood, hair, etc.).

2. Paternity Testing: Determining biological parents.

3. Identification of Missing Persons or Disaster Victims.

4. Evolutionary Studies: Tracing ancestry and species relationships.

5. Detection of Genetic Diseases.

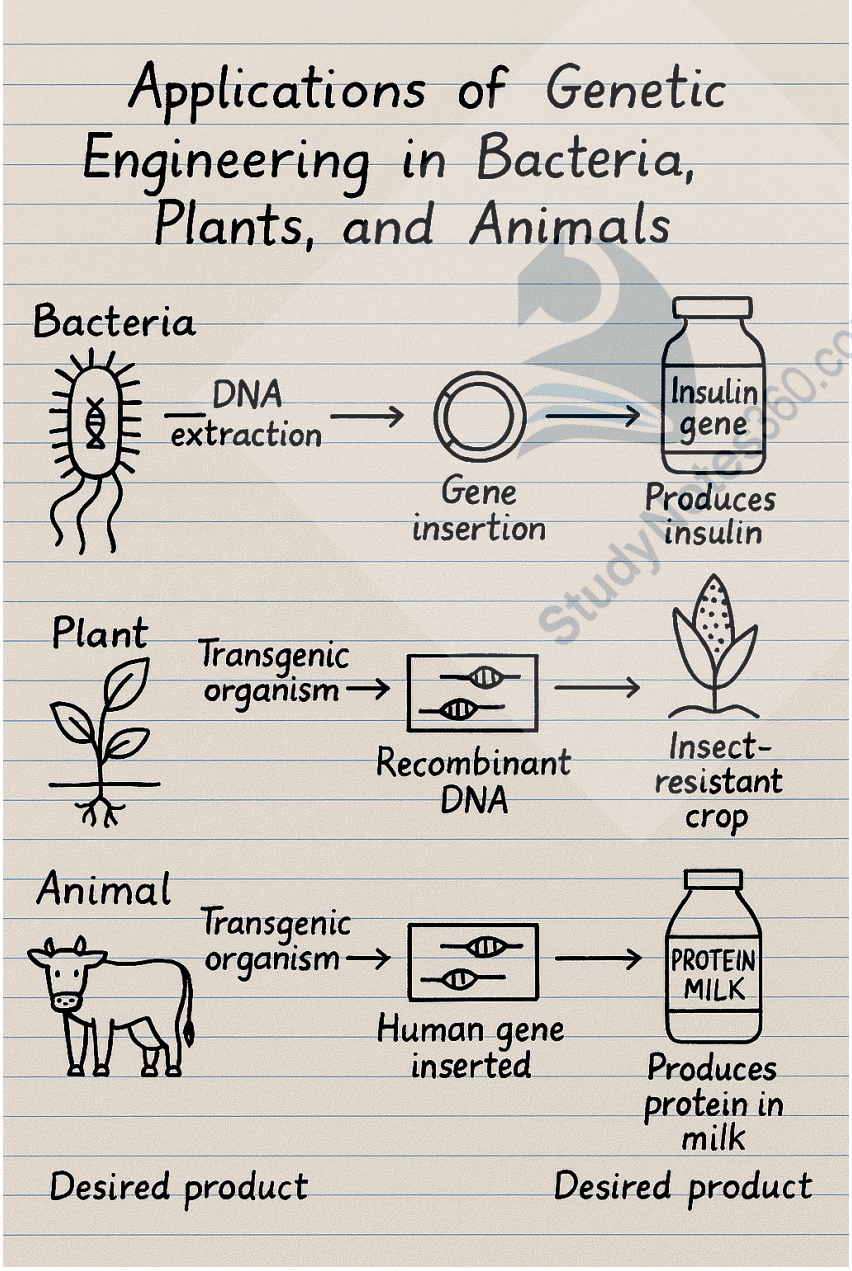
✦ **Summary:**

- DNA Fingerprinting is a powerful genetic identification tool that analyzes variations in DNA sequences.
- It involves isolating DNA, cutting it with restriction enzymes, separating fragments, and visualizing unique banding patterns using labeled probes.
- The resulting pattern serves as a genetic barcode unique to each individual.

🌟 **Q5: For what purpose have bacteria, plants, and animals been genetically altered?**

❖ **Introduction:**

- Genetic engineering (or recombinant DNA technology) allows scientists to modify the genetic material (DNA) of organisms to give them new traits or abilities.
- When bacteria, plants, and animals are genetically modified, they are called transgenic organisms.
- These modifications are made for medical, agricultural, and industrial purposes.



🔹 1. Genetically Altered Bacteria

👉 Purpose:

Bacteria are genetically modified to produce useful biological products and to assist in environmental and agricultural fields.

🔸 Examples & Uses:

1. Production of Human Insulin:

- E. coli bacteria are genetically engineered with the human insulin gene.
- They produce recombinant human insulin, which is used to treat diabetes.

2. Production of Growth Hormones and Vaccines:

- Genetically altered bacteria produce human growth hormone, interferons, and vaccines (e.g., Hepatitis B).

3. Biodegradation and Waste Treatment:

- Modified bacteria can degrade oil spills and detoxify pollutants in the environment.

4. Nitrogen Fixation Improvement:

- Some bacteria are modified to enhance nitrogen fixation, improving soil fertility.

🔹 2. Genetically Altered Plants

👉 Purpose:

Plants are genetically engineered to improve yield, resistance, and nutritional quality.

🔸 Examples & Uses:

1. Insect Resistance:

- Bt cotton and Bt corn are modified with a gene from Bacillus thuringiensis that produces a toxin against insects.

2. Herbicide Resistance:

- Some crops (e.g., soybean) are modified to tolerate specific herbicides, making weed control easier.

3. Improved Nutrition:

- Golden rice is engineered to produce vitamin A, helping reduce deficiency diseases.

4. Virus and Drought Resistance:

- Transgenic plants are made resistant to viral infections, drought, and salinity, improving productivity.

5. Edible Vaccines:

- Some plants are modified to produce vaccine components, e.g., banana or tomato containing Hepatitis B antigen.

🔹 3. Genetically Altered Animals

👉 Purpose:

Animals are genetically engineered for biomedical research, pharmaceutical production, and agricultural improvement.

🔸 Examples & Uses:

1. Production of Medicines (Pharming):

- Transgenic cows, goats, and sheep produce therapeutic proteins (like antithrombin or human lactoferrin) in their milk.

2. Disease Models for Research:

- Mice are genetically modified to study human diseases such as cancer, Alzheimer's, and diabetes.

3. Improved Growth and Meat Quality:

- Transgenic fish and livestock are engineered for rapid growth and better meat yield.

4. Organ Transplantation (Xenotransplantation):

- Pigs are genetically altered to produce organs that are more compatible with the human immune system.

✦ **Summary:**

Genetically altered organisms are created to improve health, agriculture, and industry.

- Bacteria → Produce medicines, degrade waste, fix nitrogen.

- Plants ⟶ Resist pests, tolerate herbicides, improve nutrition.
- Animals ⟶ Produce drugs, enhance growth, and assist in medical research.
- These advances have revolutionized biotechnology and improved the quality of human life.

Note:

This chapter is designed to provide a solid foundation of knowledge, with the goal of deepening understanding and encouraging further exploration of the subject. The content has been carefully selected to support effective learning and inspire students to engage with the topic more deeply.

Author: Muhammad Asghar

Purpose: To contribute to education by offering insightful, valuable content that enhances learning and understanding.