Chapter – 11

Biotechnology: Principles and Processes

NCERT Back Exercises:

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

Ans 1: The recombinant proteins used in medical practice are obtained from the recombinant DNA technology. Inthis technology, particular genes are transferred from one organism to another with the help of vectors andrestriction enzymes as molecular tools.

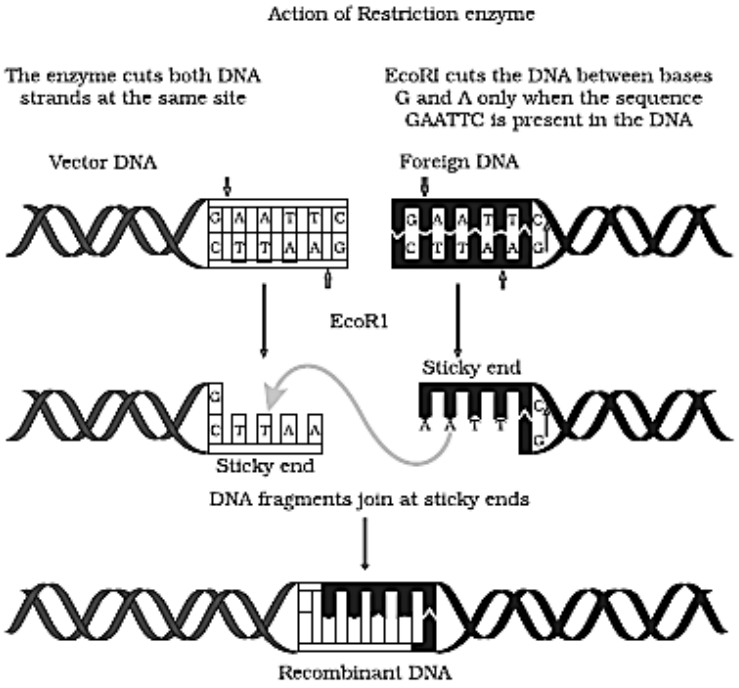
Listed below are 10 recombinant proteins:

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

Ques 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 2: Steps in the formation of recombinant DNA by action of restriction endonuclease enzyme – EcoRI

It can be diagrammatically represented as follows:



Ques 3: From what you have learnt, can you tell whether enzymes are bigger or DNA is bigger in molecularsize? How did you know?

Ans 3: Compared to DNA molecules, enzymes are smaller in size. We can say this as DNA comprises of genetic material, essential for the normal development and functioning of living entities. A DNA molecule consist ofinstructions required for the synthesis of DNA molecules and proteins. Whereas enzymes are the proteins that are synthesized from genes – a small fragment of DNA. These are crucial in the production of polypeptide chain.

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

Ans 4: The molar concentration of human DNA in a human cell can be given as:

$6.023 \times 10^{23} \times Total\ number\ of\ chromosomes$
$6.023 \times 10^{23} \times 46$
$2.77 \times 10^{23}\ moles$

Therefore, $2.77 \times 10^{23}\ moles$ is the molar concentration of DNA in each of the diploid cell in humans.

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

Ans 5: No, eukaryotic cells do not have restriction endonucleases as the DNA of eukaryotes is highly methylated by methylase – a modification enzyme. This methylation safeguards the DNA from the action of restriction enzymes. In prokaryotic cells, these enzymes are present where they aid in preventing the invasion of DNA by virus.

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

Ans 6: Stirred tank bioreactors are developed for a large-scale production of biotechnology products whereas the shake flask method is applied for a small-scale production of biotechnological products carried out in a laboratory.

The stirred tank bioreactor has few advantages over shake flasks. They are:

(i) For testing and sampling process, small amounts of culture can be drawn out from the reactor

(ii) Presence of a control system to regulate the pH and temperature

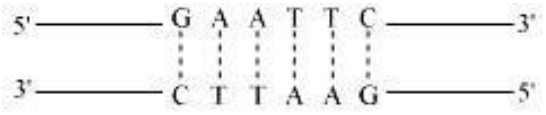
(iii) To regulate the foam, the stirred tank bioreactors have a foam breaker

Ques 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 7: A sequence of DNA that reads the same whether read from 5' –> 3' or from 3' –> 5' direction is a palindromic sequence. These are the sites for action of restriction enzymes. Almost all of the restriction enzymes are palindromic sequences.

Listed below are 5 examples of palindromic sequences, they are:

(1)



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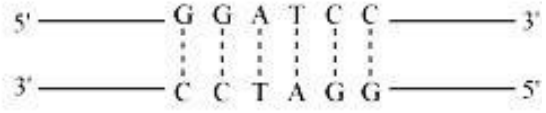

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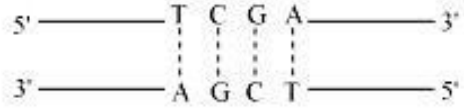

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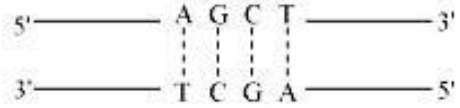

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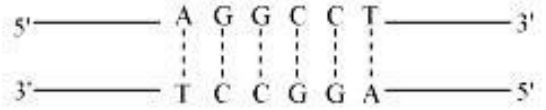


Ques 8: Can you recall meiosis and indicate at what stage a recombinant DNA is made?

Ans 8: A process involving a reduction in the quantity of genetic material is termed as Meiosis, which is a type of cell division. It occurs in two phases, namely – meiosis I and meiosis II.

In the pachytene event of prophase I, chromosomes cross-over wherein the exchange of segments betweennon-sister chromatids of homologous chromosomes occurs. This leads to the formation of recombinant DNAin the process of meiosis.

Ques 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 9: To monitor the transformation of host cells by foreign DNA, a reporter gene can be used. They serve as a selectable marker to find out if the host cell has used up the foreign DNA else the foreign gene is expressed in the cell. The reporter gene and the foreign gene are placed by scientists in the same DNA construct. This collective DNA construct is introduced into the cell where the reporter gene is used as a selectable marker to discover the successful uptake of foreign genes or the genes of interest. In a jelly fish, lac Z gene is an example of reporter gene that encodes for a green fluorescent protein.

Ques 10: Describe briefly the following:

(i) Origin of replication

(ii) Bioreactors

(iii) Downstream processingSolution:

(iv) Origin of replication

Ans 10:

(i) Origin of replication: It can be defined as a DNA sequence in a genome where replication is initiated. The process of replication initiation can either be uni-directional or bi-directional. Any piece of DNA when linked to this sequence can be made to replicate within the host cells. The sequence is also responsible to control the copy of number of the linked DNA. Hence, to recover many copies of target DNA, it should be cloned in a vector whose origin supports high copy number.

(ii) Bioreactor: They are large vessels that are used for the large-scale production of biotechnological products from raw resources. In order to obtain the required product, these bioreactors offer optimal conditions by supplying optimum pH, temperature, vitamin, oxygen etc. They have an oxygen delivery system, a foam control system, a temperature and pH control system. Also, it consists of a sampling port to withdraw a small quantity of culture for the purpose of sampling.

(iii) Downstream processing: It is a method of separating and purifying foreign gene products once the biosynthetic stage is completed. Then, the product is exposed to different procedures to separate and purify the product. Once the process is completed, the product is formulated and made to undergo several clinical trials for quality check and other related assessments.

Ques 11: Explain briefly

(i) PCR

(ii) Restriction enzymes and DNA

(iii) Chitinase

Ans 11:

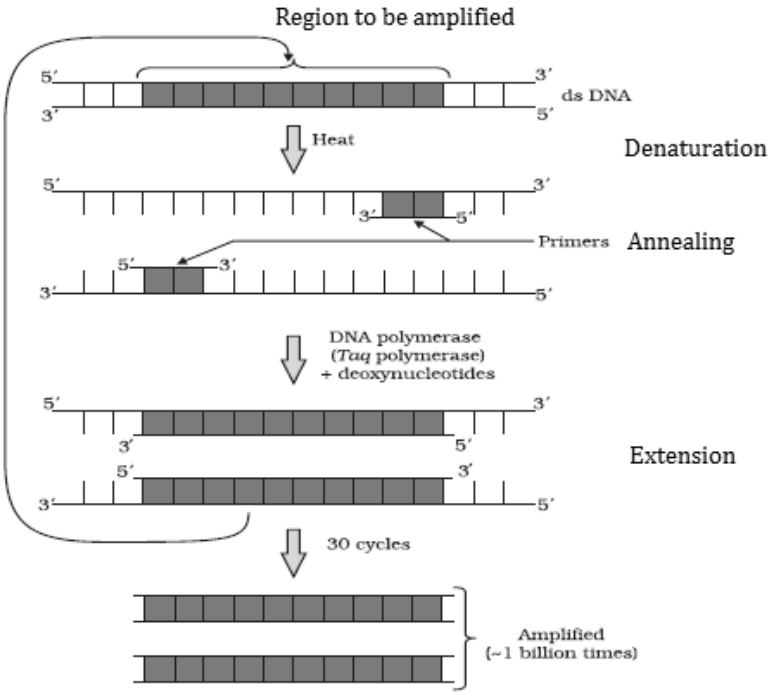
(i) PCR: In molecular biology, PCR or polymerase chain reaction is a technique to amplify a gene or a fragment of DNA in order to get multiple copies. It is widely used in the gene manipulation process. The phenomena involves the in vitro synthesis of sequences with the help of a template strand, a primer and a thermostable DNA polymerase enzyme produced by a bacterium known as Thermus aquaticus. The enzyme uses the building blocks deoxynucleotides (dNTPs) in order to extend the primer.

The following are the three steps in PCR:

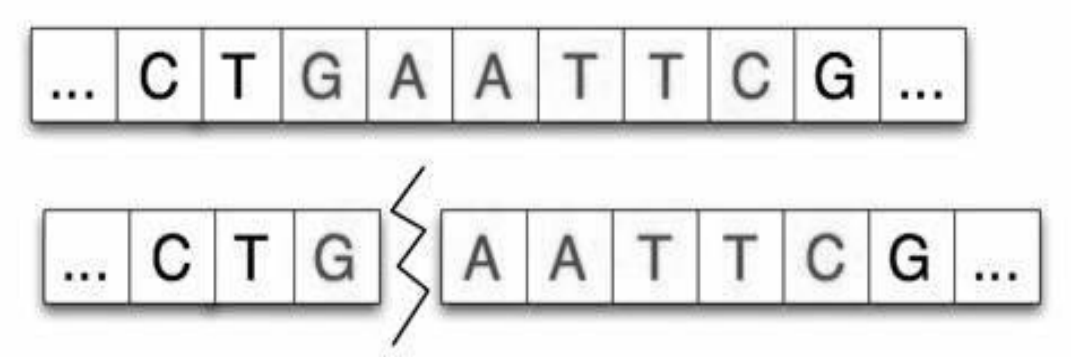
(a) Initially, the double stranded DNA molecules are heated to a high temperature to separate the two strands into a single stranded DNA molecule. This process is referred to as denaturation.

(b) This DNA molecule is then used as a template strand to synthesize a new strand by the DNA polymerase enzyme. The process is termed as annealing that leads to the replication of the original DNA molecule, and the process is carried out for multiple cycles to obtain multiple copies of the rDNA fragment.

(c) Primer is extended by Taq DNA polymerase isolated from Thermits aquatics.



(ii) Restriction enzymes and DNA: In molecular biology, restriction enzymes are molecular scissors used to cut DNA sequences from a particular sites. It has critical role to plau in the gene manipulation process. These enzymes identify a particular six-base pair sequence referred to as the recognition sequence and snip the sequence at specific sites. For instances , the recognition site for the ECORI enzyme is given below:



Restriction enzyme are grouped into two types:

(a) Endonuclease – it is a type of restriction enzyme that cuts within the DNA at specific sites. It serves as asignificant tool in genetic engineering. Typically, it is used to make a snip in the sequence to get DNA fragments possessing sticky ends. These ends are later fused by the enzyme DNA ligase.

(b) Exonuclease – this type of restriction enzyme removes the nucleotides either from 3' or 5' ends of theDNA molecule.

(c) Chitinase - It is a class of enzyme that is used for degradation of chitin, that forms the main component of the cell wall offungi. Hence, to isolate the DNA enveloped within the cell membrane of the fungus, the Chitinase enzyme is used to break the cell to release its genetic material.

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

(i) Plasmid DNA and Chromosomal DNA

(ii) RNA and DNA

(iii) Exonuclease and Endonuclease

Ans 12: The differences are as follows:

(i) Plasmid DNA and Chromosomal DNA

Plasmid DNA	Chromosomal DNA
It is an extra chromosomal DNA molecule found in bacteria, capable of replicating and is independent of chromosomal DNA	It forms the complete DNA of an entity found inside the chromosomes

(ii) RNA and DNA

RNA	DNA
Single-stranded molecule	Double-stranded molecule
Cannot replicate by themselves	Have the potential to replicate
Consist of ribose sugar	Consists of deoxyribose sugar
Pyrimidines are uracil and adenine	Pyrimidines are thymine and adenine
It is a component of ribosomes	It is a component of chromosomes

(iii) Exonuclease and Endonucleas

Exonuclease	Endonuclease
It is a kind of restriction enzyme which removes the nucleotides from 5' or 3' terminals of the DNA molecule	It is a kind of restriction enzyme that snips within the DNA at particular sites to produce sticky ends