Short Answer Questions-I (PYQ)

[2 Marks]

Q.1. What is *Eco*RI? How does *Eco*RI differ from an exonuclease?

Ans. *Eco*RI is restriction endonuclease enzyme. Exonuclease removes nucleotides form the ends of DNA while *Eco*RI makes cut at specific position within the DNA.

Q.2. Explain with the help of an example the relationship between restriction endonuclease and a palindromic nucleotide sequence.

Ans. Restriction endonuclease recognises a specific palindromic nucleotide sequence in the DNA. Restriction endonuclease cuts the strand of DNA a little away from the centre of palindromic nucleotide sequence but between the same two bases on the opposite strands, leaving single stranded portions at the end or sticky ends.

Q.3. Explain palindromic nucleotide sequence with the help of a suitable example.

Ans. The palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same. For example, the following sequences reads the same on the two strands in 5′ → 3′ direction. This is also true if it is read in the 3′ → 5′ direction.

5′ — — GAATTC — — 3′
3′ — — CTTAAG — — 5′

Q.4.

a. **What are "molecular scissors"? Give one example.**
b. **Explain their role in recombinant DNA technology.**

OR

Why are molecular scissors so called? Write their use in biotechnology.

Ans.

a. The restriction endonucleases are called molecular scissors, as they cut the DNA segments at particular locations, *e.g.*, *Eco*RI.
b. The restriction enzymes cut the DNA strands a little away from the centre of the palindromic sites, but between the same two bases on the opposite strands. This

leaves single stranded portions with overhanging stretches called sticky ends on each strand as they form hydrogen bonds with their complementary cut counterparts. This stickiness at the ends facilitates the action of the enzyme DNA ligase.

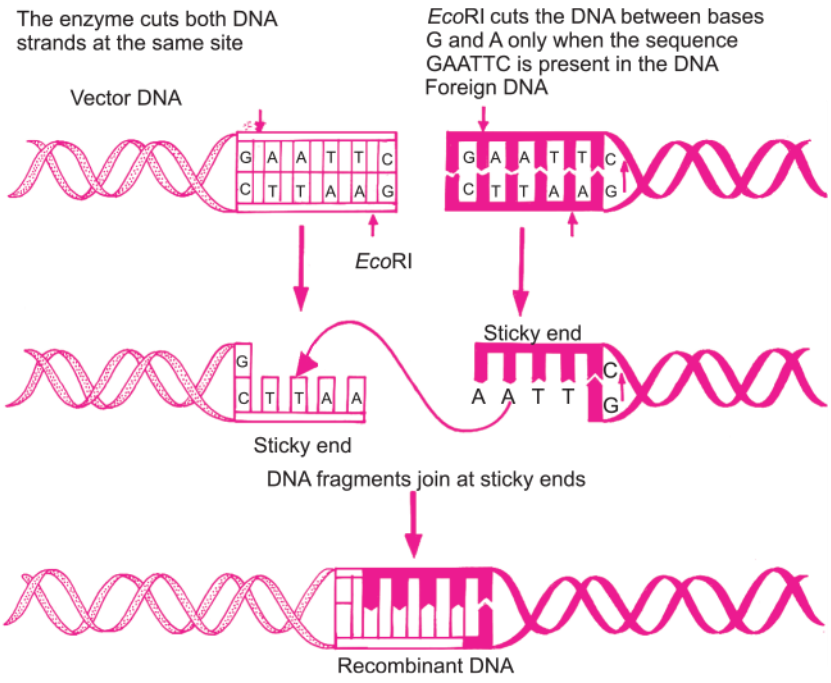
Q.5. Explain the role of the enzyme *EcoRI* in recombinant DNA technology.

OR

Explain the role of restriction endonucleases in recombinant DNA technology. Name the endonuclease that was first discovered.

Ans. *Eco*RI inspects length of DNA and recognises specific palindromic nucleotide sequences. It then binds with DNA and cuts each of the two strands of double helix at specific points.

Mechanism of Action of Endonucleases



Steps in formation of recombinant DNA by action of restriction endonuclease enzyme *EcoRI*

- Every endonuclease inspects the entire DNA sequence for the palindromic recognition sequence.
- On finding the palindrome, the endonuclease binds to the DNA.
- It cuts the opposite strands of DNA in the sugar–phosphate backbone; a little away from the centre of the palindrome sites but between the same bases on both strands.
- This results in the formation of single stranded overhanging stretches at the end of each strand called sticky ends.

- The sticky ends facilitate the action of the enzyme DNA ligase by readily forming hydrogen bonds with complementary strands.
- In genetic engineering, DNA from different sources are cut with the same restriction enzymes so that both DNA fragments have same kind of sticky ends.
- These sticky ends are complementary to each other and thus can be joined by DNA ligase (end-to-end).

The first endonuclease discovered was *Hin*dII.

Q.6. Write the convention used for naming restriction enzymes.

OR

Explain with the help of a suitable example the naming of a restriction endonuclease.

Ans. The convention for naming restriction enzymes is that the first letter to the name comes from the Genus and the second two letters come from species and third letter indicates the strain of the prokaryotic cell from which they are isolated *e.g., Eco*RI comes from *Escherichia coli* RI, here R stands for the strain and I follows the order in which the enzyme was isolated.

Q.7. Name the natural source of agarose. Mention one role of agarose in biotechnology.

Ans. The natural source of agarose is sea weed. Agarose is a natural polymer. It is used to develop the matrix for gel electrophoresis. It helps in the separation of DNA fragments based on their size.

Q.8. Write any four ways used to introduce a desired DNA segment into a bacterial cell in recombinant technology experiments.

Ans.

i. The desired DNA segment is inserted into a cloning vector and the bacterial cell can be made to take it up after making them competent by treating them with specific concentration of divalent cations such as calcium.
ii. Microinjection
iii. Biolistics
iv. Disarmed pathogen vector

Q.9. What are 'cloning sites' in a cloning vector? Explain their role. Name any two such sites in pBR322.

Ans. Cloning sites are the recognition sites on plasmid. The restriction enzymes recognise these sites for cutting and ligation of alien DNA at this place. For example, *Eco*RI, *Bam*HI.

Q.10.

a. **Mention the difference in the mode of action of exonuclease and endonuclease.**
b. **How does restriction endonuclease function?**

Ans.

a. Exonuclease removes nucleotides from the ends of DNA whereas endonuclease cuts at specific positions within DNA at specific positions.
b. Restriction endonuclease recognises and cuts specific palindromic nucleotide sequences in the DNA.

Q.11. How does a restriction nuclease function? Explain.

Ans. Restriction nuclease cuts DNA at specific sites. Nucleases are of two types exonuclease and endonuclease.
Exonuclease cuts DNA at the ends, whereas endonuclease cuts at specific sites within DNA.

Q.12. How are 'sticky ends' formed on a DNA strand? Why are they so called?

Ans. Restriction enzymes cut the strands of the DNA, a little away from the centre of the palindromic sites, but between the same two bases on opposite strands. This leaves called sticky single stranded position at the ends. These overhanging stretches are aids. These are named so because they form hydrogen bonds with their complementary cut counterparts.

Q.13. How can DNA segments, separated by gel electrophoresis, be visualised and isolated?

Ans. The separated DNA molecules are visualised only after staining DNA with ethidium bromide followed by exposure to UV radiation. They appear as bright orange coloured bands. The separated bands of DNA (on the gel) are cut from the agarose gel and extracted from the gel piece. This process is called elution.

Q.14. Why does the 'insertional inactivation' method to detect recombinant DNA is preferred to 'antibiotic resistance' procedure?

Ans. In insertional inactivation method, the presence of a chromogenic substrate gives blue coloured colonies in absence of an insert. Presence of an insert in the enzyme site do not produce colour. This is because insertional inactivation of the β-galactosidase has taken place due to the insert. Antibiotic resistance method requires duplicate plating. It is a cumbersome procedure to perform.

Q.15. Why and how bacteria can be made 'competent'?

Ans. Bacteria are made competent to accept the DNA or plasmid molecules. This is done by treating them with specific concentration of a divalent cation such as calcium to increase pore size in cell wall. The cells are then incubated with recombinant DNA on ice, followed by placing them briefly at 42°C and then putting it back on ice.

Q.16. Name the source of the DNA polymerase used in PCR technique. Mention why it is used.

Ans. The source is the bacterium *Thermus aquaticus*. It is used because it is thermostable and do not denature at high temperatures.

Q.17. Name the source organism that possesses *Taq* polymerase. What is so special about the function of this enzyme?

OR

Name the organism from where the thermostable DNA polymerase is isolated. State its role in genetic engineering.

Ans. Source organism: *Thermus aquaticus*
The enzyme can tolerate high temperature and is thus thermostable. It does not get denatured during PCR at high temperature.

Q.18. How are recombinant vectors created? Why is only one type of restriction endonuclease required for creating one recombinant vector?

Ans. The construction of recombinant DNA is done by linking a gene encoding antibiotic resistance with a native plasmid. These plasmid DNA act as vectors to transfer the piece of DNA attached to it.
Only one type of restriction endonuclease is required for creating recombinant vector because when cut by the same enzyme, the resultant DNA fragments have the same sticky ends, which can be joined together using DNA ligases.

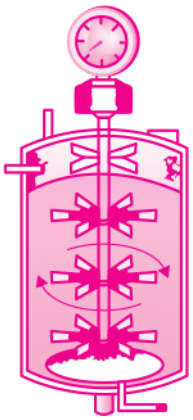
Q.19. How is DNA isolated in purified form from a bacterial cell?

Ans. DNA, a genetic material is isolated in purified form by treating the bacterial cells with the enzymes such as lysozyme to remove the cell wall. The RNA thus released can be removed by treating them with ribonuclease and enzyme proteases is added to remove proteins. Finally, chilled ethanol is added to precipitate the purified DNA.

Q.20. What are recombinant proteins? How do bioreactors help in their production?

Ans. The protein produced by genetically altered gene in a host is called recombinant protein. Bioreactors are vessels in which raw materials are biologically converted into specific products by microbes. It provides optimum growth conditions such as temperature, pH, substrate, vitamins, oxygen and salts.

Q.21. Name the type of bioreactor shown. Write the purpose for which it is used.



Ans. The given bioreactor is the simple stirred tank bioreactor.
Its purpose is large scale production of recombinant protein or enzymes, using microbial plants/ animals/human cells.

Q.22.

a. **Explain how to find whether an E. coli bacterium has transformed or not when a recombinant DNA bearing ampicillin resistant gene is transferred into it.**
b. **What does the ampicillin resistant gene act as in the above case?**

Ans.

a. *E. coli* bearing transferred recombinant DNA are first grown on ampicillin containing medium and then transferred on to a medium containing tetracycline. The transformants will grow only in ampicillin containing medium and not in tetracycline containing medium. The non-transformants, on the other hand, will grow in both the mediums.
b. Ampicillin resistant gene acts as a selectable marker and helps in selecting the transformants.

Q.23. Write the role of '*ori*' and 'restriction' site in a cloning vector pBR322.

Ans. *ori* is the site where replication starts. This site is responsible for controlling the copy number a vector.

Restriction site is the site of ligation of alien/foreign DNA in the vector, in one of the two **antibiotic resistance site or coding sequence of α-galactosidase.**

Q.24.

a. **A recombinant vector with a gene of interest inserted within the gene of α-galactosidase enzyme, is introduced into a bacterium. Explain the method**

that would help in selection of recombinant colonies from non-recombinant ones.

b. Why is this method of selection referred to as "insertional inactivation"?

Ans.

a. Bacteria is grown in a medium with chromogenic substrate, blue coloured colonies show no recombinations and colonies with no blue colour show presence of recombinants.
b. Gene for the enzyme is inactivated by insertion of foreign DNA.

Q.25. How is insertional inactivation of an enzyme used as a selectable marker to differentiate recombinants from non-recombinants?

Ans. When a recombinant DNA is inserted within the coding sequence of an enzyme β-galactosidase, it results into inactivation of the enzyme. The bacterial colonies having inserted plasmid, show no colouration while those without inserted plasmid show blue colour.

Q.26. Rearrange the following in the correct sequence to accomplish an important biotechnological reaction:

a. *In vitro* synthesis of copies of DNA of interest
b. Chemically synthesised oligonucleotides
c. Enzyme DNA-polymerase
d. Complementary region of DNA
e. Genomic DNA template
f. Nucleotides provided
g. Primers
h. Thermostable DNA-polymerase (from Thermus aquaticus)
i. Denaturation of *ds*DNA

Ans. Correct sequence is

i → e → b/g → g/b → c/b → h/c → f → d → a

Q.27. Name the source organism from which *Ti* plasmid is isolated. Explain the use of this plasmid in biotechnology.

Ans. *Ti* plasmid is isolated from *Agrobacterium tumifaciens*. *Ti* plasmid of *Agrobacterium tumifaciens* has been modified into a cloning vector, which is not pathogenic to plants but still is able to use the mechanisms to deliver genes of interest into plants.

Q.28. Explain the work carried out by Cohen and Boyer that contributed immensely in biotechnology.

Ans. Stanley Cohen and Herbert Boyer in 1972 constructed the first recombinant DNA. They isolated the antibiotic resistance gene by cutting out a piece of DNA from the plasmid of a bacterium which was responsible for conferring antibiotic resistance. The cut piece of DNA was then linked with the plasmid DNA of *Salmonella typhimurium* and transferred to E. coli for transformation.

Q.29. State how has Agrobacterium tumefaciens been made a useful cloning vector to transfer DNA to plant cells.

Ans. *Agrobacterium tumifaciens* is known to be a natural vector and consists of a pathogenic plasmid. It is capable of passing its DNA to plants and induce tumour by integrating its DNA with host genome. The tumour causing gene in the plasmid of this bacteria is replaced by gene of interest and is now used as a cloning vector to transfer the DNA into plant cells.

Q.30. How can the following be made possible for biotechnology experiments?

a. **Isolation of DNA from bacterial cell.**
b. **Reintroduction of the recombinant DNA into a bacterial cell.**

Ans.

a. By treating cell with lysozyme
b. Microinjection/gene gun

Short Answer Questions-I (OIQ)

[2 Mark]

Q.1. Define genetic engineering. Name one natural genetic engineer of plants.

Ans. Genetic engineering is the manipulation of genetic materials which can be introduced into host organisms and thus change the phenotype of the host organism.

The natural genetic engineer of plant is *Agrobacterium tumifaciens*.

Q.2. Explain any two methods of vector less gene transfer.

Ans. The two methods of vectorless gene transfer are:

i. **Micro-injection:** The technique of introducing foreign gene in a target cell by injecting the DNA, directly into the nucleus, by micro-needle is called micro-injection.
ii. **Electroporation:** It is the process in which transient holes are produced in the plasma membrane of the target cell, to incorporate foreign DNA.

Q.3. What is meant by gene cloning?

Ans. Gene cloning refers to a process in which a gene of interest is ligated to a vector. The recombinant DNA thus produced is introduced in a host cell by transformation. Each cell gets one DNA molecule and when the transformed cell grows to a bacterial colony, each cell in the colony has a copy of the gene.

Q.4. What is the role of lysing enzyme in biotechnology?

Ans. Lysing enzymes are used to open up the cell to obtain DNA along with other macromolecules for genetic experiments. Bacterial cells are treated with lysozyme, plant cells are treated with cellulase, and fungal cells are treated with chitinase for lysing.

Q.5. What do you mean by a clone?

Ans. The cell or organism derived from the same parents by asexual mean which are genetically identical to each other and to the parent are called clones.

Q.6. What is elution?

Ans. The separated band of DNA (after gel electrophoresis) are cut out from agarose gel and DNA is extracted from the gel piece. This step is called elution.

Q.7. Differentiate between *r*DNA and *c*DNA.

Ans.

***r*DNA**	***c*DNA**
It is the DNA which is formed by joining together the DNA from two different organisms.	It is the DNA which is obtained from RNA template catalysed by reverse transcriptase enzyme.

Q.8. Why are cloning vectors necessary in cloning? Name any two such vectors that are used in experiment with *E. coli*.

Ans. DNA being hydrophilic in nature cannot pass through the cell membranes into the host. Therefore, cloning vectors are required to transfer the DNA into the host by attaching the desired DNA to it.
The two cloning vectors that are used are plasmids and bacteriophages.

Q.9. Why is *Agrobacterium tumifaciens* a good cloning vector? Explain.

Ans. *Agrobacterium tumifaciens* is a soil bacterium which causes disease in many dicot plants. It is able to deliver a piece of DNA known as T-DNA, to transform the normal cells into tumour cells and direct these tumour cells to produce the chemicals required by the pathogen. The tumour inducing (Ti) plasmid of *Agrobacterium tumifaciens* has

now been modified into a cloning vector which is no more pathogenic to the plants but still deliver genes of interest into a variety of plants.

Q.10. Why is *Agrobacterium*-mediated genetic transformation described as natural genetic engineering in plants?

Ans. *Agrobacterium tumifaciens* is a pathogen of several dicot plants which exhibit natural genetic engineering in plant.

Reasons:

i. It is able to deliver a piece of DNA called 'T-DNA' to transform normal plant cell into a tumour cell.
ii. The DNA transforms the normal cells into tumour cells which direct them to produce the chemical essential for the pathogen.

As this process occurs in nature it is called natural genetic engineering.

Q.11. When a foreign DNA is introduced into an organism, how is it maintained in the host and how is it transferred to the progeny of the organism?

Ans. Foreign gene is usually ligated to a plasmid vector and introduced in the host. As plasmid replicates and makes multiple copies of itself, foreign gene also gets replicated and its copies are also made. When the host organism divides, its progeny also receives the plasmid DNA containing the foreign gene.

Q.12. What modification is done in the *Ti* plasmid of *Agrobacterium tumefaciens* to convert it into a cloning vector?

Ans. T-DNA is the only essential part required to make *Ti* plasmid a cloning vector. The plasmid is disarmed by deleting the tumour inducing genes in the plasmid so that it becomes an effective cloning vector and remove it harmful effect.

Q.13. What does 'competent' refer to in competent cells used in transformation?

Ans. Competent means bacterial cells which by various methods like treatment with $CaCl_2$ are made capable of taking up foreign DNA.

Q.14. Describe the role of $CaCl_2$ in preparation of competent cells.

Ans. $CaCl_2$ is known to increase the efficiency of DNA uptake to produce transformed bacterial cells. The divalent Ca^{2+} ions supposedly create transient pores in the bacterial cell wall, by which the entry of foreign DNA is facilitated into the bacterial cells.

Q.15. What is the significance of adding proteases at the time of isolation of genetic material (DNA)?

Ans. Role of proteases is to degrade the proteins present inside a cell (from which DNA is being isolated). If the proteins are not removed from DNA preparation then they could interfere with any downstream treatment of DNA.

Q.16. While doing a PCR, 'denaturation' step is missed. What will be its effect on the process?

Ans. If denaturation of double-stranded DNA does not take place, then primers will not be able to anneal to the template, no extension will take place, hence no amplification will occur.

Q.17. Illustrate briefly downstream processing.

Ans. Downstream processing

- All the processes to which a product is subjected to before being marketed as a finished product are called downstream processing.
- It includes:
    a. Separation of the product from the reactor.
    b. Purification of the product.
    c. Formulation of the product with suitable preservatives.
    d. Quality control testing and clinical trials in case of drugs.

Q.18. What would happen when you grow a recombinant in a bioreactor but forget to add antibiotic to the medium in which the recombinant is growing?

Ans. In the absence of antibiotic, there will be no pressure on recombinants to retain the plasmid (containing the gene of your interest). Since, maintaining a high copy number of plasmids is a metabolic burden to the microbial cells, it will thus tend to lose the plasmid.

Q.19. A wine maker and a molecular biologist who has developed a recombinant vaccine, both claim themselves to be biotechnologist. Who in your opinion is right?

Ans. Both are right because biotechnology is a very wide area which deals with techniques of using a 'natural' organism (or its parts) as well as genetically modified organism to produce products and processes useful for mankind. A wine maker employs a strain of yeast to produce wine by fermentation (a natural phenomenon), while the molecular biologist has cloned gene for the antigen (that is used as vaccine) in an organism which allows the production of the antigen in large amount.

Q.20. For producing a recombinant protein (for therapeutic purpose) in large scale, which vector would you choose—a low copy number or high-copy number?

Ans. High-copy number, because higher the copy number of vector plasmid, higher the copy number of gene and consequently, protein coded by the gene is produced in high amount.

Q.21. Would you like to choose an exonuclease enzyme while producing a recombinant DNA molecule?

Ans. No, as exonuclease acts on the free ends of linear DNA molecule. Therefore, instead of producing DNA fragments with sticky ends, it will shorten or completely degrade the DNA fragment containing the gene of interest, and the circular plasmid (vector) will not get cut as it lacks free ends.

Q.22. You have created a recombinant DNA molecule by ligating a gene to a plasmid vector. By mistake, your friend adds exonuclease enzyme to the tube containing the recombinant DNA. How will your experiment get affected as you plan to go for transformation now?

Ans. The experiment will not likely be affected as recombinant DNA molecule is circular and closed, with no free ends. Hence, it will not be a substrate for exonuclease enzyme which removes nucleotides from the free ends of DNA.

Q.23. DNA being hydrophilic cannot pass through the cell membrane of a host cell. Explain how does recombinant DNA get introduced into the host cell to transform the latter.

Ans. The cell is made competent by the following methods:

a. **Chemical method**

- The cell is treated with specific concentration of a divalent cation such as calcium to increase 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 treatment.
- The bacteria now take up the recombinant DNA.

b. **Physical methods**

The physical methods include

- **Micro-injection method:** Recombinant DNA is directly injected into the nucleus of an animal cell.
- **Biolistics or gene gun method:** Cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in plants.

Q.24. PCR is a useful tool for early diagnosis of an infectious disease. Comment.

Ans. PCR is a very sensitive technique which enables the specific amplification of desired DNA from a limited amount of DNA template. Hence, it can detect the presence of an infectious organism in the infected patient at an early stage of infection (even before the infectious organism has multiplied to large number).

Q.25. Restriction enzymes present in the cloning site of a vector should not have more than one recognition site. Comment.

Ans. If the restriction enzymes have more than one recognition site in a vector, then the vector itself will get fragmented on treatment with the restriction enzyme.

Q.26. You have chosen a plasmid as vector for cloning your gene. However this vector plasmid lacks a selectable marker. How would it affect your experiment?

Ans. In a gene cloning experiment, first a recombinant DNA molecule is constructed, where the gene of interest is ligated to the vector and introduced inside the host cell (transformation). Since, not all the cells get transformed with the recombinant/plasmid DNA, in the absence of selectable marker, it will be difficult to distinguish between transformants and non-transformants, because role of selectable marker is in the selection of transformants.

Q.27. Write the use of the following in biotechnology.

Q. Chilled ethanol

Ans. It is added to precipitate the purified DNA to isolate it.

Q. Microinjection

Ans. It is used to inject the foreign gene into a host cell, directly.

Q. Bioreactor

Ans. It is the set up to culture large volumes of transgenic bacteria to get large quantities of the product protein.

Q. Plasmid

Ans. It is the vector to transform a foreign gene.

Q.28. Is there any difference between recombinant DNA and recombinant protein? Support your answer.

Ans. *r*DNA is the plasmid vector containing the foreign DNA whereas recombinant protein is the product of transgenic gene in the host body or cell.

Q.29. Where and why do we use *Taq* polymerase enzyme when it works exactly as DNA polymerase?

Ans. In PCR, because it is a thermostable DNA polymerase enzyme, gets isolated from bacteria Thermus aquaticus from hot water springs, and it does not get denatured at high temperature which is required during PCR and works as normal DNA polymerase enzyme (whereas the normal DNA polymerase gets denatured at high temperature).

Q.30. A vector is engineered with three features which facilitate its cloning within the host cell. List the three features and explain each one of them.

Ans.

i. **Origin of replication/*ori* site—**From here the replication starts (and any piece of DNA when linked, can be made to replicate within the host cell).
ii. **At least two Selectable markers—**Helpful in identifying and eliminating non-transformants.
iii. **Unique Restriction sites for more than one restriction enzymes—**The foreign DNA links to this region of the plasmid.