BIOTECHNOLOGY: PRINCIPLES AND PROCESSES

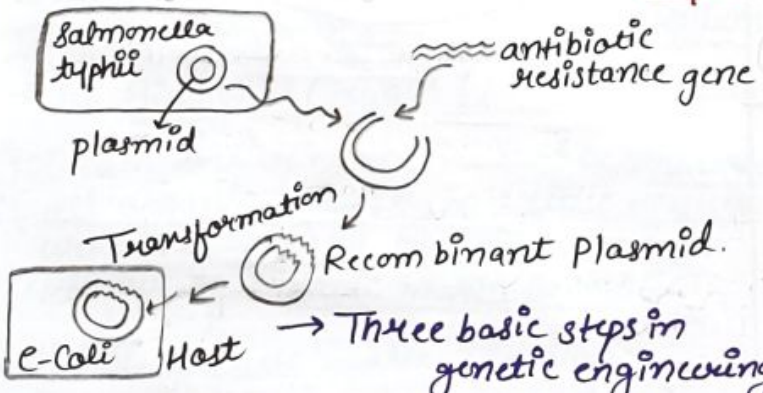
European Federation of Biotechnology (EFB) →
The integration of natural science and organisms, cells, part thereof, and molecular analogues for products and services.

Principles of Biotechnology →

(1) **Genetic engineering**
Technique to alter chemistry of DNA/RNA to introduce these into host organisms.

(2) **Bioprocess Engineering**
Maintainence of sterile conditions to enable growth of only desired microbes.

Construction of first recombinant DNA → (1972) ⊛
Done by Stanley Cohen and Herbert Boyer



→ Three basic steps in genetic engineering.

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

Isolation of Genetic Material

→ To break the cell walls.

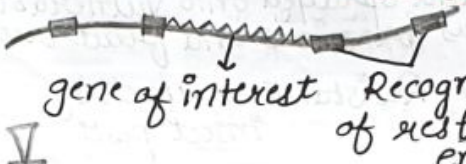
Bacterial Cells → Lysozyme
Plant Cells → Cellulase
Fungal Cells → Chitinase

→ Then cells are treated with → protease, ribonuclease, Lipase.

→ **Spooling** → Treatment with chilled ethanol to collect DNA.

Separated DNA is treated with **restriction endonuclease**

endonuclease → Cuts nucleotide from middle
exonuclease → Cuts from ends



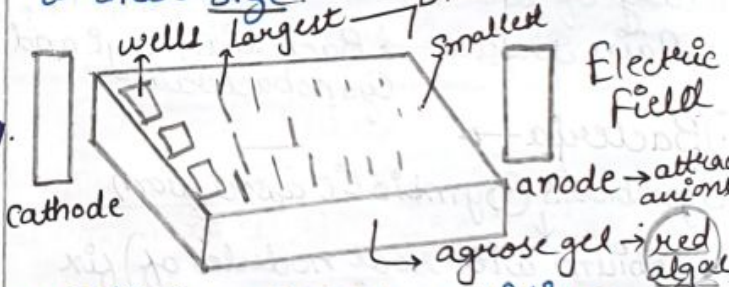
→ pieces of DNA.

→ **Separation and Isolation of DNA fragments** →

→ **Gel Electrophoresis.**

→ Negatively charged DNA pieces can be forced to move towards the cathod (+ve) under an electric field through a medium (agarose).

→ DNA fragments separate according to their size.



→ DNA is stained by ethidium bromide followed by UV Ray exposure ⊛ (Orange Colour) ⊛

→ **elution**: Extraction of DNA piece from agarose gel ✓

#selving effect

Polymerase Chain Reaction

↳ To make billions of copies of segment of DNA/gene.

→ Kary Mullis.

→ Taq polymerase → Heat resistance → from thermus aquaticus.

→ Template separate.

→ Primer

→ Nucleotides

30 cycles

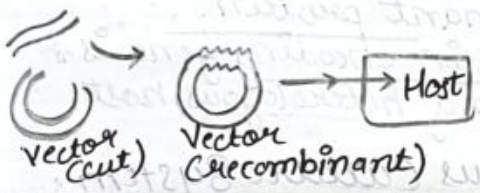
→ 3 Steps: →

① Denaturation (Strands get separated) 5' 3' / 3' 5' . 90°C temp

② Annealing 5' 3' / primer / 3' 5' . 50°C temp

③ Extension. (Taq polemerase) 5' 3' / 3' 5' . 70°C temp

amplified (~1 billion) - time...



Tools of Recombinant DNA Technology

↳ Restriction Endonuclease (nuclease → DNAase, RNAase)

→ In 1963, 2 enzymes responsible for restricting growth of bacteriophage in E.Coli were isolated.

- Methylase
- Restriction Endonuclease

→ 1st restriction endonuclease → Hind II (has recognition sequence of 6 base pair) ⊛

→ This sequence is called recognition sequence.

→ Recognition sequence are palindrome

→ 900 restriction enzymes have been isolated, from over 230 strains

→ Restriction endonuclease forming sticky ends are used in genetic engineering, while those producing blunt/flush ends are not used.

→ Naming of Restriction enzymes

EcoRI → order in which enzyme was isolated from particular strain of bacteria.

- Eco → Escherichia (Genus)
- Co → Coli (Species)
- R → RY13 (Strain)
- I

EcoRI → 5' GAATTC 3' / 3' CTTAAG 5' Vimp

Competent Host

→ Making Host Cells competent to take up DNA.

① Treat with Ca^{+2}

→ Ice → 42°C → Ice

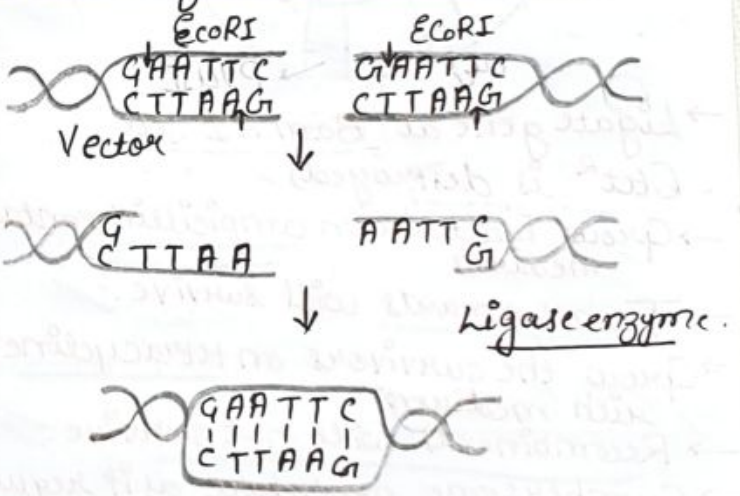
Heat Shock

② Microinjection: → DNA is directly injected in nucleus of an animal cell with help of a micropipette.

③ Biolistics/Gene Gun → Plant cells are bombarded with high velocity microparticles of gold or tungsten coated with DNA.

④ Disarmed pathogen vectors

Action of Restriction Enzyme →



Cloning Vectors

→ Features of Cloning Vectors

① Origin of Replication (ORI)

→ Replication starts from this sequence.

→ It controls the copy number

Vector – Bacteria – plasmid.

ds DNA, extra chromosomal, self replicating, only small amt of DNA transfer

② Cloning Sites / Recognition Sites

→ Recognition sequences of restriction endonuclease are called cloning sites.

→ Good Vector has recognition sequence of many restriction endonuclease, but single recognition sequence for each restriction endonuclease.

③ Selectable Marker

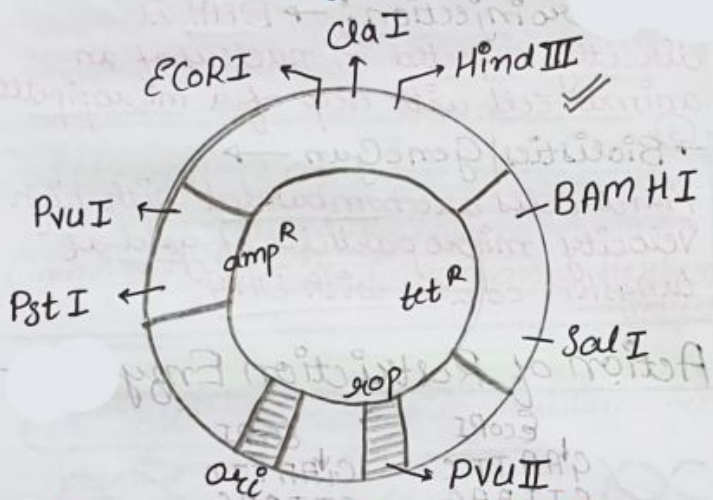
→ To select transformants from non-transformants.

→ To select recombinants from non-recombinants.

→ e.g. → Gene coding resistance to anti-biotics.

→ e.g. → pBR 322 → plasmid number

plasmid — Bolivar and Rodrigues



→ Ligate gene at BamHI site (tet^R is destroyed)

→ Grow bacteria on ampicillin containing medium

→ Transformants will survive.

→ Grow the survivors on tetracycline rich medium

→ Recombinants will not survive.

→ Cumbersome procedure as it requires two plating

→ Bacteria is plated in ampicillin killing off all non-transformants.

→ Also, X-gal is added which gives blue colour with β-galactosidase.

→ So, desired colonies are white/colourless

Acid/B for pH

PUC Vectors

→ Insertional Inactivation

→ PUC vector has both ampicillin resistance gene and gene for β-galactose ✱

→ Gene is ligated in such a way that β-galactosidase gene is inactivated.

Vectors for Cloning in plants and Animals

→ Dicot plants → Agrobacterium tumifaciens

It has Ti plasmid → Tumor Inducing (cause crown gal disease)

→ Animals → RetroViruses ✱

Obtaining a foreign gene product

→ Recombinant protein.

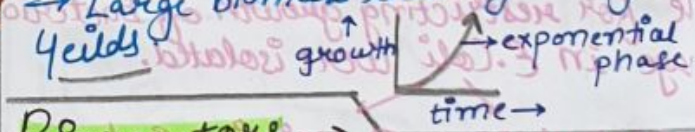
If any protein encoding gene is expressed in a heterologous host.

① Continuous Culture System

→ Used medium is drained out from one side while fresh medium is added from the other

→ Culture is maintained in log / exponential phase ✱

→ Large biomass leading to higher yields



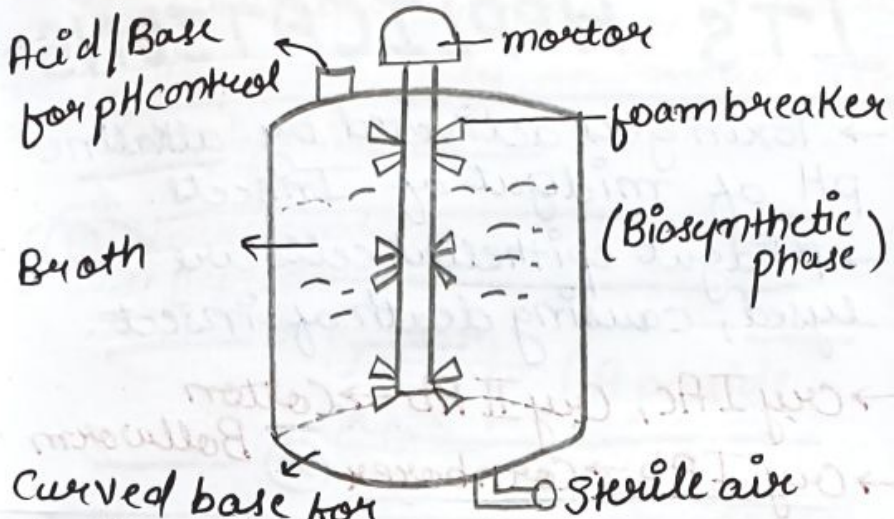
Bioreactors →

Containers in which, large volume of culture can be processed (100-1000 Lts)

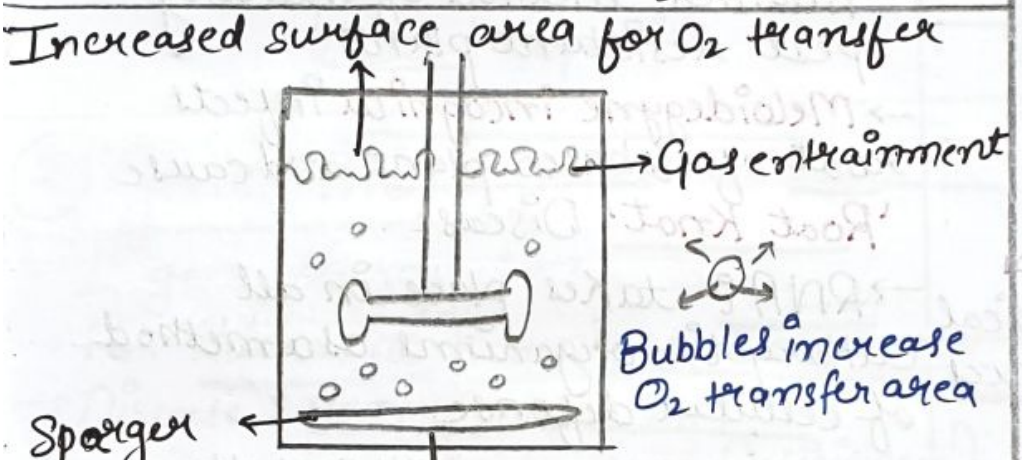
→ Bioreactors are Vessels in which raw materials are biologically converted in specific products ✱

Bioreactors

- Simple Stirred tank Bioreactor
- Sparged Stirred Tank bioreactor



→ Simple Stirred Tank Bioreactor



– Sparged stirred Bioreactor

Downstreaming Process

→ Separation
→ Purification
→ Preservatives

} Downstreaming process

NEET SLAYER