PROCESS OF RECOMBINANT DNA TECHNOLOGY

Isolation of the Genetic Material (DNA)-

- The cells are broken and opened to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids which can be achieved by treating the cells with enzymes such as **lysozyme** (bacteria), **cellulase** (plant cells), **chitinase** (fungus).
- The RNA can be removed by treatment with **ribonuclease** whereas proteins can be removed by treatment with **protease** and purified DNA ultimately precipitates out after the addition of chilled ethanol which can be seen as collection of fine threads in the suspension.

Fig. DNA precipitate

Cutting of DNA at Specific Location-

- Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme, at the optimal conditions for that specific enzyme which results in the fragments of DNA.
- The fragments are 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 agarose.
- The DNA fragments separate according to their size through sieving effect provided by the agarose gel.
- The smaller the fragment size, the farther it moves and the separated DNA fragments can be visualized only after staining the DNA with a compound known **as ethidium bromide** followed by exposure to UV radiation.
- Bright orange coloured bands of DNA can be observed in an ethidium bromide stained gel exposed to UV light.
- The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece by the process known as

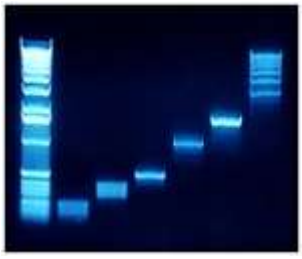


Fig . DNA bands

Amplification of Gene of Interest using PCR

- PCR stands for Polymerase Chain Reaction.
- Multiple copies of the gene of interest is synthesized in vitro using two sets of primers and the enzyme DNA polymerase.
- Primers are small chemically synthesized oligonucleotides that are complementary to the regions of DNA.
- PCR includes three major steps-

1. Denaturation
2. Annealing
3. Extension

- Denaturation is the process of heating of target DNA at 94^{o}C to seperate the two strands of DNA.
- Annealing is the process of pairing of primers with complimentary base sequences of the two separated strands.
- Extension is the process of adding complimentary deoxyribonucleotides one by one to the 3′OH ends of primers by the activity of DNA polymerase and as a result new DNA strand is synthesized.
- If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times by the use of a thermostable DNA polymerase isolated from a bacterium, *Thermus aquaticus.*
- The amplified fragment can be used to ligate with a vector for further cloning.

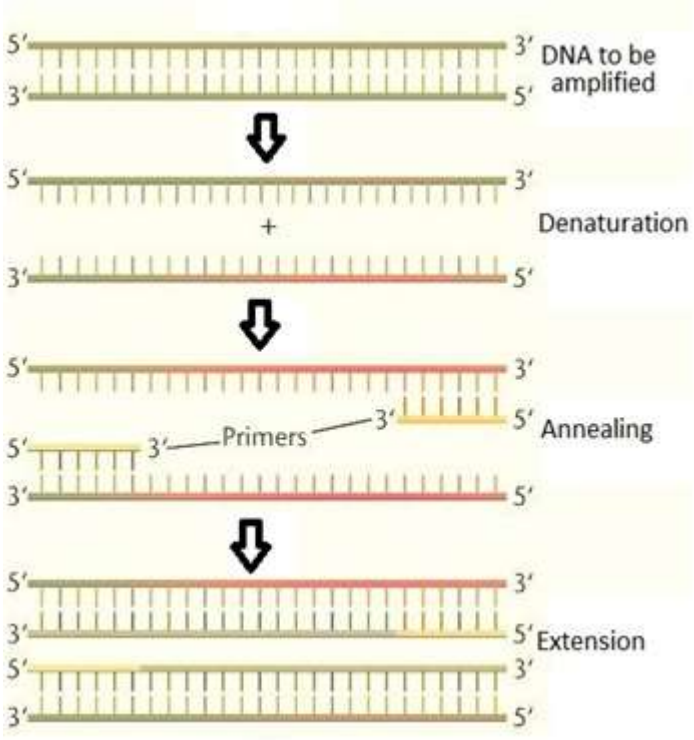


Fig. polymerase chain reaction

Insertion of Recombinant DNA into the Host Cell/Organism

- Recipient cells after making them 'competent' to receive, take up DNA present in its surrounding.
- If a recombinant DNA bearing gene for resistance to an antibiotic is transferred into E. coli cells, the host cells become transformed into ampicillin-resistant cell.

Obtaining the Foreign Gene Product

- The foreign gene when gets expressed under appropriate conditions, produces desirable proteins.
- If any protein encoding gene is expressed in a heterologous host, is called a **recombinant protein**.
- The cells harboring cloned genes of interest may be grown on a small scale in the laboratory or on a large scale in a bioreactor.

BIOREACTORS

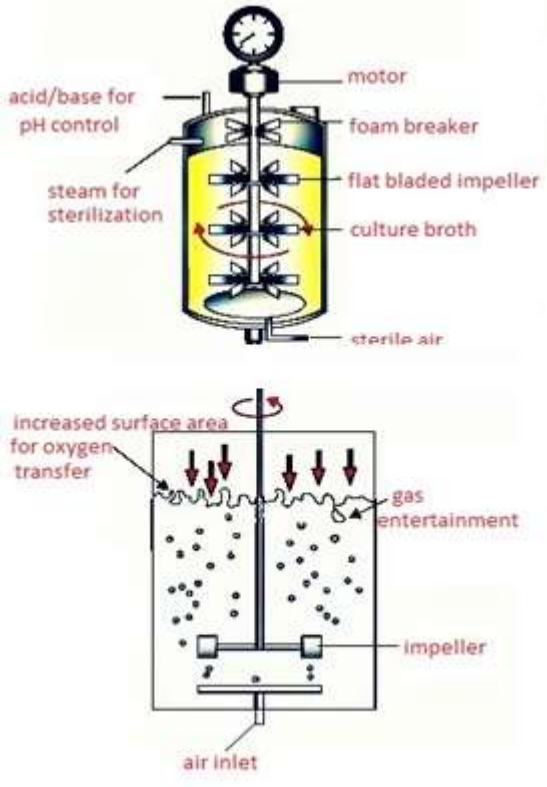
- Bioreactor is the cylindrical vessel in which biological processes is carried out on a large scale.
- The recombinant cells can be multiplied in a continuous culture system wherein the used medium is drained out from one side while fresh medium is added from the other to maintain the cells.
- Bioreactors vessels in which raw materials are biologically converted into specific products, individual enzymes, etc., using microbial plant, animal or human cells.
- A bioreactor provides the optimal conditions for achieving the desired product by providing optimum growth conditions such as temperature, pH, substrate, salts, vitamins, oxygen.
- Bioreactors are of two types-

1. Simple stirred tank bioreactor

2. Sparged stirred-tank bioreactor

- A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents and the stirrer facilitates even mixing and oxygen availability throughout the bioreactor.
- In sparged stirred-tank bioreactor sterile air is sparged through the reactor.
- The bioreactor has an agitator system, an oxygen delivery system and a foam control system, a temperature control system, pH control system and sampling ports so that small volumes of the culture can be withdrawn periodically.

- Fig. simple stirred-tank bioreactor and sparged stirred-tank bioreactor



Downstream Processing

- Downstream processing is the separation and purification of the product.
  - The product has to be formulated with suitable preservatives and the formulation has to undergo thorough clinical trials.

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