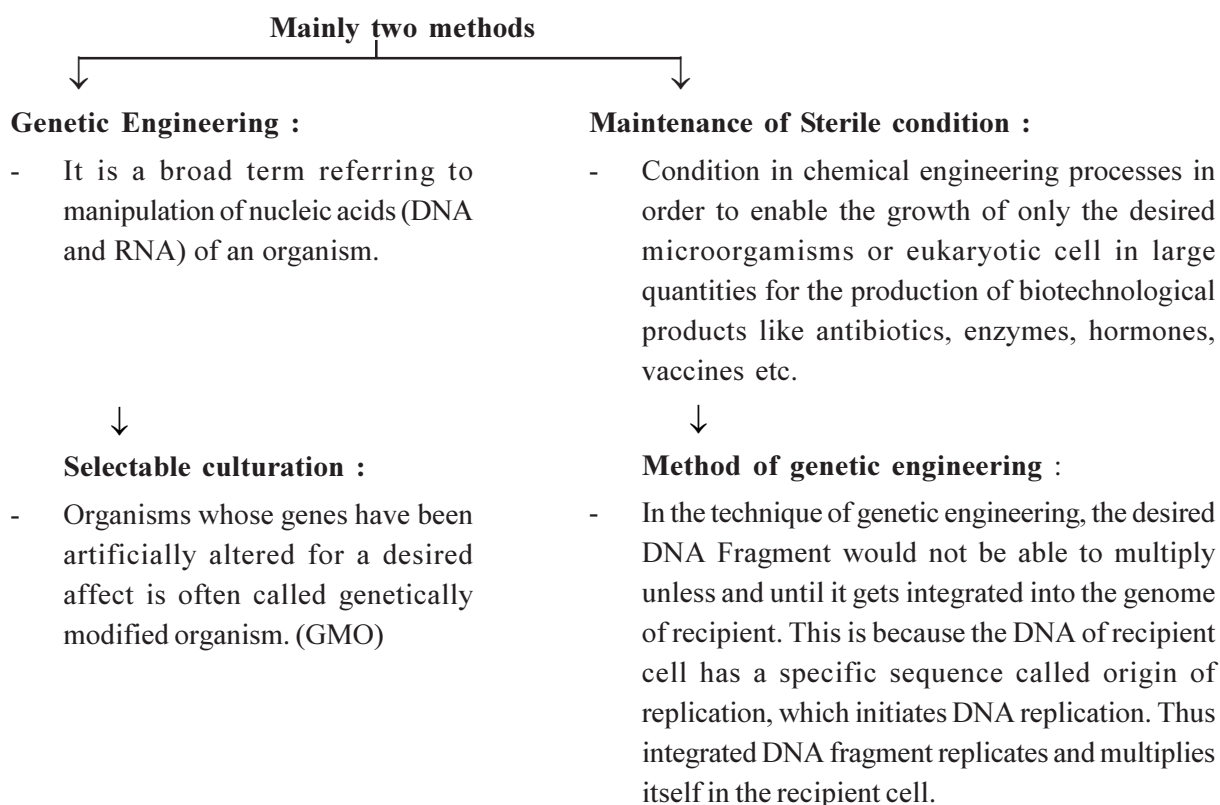


● **Principles and processes :**

- General introduction about Biotechnology :
- Biotechnology may be defined as the use of micro organisms, animals or plant cells or their components to generate products and services useful to human being.
- Biotechnology does not mean hunting and gathering animals and plants for food; however domesticating animals such as sheep and cattle for use as livestock is classical example of biotechnology.
- Our early ancestors also took advantage of micro organisms and used fermentation to make breads, cheeses, yogurts and alcoholic beverages such as beer and wine. During fermentation some strains of yeast decompose sugars to derive energy; and in the process they produce ethanol as a waste product.
- Biotechnology is also associated with medical therapies to treat diabetes, Hepatitis B, Hepatitis C, Cancer, Arthritis, Haemophilia, Bone fracture etc.

Principles of Biotechnology :



- The basic principles of recombinant DNA Technology are very simple.

-
- (1) Advantage of what has been taken up by our ancestors to produce bread, cheeses, Beer and alcoholic beverages by fermentation ?
- (A) Micro organisms (B) Plants
- (C) Sheeps and cattle like animals (D) Tissue culture
- (2) What is decomposed by yeast to derive energy during fermentation ?
- (A) Polysaccharide (B) Lipids (C) Sugar (D) Disaccharide
- (3) What is produced when sugar is decomposed ?
- (A) Alcohol (B) Ethane (C) Methanol (D) Ethanol
-

- (4) Which information is preserved by Asexual reproduction ?
 (A) Undesirable genes (B) Exact gene information
 (C) Desirable hybridization (D) Sexual Reproduction diversity
- (5) Which fragment would not be able to multiply unless and until it gets integrated into the genome of recipient ?
 (A) mRNA (B) DNA (C) RNA (D) Plasmid
- (6) How can we introduce Bt - cotton ?
 (A) Transgenic animals (B) Selective breeding (C) Domestic animals (D) Micro organisms
- (7) What is the full form of EFB.
 (A) Environmental Federation of Britan
 (B) European Federation of Biotechnology
 (C) Ecological Federation Board
 (D) European Foundation of biotechnology

Answers : (1-A), (2-C), (3-D), (4-B), (5-B), (6-A), (7-B)
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- **Useful tools of Recombinat DNA technology :**
 (1) Restriction enzymes (2) Cloning vectors and (3) Competent host
- **Restriction Enzymes :** The technique involved in recombinant DNA technology is to cut the desired DNA segment and introduce it into a vector (e.g. plasmid)
- This is achieved using specific bacterial enzymes called restriction emzymes or restroction endonucleases.
- We known more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria. These enzymes are named according to their bacteria which is gained.
- **Nucleases are of two types :** (1) Exonucleases and (2) Endonucleases.
- Exonucleases remove nucleotides from the ends of DNA where as, endonucleases make cuts at specific site within DNA.
- Restriction endonucleases recognize short palindromic sequences and cut at specific sites.
- Palindromic sequence is a sequence of base pairs in double stranded DNA that reads the same backwards and forward across the double strand.
- **Cloning vectors :** Vectors are the DNA molecules which can carry a foreign DNA Fragment to be cloned.
- The most important vectors are plasmids and bacteriophages which can replicate in the bacterial cells, indepndent of the central of chromosomal DNA.
- The following features are required to facilitate cloning into a vector.
- (1) **Origin of replication :** The origin of replication (also called the replication origin) is a particular sequence in a genome at which replication is initiated. Any fragment of DNA when linked to this sequence can be made to replicate within the host cell.
- (2) **Selectable Marker :** Beside origin of replication, the vector should have a selectable marker. Selectable markers are the genes which help in identifying and eliminating nontransformants and selectively permitting growth of the transformants.
- (iii) **Cloning sites :** In order to link the desired DNA, the vector should have very few, preferably single recognition site for the commonly used restriction enzymes. The plasmid P^{BR322} Carries within its sequences an origin of replication and two antibiotic resistance genes.
 (1) amp^R (ampicillin resistance) (2) tet^R (Tetracycline resistance)

- (iv) **Vectors for cloning Genes in plants and Animals** : A cloning vector is a carrier DNA molecule in which a desired DNA fragment can be integrated in such a way that the carrier molecule does not lose its capacity for self replication. It is used to introduce desired (donor) DNA into host cells.
- (3) **Competent Host** : DNA usually can get across cell membrane since it is a hydrophilic molecule. In order to force bacteria to take up the recombinant DNA, the bacterial cells must be made competent to take up DNA.
- **Electroporation** : Cells are exposed to rapid pulse of high voltage current to render the plasma membrane permeable to recombinant DNA.
 - **Micro-injection** : Recombinant DNA is directly injected to the nucleus of animal cell through micro-injection.
 - **Lipofection** : Recombinant DNA is coated with lipid which allows it to pass through the plasma membrane.
 - **Particle Bombardments** : The high velocity particles of tungsten or gold are coated with recombinant DNA and then shot into cells. This method is also known as biolistics or gene gun.
-
- (8) Which enzyme helps to join cut pieces of DNA ?
 (A) RNA - Ligase (B) RNA - Protease (C) DNA - Ligase (D) DNA - Polymerase
- (9) has capacity to transform normal cells into cancer cell.
 (A) TMV (B) Retrovirus (C) DNA - ligase (D) DNA - Polymerase
- (10) In which of the following process, to make easy entry of DNA and permeability of cell membrane cells are given high voltage current ?
 (A) Micro - injection (B) Lipofection (C) Particle bombardment (D) Electroporation
- (11) Which coating on DNA helps to enter in cells membrane ?
 (A) Protein (B) Lipid (C) Carbohydrate (D) Cholesterol
- (12) When the process of cloning is possible ?
 (A) DNA of one species gets fixed into another cell.
 (B) Recipient cell DNA is destroyed.
 (C) Integrated DNA in recipient cell replicates itself.
 (D) Need for cloning vector.

Answers : (8-C), (9-B), (10-D), (11-B), (12-C)

- **Process of recombinant DNA Technology** :
 - Recombinant DNA is DNA created artificially by combining the DNA from two or more organisms into a single recombinant molecule.
 - The process involves a sequence of steps as set below.
- (1) **Isolation of the Genetic Material (DNA)** :
- Recombinant DNA technology begins with the isolation of a gene of interest i.e the donor DNA. This can be achieved by cutting the DNA with the enzymes. In order to cut DNA with restriction enzymes, it needs to be in pure form, free from other macromolecules.

- As in eukaryotes, DNA is enclosed within the membranes, we have to break membranes to release DNA along with other molecules like RNA, proteins, carbohydrates and also lipids.
- (2) **Cutting of DNA at specific Locations :**
- To do this, a known DNA sequence from the donor cell is identified and removed with a restriction enzyme.
 - When cut, each fragment of DNA has an overhanging piece of single stranded DNA on its ends called sticky ends.
- (3) **Amplification of Gene of Interest using PCR :**
- (1) Denaturation
 - (2) Annealing
 - (3) Extension
- (4) **Insertion of Recombinant DNA into the Host cell / organism :**
- The recombinant DNA is introduced into the host cell in a process called 'transformation.' Recipient cells after making them competent to receive, take up DNA present in its surrounding. If a recombinant DNA possessing antibiotic resistant gene e.g ampicillin is transferred into ampicillin resistant cells.
- (5) **Obtaining the foreign Gene product :** As the ultimate aim of recombinant technology is to produce a desirable protein, the gene introduced in a recipient cell should be allowed to express.
- (6) **Down stream processing :**
- The processes like separation and purification of the products are collectively referred to as downstream processing.
-
- (13) What is produced by combination of DNA from one or more living organisms in recombinant DNA ?
 (A) Artificial DNA (B) Natural RNA (C) Artificial RNA (D) Natural DNA
- (14) What can be removed by treatment of Ribonuclease enzyme ?
 (A) DNA (B) RNA (C) mRNA (D) rRNA
- (15) What is precipitated by chilled ethanol ?
 (A) RNA (B) r RNA (C) mRNA (D) DNA
- (16) Which enzyme enables sticky ends to bind with complementary DNA ?
 (A) Ligase (B) Protease (C) Lipase (D) Restriction
- (17) Which pieces can be separated by agarose gel electrophoresis ?
 (A) RNA (B) mRNA (C) DNA (D) rRNA
- (18) Which group is responsible negative charge of DNA ?
 (A) Carbon (B) Phosphate (C) Sulphur (D) Iodine
- (19) What is used to create New copies of DNA ?
 (A) GMO (B) EFB (C) REE (D) PCR

Answers : (13-A), (14-B), (15-D), (16-A), (17-C), (18-B), (19-D)

• **A - Assertion R - Reason**

Answer the following questions, by choosing the appropriate option.

(A) A and R both are true and R is a explanation of A.

(B) A and R both are true and R is not a explanation of A.

(C) A is true and R is false

(D) A is false and R is true

- (20) Assertion A : DNA fragment would not be able to multiply unless and until it gets integrated into the genome of recipient.

Reason R : Recipient cells possess specific sequence.

(A) (B) (C) (D)

- (21) Assertion A : During fermentation some strains of yeast decompose sugars to derive energy.

Reason R : In this process ethanol is produced as waste product.

(A) (B) (C) (D)

- (22) Assertion A : When both vector (plasmid) and desired DNA cut by the same DNA.

Reason R : The resultant DNA fragments have the same kind of sticky ends.

(A) (B) (C) (D)

- (23) Assertion A : Bread is sweet in taste.

Reason R : When bread is produced by yeast, it produces carbohydrates.

(A) (B) (C) (D)

- (24) Assertion A : Exonucleases remove nucleosides from the ends of DNA.

Reason R : Endonucleases make cuts at specific site within DNA.

(A) (B) (C) (D)

- (25) Assertion A : Treating cells and the DNA with high Ca^{+2} which allows DNA uptake by cells.

Reason R : High Ca^{+2} causes membrane changes that reduce barriers to DNA movement.

(A) (B) (C) (D)

- (26) Assertion A : The plasmid pBR322 Carries within its sequence an origin of replication and two antibiotic resistance genes.

Reason R : The tet^R gene has Pst - I recognition site.

(A) (B) (C) (D)

- (27) Assertion A : Resistance to tetracyclin becomes inactivated.

Reason R : Bam H I cuts the plasmid in the gene that codes for resistance to tetracyclin.

(A) (B) (C) (D)

Answers : (20-A), (21-B), (22-A), (23-C), (24-B), (25-A), (26-C), (27-A)

• **T - F type Questions :**

Choose the correct option for given statements are true or false.

- (28) Choose the correct option for important vector :

(i) Plasmid (ii) Endonuclease (iii) Bacteriophage (iv) Ampicillin
(A) TTF (B) TFTF (C) FTFT (D) FFTT

- (29) Which of the following option is correct for restriction enzyme ?
- (i) More than 9000 restriction enzymes have been identified.
 - (ii) Nomenclature is done from the bacteria they were isolated.
 - (iii) Restriction enzymes are included in major class of enzymes.
 - (iv) Restriction endonucleases works on palindromic sequences and breaks both DNA strands.
- (A) FTFT (B) TTTT (C) TTFT (D) FTTT
- (30) Choose the correct option in the context of gene isolation ?
- (i) Donor DNA enzyme can break RNA.
 - (ii) In order to cut DNA with restriction enzymes, it needs to be in pure form.
 - (iii) RNA can be removed by treating with ribonuclease.
 - (iv) Pure DNA can be precipitated by adding hot ethanol.
- (A) FTFT (B) FTTF (C) TTTF (D) TFTF
- (31) Choose correct option in reference to produce identical copies of short DNA sequences by PCR.
- (i) A DNA molecule of interest is denatured by heating at 90 - 95°C.
 - (ii) The primers are complementary to either end of the target sequence but lie on opposite strand.
 - (iii) As the mixture cools at lower temperature 50-65°C each sequence of DNA molecule becomes annealed with an oligonucleotide primer.
 - (iv) DNA polymerase is then added and complementary strands are synthesized.
- (A) TTTF (B) FTTT (C) TTTT (D) TFTT
- (32) Choose the correct option for competent Host.
- (i) High Ca^{+2} causes membrane changes.
 - (ii) Barriers are reduced for DNA movement.
 - (iii) Recombinant DNA can be forced into bacterial cells by incubating the cells with recombinant DNA on ice followed by placing them at high temp.
 - (iv) Treated cells are put at 100°C and then again placed on ice.
- (A) TTFF (B) TFTT (C) TTTT (D) TTTF

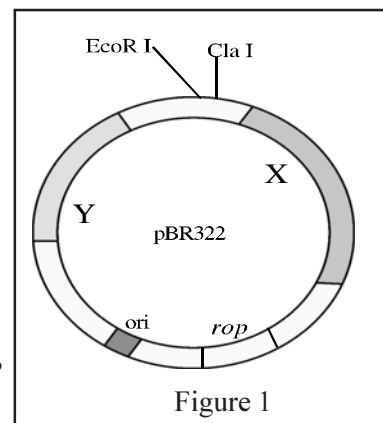
Answers : (28-B), (29-D), (30-A), (31-C), (32-D)

- (33)
- | Column - I | Column - II |
|---------------------------------|---|
| (i) Electroporation | (p) Recombinant DNA is coated with lipid. |
| (ii) Micro - injection | (q) Tiny high velocity particles of tungsten or gold are coated with recombinant DNA. |
| (iii) Lipofection | (r) Cells are exposed to rapid pulse of high voltage current. |
| (iv) Particle Bombardments | (s) Directly injected to the nucleus of animal cell through micro injection. |
| (A) (i-r) (ii-q) (iii-p) (iv-s) | (B) (i-r) (ii-s) (iii-p) (iv-q) |
| (C) (i-r) (ii-q) (iii-p) (iv-s) | (D) (i-p) (ii-s) (iii-q) (iv-r) |

- (34) **Column - I** **Column - II**
- | | | |
|------------------------|---|---------------------------------|
| (i) pBR322 | (p) Cuts plasmid | (A) (i-r) (ii-s) (iii-q) (iv-p) |
| (ii) amp ^R | (q) Recognition sites for Hind - III, Bam H I and Sal I | (B) (i-p) (ii-q) (iii-r) (iv-s) |
| (iii) tet ^R | (r) Carries two antibiotic resistance genes | (C) (i-q) (ii-p) (iii-s) (iv-r) |
| (iv) Bam H I | (s) Recognition site for Pst - I | (D) (i-s) (ii-r) (iii-p) (iv-q) |
- (35) **Column - I** **Column - II**
- | | | |
|-----------------------------------|---|---------------------------------|
| (i) Exonuclease | (p) Make cuts at specific site within DNA | (A) (i-p) (ii-r) (iii-q) (iv-s) |
| (ii) Endonuclease | (q) Can separate DNA pieces | (B) (i-q) (ii-r) (iii-p) (iv-s) |
| (iii) Agarose gel electrophoresis | (r) Synthesizes complementary chains | (C) (i-s) (ii-p) (iii-r) (iv-q) |
| (iv) DNA polymerase | (s) Remove nucleotides from ends of DNA | (D) (i-s) (ii-p) (iii-q) (iv-r) |
- (36) **Column - I** **Column - II**
- | | | |
|-------------------------|-------------------------|---------------------------------|
| (i) Restriction enzymes | (p) Eco RI | (A) (i-q) (ii-s) (iii-r) (iv-p) |
| (ii) Restriction | (q) Selectable marker | (B) (i-s) (ii-p) (iii-r) (iv-q) |
| (iii) Plasmid | (r) Important vectors | (C) (i-r) (ii-q) (iii-p) (iv-s) |
| (iv) Ampicillin | (s) Works on palindrome | (D) (i-p) (ii-s) (iii-r) (iv-q) |

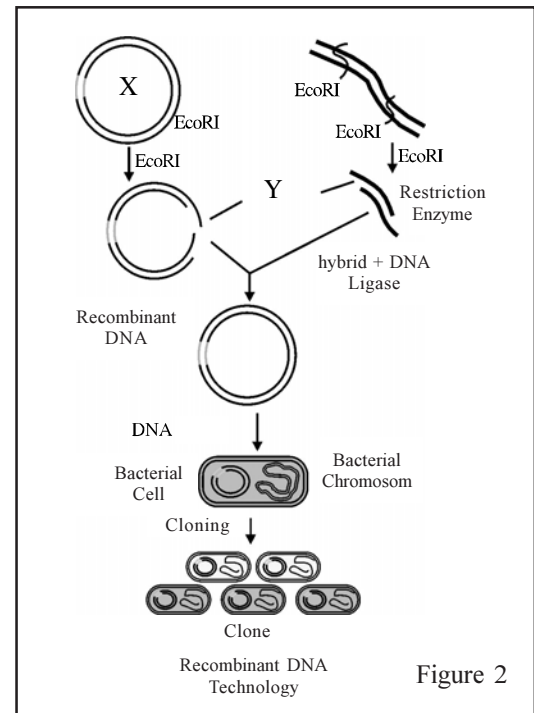
Answers : (33-B), (34-A), (35-D), (36-D)

- (37) What is indicated as 'X' and 'Y' in the following diagram 1 ?
- (A) X-amp^R, Y-tet^R
 (B) X-amp^R, Y-Pvu II
 (C) X-tet^R, Y-amp^R
 (D) X-tet^R, Y-Pvu II
- (38) Which recognition sites are indicated as : 'X' and 'Y' in diagram 1 ?
- (A) X-Sal I, Y-Pst I
 (B) X-Bam HI, Y-Sal I
 (C) X-Pst I, Y-Sal I
 (D) X-Hind III, Y-Sal I



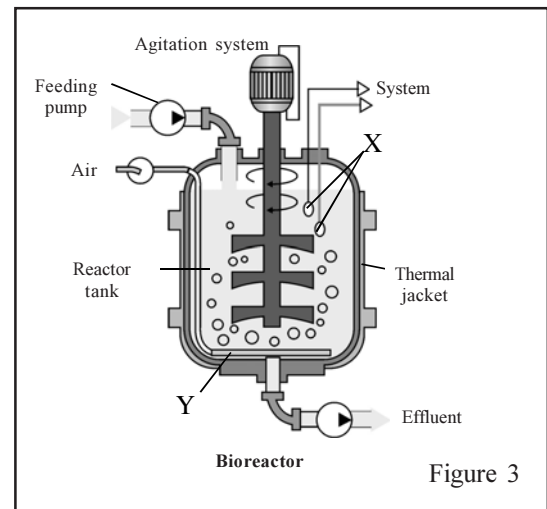
(39) What 'X' and 'Y' indicateds in given diagram 2 ?

- (A) X - Foreign DNA Y - Sticky ends
- (B) X - Plasmid, Y - Foreign DNA
- (C) X - Sticky ends, Y - foreign DNA
- (D) X - Plasmid and Y - Sticky ends



(40) What is indicated as 'X' and 'Y' in given diagram 3 ?

- (A) X - Monitor, Y - Submerged aerator
- (B) X - Submerged aerator, Y - Sensor probes
- (C) X - Sensor probes, Y - Submerged aerator
- (D) X - Monitor, Y - Sensor probes



(41) What is used to join antibiotic resistance gene to plasmid ?

- (A) DNA ligase
- (B) Endonuclease
- (C) DNA polymerase
- (D) Exonuclease

(42) For what polythene glycol method is used ?

- (A) To prepare biodiesel
- (B) To create seedless fruits
- (C) To produce energy from sewage
- (D) All of above

(43) Find the correct statement :

- (A) Biogas can be prepared using aerobic bacteria and animal waste.
- (B) Methanogens are anaerobic bacteria.
- (C) Biogas known as gobargas is pure methane.
- (D) Activated sludge in settling tank is the rich source of aerobic bacteria in STP.

(44) What is used as cloning vector in higher living organisms ?

- (A) Baculo virus
- (B) *Salmonella typhimureum*
- (C) *Rhizopus nigricans*
- (D) Retrovirus

- (45) Which nitrogen base sequence can be broken from the middle by restriction enzymes in DNA molecule ?
- (A) 5'..... CGTTCG 3'
3'..... ATGGTA 5'
- (B) 5'..... GATATG 3'
3'..... CTACTA..... 5'
- (C) 5'..... GAATTC 3'
3'..... CTTAAG 5'
- (D) 5'..... CACGTA 3'
3'..... CTCAGT 5'
- (46) The part of DNA or RNA connected with Radio active molecule is known as
- (A) Vector (B) Probe (C) Clone (D) Plasmid
- (47) Enzyme restriction endonucleases
- (A) Cuts DNA molecule at specific sites
- (B) Identify specific nucleotide sequence to connect DNA ligase
- (C) Prevents the process of enzyme DNA polymerase
- (D) Remove nucleotides from ends of DNA molecule
- (48) What is appropriate for corestriction endonucleases in *eco RI* ?
- (A) Colon (B) Coelom (C) Coenzyme (D) Coli
- (49) What is the use of agarose extracted out from sea weeds ?
- (A) Spectrometry (B) PCR (C) Tissue culture (D) Gel electrophoresis
- (50) Crystalline insecticidal protein prepared by *Bacillus thurengiensis*.
- (A) Binds to the surface of midgut epithelial cells and destroys the pest at the end.
- (B) Some genes possessing cay gene are transcribed.
- (C) Protein is activated by acidic pH of foregut.
- (D) It does not destroy the vector bacteria which possess resistance to toxin.

Answers : (37-C), (38-A), (39-D), (40-C), (41-A), (42-D), (43-B and D), (44-D), (45-C), (46-B), (47-A), (48-D), (49-D), (50-A)

