Biotechnology Principles and Processes

9 Chapter

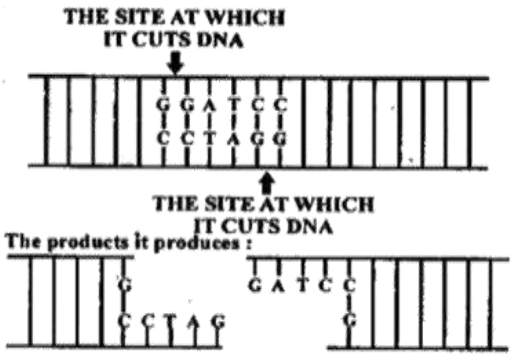
1. Can you list 10 recombinant proteins which are used in medical practice? Find out where they are used as therapeutics (use the internet).

Ans:

(i) Diabetes due to human insulin
(ii) Effective treatment of dwarfism using human growth hormone
(iii) Y1H/IX-hemophilia blood clotting factor
(iv) TPA (tissue plasminogen activator) - Heart attacks/stroke
(v) PDGF (platelet-derived growth factor) promotes healing.
(vi) Interferon - Used to treat viral infections.
(vii) Interleukin - Boosts the immune system,
(viii) Vaccine against hepatitis B virus - Preventing infection caused by the disease.
(ix) Vaccinate for Herpes - Protection against infectious diseases.
(x) Treatment for cystic fibrosis with DNase I.

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: This Restriction Endonuclease has the chemical name Bam H1. An attachment point is at 5'-GGATCC-3'. The cleavage occurs just after a G in both strands of this DNA sequence. It acts on DNA substrates –



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

Ans: Proteins and enzymes are more prominent than DNA since amino acids, smaller than nucleotides, make up proteins. After undergoing certain modifications, a polypeptide chain becomes a protein. A gene controls the synthesis of an enzyme, which is a protein. The codon is a sequence of three nucleotides that helps code one amino acid, which is then polymerized to form a polypeptide chain, eventually becoming a protein after undergoing certain modifications.

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

Ans: DNA is present at a molar concentration of 2 mg/ml in human cells.

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

Ans: The DNA molecules in eukaryotic cells are highly methylated, which means that restriction endonucleases do not exist. DNA is protected from restriction enzymes by methylation. Different strains of bacteria and prokaryotic cells have been isolated as sources of restriction endonucleases.

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

Ans: For small-scale growth and mixing of materials in laboratories, shake flasks are commonly used. Using biological reactors, biotechnological products can be produced in large quantities. In addition to better aeration and mixing properties, bioreactors also have the following characteristics advantages.

(i) The bioreactor is periodically sampled for small volumes of cultures.
(ii) The foam breaker system uses foam as a control device and the pH control system, which regulates the pH of the fluid during the process.
(iii) With the help of baffles, the bioreactor can perform even mixing and oxygen supply.

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

Ans: The DNA molecule contains palindrome nucleotide sequences, identical when read in both directions as 5'-3' or 3'-5'. Restriction enzymes act at these sites.

Below are five palindromic DNA sequence examples:

i. 5' GGATCC 3' ---------3' CCTAGG 5' ---------

ii. 5' AAGCTT 3' ---------3' TTCGAA 5' ---------
iii. 5'ACGCGT 3' ---------3' TGCGGA 5'--------- 5' ACGCGT 3' -------- 3'---------- TGCGCA 5'
iv. 5' ACTAGT 3' --------- 3' TGATCA 5' ---------
v. 5' AGGCCT 3' --------- 3' TCCGGA 5' ---------

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

Ans: Meiosis I occurs during Prophase I, when recombination nodules form after the formation of synaptonemal complexes.

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?

Ans: Transformed cells can be differentiated by detecting their co-response gene (receptor gene) through the activity of a reporter enzyme. Specifically, (3-galactosidase (Lac Z) activity is absent in transformed cells, causing them to appear white. Those that are blue seem to be free of foreign DNA.

10. Describe briefly the followings:
(a) Origin of replication
(b) Bioreactors
(c) Downstream processing

Ans:

a) Origin of Replication: In the genome, there is a specific sequence from which replication commences, so any DNA fragment linked to this sequence can be made to replicate within the host. The linked DNA contains this sequence as well as a control mechanism for its copy number. Thus, to clone DNA from a target in many copies, one should use a vector whose origin supports cloning multiple copies.
b) Bioreactor: A bioreactor is a vessel that allows microbes, plants, and animals to convert raw materials into specific products through the action of their enzymes. By using a bioreactor, optimal growing conditions can be achieved, and desired outcomes are achieved. Bioreactors of the stirring type are the most common. Stirred tank bioreactors are typically cylindrical vessels or vessels with curved bases to facilitate mixing. The fanning of sterile air bubbles in the stirred tank bioreactor is responsible for providing clean air to the cells. The stirrer facilitates mixing and oxygenation within the bioreactor. There are several bioreactor components, such as an agitator system, an

oxygen delivery system, a foam control system, a temperature control system, and a pH control system.

c) Downstream Processing: A series of processes are applied to the product before it is made into a ready for marketing product. Separation and purification are two of the main functions. Preservatives are then added to the product. Clinical trials must be conducted on such preparations, as with drugs.

11. **a) PCR**
b) Restriction enzymes and DNA
c) Chitinase

Ans:

a) PCR: The polymerase chain reaction (PCR) is an in vitro molecular biology method that combines enzymatic amplification of a single DNA strand and creating millions of copies of the specified DNA sequence in just a few hours (about 2 hours).

PCR consists of three steps:

i. Denaturing (at 96°C) the double-stranded DNA into single-stranded DNA.
ii. Annealing of the primer (at 55-65 oC) to ssDNA (single standard).
iii. Taq DNA polymerase, an enzyme isolated from Thermus aquaticus, synthesized new strands (at 72 oC).

An amplified gene is used to clone the desired gene.

Advantage – Higher productivity, higher efficiency, reduced error proneness, and fewer human errors, cyclic and automated.

b) Restriction enzymes and DNA – Restriction enzymes cleave DNA by cleaving a specific sequence of bases known as the recognition site, where the restriction enzyme cleaves the DNA strands.

i. It prevents foreign DNA from entering normal cells by digesting it at various recognition sites. Sites of recognition are palindromic.
ii. Both endonucleases and exonucleases are present.
iii. They produce sticky endings. There is a difference between the recognition site and the cleavage site. The restriction enzymes that bacteria produce are therefore thought to serve as a mechanism for defending themselves against viral attacks and removing viral sequences that interfere with their survival.

c) Chitinase is an enzyme that breaks down the glycosidic bonds in chitin present in fungi and the exoskeleton of certain arthropods and worms to facilitate their digestion or transformation.

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:

a) Plasmid DNA and Chromosomal DNA

Ans:

Plasmid DNA	Chromosomal DNA
Self-replicating	Replicates under nuclear control
Double stranded in a circular pattern	Linear, circular, or double stranded
Does not attach to histones	The protein is associated with histones.
Contains a few genes for characteristics such as antibiotic resistance and fertility factor.	Controls the metabolism of cells by controlling the characters and proteins for that purpose.

b) RNA and DNA

Ans:

RNA	DNA
Ribonucleic acid, with Pentose sugar in the form of Ribose	The sugar originating from Deoxyribonucleic Acid is called Deoxyribose.
Single stranded	Double stranded
Transfer information from DNA to proteins	The genetic material that makes up the human body
Includes uracil and cytosine as Pyrimidine bases	The thymine and cytosine bases are Pyrimidines
Stability reduced by the presence of 2′- OH group of RNA nucleotides	More stable because of double stranded nature and the presence of Thymine
RNA polymerase is responsible for synthesis	The product of DNA polymerase

c) Exonuclease and Endonuclease

Ans:

Exonuclease	Endonuclease
Remove nucleotides from the ends of the DNA.	Eliminate nucleotides at specific sites within DNA
Produce blunt ends.	Creates sticky ends.
Pathogens aren't being restricted from entering.	Limit the entry of pathogens.

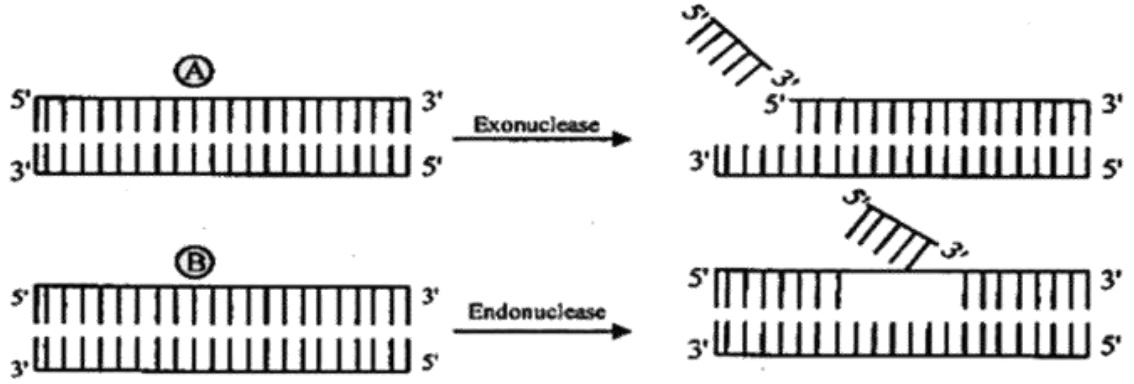


Fig. (A) Action of exonuclease, (B) Action of endonuclease