CHAPTER

11

Biotechnology: Principle and Processes

Biotechnology

Biotechnology is a field that brings together various aspects of biology to develop techniques that utilize live organisms or enzymes extracted from organisms. The purpose is to create products and processes that are valuable to humans. The term "biotechnology" was first introduced by **Karl Ereky** in 1917 and encompasses both traditional and modern approaches to this field.

Principles of biotechnology

Biotechnology, a field that has revolutionized the way we live, has its roots in two fundamental techniques: **Genetic Engineering** and **Sterilisation Methods**.

Genetic Engineering involves the manipulation of the genetic material, DNA or RNA, to alter the characteristics of an organism by introducing the modified genetic material into a host organism. This technique, pioneered by Paul Berg, has paved the way for significant advancements in biotechnology.

Sterilisation Methods, on the other hand, are crucial in maintaining a *contamination-free environment* in chemical engineering processes. These methods allow for the selective growth and manipulation of desired microbes or cells, leading to the production of high volumes of products such as vaccines, enzymes, and antibiotics.

Together, these techniques have played a pivotal role in the birth and growth of modern biotechnology, enabling ground breaking innovations and discoveries in the field.

Recombinant DNA Technology (RDT)

Recombinant DNA Technology (RDT) is a genetic engineering technique that involves the combination of genes from two different sources to create a new recombinant gene. The process was first developed by **Stanley Cohen** and **Herbert Boyer** in 1972. RDT employs various enzymes to simplify the complex process, including restriction enzymes, DNA ligases, alkaline phosphatase, and DNA polymerases.

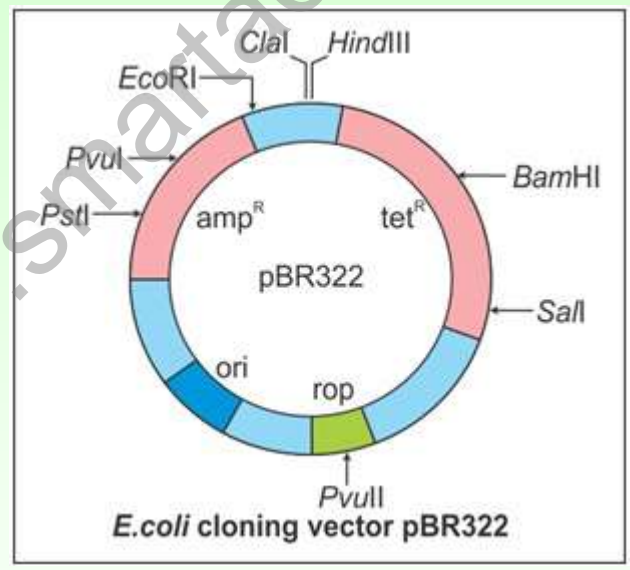
Restriction enzymes, also known as ***molecular scissors***, were discovered by Arber, Smith, and Nathan. These enzymes are used to cut DNA at specific locations during DNA technology. They belong to a larger class of enzymes called nucleases, which are of two types: **exonucleases** and **endonucleases**. **Exonucleases** remove nucleotides from the ends of the DNA in one strand of duplex, while **endonucleases** make cuts at specific positions within the DNA.

DNA ligases, or **molecular glue**, repair broken DNA fragments by joining two nucleotides. Alkaline phosphatase (AP) removes the phosphate group from the 5' end of a DNA molecule, leaving a free 5' hydroxyl group. This prevents unwanted self-ligation of vector DNA molecules during the formation of recombinant DNA. DNA polymerases help in the in vitro synthesis of complementary DNA (cDNA) strands on DNA templates.

Cloning Vectors

In recombinant DNA technology, a **vector** refers to a DNA molecule capable of carrying a foreign DNA segment and replicating itself inside a host cell. Vectors have the unique ability to replicate within bacterial cells independently of the control of chromosomal DNA.

There are various types of vectors used in recombinant DNA technology, but two common ones are **plasmids** and **bacteriophages**. **Plasmids** are small, circular, double-stranded DNA molecules that replicate autonomously and are found in many bacteria and some yeasts. For example, E. coli contains a vector called pBR322.



E.coli cloning vector pBR322

On the other hand, **bacteriophages** are viruses that infect bacteria and are used as vectors due to their high copy number within bacterial cells.

Overall, these cloning vectors are essential tools in recombinant DNA technology as they allow the transfer of desired DNA segments into host cells, making it possible to produce specific proteins or manipulate genetic traits.

To enable cloning into a vector, several features are required. Firstly, an **origin of replication (Ori**) is needed as a starting point for replication. Secondly, **selectable markers** are necessary to distinguish non-transformants from transformants during the transformation process. Thirdly, **cloning sites (recognition sites)** are typically required to link foreign DNA with the vector. This is done by ligating the foreign DNA at a restriction site found within one of the antibiotic resistance genes, which results in the inactivation of the antibiotic resistance gene (known as insertional inactivation).

In plants, the **Tumour inducing (Ti) plasmid** of Agrobacterium tumefaciens is commonly used as a cloning vector, while the proper vector for cloning in animal cells is SV40. Additionally, **retroviruses**, **adenoviruses**, and **papillomaviruses** are also used as cloning vectors in animals due to their ability to transform **normal cells** into **cancerous cells**.

To transform bacteria with recombinant DNA, the bacteria must first be made **competent** as DNA cannot pass through cell membranes. This can be achieved through methods such as heat shock, microinjection, or biolistic/gene gun.

The following are some methods to make cells competent:

Chemical transformation: This method involves treating cells with chemicals such as **calcium chloride or rubidium chloride** that disrupt the cell membrane, allowing DNA to enter. The cells are then subjected to a heat shock to further facilitate the uptake of DNA.

Electroporation: This method uses an electrical field to create temporary pores in the cell membrane, allowing DNA to enter. The cells are subjected to a brief electrical pulse that causes the cell membrane to become temporarily permeable.

Biolistic transformation: In this method, DNA-coated metal particles are fired into the cells using a gene gun. The particles penetrate the cell wall and deliver DNA directly into the cell.

Protoplast fusion: This method is used for certain types of cells that do not have a cell wall, such as plant cells. Protoplasts from two different cells are treated with a chemical that causes them to fuse, resulting in a hybrid cell that has taken up DNA from both cells.

The specific method used to make cells competent depends on the type of cells being used and the application for which they are being used

Processes of Recombinant DNA Technology

Recombinant DNA technology is a technique that allows scientists to **manipulate DNA** to create new **genetic combinations**.

It involves several steps that must be performed in a specific sequence.

The **first step** Is the **isolation of genetic material**, which is usually DNA. This can be achieved by treating bacterial cells, plant or animal tissues with enzymes such as **lysozyme (for bacteria), cellulase (for plant cells), or chitinase (for fungus)**. Ribonuclease and protease enzymes can be used to remove RNA and proteins, respectively.

Once DNA has been isolated, it can be **cut at specific locations using restriction enzymes**. These enzymes are incubated with purified DNA molecules under optimal conditions for that specific enzyme.

After cutting the DNA, the fragments are separated and isolated using a technique called **gel electrophoresis**. The separated DNA fragments are visualized by staining with **Ethidium Bromide (EtBr) and exposing to UV radiation**, resulting in bright orange colored bands of DNA.

In the next step, gene amplification is performed using **Polymerase Chain Reaction (PCR)**, a method developed by **Kary Mullis** in 1985. PCR involves three processes: denaturation, annealing, and extension, which result in the synthesis of several replicas of useful genes.

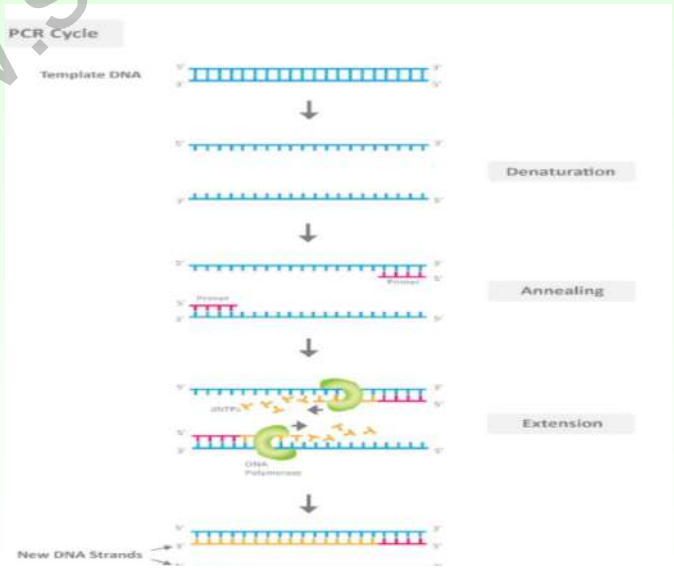
The basic steps In PCR are as follows:

Denaturation: Heat the DNA to 95°C to denature the double-stranded DNA into single strands.

Annealing: Lower the temperature to a high annealing temperature to allow the primers to anneal to the template DNA.

Extension: Raise the temperature to the optimal temperature for Taq polymerase to extend the primers and synthesize new DNA strands.

Final Extension: After the final cycle, extend the DNA strands for an additional 5-10 minutes to ensure complete extension of all PCR products.



Ligation is the next step, which involves the insertion of the DNA fragment into a vector. **Sticky ends** are obtained by cutting both the vector DNA and source DNA with the same endonuclease. Then, both are ligated by mixing vector DNA, the gene of interest, and enzyme DNA ligase to form recombinant DNA/hybrid DNA.

To insert the recombinant DNA into a host cell/organism, the recipient cells must be made **competent** to receive DNA. This can be achieved by several methods, after which a recombinant DNA containing a gene for resistance to an antibiotic is transferred to host cells to produce ampicillin resistant cells that contain an **ampicillin resistant gene** and are called **selectable markers**.

Finally, the desired protein encoded gene is expressed in the heterologous host, which is called **recombinant** protein. The cells containing cloned genes of interest are grown on a small scale in the laboratory, and these cell cultures are used to extract the desired protein using various separation techniques.

Bioreactor

Bioreactors are vessels that are designed to provide the ideal environment for growing desired products. These vessels typically have a **large volume**, ranging from 100 to 1000 liters. The ***optimal growth conditions*** are achieved by controlling factors such as temperature, pH, substrate, salts, vitamins, and oxygen levels.

A **bioreactor** typically consists of "various components such as an *agitator system, oxygen delivery system, foam control system, temperature control system, pH control system, and a sampling part to withdraw culture periodically.* **Stirring type bioreactors**, simple stirred-tank bioreactors, and sparged- tank bioreactors are the most commonly used types of bioreactors.

Downstream processing

Once the biosynthetic phase is completed, the product needs to undergo a series of processes before it can be marketed as a finished product. This series of processes is collectively referred to as **downstream processing**. The downstream processing typically involves the **separation and purification of products**.

Biotechnology : Principles and Processes

Definition

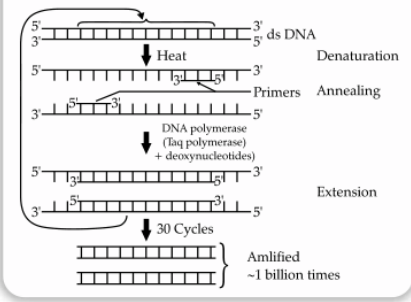
It is the integration of natural science and organisms, cells, parts thereof, and molecular analogues for product and services.

Principles of Genetic engineering

- rDNA
- Gene cloning
- Gene transfer

Processes of rDNA Technology

- Isolation of DNA
- Obtaining foreign gene product by bioreactors.
- Selection of recombinants:
  (a) By antibiotic resistance method.
  (b) By Insertional inactivation method
- Inserting of DNA into host cells. Host cells are made competent to take up DNA
- Amplification of gene by PCR



- Cutting of DNA at specific locations by restriction enzymes.

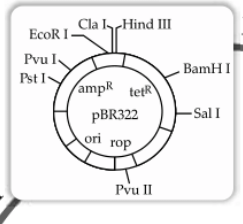
Tools of rDNA Technology

Restriction Enzymes

Exonucleases:They remove nucleotides from the ends of the DNA.
Endonucleases : They cut at specific positions within the DNA.

Cloning vector

pBR322



Features of vector

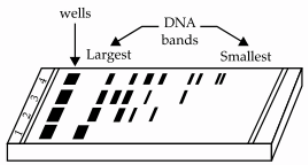
- Has *Ori*
- Has Selectable Markers
- Has fewer cloning sites
- Examples:
  Agrobacterium tumifaciens
  Retroviruses

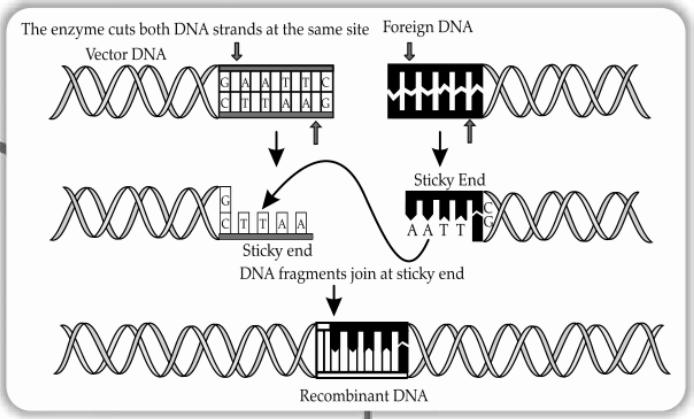
Competent host

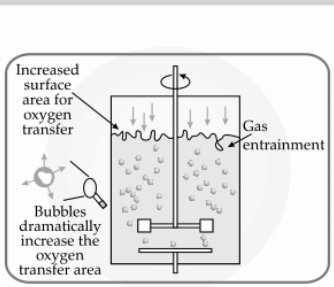
Host is made competent by:

- Biolistics
  Cell are bombarded with high velocity micro particles of gold or tungsten coated with DNA
- Microinjection
  rDNA is directly injected into the nucleus of an animal cell
- pathogen vector
  Disarmed
  Vectors like *Agrobacterium* when infects the cell transfer their combinant DNA into the host

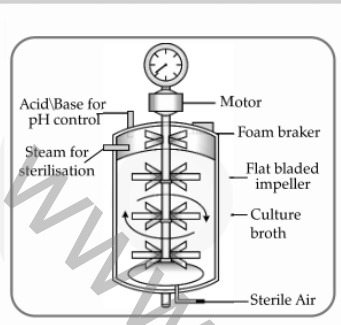
Cut DNA fragments are separated by Gel electrophoresis.







Stirred-tank bioreactor



Sparged stirred-tank bioreactor

Trace the Mind Map

- First Level
- Second Level
- Third Level

Practice Questions

1.Which technique can be used to transfer a gene from one organism to another?

a) Genetic engineering b) Tissue culture c) Transformation d) None of these

2.What do you call an environmental agent that triggers transcription from an operon?

a) Depressor b) Inducer c) Regulator d) Controlling element

3.Recombinant DNA consists of an integrated fragment of:

a) Antibiotic resistant gene b) Diseases resistant gene

c) Allergy resistant gene d) All of these

4.What do you call the ampicillin resistant gene in recombinant DNA that allows for selection of transformed cells?

a) Vectors b) Plasmid c) Selectable marker d) Cloning sites

5.What is the known sequence of DNA used to find complementary DNA strand?

a) Vector b) Plasmid c) DNA probe d) Recombinant DNA

6.How are proteins removed?

a) Ribonuclease b) Chitinase c) Cellulase d) Protease

7.What makes plasmid the vector in genetic engineering?

a) Resistance to antibiotics b) Resistance to restriction enzymes

c) Ability to carry a foreign gene d) Ability to cause infection in the host

8.Which of the following is an application of biotechnology?

(a)Human cloning (b) Renewable energy production

(c) Oil drilling (d) Deforestation

9.What is it called when scientists introduce foreign genes into an animal to make it genetically superior?

a) Tissue culture b) Biotechnology c) Genetic engineering d) Immunisation

9.How are many copies of a DNA molecule produced in a test tube?

a) Polymerase chain reaction (PCR) b) Molecular chain reaction (MCR)

c) Ephemeral chain reaction (ECR) d) All of the above

10.How was a "giant mouse" produced in the laboratory?

a) Gene mutation b) Gene duplication c) Gene synthesis d) Gene manipulation

11.What does the downstream process include?

a) Separation of the product from the reactor

b) Purification of the product

c) Formation of the product with suitable preservatives

d) Quality control testing and clinical trials in case of drugs

12.What is responsible for more advancements in genetic engineering?

a) Restriction endonuclease b) Reverse transcription

c) Protease d) Zymase

13.Why are plasmids suitable vectors for gene cloning?

a) They are small circular DNA molecules that can integrate with host chromosomal DNA.

b) They are small circular DNA molecules with their own replication origin site.

c) They can shuttle between prokaryotic and eukaryotic cells.

d) They often carry antibiotic resistance genes.

14.What is polymerase chain reaction useful for?

a) DNA synthesis b) DNA amplification c) Protein synthesis d) Amino acid synthesis

15.Which of the following describes a bioreactor?

a) A hybridoma

b) A culture containing radioactive isotopes

c) A culture for the synthesis of new chemicals

d) A fermentation tank

16.Which technique can be used to detect genetic disorders in humans?

a) Polymerase Chain Reaction (PCR) b) Gel electrophoresis

c) Spectroscopy d) All of the above

17.What is the special sequence in DNA recognized by restriction endonucleases called?

a) Restriction nucleotide sequence b) Palindromic nucleotide sequence

c) Recognition nucleotide sequence d) All of the above

18.What are primers?

a) Small chemically synthesized oligonucleotides of about 10-18 nucleotides that are complementary to the region of template DNA

b) Chemically synthesized oligonucleotides of about 10-18 nucleotides that are not complementary to the region of template DNA

c) The double-stranded DNA that needs to be amplified

d) Specific sequences present on recombinant DNA

19.Which method is used to find a gene when researchers know very little about it, resulting in a complete gene library?

a) Cloning b) Shotgun cloning c) Gene synthesis d) Cloning

20.Which statements about PCR are correct?

I. Polymerase Chain Reaction (PCR) is a technique for synthesizing multiple copies of a desired gene.

II. This technique was developed by Kary Mullis in 1985.

III. A single PCR amplification cycle involves three basic steps: denaturation, annealing, and extension.

a) I and II b) I and III c) II and III d) I, II, and III

21.Which of the following is an example of genetically modified organism (GMO)?

A) Apple grown using organic farming methods

B) Cow bred through selective breeding for higher milk production

C) Corn plant engineered to resist insect pests

D) Wheat crop cultivated using traditional farming techniques

22.What is the definition of plasmids?

a) Autonomously replicating circular extrachromosomal DNA.

b) Not replicating circular DNA.

c) Sometimes autonomously replicating circular extrachromosomal DNA.

d) Neither autonomously replicating circular extrachromosomal DNA nor non-replicating circular DNA.

23.What makes genetic engineering possible?

a) Understanding the phenomenon of transduction in bacteria

b) Visualizing DNA with an electron microscope

c) Cutting DNA at specific sites with endonucleases like DNA ase I

d) Using restriction endonucleases purified from bacteria in vitro

24.Which of the following is involved in a single PCR amplification cycle?

a) Denaturation b) Annealing c) Extension d) All of the above

25.What does DNA fingerprinting involve?

a) Molecular analysis of profiles of DNA samples

b) Analysis of DNA samples using imprinting devices

c) Techniques used for molecular analysis of different specimens of DNA

d) Techniques used in identification of fingerprints of different persons

26.What is the basis of DNA fingerprinting?

a) The double helix b) Errors in base sequence

c) Polymorphism in sequence d) DNA replication

27.In genetic engineering, what is the term "vector" used to describe?

a) Plasmid b) Sources of DNA c) Cell which receives d) Virus

28.Which term refers to the controlled use of biological agents for beneficial purposes?

a) Biochemistry b) Molecular biology c) Biotechnology d) Microbiology

29.What is Humulin?

a) Insulin obtained from pigs b) Insulin identical to human insulin

c) Insulin obtained from a virus d) A type of human clone

30.Which statement is incorrect?

a) Gene therapy involves replacing defective genes with normal ones to treat disease at the molecular level

b) Calcitonin is a recombinant product used in the treatment of infertility

c) Bt toxin is a biodegradable insecticide derived from Bacillus thuringiensis

d) Trichoderma sp. Is a biocontrol agent used to combat fungal diseases in plants

31.What is the nature of plasmids, extrachromosomal circular DNA molecules?

a) They have their own point of replication and can replicate independently

b) They have their own point of replication but cannot replicate independently

c) They do not have their own point of replication and cannot replicate independently of bacterial chromosomal DNA

d) None of the above options are correct.

32.What method can be used for gene amplification using primers?

a) Microinjection b) ELISA c) Polymerase chain reaction d) Gene gun

33.What is the purpose of using the polyethylene glycol method in genetic engineering?

a) Biodiesel production b) Seedless fruit production

c) Energy production from sewage d) Gene transfer without a vector

34.What are the commonly used enzymes in genetic engineering?

a) Restriction endonuclease and polymerase b) Endonuclease and ligase

c) Restriction endonuclease and ligase d) Ligase and polymerase

35.Which technique has helped in solving many mysteries involving murders, robberies and rapes?

a) Gene splicing b) Computer technology

c) DNA fingerprinting d) Gene cloning

36.Which of the following techniques is commonly used in DNA fingerprinting?

A) Polymerase chain reaction (PCR) B) Fermentation

C) Photosynthesis D) Hybridization

37.What is cDNA?

a) Circular DNA

b) Cloned DNA

c) DNA produced from reverse transcription of RNA

d) Cytoplasmic DNA

38.Who developed PCR, and in what year did he receive the Nobel Prize for chemistry?

a) Kary Mullis 1990 1997

b) Flemming 1985 1993

c) Kary Mullis 1985 1993

d) Flemming 1990 1997

39.What are the enzymes commonly known as molecular scissors used for in genetic engineering?

a) A-Tu ligases; B-Molecular glu

b) A-Restriction enzyme; B-Molecular scissors

c) A-Joining enzyme; B-Molecular glu enzymes

d) A-DNA polymerases; B-Synthesising

40.In a genetic engineering experiment, which type of DNA fragment can be cut using restriction enzymes?

a) Bacterial DNA only

b) Viral DNA only

c) Any DNA fragment

d) Eukaryotic DNA only

41.When a recombinant DNA carrying an antibiotic resistance gene is transferred into an E. coli cell, the host cell becomes resistant to that antibiotic. What is the name of the antibiotic resistance gene in this case?

(a) Vectors (b) Plasmid (c) Selectable marker (d) Cloning sites

42.Which method(s) is/are used to distinguish between recombinant and non-recombinant cells?

(a) Antibiotic resistance genes

(b) Insertional inactivation

c) Gene cloning

(d) Both (a) and (b)

43.In insertional inactivation, where is the recombinant DNA inserted?

(a) Within the coding sequence of b-galactosidase (b) Within the tetracycline resistant gene

(c) Within a restriction enzyme (d) Within the ampicillin resistant gene

44.What is the name of the piece of DNA that Agrobacterium tumefaciens delivers into a dicot plant?

(a) rDNA (b) T-DNA (c) mDNA (d) cDNA

45.What is the effect of treating a host cell with divalent cations?

(a) It changes the permeability of the DNA.

(b) It increases the efficiency with which DNA enters the bacterium.

c) It decreases the efficiency with which DNA enters the bacterium.

(d) It changes the permeability of the host cell.

46.Which of the following processes are involved in a single PCR amplification cycle?

a. Denaturation b. Extension c. Annealing d. All of the above

47.What is the term used for a protein encoding gene that is expressed in a heterologous host?

a. Foreign protein b. Heterologous protein c. Recombinant protein d. Alien protein

48.Stirred-tank bioreactors are primarily designed for which purpose?

a. Purification of the product

b. Addition of preservatives to the product

c. Availability of oxygen throughout the bioreactor

d. Ensuring anaerobic conditions in the culture vessel

49.What is the advantage of using stirred-tank bioreactors over shake flasks?

a. They provide high temperature and pH

b. They provide better aeration and mixing properties

c. They do not allow the entry of CO2

d. They are easy to operate

50.Which of the following components are typically found in a bioreactor?

a. An agitator system

b. An oxygen delivery system

c. A foam control system

d. A temperature control system

e. A pH control system

f. Sampling ports to withdraw cultures periodically

Choose the correct option:

a. I, II, III, IV, and V

b. II, IV, V, and VI

c. I, II, III, IV, and VI

d. All of the above

Answers

1.Answer: a) Genetic engineering

Explanation: Genetic engineering is a technique used to manipulate an organism's genes, including the transfer of genes from one organism to another. This is typically achieved through the use of recombinant DNA technology.

2.Answer: b) Inducer

Explanation: An inducer is an environmental agent that triggers the transcription of a gene or set of genes from an operon. Inducers typically bind to regulatory proteins that control the expression of genes in the operon, causing a conformational change that allows transcription to occur.

3.Answer: d) All of these

Explanation: Recombinant DNA is a type of DNA molecule that has been artificially created by combining DNA fragments from different sources. These fragments can include genes that confer resistance to antibiotics, diseases, or allergies, among others.

4.Answer: c) Selectable marker

Explanation: The ampicillin-resistant gene in recombinant DNA is commonly used as a selectable marker, allowing scientists to identify cells that have successfully taken up the recombinant DNA by selecting cells that are able to grow in the presence of ampicillin.

5.Answer: c) DNA probe

Explanation: A DNA probe is a short, single-stranded sequence of DNA that is used to detect the presence of a complementary DNA sequence in a sample. By labeling the probe with a detectable marker, scientists can visualize the location of the complementary DNA sequence.

6.Answer: d) Protease

Explanation: Proteases are enzymes that break down proteins by cleaving the peptide bonds that link amino acids together. Proteases are commonly used to remove proteins from biological samples, such as cell lysates or purified protein preparations.

7.Answer: c) Ability to carry a foreign gene

Explanation: A vector is a DNA molecule used to transfer foreign genetic material into another cell. Plasmids are commonly used as vectors in genetic engineering due to their ability to carry foreign DNA sequences and replicate independently within the host cell.

8.The correct answer Is B) Renewable energy production.

Biotechnology involves the use of living organisms or their products to develop or modify useful products or processes. One of the applications of biotechnology is in renewable energy production. Biotechnological processes can be used to harness energy from renewable sources such as biomass, biofuels, and biogas. These methods help in reducing dependence on non-renewable energy sources and contribute to sustainable energy production.

9.Answer: c) Genetic engineering

Explanation: Genetic engineering involves the manipulation of an organism's genetic material to achieve a desired outcome. This can include the introduction of foreign genes into an animal to confer desirable traits or characteristics.

10.Answer: d) Gene manipulation

Explanation: A "giant mouse" could be produced in the laboratory through gene manipulation, such as overexpressing genes involved in growth and development. By altering the expression of specific genes, scientists can manipulate an organism's phenotype, including size and growth rate.

11.Answer: b) Purification of the product

Explanation: The downstream process involves the purification and recovery of the product from the reactor or fermentation vessel. This process usually includes several steps such as filtration, chromatography, and precipitation to remove unwanted impurities and isolate the desired product in a pure form.

12.Answer: a) Restriction endonuclease

Explanation: Restriction endonucleases are enzymes that cut DNA at specific recognition sequences, which have led to significant advancements in genetic engineering. These enzymes have allowed scientists to cut and paste DNA fragments from different sources, creating recombinant DNA molecules with new combinations of genes.

13.Answer: b) They are small circular DNA molecules with their own replication origin site.

Explanation: Plasmids are small, circular DNA molecules that can replicate independently from the host chromosome. They are also able to carry foreign DNA and transfer it between cells. These features make plasmids suitable vectors for gene cloning, as they can be easily manipulated in the laboratory and introduced into host cells for the expression of recombinant proteins.

14.Answer: b) DNA amplification

Explanation: Polymerase Chain Reaction (PCR) is a technique used to amplify or make many copies of a specific DNA sequence in vitro. It is useful for a variety of applications such as gene cloning, DNA sequencing, and diagnostic testing for genetic disorders.

15.Answer: d) A fermentation tank

Explanation: A bioreactor is a vessel used to carry out biological reactions, such as fermentation or cell culture, under controlled conditions. A fermentation tank is a type of bioreactor used for the production of microorganisms, enzymes, and other biological products.

16.Answer: d) All of the above (Polymerase Chain Reaction (PCR), Gel electrophoresis, and Spectroscopy)

Explanation: Polymerase Chain Reaction (PCR), gel electrophoresis, and spectroscopy are all techniques that can be used to detect genetic disorders in humans. PCR can be used to amplify specific DNA sequences for diagnosis, while gel electrophoresis and spectroscopy can be used to analyze DNA fragments and detect genetic mutations.

17.Answer: b) Palindromic nucleotide sequence

Explanation: The special sequence in DNA recognized by restriction endonucleases is called a palindromic nucleotide sequence. This is a sequence that reads the same forward and backward on complementary strands of DNA.

18.Answer: a) Small chemically synthesized oligonucleotides of about 10-18 nucleotides that are complementary to the region of template DNA

Explanation: Primers are short, chemically synthesized oligonucleotides that are complementary to a specific region of DNA. They are used in PCR to initiate DNA synthesis and amplify a specific DNA sequence.

19.Answer: b) Shotgun cloning

Explanation: Shotgun cloning is a method used to create a gene library when very little is known about the gene of interest. In this method, the DNA is randomly fragmented and cloned, resulting in a complete gene library that can be screened for the desired gene.

20.Answer: d) I, II, and III (Polymerase Chain Reaction (PCR) is a technique for synthesizing multiple copies of specific sequence of DNA.

21.The correct answer Is C) Corn plant engineered to resist insect pests.

A genetically modified organism (GMO) refers to an organism whose genetic material has been altered using biotechnology techniques. In this case, a corn plant engineered to resist insect pests has had its genetic material modified to incorporate specific genes that provide resistance against pests. This modification enables the plant to defend itself against harmful insects, reducing the need for chemical insecticides.

22.Answer: a) Autonomously replicating circular extrachromosomal DNA.

Explanation: Plasmids are circular pieces of DNA that exist separately from the chromosomal DNA in bacteria and some other organisms. They are typically small in size and contain genetic information that is not essential for the survival of the host organism. Plasmids can replicate independently of the host chromosome and can be transferred between different cells, allowing them to play important roles in the spread of antibiotic resistance and other traits.

23.Answer: d) Using restriction endonucleases purified from bacteria in vitro

Explanation: Genetic engineering is the process of manipulating the genetic material of an organism in order to modify its traits. This is made possible by a number of different tools and techniques, including the use of restriction endonucleases, which are enzymes that can cut DNA at specific sites. These enzymes are often purified from bacteria and can be used in vitro to create specific DNA fragments that can be used for cloning and other applications.

24.Answer: d) All of the above

Explanation: PCR (polymerase chain reaction) is a technique used to amplify DNA sequences. In a single PCR cycle, the DNA template is denatured (separated into single strands), annealed (primers bind to the complementary sequences on the template), and extended (DNA polymerase adds new nucleotides to the growing strand). This process is repeated multiple times to generate large amounts of the target DNA sequence.

25.Answer: a) Molecular analysis of profiles of DNA samples

Explanation: DNA fingerprinting is a technique used to analyze and compare the DNA sequences of different individuals. It involves the generation of a molecular profile of an individual's DNA by analyzing specific regions of the genome that contain variable numbers of short tandem repeats (VNTRs) or other polymorphic sequences. These profiles can be used for a variety of applications, including forensic analysis and paternity testing.

26.Answer: c) Polymorphism in sequence

Explanation: DNA fingerprinting is based on the fact that the DNA sequences of different individuals can vary in small but detectable ways. These variations, which are caused by polymorphisms in the DNA sequence, can be used to generate unique molecular profiles that can be used for identification purposes.

27.Answer: a) Plasmid

Explanation: In genetic engineering, a vector is a DNA molecule that is used to carry a foreign DNA sequence into a host cell, where it can be replicated and expressed. Plasmids are commonly used as vectors in bacterial systems, while other types of vectors, such as viruses and artificial chromosomes, are used in other organisms.

28.c) Biotechnology

Explanation: Biotechnology refers to the application of technology to manipulate living organisms or their components to produce useful products or services. It involves the controlled use of biological agents, such as cells or enzymes, for beneficial purposes such as the production of medicines, foods, and fuels.

29.b) Insulin identical to human insulin

Explanation: Humulin is a brand name for a type of insulin that is identical to human insulin. It is produced using recombinant DNA technology, which involves inserting the human insulin gene into bacteria, which then produce the insulin protein.

30.b) Calcitonin is a recombinant product used in the treatment of infertility

Explanation: Calcitonin is a hormone that is produced by the thyroid gland and is involved in regulating calcium levels in the body. It is not used in the treatment of infertility. The other statements are correct: gene therapy involves replacing defective genes with normal ones, Bt toxin is a biodegradable insecticide, and Trichoderma sp. Is a biocontrol agent used to combat fungal diseases in plants.

31.a) They have their own point of replication and can replicate independently

Explanation: Plasmids are small, circular DNA molecules that are found in many types of bacteria. They contain their own origin of replication and can replicate independently of the bacterial chromosome. Plasmids can also carry genes that provide various benefits to the bacteria, such as antibiotic resistance.

32.c) Polymerase chain reaction

Explanation: Polymerase chain reaction (PCR) is a method used to amplify a specific segment of DNA using primers, which are short DNA sequences that are complementary to the regions of DNA that flank the target sequence. PCR involves cycles of heating and cooling to denature the DNA, anneal the primers, and allow DNA polymerase to synthesize new DNA strands.

33.d) Gene transfer without a vector

Explanation: The polyethylene glycol (PEG) method is a technique used to transfer DNA into cells without the use of a viral or bacterial vector. PEG is a chemical that can disrupt the cell membrane, allowing DNA to enter the cell. This method is often used in plant genetic engineering.

34.c) Restriction endonuclease and ligase

Explanation: Restriction endonucleases (also known as restriction enzymes) are enzymes that cut DNA at specific recognition sites, while ligase is an enzyme that can join two DNA fragments together. These enzymes are commonly used in genetic engineering to cut and splice DNA molecules to create recombinant DNA.

35.c) DNA fingerprinting

Explanation: DNA fingerprinting is a technique used to identify individuals based on their DNA profiles, which are unique to each individual (except identical twins). DNA fingerprinting has been used in criminal investigations to link suspects to crime scenes, identify victims, and exonerate the wrongly accused.

36.The correct answer is A) Polymerase chain reaction (PCR).

DNA fingerprinting, also known as DNA profiling or genetic fingerprinting, is a technique used to analyze and compare DNA samples from different individuals. Polymerase chain reaction (PCR) is an essential component of this technique. PCR amplifies specific regions of the DNA to obtain sufficient material for analysis. It allows for the replication of DNA sequences in a controlled and efficient manner, enabling the detection of unique genetic markers that can be used for identification purposes.

37.c) DNA produced from reverse transcription of RNA – cDNA stands for complementary DNA, which is produced through reverse transcription of RNA using an enzyme called reverse transcriptase. This process allows scientists to make a DNA copy of an RNA molecule, which can then be cloned and studied.

38.a) Kary Mullis 1990 – Kary Mullis developed the polymerase chain reaction (PCR) in 1983, which allows for the amplification of DNA sequences in vitro. He was awarded the Nobel Prize in Chemistry in 1993 for his work on PCR.

39.b) A-Restriction enzyme; B-Molecular scissors – Restriction enzymes, also known as molecular scissors, are used in genetic engineering to cut DNA at specific locations. This allows scientists to create fragments of DNA that can be manipulated and recombined with other DNA sequences.

40.c) Any DNA fragment – Restriction enzymes can cut any DNA fragment, regardless of its source, as long as it contains the specific recognition site for the enzyme being used.

41.© Selectable marker – The antibiotic resistance gene in this case is a selectable marker, which allows scientists to easily identify cells that have taken up the recombinant DNA by exposing them to the antibiotic and selecting for the cells that are able to survive.

42.d) Both (a) and (b) – Antibiotic resistance genes and insertional inactivation can both be used to distinguish between recombinant and non-recombinant cells.

43.a) Within the coding sequence of b-galactosidase – In insertional inactivation, the recombinant DNA is inserted into the coding sequence of a gene, such as b-galactosidase, which disrupts its function and allows scientists to easily identify cells that have undergone recombination.

44.b) T-DNA – Agrobacterium tumefaciens is a bacterium that is commonly used in genetic engineering to transfer DNA into plant cells. The piece of DNA that is delivered by the bacterium is called T-DNA, or transfer DNA.

45.b) It increases the efficiency with which DNA enters the bacterium – Divalent cations, such as calcium ions, can increase the efficiency with which DNA enters a bacterium during transformation by neutralizing the negative charge on the DNA and the bacterial cell wall.

46.d) All of the above – A single PCR amplification cycle typically involves three steps: denaturation, in which the double-stranded DNA template is heated to separate the strands; annealing, in which primers bind to complementary sequences on the template DNA; and extension, in which a DNA polymerase enzyme extends the primers to create new DNA strands.

47.b) Heterologous protein – A heterologous protein is a protein that is produced in a host organism using genetic material from a different organism. This is a common practice in genetic engineering, where scientists may express a protein from one organism in a different host organism to study its function or produce it on a large scale.

48.c) Availability of oxygen throughout the bioreactor

Stirred-tank bioreactors are primarily designed to provide a uniform distribution of nutrients and oxygen throughout the bioreactor. The impeller system in the stirred-tank bioreactor creates a turbulent flow that ensures efficient mixing and homogenization of the culture medium. This enables oxygen to be distributed evenly throughout the bioreactor, which is essential for the growth and metabolism of aerobic microorganisms.

49.b) They provide better aeration and mixing properties

Stirred-tank bioreactors provide better aeration and mixing properties than shake flasks. Shake flasks rely on shaking or agitation to mix the culture medium and provide oxygen to the microorganisms. However, this method can lead to uneven mixing, poor oxygen transfer, and lower yields compared to a stirred-tank bioreactor. In contrast, stirred-tank bioreactors use an impeller system to provide a more uniform and efficient mixing of the culture medium, which leads to better aeration and growth of the microorganisms.

50. d) All of the above

Bioreactors typically contain all of the following components:

An agitator system (I) to provide mixing of the culture medium

An oxygen delivery system (II) to supply oxygen to the microorganisms

A foam control system (III) to prevent excessive foaming of the culture

A temperature control system (IV) to maintain a constant temperature within the bioreactor