BIOTECHNOLOGY: PRINCIPLES AND PROCESSES

PROCESSES OF RECOMBINANT DNA TECHNOLOGY

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The main steps of rDNA technology are as follow

(1) Isolation of the genetic material (DNA)

(2) Fragmentation of DNA at specific locations by restriction endonuclease

(3) Separation and Isolation of DNA fragments

(4) Gene amplification by PCR

(5) Insertion of recombinant DNA into the host cell / Organism

(6) Culturing the host cells in a nutrient medium at a large scale for obtaining the foreign gene product

(7) Extraction of the desired product utilises downstream processing

1. Isolation of DNA :-

The DNAs which are to be used as passenger DNA and the vehicle DNA are extracted out of their cells by lysing the cells with the suitable enzyme. The DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA. proteins, polysaccharides and also lipids. This can be achieved by treating the bacterial cells/plant or animal tissue with enzymes such as lysozyme (bacteria), cellulase (plant cells), chitinase (fungus). You know that genes are located on long molecules of DNA interwined with proteins such as histones. The RNA can be removed by treatment with ribonuclease whereas proteins can be removed by treatment with protease. Other molecules can be removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethanol. This can be seen as collection of fine threads in the suspension. DNA that separates out can be removed by spooling method.

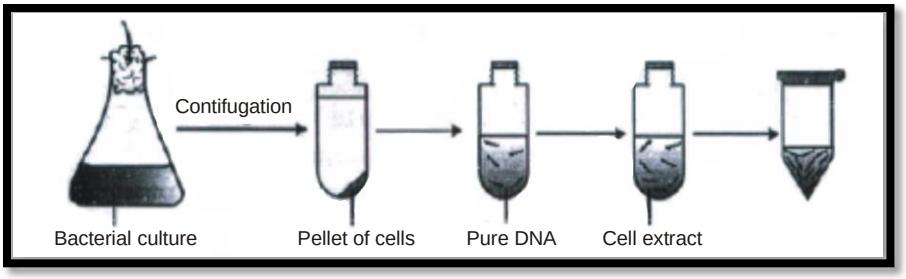


Fig.

The basic steps in preparation of total cell DNA from a culture of bacteria

(2) Fragmentation of DNA at Specific Locations by RE

Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme. Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion. Both vector and insert should be digested with the same restriction enzyme.

(3) Separation and Isolation of DNA Fragments

A Separation of DNA fragments by gel electrophoresis

The cutting of DNA by restriction endonucleases results in the fragments of DNA. These 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 positive electrode anode under an electric field through a medium/matrix,

Nowadays, 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.

Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion. This process is repeated with the vector DNA also.

The separated DNA fragments can be visualised only after staining the DNA with a compound such as Ethidium bromide or Acridine orange, followed by exposure to

UV radiation (you cannot see pure DNA fragments in the visible light and without staining). You can see bright orange coloured bands of DNA in a ethidium bromide stained gel exposed to UV light.

B Isolation of desired DNA fragment

The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as elution. The DNA fragments purified in this way are used in constructing recombinant DNA by joining them with cloning vectors.

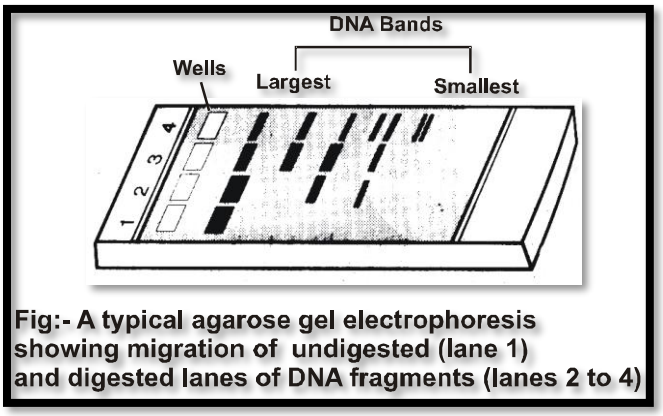


Fig:- A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested lanes of DNA fragments (lanes 2 to 4)

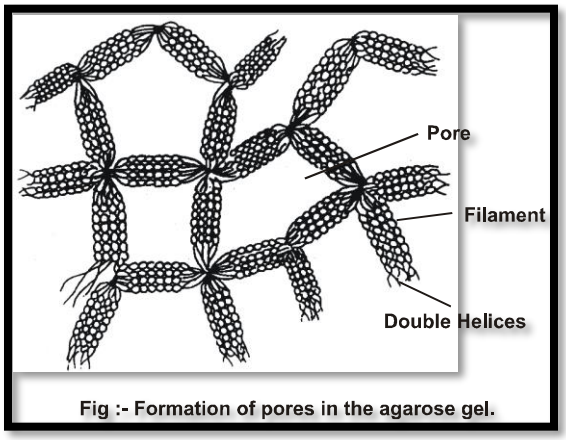


Fig :- Formation of pores in the agarose gel.

3. Amplification of gene of interest using PCR

Polymerase chain reaction technology (PCR-technology)

This technique was invented by Kary mullis (1983).

In 1993 Karry Mullis got nobel prize for PCR(for chemistry)

PCR is a method for amplifying a specific region of DNA molecule without the requirement for time consuming cloning procedures.

PCR reaction takes place in Eppendorf tube.

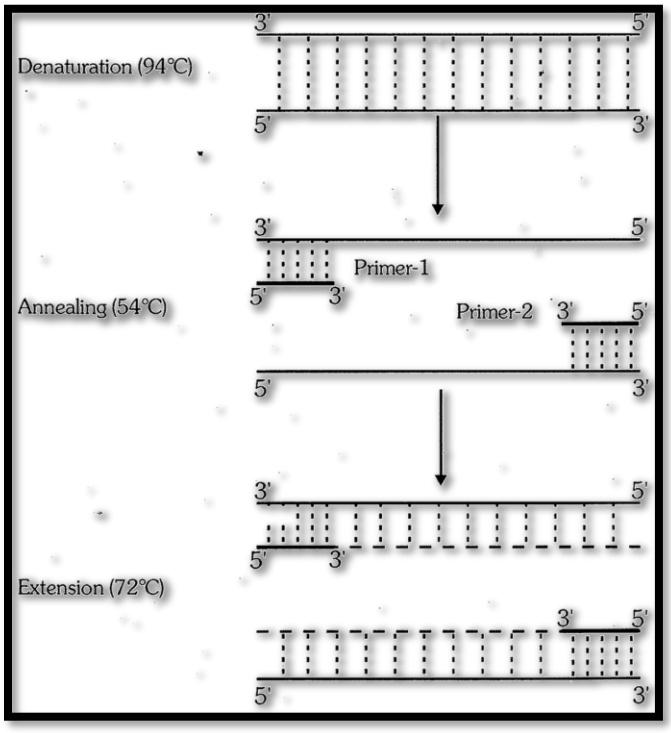
Using PCR-technique very low content of DNA available from samples of blood or semen or any other tissue or hair cell can be amplified many times and analysed. In this technique Taq-Polymerase is used. Taq polymerare enzyme is used in PCR which is a special type of DNA polymerare enzyme which is resistant to high temperature.

Taq Polymerase is isolated from Thermus aquaticus bacterium.

Some other examples of polymerase which are used in PCR are -

Pflu Polymerase - Isolated from Pyrococus furiosus bacterium.

Vent Polymerase - Isolated from Thermococcus litoralis bacterium.



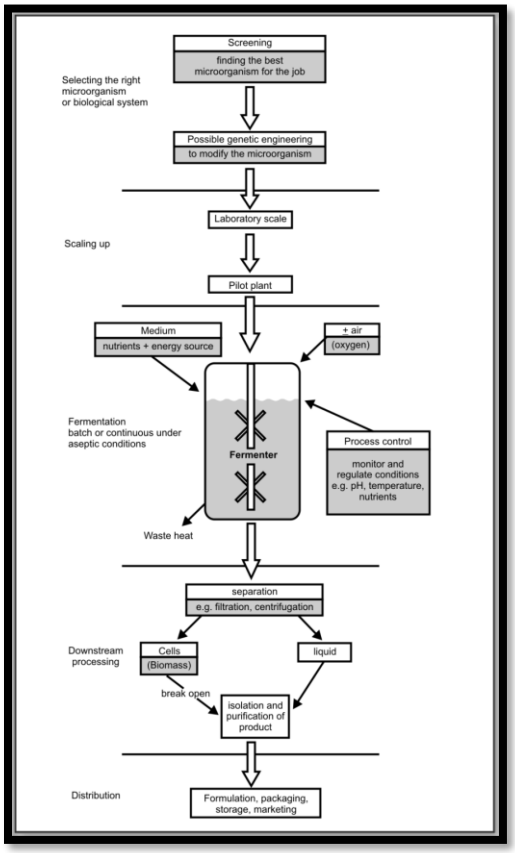
(i) **Denaturation (94°)-** In this step a double stranded DNA molecule is placed at 94°C. So double stranded DNA becomes single stranded & each single stranded DNA functions as a template.

(ii) **Annealing/Cooling (54 °)-** In this step two primer DNA are attached at 3' end of single stranded DNA

(iii) **Extension (72°)-** In this process Taq polymerase enzyme synthesize DNA strain over template.PCR is automatic process because Taq. polymerase enzyme is heat resistant.

(5) **Insertion of Recombinant DNA into the Host Cell/Organism**

There are several methods of introducing the ligated DNA into recipient cells. Recipient cells after making them 'competent' to receive, take up DNA present in its surrounding. So, if a recombinant DNA bearing gene for resistance to an antibiotic (e.g., ampicillin) 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 will grow, untransformed recipient cells will die. Since, due to ampicillin resistance gene, one is able to select a transformed cell in the presence of ampicillin. The ampicillin resistance gene in this case is called a selectable marker.



(6) **Insertion of Recombinant DNA into the Host Cell/Organism**

After initial investigations using ordinary laboratory apparatus it is usual to make a 'pilot plant' that involves use of a small 'fermenter' such as large shake flasks in laboratories. Fermenter is the tank or vessel in which the process will be carried out. Optimum nutrient and physical condltions for maximum yield must be determined.

New factors come into play when the process has to scaled up from pilot production to full-scale (100-1000 *l*). Some of the important factors are as follows:

Maintaining aseptic conditions. It is easy to contaminate both inputs and outputs to the main fermenter.

Physical factors, such as mixing and aerating the media and getting rid of waste heat, create the biggest problems in moving from one scale to another.

To supply enough oxygen in large-scale cultures, air must be forced through the medium because the simple agitation used at the laboratory scale is inadequate. Small bubbles are more effective than large bubbles, so a sparger is used (a tube with small holes). The mixture may also be stirred.

Anti-foaming agents are required to reduce the foaming caused by stirring and aeration.

Heat is produced by the activity of microorganisms and large-scale production. Cooling water must be circulated around the fermenter.

To keep conditions constant, such as supply of nutrients, pH and oxygen concentration, throughout the medium on a large scale. Sophisticated monitoring devices and control processes are needed.