EXERCISE QUESTIONS

CHAPTER-11 BIOTECHNOLOGY : PRINCIPLES AND PROCESSES

1. **Can you list 10 recombinant proteins which are used in medical practice? Find out where they are used as therapeutics (use the internet).**

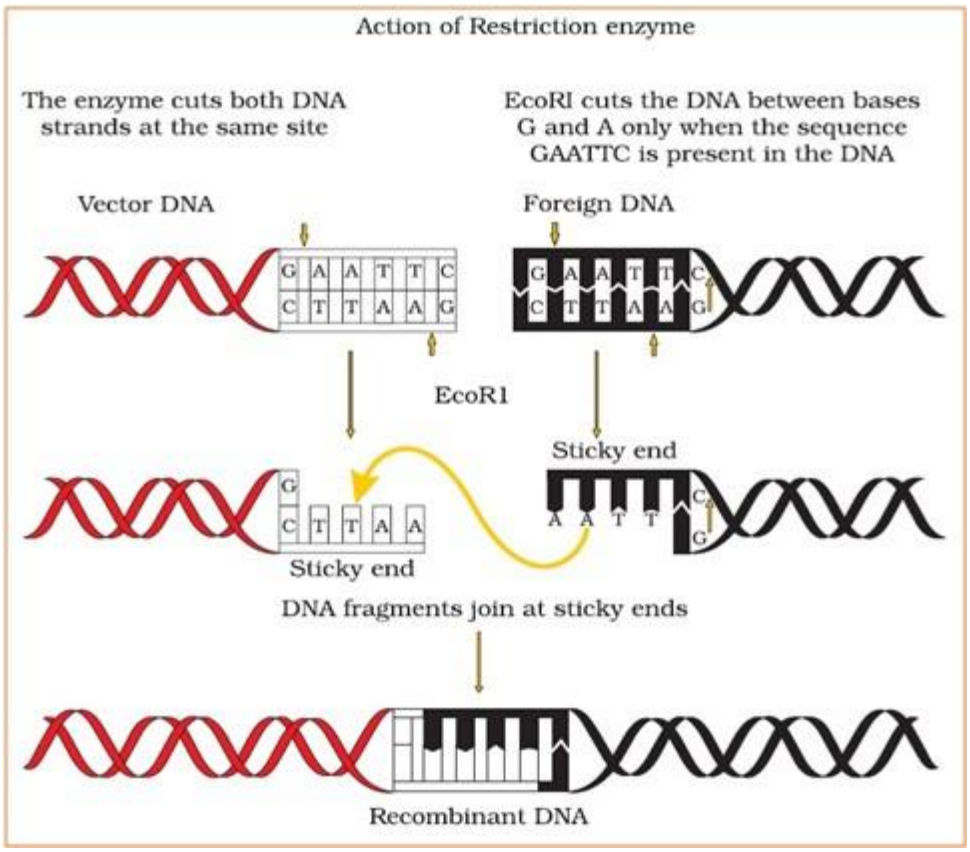
Ans - (i) Human growth hormone – Dwarfism cure

(ii) Human insulin – Diabetes
(iii) Blood clotting factor Y1H/IX-Haemophilia
(iv) TPA (tissue plasminogen activator) – Heart attack/strokes
(v) PDGF (platelet derived growth factor) – Stimulates wound healing.
(vi) Interferon – Treatment of viral infection.
(vii) Interlinking – Enhances immune reaction,
(viii) Hepatitis B vaccine – Prevention of infectious disease.
(ix) DNase I – Treatment of cystic fibrosis.

(x) Herpes Vaccine – Prevention of infectious disease.

2. Make a chart (with diagrammatic representation) showing a restriction enzyme, the substrate DNA on which it acts, the site at which it cuts DNA and the product it produces.

Ans - The following chart shows the action of the restriction enzyme EcoRI, the substrate DNA on which it acts and the site where it cuts



3. From what you have learnt, can you tell whether enzymes are bigger or DNA is bigger in molecular size? How did you know?

Ans - Enzymes are smaller in size than DNA molecules. We know this because DNA contains genetic information for the development and functioning of all living organisms. It contains instructions for the synthesis of proteins and DNA molecules. On the other hand, enzymes are proteins which are synthesised from a small stretch of DNA known as ‘genes’, which are involved in the production of the polypeptide chain.

4. What would be the molar concentration of human DNA in a human cell? Consult your teacher.

Ans - The molar concentration of human DNA in a human diploid cell is as follows:

⇒ Total number of chromosomes × 6.023 × 10^23

⇒ 46 × 6.023 × 10^23

⇒ 2.77 × 10^23 moles

Hence, the molar concentration of DNA in each diploid cell in humans is 2.77 × 10^23 moles.

5. Do eukaryotic cells have restriction endonucleases? Justify your answer.

Ans - No, eukaryotic cells do not have restriction endonucleases. This is because the DNA of eukaryotes is highly methylated by a modification enzyme, called methylase. Methylation protects the DNA from the activity of restriction enzymes .These enzymes are present in prokaryotic cells where they help prevent the invasion of DNA by virus.

6. Besides better aeration and mixing properties, what other advantages do stirred tank bioreactors have over shake flasks?

Ans - The advantages of stirred tank bioreactors over shake flasks are as follows:

1. Stirred tank bioreactors are utilised for large-scale production of biotechnological products, unlike the shake flask method which is used for small-scale production of products.

2. In stirred tank bioreactors, a small sample can be taken out for testing.

3. Stirred tank bioreactors have foam breakers to control the foam.

4. Stirred tank bioreactors have temperature and pH control systems.

7. Collect 5 examples of palindromic DNA sequences by consulting your teacher. Better try to create a palindromic sequence by following base-pair rules.

Ans - Palindrome nucleotide sequences in the DNA molecule are groups of bases that form the same sequence when read both forward and backward. Five examples of palindromic DNA sequences are as follows:
(i) 5′-————————GGATCC————————3’
3′————————CCTAGG————————5′
(ii) 5’————————AAGCTT————————3′
3′————————TTCGAA ————————-5′
(iii) 5′————————ACGCGT————————3′
3′————————TGCGGA———————— 5′
(iv) 5′———— ACTAGT————3′
3′————————TGATCA————5′
(v) 5′————————AGGCCT————————3′
3′————————TCCGGA————————5′

8. Can you recall meiosis and indicate at what stage a recombinant DNA is made?

Ans - Meiosis is a process that involves the reduction in the amount of genetic material. It is two types, namely meiosis I and meiosis II. During the pachytene stage of prophase I, crossing over of chromosomes takes place where the exchange of segments between non-sister chromatids of homologous chromosomes takes place. This results in the formation of recombinant DNA.

9. Can you think and answer how a reporter enzyme can be used to monitor transformation of host cells by foreign DNA in addition to a selectable marker?

Ans - A reporter gene can be used to differentiate transformed cells by tracking down the activity of its co-responding genes (receptor gene). It can be used to monitor the transformation of host cells by foreign DNA. They act as a selectable marker to determine whether the host cell has taken up the foreign DNA or the foreign gene gets expressed in the cell. The researchers place the reporter gene and the foreign gene in the same DNA construct. Then, this combined DNA construct is inserted in the cell. Here, the reporter gene is used as a selectable marker to find out the successful uptake of genes of interest (foreign genes). An example of reporter genes includes lac Z gene, which encodes a green fluorescent protein in a jelly fish. The others, which appear blue in colour, indicate that cells do not carry foreign DNA.

10. Describe briefly the following:

(a) Origin of replication

(b) Bioreactors

(c) Downstream processing

Ans - (a) Origin of Replication: This is 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 sequence is also responsible for controlling the copy number of the linked DNA. So, if one wants to recover many copies of the target DNA it should be cloned in a vector whose origin support high copy number.

b) Bioreactor: A bioreactor has an agitator system, an oxygen delivery system, a foam control system, a temperature control system, pH control system and sampling ports. Bioreactors can be thought of as vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cell and/or their enzymes. The bioreactor provides optimum growth conditions and facilitates achieving the desired products. The most commonly used bioreactor is of stirring type. A stirred tank bioreactor is usually a cylindrical vessel or vessel with a curved base to facilitate mixing of the contents. In the sparged stirred tank bioreactor, sterile air bubbles are sparged.

(c) Downstream Processing : Such formulations have to undergo clinical trials, in case of drugs. The product obtained is subjected to a series, of processes collectively called downstream processing before it is made into a finished product ready for marketing. The two main processes are separation and purification.

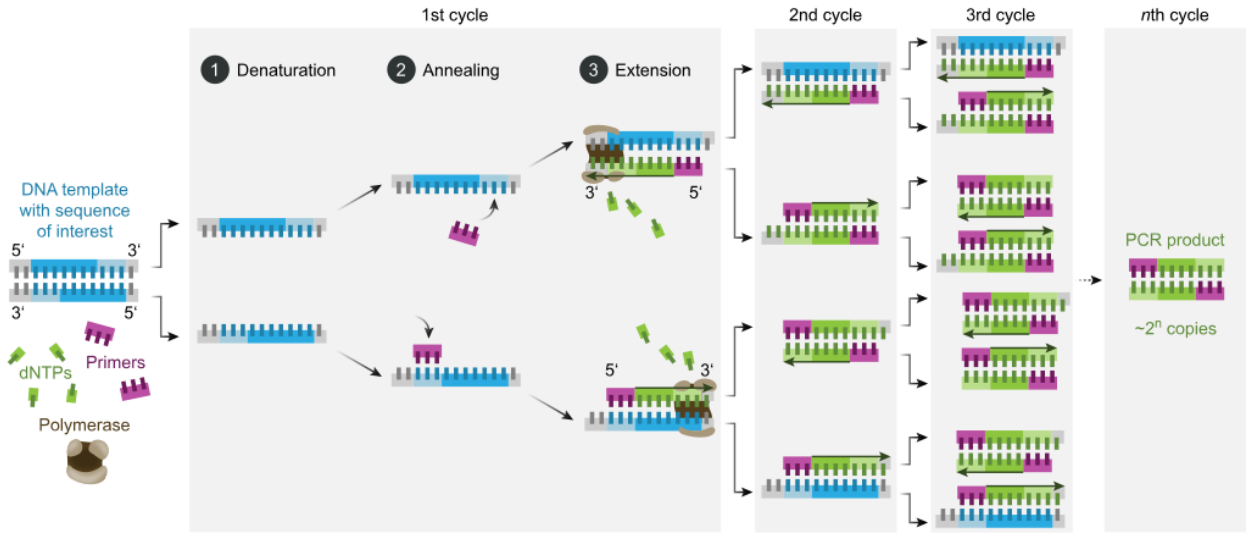
11. Explain briefly

(a) PCR

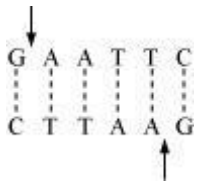
(b) Restriction enzymes and DNA

(c) Chitinase

Ans - (a) PCR: - Polymerase chain reaction (PCR) is a technique in molecular biology to amplify a gene or a piece of DNA to obtain its several copies. It is extensively used in the process of gene manipulation. The process involves *in-vitro* synthesis of sequences using a primer, a template strand, and a thermostable DNA polymerase enzyme obtained from a bacterium, called *Thermus aquaticus*. The enzyme utilizes building blocks dNTPs (deoxynucleotides) to extend the primer. In the first step, the double stranded DNA molecules are heated to a high temperature so that the two strands separate into a single stranded DNA molecule. This process is called denaturation. Then, this ssDNA molecule is used as a template strand for the synthesis of a new strand by the DNA polymerase enzyme and this process is called annealing, which results in the duplication of the original DNA molecule.



(b) Restriction enzymes are molecular scissors used in molecular biology for cutting DNA sequences from a specific site. It plays an important role in gene manipulation. The enzymes recognize a specific six-box pair sequence known as the recognition sequence and cut the sequence at a specific site. For example, the recognition site for enzyme ECORI is as follows:



Restriction enzyme are categorized into two types −

(i) Exonuclease − It is a type of restriction enzyme that removes the nucleotide from either 5' or 3' ends of the DNA molecule.

(ii) Endonuclease − It is a type of restriction enzyme that makes a cut within the DNA at a specific site. This enzyme acts as an important tool in genetic engineering. It is commonly used to make a cut in the sequence to obtain DNA fragments with sticky ends, which are later joined by enzyme DNA ligase.

(c) Chitinase − Chitinase is a class of enzymes used for the degradation of chitin, which forms a major component of the fungal cell wall. Therefore, to isolate the DNA enclosed within the cell membrane of the fungus, enzyme chitinase is used to break the cell for releasing its genetic material.

12. Discuss with your teacher and find out how to distinguish between

(a) Plasmid DNA and Chromosomal DNA

(b) RNA and DNA

(c) Exonuclease and Endonuclease

Ans -The differences between plasmid DNA and chromosomal DNA are as follows:

Plasmid DNA	Chromosomal DNA

Circular, extra-chromosomal DNA which is capable of self-replication and is found in bacteria is called plasmid DNA.	The entire DNA (excluding extrachromosomal DNA) present in the cell constitutes chromosomal DNA
It is found only in bacteria	IT is found in both bacteria and other eukaryotic cells.

(b) RNA and DNA

The differences between RNA and DNA are as follows:

RNA	DNA
RNA contains ribose sugar	DNA contains deoxyribose sugar
In RNA, adenine and uracil are found as pyrimidines	In DNA, adenine and uracil are found as pyrimidines
It has catalytic properties and is less stable than DNA	DNA is non-catalytic and is stable than RNA

(c) Exonuclease and Endonuclease

The differences between exonuclease and endonuclease are as follows:

Exonuclease	Endonuclease
These are nuclease (enzymes) that cut DNA from its ends.	These are nucleases that cut DNA from internal sites on DNA