Term-II

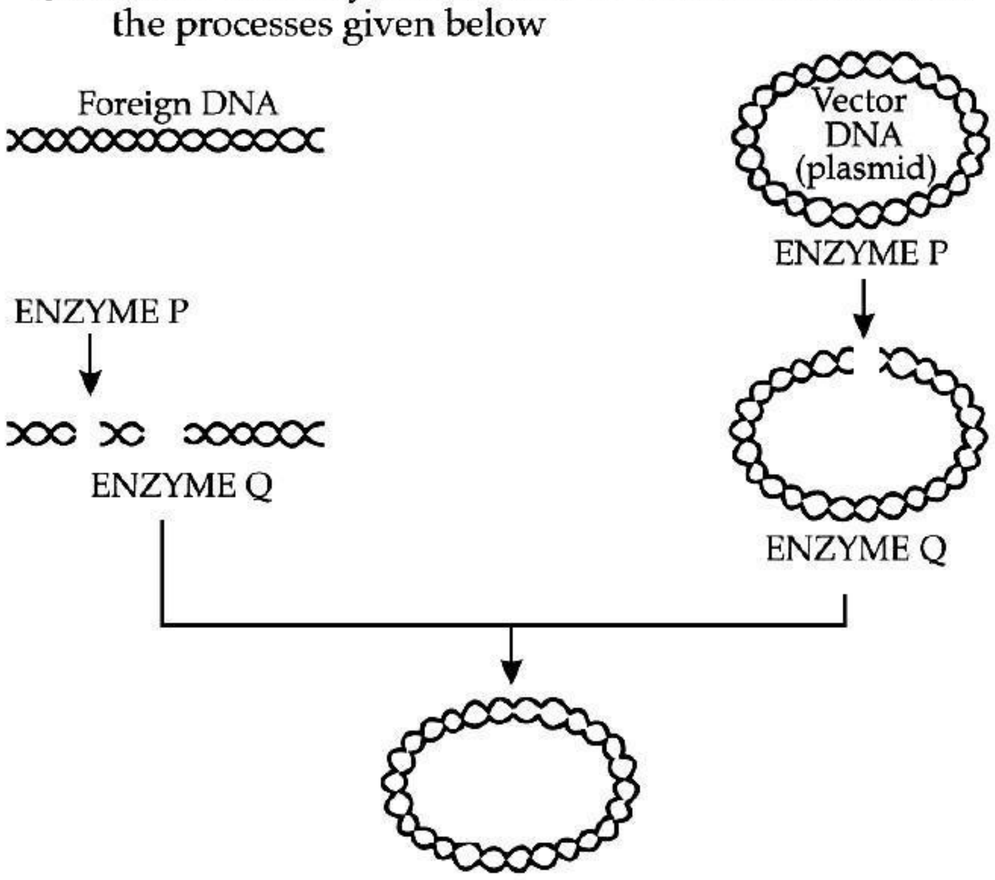
BIOTECHNOLOGY : PRINCIPLES AND PROCESSES

Syllabus

- *Genetic Engineering (Recombinant DNA Technology).*

STAND ALONE MCQs (1 Mark each)

Q. 1. Name the enzymes 'P' and 'Q' that are involved in the processes given below



(A) Enzyme P-Exonuclease and Enzyme Q-Permease

(B) Enzyme P- Exonuclease and Enzyme Q- Ligase

(C) Enzyme P-Endonuclease and Enzyme Q-Permease

(D) Enzyme P-Restriction endonuclease and Enzyme Q- Ligase

R [CBSE SQP, 2020]

Ans. Option (C) is correct.

> ***Explanation :*** Enzyme P is restriction endonuclease that cuts the DNA into fragments while enzyme Q join the two fragments.

Q. 2. A biotechnologist wanted to create a colony of *E.coli* possessing the plasmid pBR322, sensitive to Tetracycline. Which one of the following restriction sites would he use to ligate a foreign DNA?

(A) Sal I **(B)** Pvu I

(C) EcoRI **(D)** Hind III

U [CBSE SQP, 2020]

Ans. Option (A) is correct.

> ***Explanation :*** When an alien gene is ligated at the Sal I site of tetracycline resistance gene in the vector pBR322, the recombinant lose tetracycline resistance due to insertion of the foreign DNA.

Q. 3. What is the criterion for DNA fragments movement on agarase gel during gel electrophoresis?

(A) The larger the fragment size, farther it moves

(B) The smaller the fragment size, farther it moves

(C) Positively charged fragment move to farther end.

(D) Negatively charged fragment do not move. R

Ans. Option (B) is correct.

> ***Explanation :*** In agarose gel electrophoresis of DNA molecule smaller molecules travel faster then larger molecules. The movement of DNA fragment is inversely proportional to number of base pairs. Separation of DNA occurs by its length.

Q.4. An enzyme catalysing the removal of nucleotides from the ends of DNA is

(A) endonuclease **(B)** exonuclease

(C) DNA ligase. **(D)** Hind – II. R

Ans. Option (B) is correct.

> ***Explanation :*** Exonucleases remove nucleotides from the ends of the DNA. Endonucleases make cuts at specific positions within the DNA. DNA ligase (also called genetic gum) is a sealing enzyme which is responsible for joining of two individual fragments of DNA, whereas Hind-II is the first discovered restriction endonuclease enzyme.

Q. 5. The transfer of genetic material from one bacterium to another through the mediation of a vector like virus is termed as

(A) transduction **(B)** conjugation

(C) transformation **(D)** translation R

Ans. Option (A) is correct.

> ***Explanation :*** Transduction is the process by which genetic material (DNA) is transferred from one bacterium to another through the mediation of a vector, like virus. Bacterial conjugation is the process of transfer of genetic material (plasmid) between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. Transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material (exogenous DNA) from its surroundings and taken up through the cell membranes. Translation is the process in which cellular ribosomes create proteins. It is a part of the process of gene expression.

Q.6. 'Restriction' in Restriction enzyme refers to

(A) cleaving of phosphodiester bond in DNA by the enzyme.

(B) cutting of DNA at specific position only.

(C) prevention of the multiplication of bacteriophage in bacteria.

(D) All of the above R

Ans. Option (B) is correct.

> ***Explanation :*** Restriction enzymes (also called molecular scissors) are responsible for cutting DNA. These enzymes belong to a class of enzymes called nucleases and are of two types : (i) Exonuclease which cut DNA at the ends and (ii) endonucleases which make cuts at specific positions within the DNA. The term 'restriction' refers to the function of these enzymes in restricting the propagation of foreign DNA of bacteriophage in host bacterium, that is, cutting of DNA, at specific position only.

Q. 7. An antibiotic resistance gene in a vector usually helps in the selection of

(A) competent cells **(B)** transformed cells

(C) recombinant cells **(D)** None of the above R

Ans. Option (B) is correct.

> ***Explanation :*** Selectable markers help in identifying and eliminating non-transformants and selectively permitting the growth of the transformants. The normal E. coli cells do not carry resistance against any antibiotic. Competent bacterial cells are made capable to take foreign DNA with chemical treatment (e.g., calcium chloride).

Q. 8. Significance of 'heat shock' method in bacterial transformation is to facilitate

(A) binding of DNA to the cell wall.

(B) uptake of DNA through membrane transport proteins.

(C) uptake of DNA through transient pores in the bacterial cell wall.

(D) expression of antibiotic resistance gene. R

Ans. Option (C) is correct.

> ***Explanation :*** In chemical method, the cell is treated with specific concentration of a divalent cation such as calcium which increase the pore size in cell wall. The cells are incubated with recombinant DNA on ice, followed by placing them briefly at 42°C and then putting it back on ice. This is called heat shock method. The bacteria now take up these recombinant DNA.

Q. 9. While isolating DNA from bacteria, which of the following enzymes is not used?

(A) Lysozyme **(B)** Ribonuclease

(C) Deoxyribonuclease **(D)** Protease R

Ans. Option (C) is correct.

> ***Explanation :*** Deoxyribonuclease enzyme is not used in the process of isolating DNA from bacteria as this enzyme causes the lysis of DNA molecules

Q.10. Which of the following bacteria is not a source of restriction endonuclease?

(A) *Haemophilus influenzae*

(B) *Escherichia coli*

(C) *Agrobacterium tumefaciens*

(D) *Bacillius amyloli* R

Ans. Option (C) is correct.

> ***Explanation :*** *Agrobacterium tumefaciens* is a pathogen of several dicot plants. It delivers a piece of DNA known as T-DNA in the Ti plasmid which transforms normal plant cells into tumour cells to produce chemicals required by pathogens. The restriction enzyme Eco Rl, is isolated from E. coli RY13. The first restriction enzymes Hind II was isolated from bacterium *Haemophilus influenzas*. The restriction enzyme Bam HI is isolated from *Bacillus amyloli.*

Q. 11. The correct order of step in polymerase chain reaction (PCR) is :

(A) Extension, Denaturation, Annealing

(B) Denaturation, Annealing, Extension

(C) Denaturation, Extension, Annealing

(D) Annealing, Extension, Denaturation R

Ans. Option (B) is correct.

> ***Explanation :*** A single PCR amplification cycle involves three basic steps : denaturation, annealing and extension. PCR stands for polymerase chain reaction in which multiple copies of the gene, for DNA of interest is synthesised *in vitro.*

Q.12. The process of separation and purification of expressed protein before marketing is called :

(A) Upstream processing

(B) Downstream processing

(C) Bio processing

(D) Post production processing

Ans. Option (B) is correct.

> ***Explanation :*** After completion of bio synthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product. The processes include separation & purification, which are collectively referred to as downstream processing.

Q. 13. Stirred-tank bioreactors have been designed for.

(A) ensuring anaerobic conditions in culture vessel

(B) purification of product

(C) addition of preservatives to product

(D) availability of oxygen throughout process

Ans. Option (D) is correct.

> ***Explanation :*** The stirred tank bioreactor is well suited for large scale production of micro organism under aseptic condition for a number of days. It can be used easily in research laboratory and main advantage is an oxygen delivery system which provides oxygen without any interruption.

Q.14. Which of the following has popularised the PCR (polymerase chain reactions)?

(A) Easy availability of DNA template

(B) Availability of synthetic primers

(C) Availability of cheap deoxyribonucleotides

(D) Availability of 'Thermostable' DNA polymerase R

Ans. Option (D) is correct.

> ***Explanation :*** The polymerase chain reaction (PCR) is a reaction in which amplification of specific DNA sequences is carried out in vitro. Such repeated amplification is achieved by the using thermostable DNA polymerase (isolated from a bacterium, *Thermus aquaticus*) enzyme which remains active and stable during high temperature and induced denaturation of double-standard DNA.

Q.15. Which of the following steps are catalysed by Taq polymerase in a PCR reaction?

(A) Denaturation of template DNA

(B) Annealing of primers to template DNA

(C) Extension of primer end on the template DNA

(D) All of the above R

Ans. Option (C) is correct.

> ***Explanation :*** In polymerase chain reaction, polymerisation or extension step is catalysed by Taq polymerase enzyme. PCR is carried out in the following three steps (i) Denaturation : The double-stranded DNA is denatured by applying high temperature of 95°C for 15 seconds. Each separated single strand now acts as template for DNA synthesis. (ii) Annealing : Two sets of primers are added which anneal to the three ends of each separated strand. Primers act as initiators of replication. (iii) Extension : DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction. A thermostable DNA polymerase (Taq DNA polymerase) is used in the reaction which can tolerate the high temperature of the reaction. All these steps are repeated many times to obtain several copies of desired DNA.

Q.16. The role of DNA ligase in the construction of a recombinant DNA molecule is

(A) formation of phosphodiester bond between two DNA fragments.

(B) formation of hydrogen bonds between sticky ends of DNA fragments.

(C) ligation of all purine and pyrimidine bases.

(D) None of the above R

Ans. Option (A) is correct.

> ***Explanation :*** DNA ligase (joining or sealing enzymes) are also called genetic gum. They join two individual fragments of double-stranded DNA by forming phosphodiester bonds between them. Thus, they help in sealing gaps in DNA fragments. Therefore, they act as a molecular glue.

Q.17. Which of the given statement is correct in the context of observing DNA separated by agarose gel electrophoresis?

(A) DNA can be seen in visible light.

(B) DNA can be seen without staining in visible light.

(C) Ethidium bromide stained DNA can be seen in visible light.

(D) Ethidium bromide stained DNA can be seen under exposure to UV light. R

Ans. Option (D) is correct.

> ***Explanation :*** The separated DNA fragments (by the process of gel electrophoresis) are visualised after staining the DNA with ethidium bromide followed by exposure to ultraviolet (UV)-radiation. These fragments are seen as orange coloured bands.

Q. 18. Who among the following was awarded the Nobel Prize for the development of PCR technique?

(A) Herbert Boyer **(B)** Hargovind Khurana

(C) Kary Mullis **(D)** Arthur Kornberg R

Ans. Option (C) is correct.

> ***Explanation :*** Polymerase chain reaction technique was developed by Kary Mullis in 1985, and for this he received Nobel Prize for chemistry in 1993. It is a reaction in which amplification of specific DNA sequences is carried out in vitro. H. G. Khurana discovered DNA ligase enzyme into phage in 1969. White DNA polymerase was discovered by Arthur Kornberg and Herbert Boyer generated first recombinant DNA molecule by combining a gene from a bacterium with plasmid of E. coli in 1972.

Q.19. Which of the following statements does not hold true for restriction enzyme?

(A) It recognises a palindromic nucleotide sequence.

(B) It is an endonuclease.

(C) It is isolated from viruses.

(D) It produces the same kind of sticky ends in different DNA Molecules. R

Ans. Option (C) is correct.

> ***Explanation :*** Restriction enzymes are a protein produced by bacteria that cleaves DNA at specific sites. These are not found in viruses. They are present in bacteria to provide a type of defence mechanism (called the restriction modification system) against bacterial viruses. They are of two types : endonuclease and exonuclease. They are indispensable tools in recombinant DNA technology and genetic engineering.

Q.20. Which of the following should be chosen for best yield if one were to produce a recombinant protein in large amounts?

(A) Laboratory flask of largest capacity

(B) A stirred-tank bioreactor without in-lets and outlets

(C) A continuous culture system

(D) Any of the above R

Ans. Option (C) is correct.

> ***Explanation :*** If any protein encoding gene is expressed in a heterologous host, it is called recombinant protein. The cells harbouring cloned genes of interest may be grown on a small scale in the laboratory. The cultures may be used for extracting the desired protein and then purifying it by using different separation techniques. The cells can also be multiplied in a continuous culture system where the used medium is drained out from one side while fresh medium is added from the other to maintain the cells in their physiologically most active log/exponential phase. This type of culturing method produces a larger biomass leading to higher yields of desired protein.

ASSERTION AND REASON BASED MCQs (1 Mark each)

Directions : In the following questions a statement of assertion (A) is followed by a statement of reason (R). Mark the correct choice as :

(A) Both assertion (A) and reason (R) are true and reason (R) is the correct explanation of assertion (A).

(B) Both assertion (A) and reason (R) are true but reason (R) is not the correct explanation of assertion (A).

(C) Assertion (A) is true but reason (R) is false.

(D) Assertion (A) is false but reason (R) is true.

Q. 1. Assertion (A) : *EcoRI* is restriction endonuclease enzyme.
Reason (R) : Exonuclease removes nucleotides from the ends of DNA.

Ans. Option (B) is correct.

> ***Explanation :*** *EcoRI* is a restriction endonuclease enzyme. Exonuclease removes nucleotides from the ends of DNA.

Q. 2. Assertion (A) : Any fragment of DNA, when linked to the ori region, can be initiated to replicate.
Reason (R) : Ori is a genetic sequence that acts as the initiation site for replication of DNA.

Ans. Option (A) is correct.

> ***Explanation :*** The process of DNA replication begins at the ori sequence. The presence of ori in a DNA fragment makes it a self-replicating molecule.

AI **Q. 3. Assertion (A) :** Restriction enzymes belongs to class nucleases.
Reason (R) : Nucleases are of two kinds : exo and endonucleases. Exonucleases remove nucleotides within the DNA.

Ans. Option (C) is correct.

> ***Explanation :*** Nucleases are of two kinds : exo and endonucleases, but exonucleases remove nucleotides from the ends of the DNA.

Q.4. Assertion (A) : *E. coli* having pBR322 with DNA insert at BamH I site cannot grow in medium containing tetracycline.
Reason(R) : Recognition site for BamH I is present in TetR region of pBR322.

Ans. Option (A) is correct.

> ***Explanation :*** pBR322 carries recognition sites for number of commonly used restriction enzymes. Recognition site for BamHl is present in tetr region i.e., region responsible for tetracyline resistance. When an insert is added at the BamHl recognition site the gene for tetracycline resistance becomes non-functional and the recombinant bacteria with plasmid pBR322 that has DNA insert at BamHl lose tetracycline resistance.

Q. 5. Assertion (A) : It is essential to have few cloning sites in cloning vector.
Reason(R) : It helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants.

Ans. Option (B) is correct.

> ***Explanation :*** It is essential to have few cloning sites in cloning vector. It is because, in order to link the alien DNA, the vector needs to have very few recognition sites for the commonly used restriction enzymes. Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning. Also, the vector requires a selectable marker, which helps in

identifying and eliminating non-transformants and selectively permitting the growth of the transformants.

Q. 6. Assertion (A) : *Thermus aquaticus,* is used in PCR technique.

Reason (R) : It is a heat-stable DNA polymerase.

Ans. Option (A) is correct.

Explanation : *Thermus aquaticus,* is the source of DNA polymerase because it is a heat-stable DNA polymerase.

AI **Q. 7. Assertion (A) :** Agarose gel electrophoresis is used to check the progression of a restriction enzyme digestion.

Reason (R) : Restriction enzyme digestions are performed by incubating purified DNA molecules with restriction enzyme.

Ans. Option (B) is correct.

Explanation : In Agarose gel electrophoresis, DNAs fragments cut by restriction enzyme can be arranged according to their sizes.

Q. 8. Assertion (A) : DNA is positively charged molecule.

Reason (R) : DNA moves towards the positive electrode (anode).

Ans. Option (D) is correct.

Explanation : DNA is a negatively charged molecule, hence it moves towards the positive electrode (anode).

Q. 9. Assertion (A) : A primer is a small segment of DNA that binds to a complementary strand of DNA. Reason(R) : Primers are necessary to stop the functioning of DNA polymerase enzyme and, therefore, are necessary in polymerase chain reaction.

Ans. Option (D) is correct.

Explanation : A primer is a small segment of DNA that binds to a complementary strand of DNA. Primers are necessary to start the functioning of DNA polymerase enzyme and, therefore, are necessary in polymerase chain reaction.

Q. 10. Assertion (A) : β-galactosidase coding sequence act as a selectable marker.

Reason (R) : Enzyme galactosidase converts the galactose into lactose.

Ans. Option (A) is correct.

Explanation : Galactosidase is an enzyme that converts the galactose into lactose. This property makes this enzyme to be used as a selectable marker or reporter gene in molecular biology experiments. This property is exploited during the selection of recombinants from the non-recombinants.

CASE-BASED MCQs

Attempt any four sub-parts from each question. sub-part each carries 1 mark.

I. Read the following and answer questions from Q.1. to Q.5. given below :

Restriction endonuclease was isolated for the first time by W. Aber in 1962 in bacteria. Restriction endonucleases cut the DNA duplex at specific points therefore they are also called as molecular scissors or biological scissors. Three types of restriction endemicleases are Type 1. Type ll and Type III but only Type II restriction endonucleases are used in recombinant DNA technology. Restriction endonuclease EcoR I recognises the base sequence GAATTC In DNA duplex and cut strands between G and A.

Q. 1. Only type II restriction enzymes are used in gene manipulation because:

(A) ATP is not required for cleaving

(B) it consists of three different subunits

(C) it makes cleavage or cut in both the strands of DNA molecule

(D) both (A) and (C)

Ans. Option (D) is correct.

Explanation : Type II enzymes are simpler and don't require ATP as an energy source, unlike Type I and it makes cleavage or cut in both the strands of DNA molecule.

Q. 2. Which of the following ions are used by restriction endonucleases for restriction?

(A) Mg^{2+} ions **(B)** Mn^{2+} ions

(C) Na^{+}ions **(D)** K^{+}ions

Ans. Option (A) is correct.

Explanation : The restriction endonuclease binds two magnesium ions. One of these ions,binds to the phosphate group where the cleavage occurs and is required for catalysis.

Q. 3. Restriction endonuclease was isolated for the first time from a:

(A) plant cell **(B)** animal cell

(C) prokaryotic cell **(D)** germinal cell

Ans. Option (C) is correct.

Explanation : Restriction endonuclease was isolated for the first time from the bacterium *Haemophilus influenzae* (prokaryotic cell).

Q. 4. Restriction endonucleases are also called as molecular or biological scissors because:

(A) they cleave base pairs of DNA only at their terminal ends

(B) they cleave one or both the strands of DNA

(C) they act only on single stranded DNA

(D) none of these

Ans. Option (B) is correct.

> ***Explanation :*** Restriction endonucleases naturally target double stranded DNA and could cleave one or both strands of the same.

Q. 5. Which type of restriction endonucleases is used most in genetic engineering?

(A) Type I (B) Type II

(C) Type III (D) Type IV

Ans. Option (B) is correct.

> ***Explanation :*** Type I and Type III are complex and have only a limited role in genetic engineering. Type II restriction endonucleases are used mostly as the cutting enzymes in gene cloning.

II. Read the following text and answer the following questions on the basis of the same:

The term biotechnology refers to the use of living organisms or their products to modify human health and their human environment. For example, 'test-tube' programme, synthesis of a gene or correcting a defective gene are all part of the biotechnology. The basis of the modern biotechnology are genetic engineering and maintenance of sterile conditions. Genetic engineering is the technique that alter the chemistry of genetic material i.e. DNA and RNA, then this genetic material is introduced into host organisms, which alter the phenotype of the host organism.

Q. 1. Discovery of _____ molecule made genetic engineering possible.

(A) Restriction exonuclease

(B) Restriction exonuclease

(C) Ribozyme

(D) DNA polymerase

Ans. Option (B) is correct.

> ***Explanation :*** Restriction endonucleus act as molecular sensor that gets the DNA from specific nucleotide and gives desired fragment of DNA.

Q. 2. The recognition sequence of the first restriction enzyme isolated was _____ base pair long.

(A) four (B) six

(C) five (D) two.

Ans. Option (B) is correct.

> ***Explanation :*** ECoRI is isolated as first restiriction that GAATAC nucleotide base pair.

Q.3. The specific DNA sequence where ***EcoRI*** cuts is :

(A) GATTCG (B) GAATTC

(C) GTTCAA (D) **TTCCAA.**

Ans. Option (B) is correct.

> ***Explanation :*** ECoRI recognises GAATAC base pairs that cuts between G and A.

Q.4. The cutting of DNA at specific locations became possible with the discovery of :

(A) Ligases (B) Restriction enzymes

(C) Probes (D) Selectable markers.

Ans. Option (B) is correct.

> ***Explanation :*** Restriction enzymes are the molecular scissors that cuts the DNA from specific recognition site.

Q.5. DNA fragments are :

(A) Positively charged

(B) Negatively charged

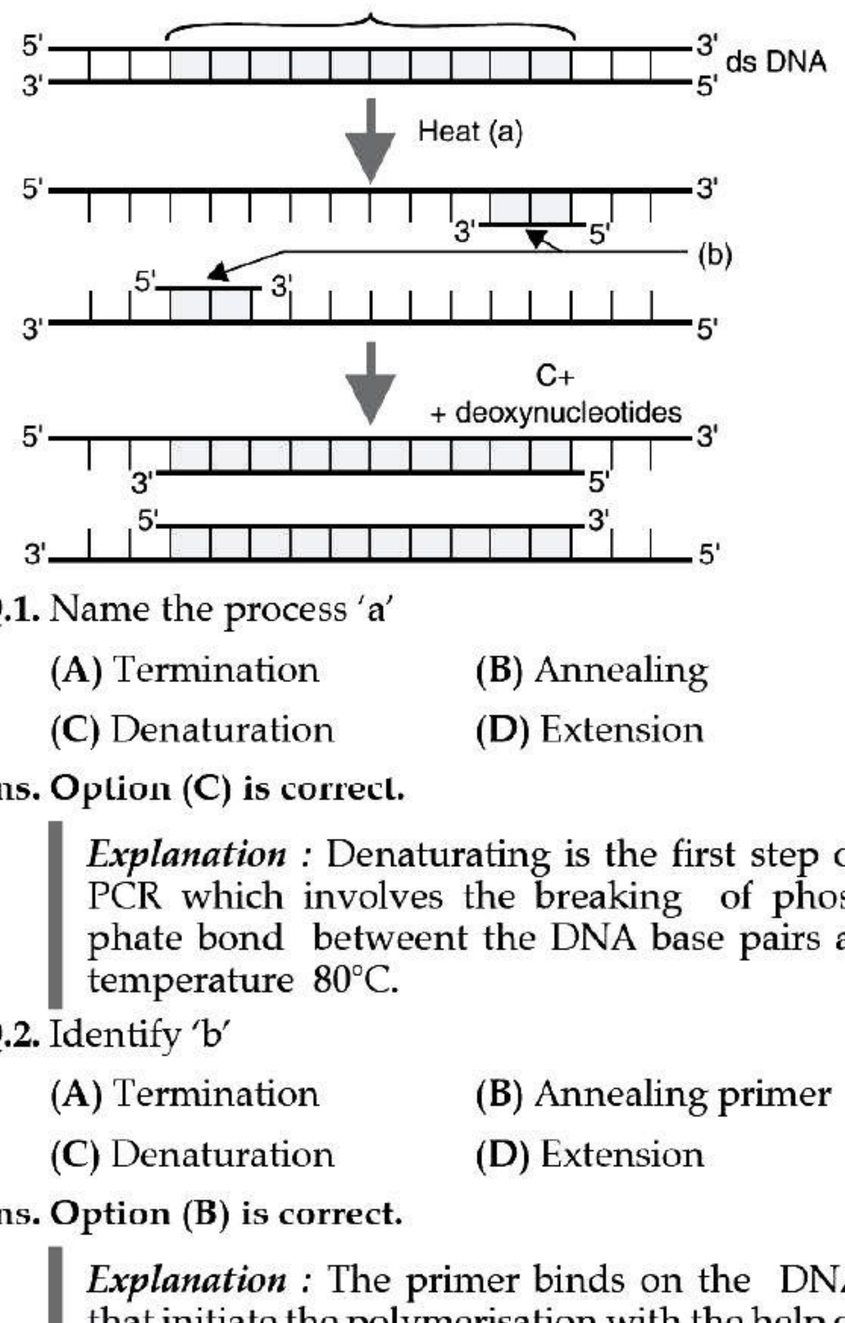
(C) Neutral

(D) Either positively or negatively charged depending on their size.

Ans. Option (C) is correct.

> ***Explanation :*** DNA have PO_4^- as negative end So, DNA is negatively charged.

III. A schematic representation of polymerase chain reaction (PCR) up to the extension stage is given below.



Q.1. Name the process 'a'

(A) Termination (B) Annealing

(C) Denaturation (D) Extension

Ans. Option (C) is correct.

> ***Explanation :*** Denaturating is the first step of PCR which involves the breaking of phosphate bond betweent the DNA base pairs at temperature 80°C.

Q.2. Identify 'b'

(A) Termination (B) Annealing primer

(C) Denaturation (D) Extension

Ans. Option (B) is correct.

> ***Explanation :*** The primer binds on the DNA that initiate the polymerisation with the help of tag polymerase enzyme at 72°C temperature.

Q.3. Which of the following has popularized the PCR (Polymerase Chain Reaction) ?

(A) Easy availability of DNA template

(B) Availability of synthetic primers

(C) Availability of cheap deoxyribonucleotides

(D) Availability of thermostable DNA polymerase.

Ans. Option (D) is correct.

> ***Explanation :*** Tag polymerase enzyme help to maintain the stability of DNA to be used repeatedly at high temperature in the PCR.

Q.4. PCR technique is best for :

(A) DNA synthesis **(B)** Protein amplification

(C) DNA amplification **(D)** DNA ligation.

Ans. Option (C) is correct.

> ***Explanation :*** PCR techniques is helpful to detect very minute traces of virus or bacterial DNA and other multiple copies by DNA amplication.

Q.5. Which among the following is not an application of PCR ?

(A) ELISA

(B) Diagnosis of pathogens

(C) DNA fingerprinting

(D) In palaeontology.

Ans. Option (A) is correct.

> ***Explanation :*** PCR techniques is used to identity traces of DNA but it can not diagnose pathogen or organism.