9 CHAPTER

Biotechnology-Principle and Processes

Level - 1 CORE SUBJECTIVE QUESTIONS

MULTIPLE CHOICE QUESTIONS (MCQs) (1 Mark)

1. Option (A) is correct
Explanation: Eco R I cuts the DNA between bases G and A only when the sequence of GAATTC is present. The number of nucleotides present in the resultant sticky ends that will be formed in each of the two strands of DNA after this enzyme cuts the DNA will be 1 & 5; 5 & 1
2. Option (A) is correct
Explanation: Restriction endonuclease Hind II recognises a specific sequence of six base pairs in the DNA and always cuts at a particular site within this sequence.
3. Option (B) is correct
Explanation: In PCR (Polymerase Chain Reaction), the number of DNA molecules doubles with each cycle. Starting with one DNA molecule, after 10 cycles, the number of new DNA molecules generated would be 210 which equal 1024.
4. Option (C) is correct
Explanation: Plasmids are double stranded, circular, self-replicating, and extra-chromosomal DNA molecules.
5. Option (A) is correct
Explanation: PCR (Polymerase Chain Reaction) requires Taq polymerase, primers, and a DNA segment to amplify, but it does not require restriction endonucleases, which are used to cut DNA at specific sequences.
6. Option (B) is correct
Explanation: In PCR, primers are short sequences of nucleotides that are complementary to the target DNA region's sequences. During the annealing step, these primers bind to the single-stranded DNA template at their complementary sites, which allows the Taq polymerase to extend the primers and synthesise the new DNA strand.
7. Option (D) is correct
Explanation: The correct matching pair is:
P. DNA Ligase- Joins the DNA fragments
Q. Restriction exonuclease – Removes nucleotides from ends of DNA
R. Taq polymerase – Extends primer on a DNA template
S. Restriction endonuclease – Cuts DNA at a specific position.
8. Option (B) is correct
Explanation: The correct sequence for constructing recombinant DNA involves the following steps:
1. Isolation of genetic material (i): The DNA containing the gene of interest is isolated.
2. Amplification of gene of interest (iv): The isolated gene is amplified using techniques such as PCR.
3. Insertion of recombinant DNA in the host cell/ organism (ii): The amplified gene is inserted into a suitable vector and then transferred into the host cell.
4. Obtaining the foreign gene product (iii): The host cell expresses the foreign gene, producing the desired protein or product.
5. Downstream processing (v): The product is purified and processed for further use.
9. Option (C) is correct
Explanation: The correct sequence of the processes carried out after the biosynthetic stage in a bioreactor is:
(iii) Separation of product
(i) Purification of product
(ii) Formulation with suitable preservative
(iv) Clinical trial of product
10. Option (B) is correct
Explanation: Plasmids are suitable vectors for gene cloning because they are small circular DNA molecules with their own origin of replication site.
11. Option (B) is correct
Explanation: The Ori site (origin of replication) is the sequence in a vector that controls the copy number of linked DNA by allowing the DNA to replicate within a host cell.
12. Option (B) is correct
Explanation:
- Step 'Q' is a denaturation of DNA strand at high temperature, followed by annealing.

- Step 'R' is an extension of DNA in presence of thermostable DNA polymerase.

13. Option (D) is correct

Explanation: Enzyme Pst-I makes a staggered cut of the DNA at its recognition sequence.

5' C T G C A G 5' Pst I → 5' C T G C A 3' 5' G 3'
3' G A C G T C 3' digest 3' G 5' 3' A C G T C 5'

3' protruding ends

14. Option (A) is correct

Explanation: The construction of the first recombinant DNA by linking a gene encoding antibiotic resistance with a native plasmid of *Salmonella typhimurium* was accomplished in 1972 by Stanley Cohen and Herbert Boyer. This rDNA was transferred in *Escherichia coli* which could replicate and produce multiple copies.

ASSERTION-REASON QUESTIONS (1 Mark)

1. Option (C) is correct
Explanation: DNA Being negatively charged practical move towards positive electrode. The small the fragment size of DNA, the faster it moves.

2. Option (C) is correct
Explanation: The stirrer in a bioreactor ensures that oxygen and nutrients are evenly distributed throughout the culture medium, promoting optimal growth conditions for microorganisms. Stirred tank bioreactors have a curved or rounded base rather than a flat one.

3. Option (C) is correct
Explanation: While it is true that specific enzymes are used to degrade cell walls to isolate DNA, cellulase specifically degrades cellulose, which is found in plant cell walls, not fungal cell walls. Fungal cell walls are primarily composed of chitin, and enzymes like chitinase are used to degrade them.

4. Option (D) is correct
Explanation: DNA is a hydrophilic molecule, which means it cannot easily pass through the hydrophobic lipid bilayer of cell membranes. Therefore, bacterial cells must be made competent (for example, by chemical treatment or electroporation) to take up DNA from their environment.

5. Option (C) is correct
Explanation: In PCR (polymerase chain reaction), synthetic oligonucleotide primers are used to initiate DNA replication. The primers are short, single-stranded DNA sequences that are designed to bind to the complementary regions of the target DNA sequence. During the annealing step of PCR, the temperature is lowered to allow the primers to bind to the single-stranded DNA template at their complementary regions. This allows the DNA polymerase to begin copying the DNA sequence.

6. Option (D) is correct
Explanation: When DNA from different sources is cut by the same restriction enzyme, the resulting fragments have the same sticky ends because the enzyme targets specific sequences. For example, if a restriction enzyme recognises 5'–GAATTC–3', all DNA cut by this enzyme will share the same sticky ends, allowing for recombination. DNA ligases then join these fragments by forming phosphodiester bonds between adjacent nucleotides, effectively sealing the sticky ends.

VERY SHORT ANSWER TYPE QUESTIONS (2 Marks)

1. Ori– It is the site where replication starts. It is responsible for controlling the copy number of the linked DNA.
Restriction site – It is the site of ligation of alien DNA or foreign DNA or desirable DNA to the vector.

2. To take up the DNA, as the DNA molecule is hydrophilic (it cannot pass through cell membrane).
By treating host cells with a specific concentration of a divalent cations (like Ca^{2+} ion) and then incubating the cells with rDNA on ice followed by placing them briefly at 42°C (Heat shock) and then putting them back on ice.

3. (i) 'A; Circular DNA/Plasmid
'B' Bacteriophage
(ii) Plasmid can carry foreign gene into the host cell/ acts as cloning vector/has selectable marker/ independent of the control of chromosomal DNA/ high copy number.
Bacteriophage cloning vector have the ability to replicate in bacterial cells / independent of the control of chromosomal DNA / high copy number per cell.

4. Treating bacteria with specific concentration of calcium (ions) which increases the efficiency with which DNA enters the bacteria through pores in its cell wall, recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42°C (heat shock), then putting them back on ice.

5. Recombinant DNA/Desired DNA is inserted into the coding sequence of an enzyme β-galactosidase. This results into inactivation of the gene for the synthesis of this enzymes, presence of chromogenic substrate gives blue coloured colonies if the plasmid in bacteria does not have the insert (Non recombinants), but presence of insert (Recombinants) leads to the growth of bacterial colonies with no colour.

6. (i) *Thermus aquaticus*
(ii) Thermostable DNA polymerase remains active during the high temperature induced denaturation of double stranded DNA. It is not required to be added every time after denaturation in every cycle.

7.

	Microinjection	Biolistics/Gene Gun
(1)	Suitable for animal cell.	Suitable for plants cell.
(2)	Recombinant DNA is directly injected/into the nucleus.	Cells are bombarded with high velocity micro-particles of gold tungsten/coated with DNA.

8. **In EcoRI:** First letter 'E' comes from the genus, the second two 'co' letters from the species, letter 'R' – is derived from the name of strain, Roman number 'I'- indicates the order in which the enzyme is isolated.

9. **(i)**
- Set up A,
- DNA fragments being negatively charged move towards the anode on applying the electric field.

(ii)
- I
- Smaller fragment will move faster as compared to longer fragments of DNA or longer fragments of DNA will move slower as compared to smaller fragments of DNA.

10. **(i)** 'ori' – a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells. This sequences is also responsible for controlling the copy number of the linked DNA.

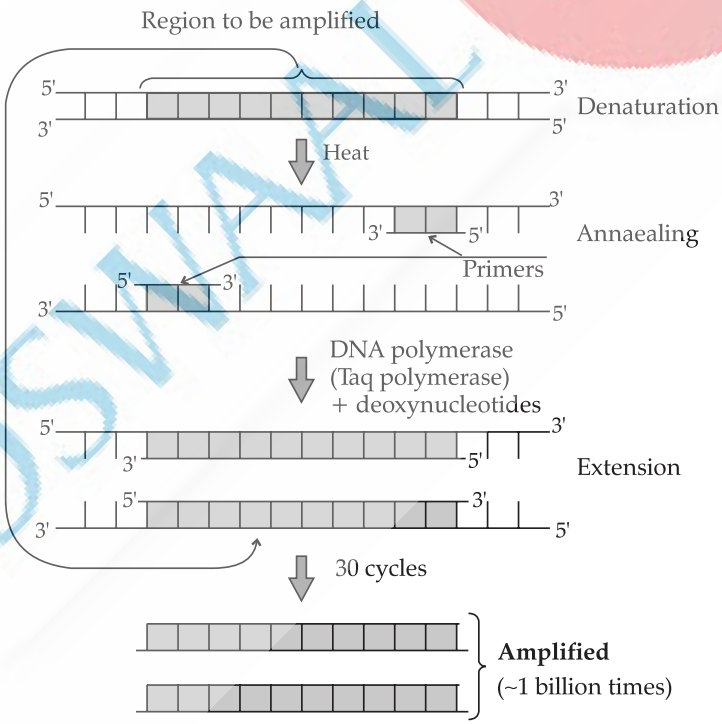
(ii) Plants – *Agrobacterium tumefaciens*
Animals – Retrovirus

11. **(i)** Micro injection, Recombinant DNA is directly injected in the nucleus of an animal cell.
Retrovirus, animal cells are infected with disarmed retrovirus.

(ii) Biolistics or Genegun, plants cell are bombarded with high velocity micro particles of gold or tungsten coated with DNA (rDNA)
Agrobacterium tumefaciens, delivering gene by disarmed pathogen.

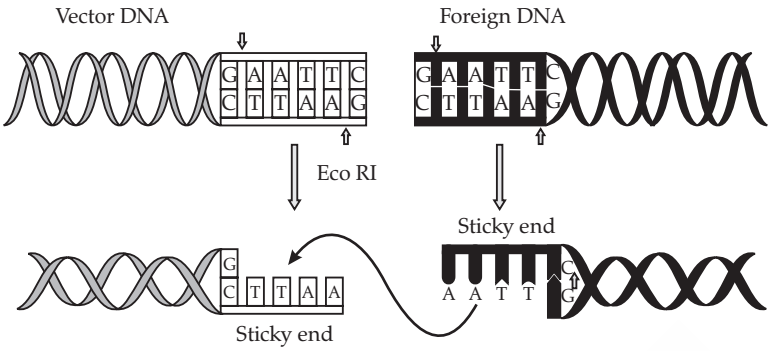
SHORT ANSWER TYPE QUESTIONS (3 Marks)

1. PCR stands for Polymerase Chain Reaction. In this reaction, multiple copies of the gene (or DNA) of interest are synthesised in vitro using two sets of primers (small chemically synthesised oligonucleotides that are complementary to the regions of DNA) and the enzyme DNA polymerase. The enzyme extends the primers using the nucleotides provided in the reaction and the genomic DNA as template.
If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times, i.e., 1 billion copies are made. Such repeated amplification is achieved by the use of a thermostable DNA polymerase (isolated from a bacterium, *Thermus aquaticus)*, which remains active during the high temperature induced denaturation of double stranded DNA. The amplified fragment if desired can now be used to ligate with a vector for further cloning.
Each cycle has three steps: (i) Denaturation, (ii) Annealing and (iii) Extensions.

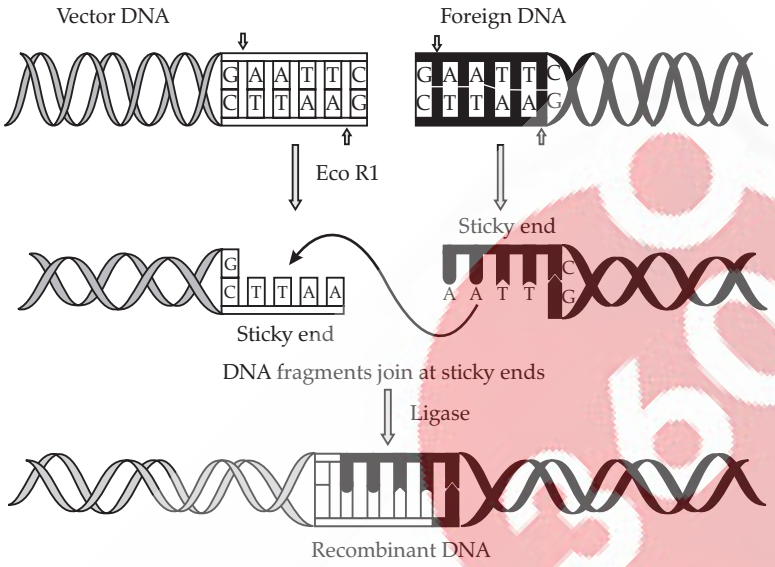


2. **(i)**
- In EcoRI (comes from *Escherichia coli* RY13)
- E represent Genus *Escherichia*,
- co represent species *coli*,
- R represents RY 13 strain,
- I represent order in which the enzyme were isolated from that strain of bacteria.

(ii)



3.



4. **(i)** Gel electrophoresis, to separate DNA fragments.

(ii) **(a)** Agarose gel, to separate DNA fragments according to their size through sieving effect.

(b) Stained with Ethidium bromide followed by exposure to UV light which can be seen as orange coloured DNA band. Separated DNA band are cut from the gel and extracted from gel piece.

5. **(i)** Helps in identifying non-transformants from transformants / Recombinants from non - recombinants.

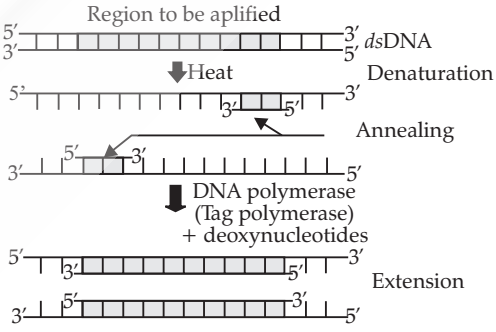
(ii) Genes encoding resistance to antibiotics such as ampicillin/tetracycline/kanamycin/ chloramphenicol / ampR / tetR

(iii) (The normal *E. coli* cells do not carry resistance against any of these antibiotics.) It helps to identify and select transformants/identification of recombinants.

6. Plasmids are extra chromosomal self-replicating (double stranded) circular DNA molecules (generally found in bacterial cell).

- Plasmid is circular extra chromosomal DNA of bacterial cells whereas cloning vector is a vehicle that carries foreign DNA into another cell.
- Bacteriophage, pBR322

7.



LONG ANSWER TYPE QUESTIONS (5 Marks)

1. **(i)**

- DNA is a hydropilic molecule and cannot pass through the cell membrane.
- A bacterial cell is made competent by treating the cacterial cell with a specific concentration of a divalent cation such as calcium, which increases efficiency with which the DNA enters through pores in its cell wall. This creates certain transient pores in its cell and increases the efficiency of the cell to take up DNA.

(ii) (1) Separation of DNA fragments.

(2) DNA fragments are negatively charged molecules, they can be separated according to their size by forcing them to move toward the anode under an electric field through agarose gel.

(3) To stain the DNA to visualise by exposure to UV radiation.

2. (i) Can act as vector/can self-replicate to form multiple copies/ have selectable markers/ small in size will facilitate insertion / presence of 'Ori'.

E. coli, *Agrobacterium tumefaciens*, *Salmonella typhi*, Bacteria, (any two)

(ii) 'Ori' – This is a sequence from where replication starts. Any piece of DNA when linked to this sequence can be made to replicate with in the host cells. It controls copy number of linked DNA.

Selectable marker helps in identifying and eliminating non-transformants, and selectively permitting the growth of transformants during recombinant DNA technology.

(iii) Restriction endonuclease identifies a specific palindromic sequence of DNA and cut the DNA at the specific sites in both the host as well in desired/foreign DNA, thereby creates "sticky ends" facilitating ligation to form a recombinant DNA.

3. (i) Optimum growth conditions:

(1) Temperature,
(2) pH,
(3) Substrate,
(4) Salts,
(5) Vitamins,
(6) Oxygen (Any four)

(ii) Lag phase / Exponential pase

(iii)
- No
- It needs separation and purification / down-streaming process / quality control testing / needs to be fomulated with suitable preservatives / clinical trials.

4. (i) (I) E - Genus
co – Species
R – Strain
I – Order of isolation of enzyme

(II) Recognition site – 'GAATTC 3'
3'CTTAAG 5'

Cleavage site – Between G and A from both sides

```
    ↓
5' GAATTC 3'
3' CTTAAG 5'
      ↑
```

(ii)
- Sticky ends
- Sticky ends hydrogen bonds with their complimentary cut counterparts, this stickiness facilitates the action of the enzyme DNA ligase.

5. (i)
- EcoRI
- 5' – GAATTC – 3'
  3' – CTTAAG – 5'
- EcoRI cuts the DNA between bases G and A from 5' end of both DNA stands.

```
    ↓
5' GAATTC 3'
3' CTTAAG 5'
      ↑
```

(ii)
- DNA molecule being negatively charged moves towards the anode/positive electrode through a medium of agrose gel under an electric field.
- DNA fragments separate according to their size/molecular weight (smaller the fragment size, the farther it moves).

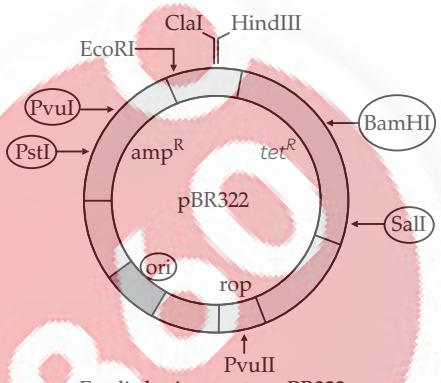
6. (i) No

Cut with same restriction endonuclease to obtain complementary sticky ends / to obtain DNA fragments with same kind of sticky ends (which can be joined together end to end)

(ii) DNA ligase

(iii) Cloning / Host Cloning / Gene Cloning.

(iv)



E.coli cloning vector pBR322

(v) Codes for the proteins involved in replication of the plasmid.

7. (i) (1) S (2) R (3) T

(ii)
- Elution is the process of cutting of separated bonds of DNA, and extracting from the agarose gel.
- Purified DNA is used in rDNA technology/ genetic experiments.

8. (i) **Advantages:**

(1) Large volumes (100 – 1000 litres) of culture can be processed,
(2) Foam control system can control foam formation,
(3) Temperature can be controlled by temperature control system,
(4) pH control system,
(5) Optimum growth conditions can be maintained,
(6) Substrate/salt/vitamins can be periodically added,
(7) Small volumes of cultures can be withdrawn periodically through sampling ports.
(any four)

(ii) Product has to be formulated with suitable preservatives, clinical trials are required in case of drugs, strict quality control testing of each product is required. (any two)

Level - 2 ADVANCED COMPETENCY FOCUSED QUESTIONS

MULTIPLE CHOICE QUESTIONS (MCQs) (1 Mark)

1. Option (B) is correct
Explanation: The origin of replication (ori) is the specific DNA sequence in a cloning vector where replication begins. It ensures that the vector can replicate inside the host cell. Any foreign DNA inserted into the vector is also copied along with it. The copy number of the plasmid can also be controlled based on the nature of the ori.
2. Option (C) is correct
Explanation: DNA ligase is the enzyme used in genetic engineering to join foreign DNA fragments with a vector (e.g., plasmid). It forms phosphodiester bonds between the sugar and phosphate groups of adjacent DNA nucleotides, sealing the "nicks" and creating a stable recombinant DNA molecule.
3. Option (C) is correct
Explanation: Taq polymerase is a thermostable DNA polymerase isolated from the bacterium *Thermus aquaticus*. It is essential in PCR (Polymerase Chain Reaction) because it synthesises new DNA strands during the extension step (~72°C), and remains active at high temperatures, unlike normal DNA polymerases which would denature.
4. Option (B) is correct
Explanation: *Agrobacterium tumefaciens* is a soil bacterium widely used in plant genetic engineering because of its Ti plasmid (Tumor-inducing plasmid). This plasmid naturally transfers a segment of DNA (T-DNA) into the plant genome, causing crown gall disease in wild conditions, but in biotechnology, scientists replace disease-causing genes with desirable genes making it an efficient and natural gene delivery system for dicot plants.
5. Option (A) is correct
Explanation: PCR (Polymerase Chain Reaction) involves the following three main steps repeated over multiple cycles:
   (1) **Denaturation (~94–95°C):** Double-stranded DNA is heated to separate it into single strands.
   (2) **Annealing (~50–65°C):** Primers bind (anneal) to the complementary sequences on the single-stranded DNA templates.
   (3) **Extension (~72°C):** Taq polymerase synthesises new DNA strands by adding nucleotides to the primers.
6. Option (B) is correct
Explanation: Insertional inactivation is a method used to identify recombinant clones. In plasmids like pUC19, the lacZ gene codes for the enzyme β-galactosidase, which can break down X-gal, a synthetic substrate, to produce a blue pigment. When a foreign gene is inserted into the lacZ gene, the gene is disrupted (inactivated).As a result, β-galactosidase is not produced, and the recombinant colonies remain white on X-gal plates. Non-recombinant colonies (with an intact lacZ gene) appear blue. This colour differentiation helps easily identify which bacterial colonies have taken up the recombinant plasmid.

ASSERTION-REASON QUESTIONS (1 Mark)

1. Option (A) is correct
Explanation: Assertion is true. Restriction endonucleases are enzymes that cut DNA at specific sites and are therefore referred to as molecular scissors in genetic engineering.
Reason is also true. These enzymes recognise and cut at specific palindromic sequences — sequences that read the same forward and backward (e.g., GAATTC).
2. Option (C) is correct
Explanation: Assertion is true. DNA ligase is an enzyme that joins DNA fragments by forming phosphodiester bonds. It is crucial in recombinant DNA technology for sealing the inserted gene into the vector.
Reason is false. DNA ligase functions in both prokaryotic and eukaryotic cells, not just prokaryotic. In fact, different types of DNA ligases (like T4 DNA ligase, human DNA ligase) are used in various organisms and in vitro system.
3. Option (C) is correct
Explanation: Assertion is true. Insertional inactivation is a technique used in recombinant DNA technology to identify recombinant colonies. When a foreign gene is inserted into a reporter gene (like lacZ), the gene is inactivated, allowing for visual identification of recombinant colonies (e.g., white colonies on X-gal plates).
Reason is false. Insertional inactivation typically targets reporter genes like lacZ, not antibiotic resistance genes.
4. Option (A) is correct
Explanation: Assertion is true. Taq polymerase is a thermostable DNA polymerase derived from *Thermus aquaticus*, a heat-loving bacterium. It does not denature at high temperatures used in PCR, making it ideal for the process.
Reason is also true. PCR (Polymerase Chain Reaction) involves repeated cycles of Denaturation (~94°C), Annealing (~50–65°C), and Extension (~72°C). These cycles involve high temperatures that would normally inactivate regular DNA polymerases.

VERY SHORT ANSWER TYPE QUESTIONS (2 Marks)

1. Biotechnology introduces a gene from ***Bacillus thuringiensis*** into cotton, producing Bt toxin. This toxin kills insect larvae (e.g., bollworms), reducing the need for external pesticide application and making farming more sustainable.
2. Recombinant human insulin is identical to natural human insulin, reducing the risk of allergic reactions. It is ethically and hygienically better and can be mass-produced using genetically modified *E. coli*, ensuring a stable supply.

3. In gene therapy, a normal functional gene is inserted into a patient's cells using a viral vector. This replaces or compensates for the defective gene, restoring normal protein function and alleviating symptoms.
4. PCR amplifies tiny DNA samples collected from crime scenes. This helps in identifying individuals by matching DNA fingerprints, even from minimal biological material like hair, blood, or saliva.
5. **Lipase** – used in detergent industry to remove oily stains.
Pectinase – used in fruit juice clarification in the food industry.

SHORT ANSWER TYPE QUESTIONS (3 Marks)

1. **(i)** The gene of interest is cut using a restriction enzyme.
(ii) The plasmid vector is also cut with the same restriction enzyme to create compatible ends.
(iii) The gene is inserted into the plasmid and joined using DNA ligase to form recombinant DNA.
(iv) The recombinant plasmid is introduced into the host cell (e.g., *E. coli*) by transformation.
2. **(1)** The gene for human insulin is inserted into *E. coli* using recombinant DNA technology. The bacteria produce insulin, which is harvested and purified.
(2) Genes coding for antigens (from pathogens) are inserted into microbes to produce recombinant vaccines (e.g., Hepatitis B vaccine produced by yeast).
3. **(i)** **Denaturation:** DNA is heated (~94°C) to separate strands.
(ii) **Annealing:** Primers bind to the target sequences (~55°C).
(iii) **Extension:** Taq polymerase synthesises new DNA strands (~72°C).
Application: Used in disease diagnosis, DNA fingerprinting, and gene cloning.
4. The Bt gene from *Bacillus thuringiensis* codes for a Cry protein that is toxic to insect larvae (e.g., fruit borers). When pests ingest the Cry protein, it damages their gut, leading to death. This reduces pesticide use, protects beneficial insects, and supports eco-friendly farming.
5. pUC19 carries the lacZ gene, which produces a blue pigment in the presence of X-gal. If a foreign gene is inserted into the lacZ region, the gene is inactivated.
Recombinants → white colonies (no lacZ activity)
Non-recombinants → blue colonies (intact lacZ gene)
This is called insertional inactivation and helps in easy screening of recombinant colonies.

CASE BASED QUESTIONS (4 Mark)

1. **(i)** Option (B) is correct
Explanation: During the exponential growth phase, bacterial cells rapidly divide and produce a larger biomass. This results in higher production of the recombinant molecule since the metabolic activity and protein synthesis are at their peak.
(ii) Option (C) is correct
Explanation: Replacing the used-up medium with fresh nutrients can help support the growth of the bacterial culture, reviving the bio-processing and restarting recombinant molecule production.
(iii) Option (B) is correct
Explanation: Sterile conditions refer to an environment free from any contaminating or unwanted microorganisms, ensuring that only the desired bacteria can grow and produce the recombinant molecule.
(iv) Option (A) is correct
Explanation: The bio-processor is sterilised before introducing the bacterial culture. This ensures that the bacteria producing the recombinant molecule are not exposed to harmful conditions during the sterilisation process.
2. **(i)** Polymerase Chain Reaction.
(ii) **(a)** Very low concentration of a bacteria or virus (during early stages of infection), can be detected by amplification of their nucleic acid by PCR.

OR

(b) Taq polymerase is heat resistant enzyme/ thermostable/ does not get denatured at high temperature.
Thermus aquaticus / Thermophilus aquaticus
(iii) Denaturation (at high temperature) → Annealing → Extension (of the primers)
3. **(i)** • Hind II–These enzymes are called restriction enzymes because they restrict infection of bacteria by certain viruses (i.e., bacteriophages), by degrading the viral DNA without affecting the bacterial DNA. Thus, their function in the bacterial cell is to destroy foreign DNA that might enter the cell.
(ii) • 5′ — GAATTC — 3′
3′ — CTTAAG — 5′
(iii) **(a)** It inspects the length of DNA sequence and binds to specific strands at specific points in the sugar - phosphate backones.

OR

(b) In EcoRI (comes from *Escherichia coli* RY 13)
- E represent Genus *Escherichia*,
- co represent species coli,
- R represent RY 13 strain,
- I represent order in which the enzyme isolated from that strain of bacteria.

4. **(i)** **(a)** Recombinant DNA technology

OR

(b) Baacterial-Lysozyme, Fungal-Chitinase

(ii) Scientist at Eli Lilly company prepared two DNA sequences, corresponding to A and B peptide chains of human insulin, and introduced them in plasmids of *E. Coli* to produce insulin chains. Chains A and B were produced separately, extracted, and combined by creating disulfide bonds to form human insulin.

5. **(i)** 3/Three fragments

5' –C–C–G–T–A–G 3'
3' G–G–C–A–T–C

5' –C–T–A–T–C–A–G 3'
3' –G–A–T–A–G–T–C 5'

5' C–T–G–G–3'
3' G–A–C–C–5'

(ii) Alu I, Alu I site is present in the given sequence and BamH I site is not given.

(iii) **(a)** Sugar phosphate backbone

Palindrome sequence/recognition site/ restriction site.

OR

(b) 5′ G-A-A-T-T-C 3′

3′ C-T-T-A-A-G 5′

6. **(i)**
- The *E.coli* DNA polymerase cannot carry out PCR at high temperature (as it becomes inactive).
- Whereas Taq polymerase being thermostable remains active even at high temperatures.

(ii)
- Primers are small chemically synthesised oligonucleotides that are comlementary to the regions of genomic DNA strand.
- Primers help in extension of complementary DNA strand.

(iii) Early detection of diseases like cancer / AIDS / genetic disorder, by amplification of desired genes (when very low concentration of bacteria or virus before setting of the disease symptoms).

LONG ANSWER TYPE QUESTIONS (5 Marks)

1. **(i)** Recombinant DNA technology.

(ii) Human insulin consists of two polypeptide chains – A and B – linked by disulfide bonds.

(iii) Genes for A and B chains are inserted into separate *E. coli* using plasmids. Each bacterium produces one chain. The chains are extracted, purified, and combined to form functional insulin.

(iv) Recombinant insulin is identical to human insulin and less likely to cause allergic reactions.

(v) Concern about the uncontrolled release of GMOs into the environment.

2. PCR is based on the principle of in vitro amplification of specific DNA segments using primers, nucleotides, and Taq polymerase.It involves three steps repeated cyclically:

Denaturation (~94°C) – DNA strands separate.

Annealing (~55°C) – Primers bind to the template.

Extension (~72°C) – Taq polymerase synthesizes new strands.

Taq polymerase is used because it is thermostable and doesn't get denatured at high temperatures.

Applications:

(1) Detecting genetic diseases

(2) DNA fingerprinting

(3) Forensic investigations

(4) Cloning and gene identification

(5) Disease diagnosis (e.g., COVID-19, TB)

3. **(i)** The Cry gene encodes a protein that becomes toxic in the alkaline gut of insects, damaging their gut lining and killing them.

(ii) The gene is cloned from *Bacillus thuringiensis* into a vector and introduced into the plant genome using *Agrobacterium* or gene gun methods.

(iii) **Advantages:**

(1) Reduces need for chemical pesticides

(2) Increases crop yield and reduces pest-related losses

Limitation: May lead to resistance in pests and affect biodiversity.

4. **(i)** Acts as a natural genetic engineer; its Ti plasmid helps transfer genes into plant cells.

(ii) Small, self-replicating DNA molecules used as vectors to carry foreign genes.

(iii) Cut DNA at specific sequences, enabling precise gene insertion.

(iv) Help identify transformed cells (e.g., antibiotic resistance genes or lacZ).

(v) Large vessels for growing genetically modified cells under controlled conditions to produce proteins/drugs at an industrial scale.