# Biotechnology: Principles and Processes

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**Class: XII** **Biology**

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## Very Short Answer Questions

**1. What is the definition of Biotechnology given by EFB?**

The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services.

**2. What is the full form of EFB?**

European Federation of Biotechnology.

**3. What is genetic engineering?**

Genetic engineering is a technique to alter the chemistry of genetic material (DNA and RNA), to introduce these into host organisms and thus change the phenotype of the host organism.

**4. What is the advantage of asexual reproduction in plants?**

Asexual reproduction preserves the genetic information, while sexual reproduction permits variation.

**5. What is the disadvantage of traditional hybridisation procedures used in plant and animal breeding?**

The disadvantage of traditional hybridisation procedures used in plant and animal breeding very often leads to **inclusion and multiplication of undesirable genes** *along with the desired genes.*

**6. What is cloning?**

The linking of alien DNA with the origin of replication in order to replicate and multiply itself in the host organism is known as cloning. This can also be called as making multiple identical copies of template DNA.

**7. What is a plasmid?**

The autonomously replicating, circular, extra-chromosomal DNA is known as plasmid.

**8. What are restriction enzymes?**

The enzymes used for cutting DNA at specific locations, are called restriction enzymes or molecular scissors.

**9. What are vectors?**

The plasmid DNAs which deliver the alien pieces of DNA into the host organisms are called as vectors.

**10. Which enzyme acts on cut DNA molecules and joins their ends?**

DNA ligase

**11. How many restriction enzymes have been isolated?**

More than 900 restriction enzymes have been isolated from over 230 strains of bacteria each of which recognise different recognition sequences.

**12. Name the first restriction endonuclease.**

The first restriction endonuclease is **Hind-II**, whose functioning depended on a specific DNA nucleotide sequence was isolated and characterised five years later.

**13. Name the class to which restriction enzymes belong to.**

Restriction enzymes belong to a larger class of enzymes called **nucleases.**

**14. What are palindromes?**

The groups of letters that form the same words when read both forward and backward, e.g. MALAYALAM

**15. What is recognition sequence?**

Restriction enzymes always cut DNA molecules at a particular point by ***recognising a specific sequence of six base pairs***. This specific base sequence is known as the recognition sequence.

**16. What is downstream Processing?**

The separation and purification of bio-products are collectively referred to as downstream processing.

## Short Answer Type Questions

**1. What is the advantage of maintaining sterile ambience in chemical engineering processes?**

It enables the growth of only the **desired microbe**/eukaryotic cell in large quantities for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.

**2. What is the advantage of genetic engineering over traditional hybridisation procedures used in plant and animal breeding?**

Genetic engineering **overcomes the inclusion and multiplication of undesirable genes** along with the desirable genes.

It helps us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into the target organism.

**3. Do you know the likely fate of a piece of DNA, which is transferred into an alien organism?**

The piece of DNA **would not be able to multiply itself** in the **progeny cells** of the organism.

But, when it gets **integrated into the genome of the recipient, it multiplies** and be inherited along with the host DNA.

This is because the alien piece of DNA has become the part of a chromosome, which has the ability to replicate.

**4. What is origin of replication? What is its importance?**

**Origin of replication** is a specific DNA sequence in a chromosome**,** which is responsible for initiating replication

Any piece of DNA when linked to this sequence can be made to replicate within the host cells.

This sequence is also responsible for **controlling the copy number** of the linked DNA.

So, if one wants to recover many copies of the target DNA it should be cloned in a vector whose origin support high copy number.

**5. What are the three basic steps involved in genetically modifying an organism?**

1. Identification of DNA with desirable genes;
2. Introduction of the identified DNA into the host;
3. Maintenance of DNA introduced in the host and transfer of the DNA to its progeny.

**6. How are enzymes named?**

The **first letter** of the name comes from the genus and the **second two letters** come from the species of the prokaryotic cell from which they were isolated, e.g., EcoRI comes from *Escherichia coli* RY 13.

In EcoRI, the letter ***R*** is derived from the name of strain.

**Roman numbers** following the names indicate the order in which the enzymes were isolated from that strain of bacteria.

**7. What are the two kinds of nucleases? Describe their function.**

The two kinds of nucleases are **exonucleases** and **endonucleases.**

Exonucleases **remove nucleotides** from the ends of the DNA whereas endonucleases make cuts at specific positions within the DNA.

Each restriction endonuclease functions by inspecting the length of a DNA sequence.

Once it finds its **specific recognition sequence**, it will bind to the DNA and cut each of the two strands of the double helix at specific points in their sugar-phosphate backbones.

**8. What are the uses of selectable markers? Give examples.**

Selectable markers are genes which help in **identifying and eliminating non-transformants** and selectively permitting the growth of the transformants.

The **genes encoding resistance to antibiotics** such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered selectable markers for *E. coli*

The normal *E. coli* cells do not carry resistance against any of these antibiotics.

**9. Why are plasmids and bacteriophages used as cloning vectors? Give an account on cloning vectors.**

**Plasmids and bacteriophages** have the ability to replicate within bacterial cells independent of the control of chromosomal DNA. So, they are used as cloning vectors.

Bacteriophages because of their **high number per cell** have **very high copy numbers** of their genome within the bacterial cells.

Some plasmids may have **only one or two copies per cell** whereas others may have **15-100 copies per cell**. Their numbers can go even higher.

If an alien piece of DNA is linked with bacteriophage or plasmid DNA, it will multiply equal to the copy number of the plasmid or bacteriophage.

Vectors used at present, are engineered in such a way that **they help easy linking** of foreign DNA and easy **selection of recombinants** from non-recombinants.

**10. Describe the role of Agrobacterium tumefaciens in transforming a plant cell.**

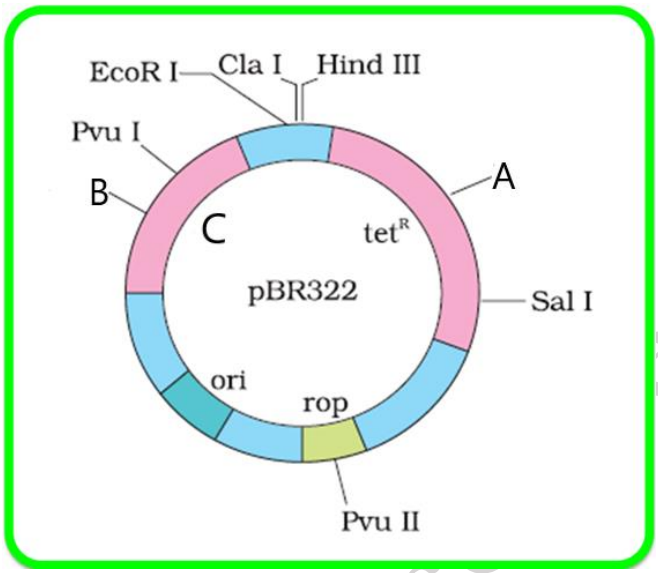
Agrobacterium tumefaciens is known to cause infection in plants, especially in dicots.

The Ti plasmid of this bacterium induces tumour formation inside the plant cell and has been exploited for cloning of the gene of interest and stably integrating them into the plant genes.

The DNA of the Ti plasmid gets incorporated with the host cell DNA.

However, to use it as a vector, the tumour-causing gene has been deleted by a molecular biologist.

**11. Name the regions marked A, B and C.**




A- Bam HI

B- Pst I

C- ampR

## Long Answer Type Questions

**1. What are bioreactors? Describe the structure and function of them.**

**OR**

**Explain how bioreactors help to produce bio-products in large quantities?**

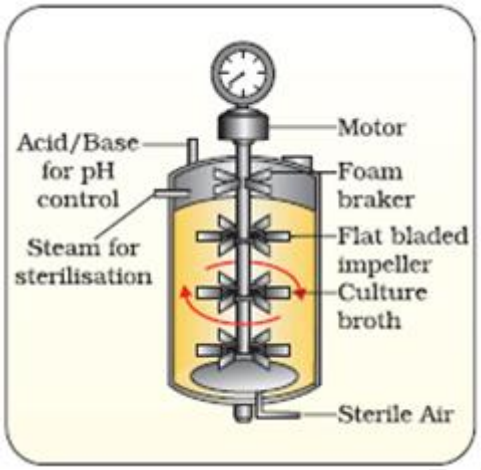
Bioreactors are vessels in which large volumes (100-1000 litres) of raw materials are biologically converted into specific products, individual enzymes, etc., using microbial plant, animal or human cells.

The bioreactor has

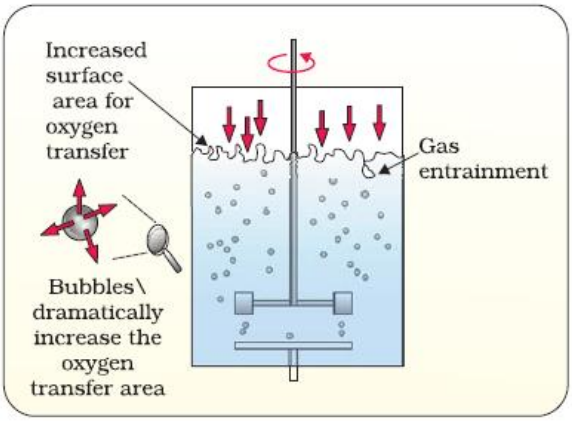
- Agitator system
- Oxygen delivery system
- Foam control system
- Temperature control system
- pH control system
- Sampling ports so that small volumes of the culture can be withdrawn periodically.

**The two types of bioreactors are**

(a) Simple stirred-tank bioreactor;




(b) Sparged stirred-tank bioreactor through which sterile air bubbles are sparged.




The most commonly used bioreactors are of stirring type.

A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents.

The stirrer facilitates even mixing and oxygen availability throughout the bioreactor. Alternatively, air can be bubbled through the reactor.

A bioreactor provides the optimal conditions for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).

## 2. Explain the process of Amplification of Gene of Interest using PCR

PCR stands for **Polymerase Chain Reaction.**

In this reaction, multiple copies of desirable gene are synthesised in vitro using **two sets of primers** (forward primer and reverse primer).

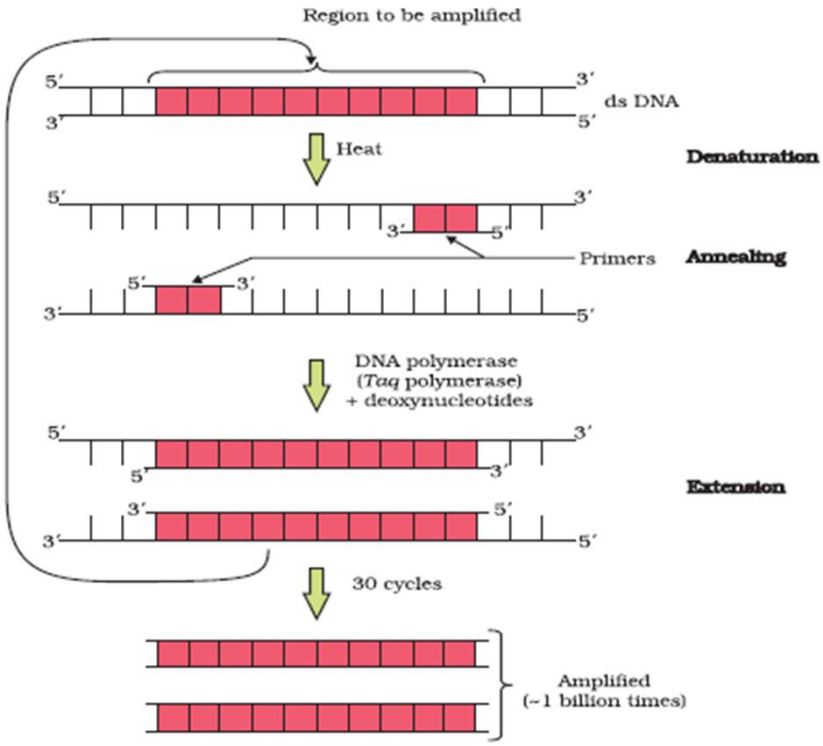
Primers are small chemically synthesised oligonucleotides that are complementary to the regions of DNA.

The enzyme **DNA polymerase** extends the primers using the nucleotides provided in the reaction and the genomic DNA as template.

If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to billion times, i.e., 1 billion copies are made.

Such repeated amplification is achieved by the use of a **thermostable DNA polymerase** (isolated from a bacterium, Thermus aquaticus), which remain active during the high temperature induced denaturation of double stranded DNA.

The amplified fragment can be used to ligate with a vector for further cloning.




### 3. What are alternative selectable markers? Describe the process of selecting recombinants from non-recombinants using alternative selectable markers.

Alternative selectable markers are screenable markers which can also be denoted as reporter genes, which allow the researcher to distinguish between desired and undesired cells, e.g. blue and white colonies of bacteria.

They differentiate recombinants from non-recombinants on the basis of their ability to produce colour in the presence of a **chromogenic substrate**.

A recombinant DNA is inserted within the coding sequence of an enzyme alpha-galactosidase.

This results into inactivation of the enzyme, which is referred to as **insertional inactivation.**

If the plasmid in the bacteria **does not have an insert,** the colonies give **blue colour** in the presence of a chromogenic substrate

If the plasmid in the bacteria **has an insert,** it results into insertional inactivation of the a-galactosidase and the colonies do not produce any colour, these are identified as recombinant colonies.

**4. Describe the techniques used for the transfer of recombinant DNA into the host cell.**

Since DNA is a hydrophilic molecule, it cannot pass through cell membranes.

In order to force bacteria to take up the plasmid, the bacterial cells must first be made competent to take up DNA.

**Heat Shock Method**

**Treatment of bacterial cell with Calcium:**

The bacterial cells are **made competent to take up DNA by treating them with a specific concentration of a divalent cation, such as calcium**, which increases the efficiency with which DNA enters the bacterium through pores in its cell wall.

**Incubation of bacteria cells with recombinant DNA on ice:**

Recombinant DNA can then be forced into the cells by **incubating the cells with recombinant DNA on ice**, followed by **placing them briefly at 42°C** (heat shock), and then putting them **back on ice.**

This enables the bacteria to take up the recombinant DNA. This is not the only way to introduce alien DNA into host cells.

**Micro-injection**

In a method known as **micro-injection,** recombinant DNA is directly injected into the nucleus of an animal cell.

**Biolistics** or **Gene gun Method**

The **cells are bombarded** with high velocity micro-particles of **gold or tungsten coated with DNA.** This method is known as biolistics or gene gun.

**Disarmed pathogen vectors**

This method uses disarmed pathogen vectors, which when allowed infecting the cell, transferring the recombinant DNA into the host.

## 5. Describe the vectors used for cloning genes in plants and animals.

**Agrobacterium tumefaciens**, a pathogen of several dicot plants is able to deliver a piece of DNA known as '**T-DNA to transform normal plant cells into a tumor** and **direct these tumor cells to produce the chemicals** required by the pathogen.

**Retroviruses** in animals have the ability to **transform normal cells into cancerous** cells.

A better understanding of the art of delivering genes by pathogens in their eukaryotic hosts has generated knowledge to transform these tools of pathogens into useful vectors for delivering genes of interest to humans.

The tumor inducing (Ti) plasmid of ***Agrobacterium tumifaciens*** **has now been modified into a cloning vector which is no more pathogenic to the plants** but is still able to use the mechanisms to deliver genes of our interest into a variety of plants.

Similarly, **retroviruses have also been disarmed** and are now **used to deliver desirable genes** into animal cells.

So, once a gene or a DNA fragment has been ligated into a suitable vector it is transferred into a bacterial, plant or animal host (where it multiplies).

## 6. Explain the techniques used in the separation and isolation of DNA fragments.

### Cutting of DNA:

The cutting of DNA by **restriction endonucleases** results in the fragments of DNA.

### Gel Electrophoresis:

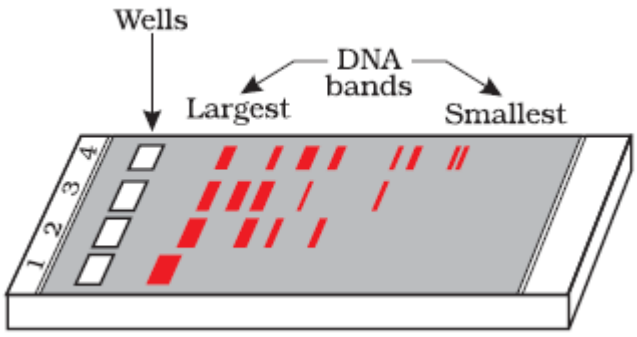
The cut fragments can be separated by a technique known as **gel electrophoresis.**

Since DNA fragments are **negatively charged molecules** they can be separated by forcing them to move towards the **anode** under an electric field through a medium/matrix.

**The most commonly used matrix is agarose** which is a natural polymer extracted from sea weeds.

**The DNA fragments separate (resolve) according to their size through sieving effect provided by the agarose gel.**

Hence, the smaller the fragment size, the farther it moves.




**Visualization:**

The separated DNA fragments can be visualised only after staining the DNA with **ethidium bromide followed by exposure to UV radiation** (you cannot see pure DNA fragments in the visible light and without staining).

A **bright orange coloured bands of DNA** is seen in an **ethidium bromide** stained gel exposed to **UV light**

**Elution:**

The process of separating the bands of DNA from the agarose gel is known as elution.

The DNA fragments purified in this way are used for constructing a recombinant DNA by joining them with cloning vectors.

**7. Why should a vector have a single recognition site in order to link the alien DNA? Explain the process of linking the alien DNA into the vector pBR322.**

**Need for single recognition site:**

In order to link the alien DNA, the vector needs to have very few, preferably single, **recognition site** for the commonly used restriction enzymes.

Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning

**Linking of alien DNA into the vector pBR322:**

The ligation of alien DNA is carried out at a restriction site present in one of the two **antibiotic resistance genes.**

If a foreign DNA is ligated at the **Bam HI** site of tetracycline resistance gene in the vector **pBR322.**

The recombinant plasmids will **lose tetracycline resistance due to insertion of foreign DNA.**

But the recombinant plasmids can be selected out from non-recombinants by plating the transformants on ampicillin medium.

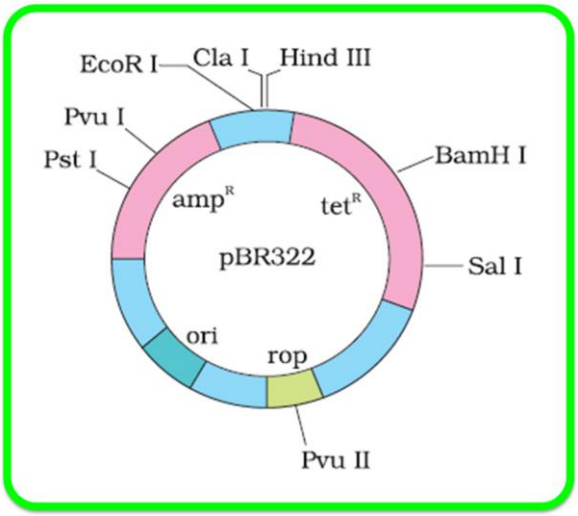
**Selection of Transformants and Recombinants:**

The transformants growing on ampicillin containing medium are then transferred to a medium containing tetracycline.

The recombinants will grow in ampicillin containing medium but not in the tetracycline medium.

But, non-recombinants will grow on the medium containing both the antibiotics.

In this case, **ampicillin resistance gene helps in selecting the transformants.** whereas the **tetracycline resistance gene** helps in **selection of recombinants.**




**8. The ampicillin resistance gene in vectors provides resistance against the antibiotic, ampicillin, and is used as a selectable marker. How does Ampicillin Resistance gene help us to select the transformants from non-transformants?**

Ampicillin resistance gene is the most commonly used selection marker.

Recipient cells after making them 'competent' to receive, take up DNA present in its surrounding.

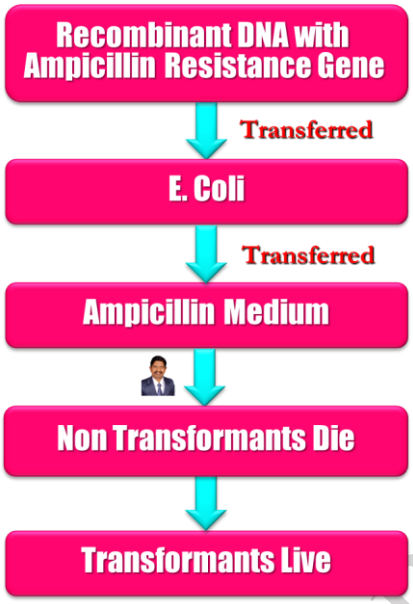
If a recombinant DNA bearing ampicillin resistance gene is transferred into E. coli cells, the host cells become transformed into ampicillin-resistant cells.

If we spread the transformed cells on agar plates containing ampicillin, only transformants grow, untransformed recipient cells die.

So, we can select transformed cells from non-transformed cells in the presence of ampicillin.

The ampicillin resistance gene in this case is used as a selectable marker.

## Ampicillin Resistance Gene as a Selectable Marker




**9. A scientist used the plasmids that contained ampicillin resistance gene and lacZ gene as selectable markers. He inserted the gene of interest within the ampicillin resistance gene instead of lacZ gene due to human error. The competent cells were allowed to take up the plasmid. This was followed by plating them on agar media containing ampicillin and X-gal. What would be the result of the experiment?**

The bacteria which took up the recombinant plasmid will not grow in ampicillin medium.
The ampicillin resistance gene provides resistance against the antibiotic, ampicillin, and is used as a selectable marker.

In this case, the scientist has accidentally inserted the gene of interest within the ampicillin resistance (AmpR) gene.

This would inactivate the AmpR gene and the host cells would lose ampicillin resistance and become sensitive to ampicillin.

So, the transformed bacteria with recombinant plasmids would not be able to grow on agar plate containing ampicillin.

The lacZ gene is used as a selectable marker in many cloning vectors to screen recombinants from non-recombinants.

It codes for the enzyme Beta-Galactosidase which hydrolyses the chromogenic substrate X-gal into a blue product.

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