Biotechnology : Principles and Processes

CHAPTER 11

NCERT FOCUS

ANSWERS

Topic 1

1. Both DNA (deoxyribonucleic acid) and enzymes are macromolecules. DNA is a polymer of deoxyribonucleotides and enzymes are proteins hence these are polymers of amino acids. But DNA is bigger in molecular size as compared to proteins because synthesis of proteins is regulated by a small segment of DNA, called genes and also a large number of proteins can be synthesised by a DNA molecule.

2. No, the eukaryotic cells do not have restriction endonucleases. All the restriction endonucleases have been isolated from various strain of bacteria. Prokaryotes/bacteria have this enzyme as a defence mechanism to restrict the growth of bacteriophages.

3. The palindromes in DNA are base pair sequences that are the same when read forward (left to right) or backward (right to left) from a central axis of symmetry. For example, the following sequences read the same on the two strands in 5′→ 3′ direction as well as 3′ → 5′ direction.

(i) 5′-G-G-A-T-C-C-3′
3′-C-C-T-A-G-G-5′

(ii) 5′-G-A-A-T-T-C-3′
3′-C-T-T-A-A-G-5′

(iii) 5′-A-A-G-C-T-T-3′
3′-T-T-C-G-A-A-5′

(iv) 5′-G-T-C-G-A-C-3′
3′-C-A-G-C-T-G-5′

(v) 5′-A-C-T-A-G-T-3′
3′-T-G-A-T-C-A-5′

4. Recombinant DNA is formed due to crossing over between non-sister chromatids of homologous chromosome. It occurs during pachytene stage of prophase of meiosis I.

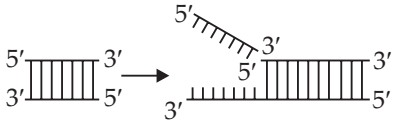
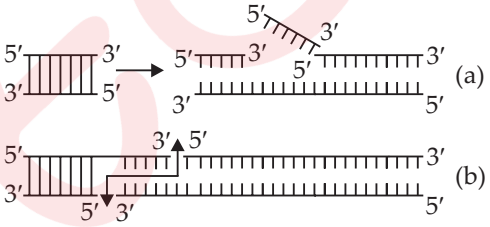
5. Transformation is a process through which a piece of DNA is introduced into a host bacterial cell. Normally, the genes encoding resistance to antibiotics such as ampicillin, tetracycline, etc., are considered as useful selectable markers to differentiate between transformed and non-transformed bacterial cells. In addition to these selectable markers, an alternative selectable marker has been developed to differentiate transformed and non-transformed bacterial cell on the basis of their ability to produce colour in the presence of a chromogenic substance. A recombinant DNA is inserted in the coding sequence of an enzyme β-galactosidase (reporter enzyme). If the plasmid in the bacterium does not have an insert, the presence of a chromogenic substance gives blue coloured colonies, presence of insert results into insertional inactivation of β-galactosidase and, therefore, the colonies do not produce any colour, these colonies are marked as transformed colonies.

6. (a) Plasmid DNA is naked double stranded DNA that forms a circle with no free ends. It is smaller than the host chromosome and can be easily separated.
Chromosomal DNA is double stranded linear DNA molecule associated with large proteins. This DNA exists in relaxed and supercoiled forms and provides a template for replication and transcription. It has free ends.
(b) Differences between DNA and RNA are given in the following table:

	DNA	RNA
(i)	It usually occurs inside nucleus and some cell organelles.	Most of the RNA is found in the cytoplasm.
(ii)	DNA is the genetic material.	RNA is not the genetic material except in certain viruses.
(iii)	It is double stranded with the exception of some viruses (*e.g.*, ϕ × 174).	RNA is single stranded with the exception of some viruses (*e.g.*, double stranded in *Reovirus*).
(iv)	DNA is of only two types; intra-nuclear and extra-nuclear.	There are atleast three types of RNAs – *m*RNA, *r*RNA and *t*RNA.
(v)	It contains deoxyribose sugar.	It contains ribose sugar.
(vi)	Nitrogen base in DNA includes – adenine, cytosine, guanine and thymine.	Thymine is replaced by uracil in RNA, the other three are similar – adenine, cytosine and guanine.
(vii)	Unusual bases are very few or absent.	Many unusual or modified bases are often present.
(viii)	Hydrogen bonds are formed between complementary nitrogen bases on the opposite strands of DNA (A –T, C–G).	Base pairing through hydrogen bonds occurs only in the coiled parts.

(ix)	DNA is spirally twisted to produce a regular helix.	The strand may get folded at places to produce a secondary helix or pseudohelix.
(x)	It replicates to form new DNA molecules.	It cannot normally replicate itself.
(xi)	DNA transcribes genetic information to RNA.	RNA translates the transcribed message for forming polypeptides.
(xii)	Its quantity is fixed for cell.	The quantity of RNA of a cell is variable.
(xiii)	Purine and pyrimidine bases are in equal number.	There is no proportionality between number of purine and pyrimidine bases.
(xiv)	It occurs in the form of prochromosome, chromatin or chromosomes.	It occurs in ribosomes or forms association with ribosomes.
(xv)	It is long lived.	Some RNAs are very short lived while others have somewhat longer life.

(c) Differences between exonucleases and endonucleases are as follows :

	Exonucleases	Endonucleases
(i)	They cleave base pairs of DNA at their terminal ends. 	They cleave DNA at any point except the terminal ends. 
(ii)	They act on single strand of DNA or gaps in double stranded DNA.	They cleave one strand or both strands of double stranded DNA.

Topic 2

1. Recombinant proteins used in medical practice as therapeutics are as follows:

(i) OKT-3, a therapeutic antibody is used for reversal of acute kidney transplantation rejection.

(ii) ReoPro is for prevention of blood clots.

(iii) Tissue plasminogen activator (TPA) is for acute myocardial infarction.

(iv) Asparaginase is for treatment of some types of cancer.

(v) DNase is for treatment of cystic fibrosis.

(vi) Insulin is used in diabetes mellitus.

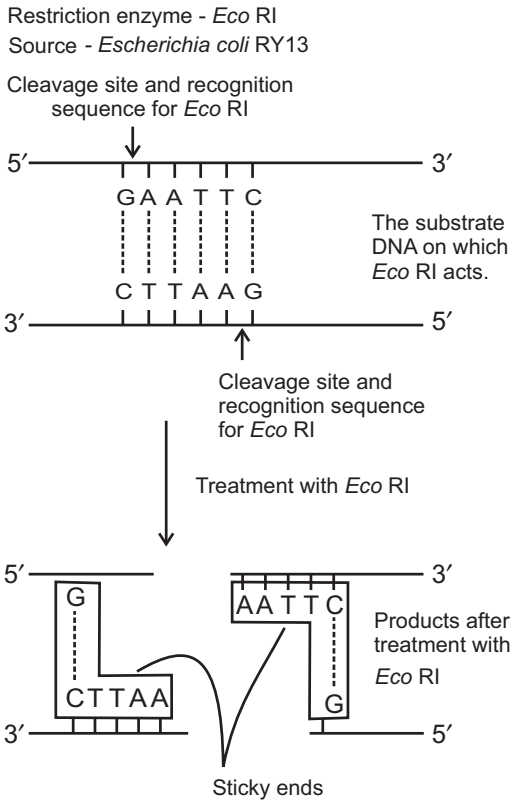
(vii) Blood clotting factor VIII is used for treatment of haemophilia A.

(viii) Blood clotting factor IX is for treatment of haemophilia B.

(ix) Hepatitis B vaccine is for prevention of hepatitis B.

(x) Platelet derived growth factor has been approved for diabetic/skin ulcers. It also stimulates wound healing.

2. The diagrammatic representation of action of restriction enzyme *Eco* RI is as follows:



3. Shake flasks are used for growing and mixing the desired materials on a small scale in the laboratory. Bioreactors are vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells and their enzymes.
Bioreactors are used for large scale production of biomass or cell products under aseptic conditions. Here large volumes (100–1000 litres) of culture can be processed. A bioreactor provides the optimal conditions for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen). The most commonly used bioreactors are of stirring type.
A bioreactor is more advantageous than shake flasks as it has an agitator system to mix the contents properly, an oxygen delivery system to make availability of oxygen, a foam control system, a temperature control system, a pH control system and a sampling port to withdraw the small volumes of the culture periodically.

4. (a) Origin of replication (*ori*) : One of the major components of a plasmid is a sequence of bases where replication starts. It is called origin of replication (*ori*). This is a specific portion of plasmid genome that serves as start signal for self-replication (to make another copy of itself). Any piece of DNA when linked to this sequence can be made to replicate within the host cells. This property is used to make a number of copies of linked DNA (or DNA insert).
(b) Bioreactors : These are vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells and their enzymes. They are allowed to synthesise the desired proteins which are finally extracted and purified from cultures. Small volume cultures are usually employed in laboratories for research and production of less quantities of products. However, large scale production of the products is carried out in 'bioreactors'. The most commonly used bioreactors is stirring type bioreactor (fermenter) that has a provision for batch culture or continuous culture.
(c) Downstream processing : After the formation of the product in the bioreactors, it undergoes through some processes before a finished product is ready for marketing. These processes include separation and purification of products which are collectively called the downstream processing. The product is then subjected to quality control testing and kept in suitable preservatives. The downstream process and quality control test are different for different products.

5. (a) Polymerase chain reaction (PCR) is a technique of synthesising multiple copies of the desired gene (DNA) *in vitro*. This technique was developed by Kary Mullis in 1985. It is based on the principle that a DNA molecule, when subjected to high temperature, splits into two strands due to denaturation. These single stranded molecules are then converted to double stranded molecules by synthesising new strands in presence of enzyme DNA polymerase. Thus, multiple copies of the original DNA sequence can be generated by repeating the process several times. The basic requirements of PCR are, DNA template, two nucleotide primers usually 20 nucleotides long and enzyme DNA polymerase which is stable at high temperature (usually *Taq* polymerase):
Working mechanism of PCR is as follows:
(i) First of all, the target DNA (DNA segment to be amplified) is heated to high temperature (94 to 96° C). Heating results in the separation of two strands of DNA. Each of the two strands of the target DNA now acts as template for synthesis of new DNA strand. This step is called denaturation.
(ii) Denaturation is followed by annealing. During this step, two oligonucleotide primers hybridise to each of single stranded template DNA in presence of excess of synthetic oligonucleotides. Annealing is carried out at lower temperature (40° – 60°C).
(iii) Third and final step is extension. During this step, the enzyme DNA polymerase synthesises the DNA segment between the primers. Usually *Taq* DNA polymerase, isolated from a thermophilic bacterium *Thermus aquaticus*, is used in most of the cases. The two primers extend towards each other in order to copy the DNA segment lying between the two primers. This step requires presence of deoxynucleoside triphosphates (dNTPs) and Mg^{2+} and occurs at 72°C.
The above mentioned three steps complete the first cycle of PCR. The second cycle begins with denaturation of extension product of first cycle and after completing the extension step, two cycles are completed. If these cycles are repeated many times, the DNA segment can be amplified to approximately billion times, *i.e.*, one billion copies of desired DNA segment are made.
(b) Restriction enzymes are used to break DNA molecules. They belong to a larger class of enzymes called nucleases. Restriction enzymes are of three types – exonucleases, endonucleases and restriction endonucleases.
(i) Exonucleases : They remove nucleotides from the terminal ends (either 5′ or 3′) of DNA in one strand of duplex.
(ii) Endonucleases : They make cuts at specific position within the DNA. These enzymes do not cleave the ends and involve only one strand of the DNA duplex.
(iii) Restriction endonucleases : These were found by Arber in 1963 in bacteria. They act as "molecular scissors" or chemical scalpels. They recognise the base sequence at palindrome sites in DNA duplex and cut its strands. Three main types of restriction endonucleases are type I, type II and type III. Out of the three types, only type II restriction enzymes are used in recombinant DNA technology because they can be used *in vitro* to recognise and cut within specific DNA sequence typically consisting of 4 to 8 nucleotides.
(c) Chitinase is a lysing enzyme that dissolves the fungal cell wall. It results in the release of DNA along with several other macromolecules.

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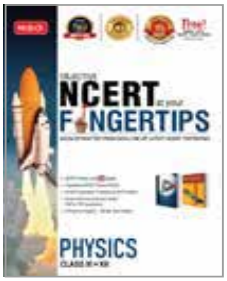

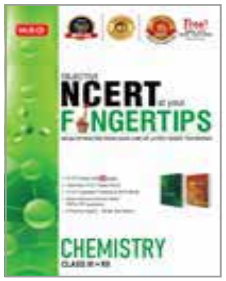

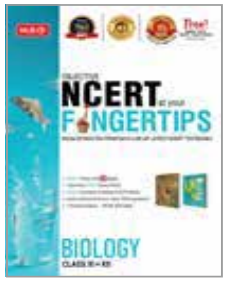

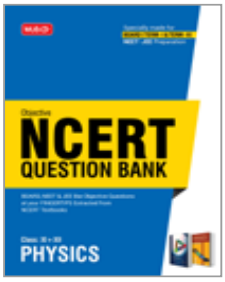

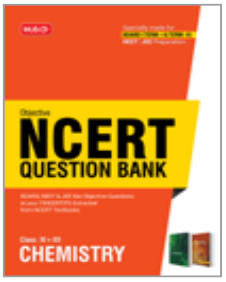

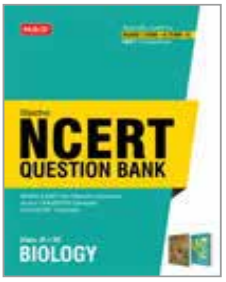

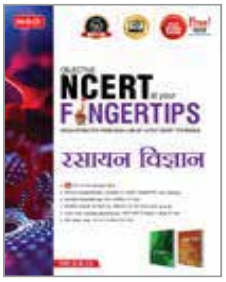

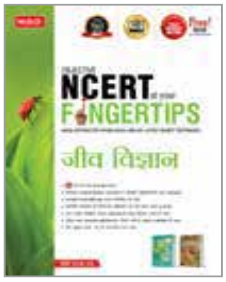

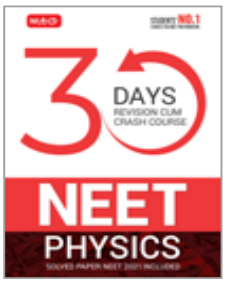

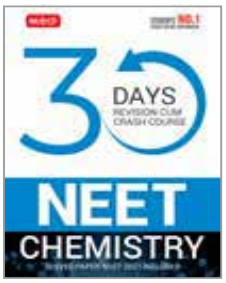

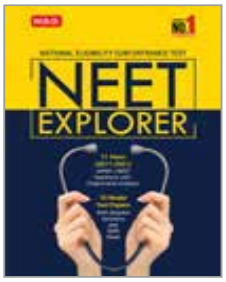

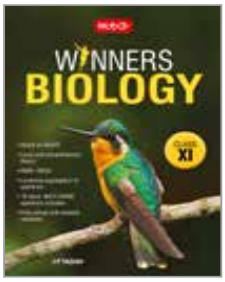

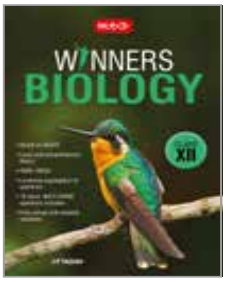

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