PRINCIPLES OF BIOTECHNOLOGY

Bioprocess Engineering

It includes rDNA, use of gene cloning and gene transfer to overcome the limitation of multiplication of undesirable genes during hybridization.

Genetic Engineering

It involves the maintenance of a sterile ambience in chemical engineering processes to enable the growth of only desired cell in large number.

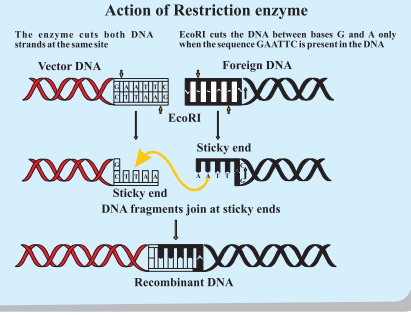
The three basic steps in genetically modifying an organism —
(i) identification of DNA with desirable genes;
(ii) introduction of the identified DNA into the host;
(iii) maintenance of introduced DNA in the host and transfer of the DNA to its progeny

9. BIOTECHNOLOGY: PRINCIPLES AND PROCESSES

TOOLS

RESTRICTION ENZYMES

- Also called molecular scissors.
- Restriction enzymes belong to a larger class of enzymes called nucleases. These are of two kinds; 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.

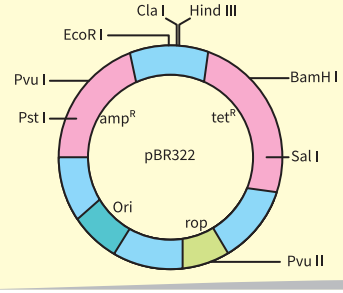


COMPETENT HOST

- Host bacterial cells must be made competent to take up the cloning vectors with recombinant DNA.
- The chemical, physical and disarmed pathogens methods are used to make the host competent.

CLONING VECTORS

- Vehicles of DNA fragments.
- DNA fragments are attached to vectors to transfer them to the host.
- Plasmid and bacteriophages are commonly used vectors.
- Vector must possess
  (1) ORI – Origin of Replication
  (2) selectable marker
  (3) Cloning sites



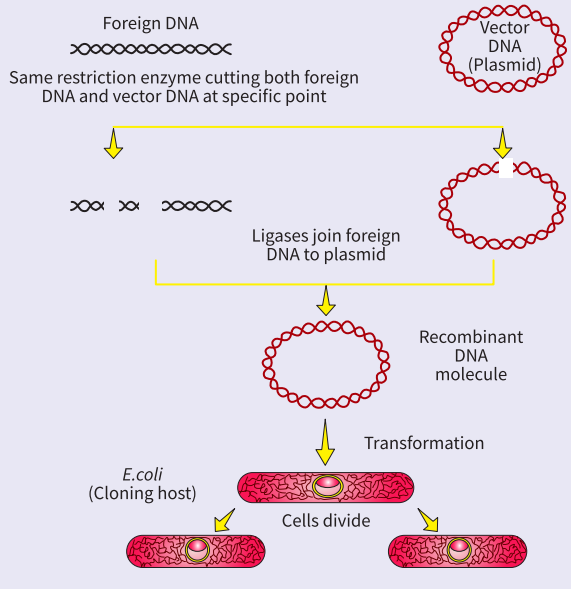
Processes of rDNA Technology

1) Isolation of DNA
- To cut the DNA with restriction enzymes, it needs to be in pure form free from other macromolecules.
- The cell is lysed, to release DNA, with chitinase, lysozyme etc

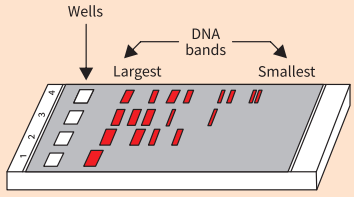
2) Fragmentation of DNA by Restricton Enzyme
- Pure DNA molecules are incubated with restriction enzyme
- Agarose gel electrophoresis is done to separate the DNA fragments.

3) Isolation of Desired DNA Fragment
- Done by Gel electrophoresis.

4) Amplification of Gene of Interest using PCR
5) Ligation of DNA Fragment into Vector
6) Transferring the Recombinant DNA into the Host
7) Culturing the Host Cells in a Medium at Larger Scale
8) Downstream Processing



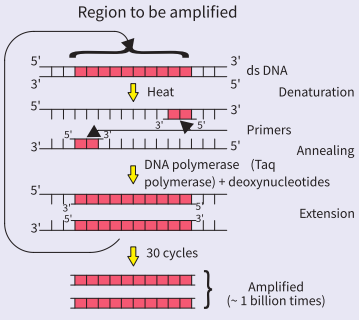
Gel electrophoresis



A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

The DNA fragments separate out according to their size because of the sieving property of agarose gel. The separated DNA fragments are visualized after staining the DNA with ethidium bromide followed by exposure to UV radiation. The separated bands of DNA are cut out and extracted from the gel piece.

Amplification of desired DNA fragment / Gene of interest using PCR



The Polymerase Chain Reaction (PCR) is a technique developed by Kary Mullis in 1985 and awarded the Nobel Prize for Chemistry in 1993. It requires a DNA template, primers, enzymes, and three steps: denaturation, annealing, and extension. The reaction is repeated many times to obtain several copies of desired DNA.

BIOREACTORS

Bioreacotors are the vessels in which raw materials are biologically converted into specific products, individual enzymes etc, using microbial plant, animal, or human cells.

Stirring Type Bioreactor

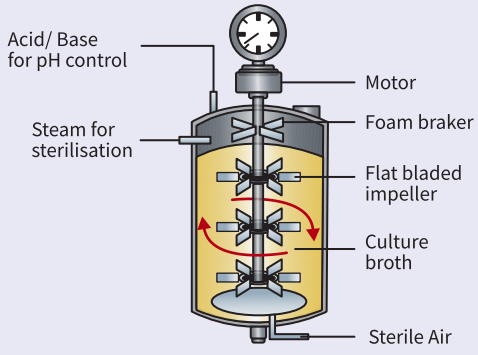
- Most commonly used bioreactors
- Usually cylindrical or with curved base to facilitate the mixing of reactor contents.

Components of Bioreactors

- Agitator system
- Oxygen delivery system
- Temperature control system
- Foam control system
- Sampling ports
- pH control system

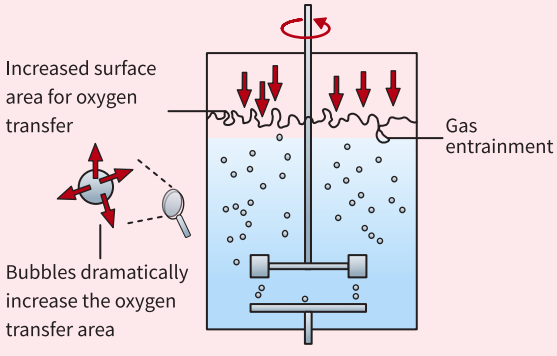
Simple stirred tank

Simple moving type reactor



Sparged stirred tank

Gas is bubbled through a liquid to remove the other dissolved gas.



Obtaining the Foreign Gene Product

All the processes to which a product is subjected to before being marketed as a finished product are called downstream processing.

It includes:

- Separation of the product from the reactor.
- Purification of the product.
- Formulation of the product with suitable preservatives.
- Quality control testing and clinical trials in case of drugs.