Chapter: Biotechnology: Principles and Processes

Exercise

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

Answer: Recombinant proteins are made using recombinant DNA technology and are employed in medical treatment. Using vectors and restriction enzymes as molecular tools, specific genes are transferred from one creature to another in this approach.

Listed below are 10 recombinant proteins:

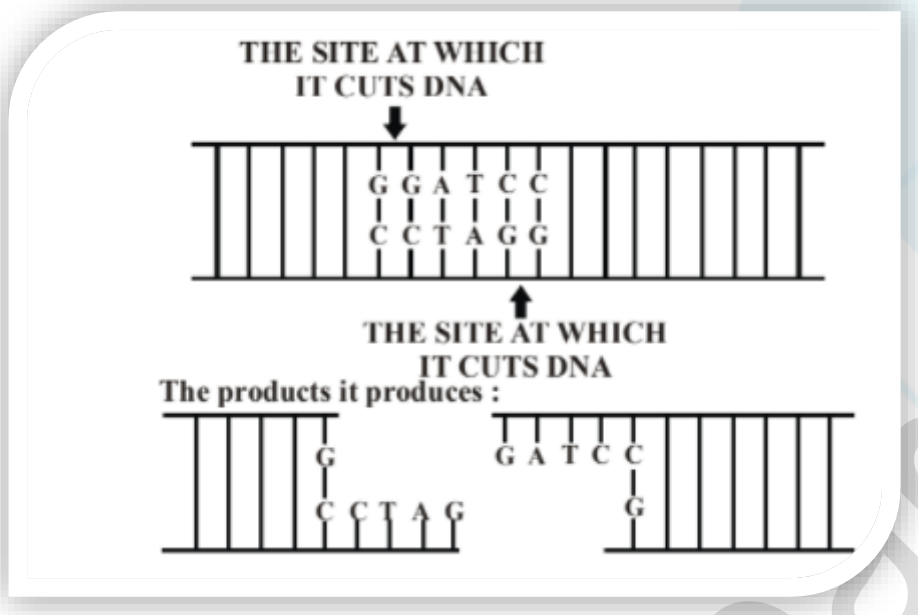
Tissue plasminogen activator	**In the treatment of acute myocardial infection**
DNAse I	In the treatment of cystic fibrosis
Coagulation factor VIII	In the treatment of hemophilia A
Antithrombin III	Blood-clot prevention
Human recombinant growth hormone	To promote growth in an individual
Coagulation factor IX	In the treatment of hemophilia B
Insulin	In the treatment of type I diabetes mellitus
Interferon-β	Use to treat herpes and viral enteritis
Recombinant protein	Therapeutic application
Interferon-α	In the treatment of chronic hepatitis C

Question 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.

Answer:

EcoRI is a restriction endonuclease enzyme that helps in the synthesis of recombinant DNA.

It can be expressed graphically as follows:



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

Answer:

Enzymes are smaller in size than DNA molecules. This is because DNA is made up of genetic material that is required for the regular growth and functioning of living organisms. The instructions for the production of DNA molecules and proteins are included in a DNA molecule. Enzymes, on the other hand, are proteins that are made from genes, which are little pieces of DNA. These are necessary for polypeptide chain synthesis.

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

Answer:

The molar concentration of human DNA in a human cell can be calculated using the following formula:

Total number of chromosomes: 6.023 x 1023

46 x 6.023 x 1023

There are 2.77 x 1023 moles in the world.

As an outcome, the molar concentration of DNA in each diploid cell in humans is 2.77 x 1023 moles.

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

Answer:

No, eukaryotic organisms lack restriction endonucleases because their DNA is heavily methylated by the modifying enzyme methylase. This methylation protects the DNA from restriction enzyme action. These enzymes are found in prokaryotic cells, where they help to prevent virus invasion of DNA.

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

Answer:

Stirred tank bioreactors are designed for large-scale biotechnology product production, whereas the shake flask method is used for small-scale biotechnological product manufacturing in the lab.

Compared to shake flasks, the stirred tank bioreactor has minimal advantages. They are as follows:

(i) Small volumes of culture can be extracted from the reactor for testing and sampling purposes.

(ii) The presence of a control system for pH and temperature regulation

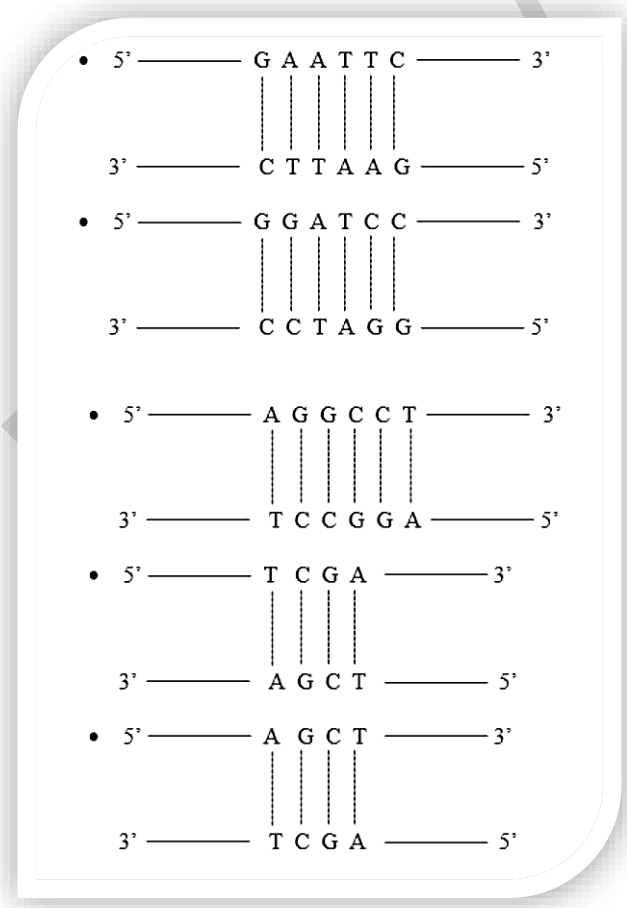
(iii) The stirred tank bioreactors contain a foam breaker to control the foam.

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

Answer:

A palindromic sequence is one that reads the same whether read from the 5' to 3' or 3' to 5' direction. Restriction enzymes can only act at these locations. Almost all restriction enzyme sequences are palindromic.

The following are five examples of palindromic sequences:



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

Answer:

Meiosis, a kind of cell division, is a process in which the amount of genetic material is reduced. During the pachytene event of prophase I, chromosomes cross across, resulting in segment exchange between non-sister chromatids of homologous chromosomes. This results in the synthesis of recombinant DNA during the meiosis phase.

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

Answer:

A reporter gene can be used to track the transformation of host cells by foreign DNA. They are employed as a selective marker to determine if the foreign DNA has been used up by the host cell or if the foreign gene is being expressed in the cell. Scientists combine the reporter gene with the foreign gene in the same DNA construct. The reporter gene is utilized as a selective marker to determine the effective uptake of foreign genes or genes of interest once this collective DNA construct is introduced into the cell. Lac Z gene, which encodes for a green fluorescent protein in jellyfish, is an example of a reporter gene.

Question 10. Describe briefly the following:

(a) Origin of replication

(b) Bioreactors

(c) Downstream processing

Answer:

(a) Origin of replication

A replication start site is a DNA sequence in a genome where replication begins. The replication initiation procedure can be either unidirectional or bidirectional. When linked to this sequence, any fragment of DNA can be made to replicate within the host cells. The sequence is also in charge of regulating the number of copies of linked DNA. To recover a large number of copies of target DNA, it should be cloned in a vector with a high copy number origin.

(b) Bioreactor

They are enormous vessels that are used to produce biotechnological goods on a massive scale from raw materials. These bioreactors provide perfect circumstances for obtaining the desired product by supplying optimum pH, temperature, vitamins, oxygen, and other factors. They have a system for delivering oxygen, controlling foam, and controlling temperature and pH. It also has a sampling port where a little amount of culture can be withdrawn for sampling.

(c) Downstream processing

Once the biosynthetic stage is complete, it is a way of isolating and purifying foreign gene products. The product is then subjected to several treatments in order to separate and purify it. After the process is finished, the product is created and put through a series of clinical studies for quality control and other evaluations.

Question 11. Explain briefly

(a) PCR

(b) Restriction enzymes and DNA

(c) Chitinase

Answer:

(a) PCR

PCR, or polymerase chain reaction, is a technique for amplifying a gene or a fragment of DNA in order to obtain many copies in molecular biology. It is commonly utilized in the process of gene manipulation. The phenomenon entails the in vitro synthesis of sequences using a template strand, a primer, and a thermostable DNA polymerase enzyme produced by the Thermus aquaticus bacteria. To lengthen the primer, the enzyme uses the building blocks deoxynucleotides (dNTPs).

The three phases of PCR are as follows:

(i) The two strands of double-stranded DNA molecules are separated into a single-stranded DNA molecule by heating them to a high temperature. Denaturation is the term for this procedure.

(ii) The DNA polymerase enzyme then uses this DNA molecule as a template strand to build a new strand. The process is called annealing, and it results in the replication of the original DNA molecule. It is repeated several times to create several copies of the rDNA segment.

(ii) Taq DNA polymerase from Thermits aquatics is used to lengthen the primer.

(b) Restriction enzymes and DNA

Restriction enzymes are molecular scissors used in molecular biology to cut DNA sequences from a specific spot. It plays a crucial part in the process of gene manipulation. These enzymes recognize a certain six-base pair sequence known as the recognition sequence and snip it at precise locations. The recognition site for the ECORI enzyme, for example, is as follows:

Restriction enzymes can be divided into two categories:

(i) Endonuclease - It is a restriction enzyme that cuts certain places in DNA. It is an important tool in the field of genetic engineering. It is typically used to make a snip in the sequence in order to obtain DNA fragments with sticky ends. The enzyme DNA ligase then joins the two ends together.

(ii) Exonuclease -It is a restriction enzyme that removes nucleotides from the 3' or 5' ends of a DNA molecule.

(c) Chitinase

It is a kind of enzyme that is used to break down chitin, which is the major component of fungi's cell walls. As a result, the Chitinase enzyme is employed to split the cell membrane of the fungus and liberate its genetic material, allowing the DNA to be isolated.

Question 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

Answer:

The differences are as follows:

(a) Plasmid DNA and Chromosomal DNA

Plasmid DNA	Chromosomal DNA
It is a replicating extrachromosomal DNA molecule found in bacteria that is not dependent on chromosomal DNA.	It is the whole DNA of an organism located within the chromosomes.

(b) RNA and DNA

RNA	DNA
A single-stranded molecule is one that has only one strand.	DNA molecule with two strands
It's made up of ribose sugar.	It's made up of deoxyribose sugar.
Uracil and adenine are pyrimidines.	Thymine and adenine are pyrimidines.
It's a ribosomal component.	It is a chromosomal component.

(c) Exonuclease and Endonuclease

Exonuclease	Endonuclease
It's a restriction enzyme that removes nucleotides from the DNA molecule's 5' or 3' terminals.	It's a restriction enzyme that creates sticky ends by snipping DNA at specific locations.