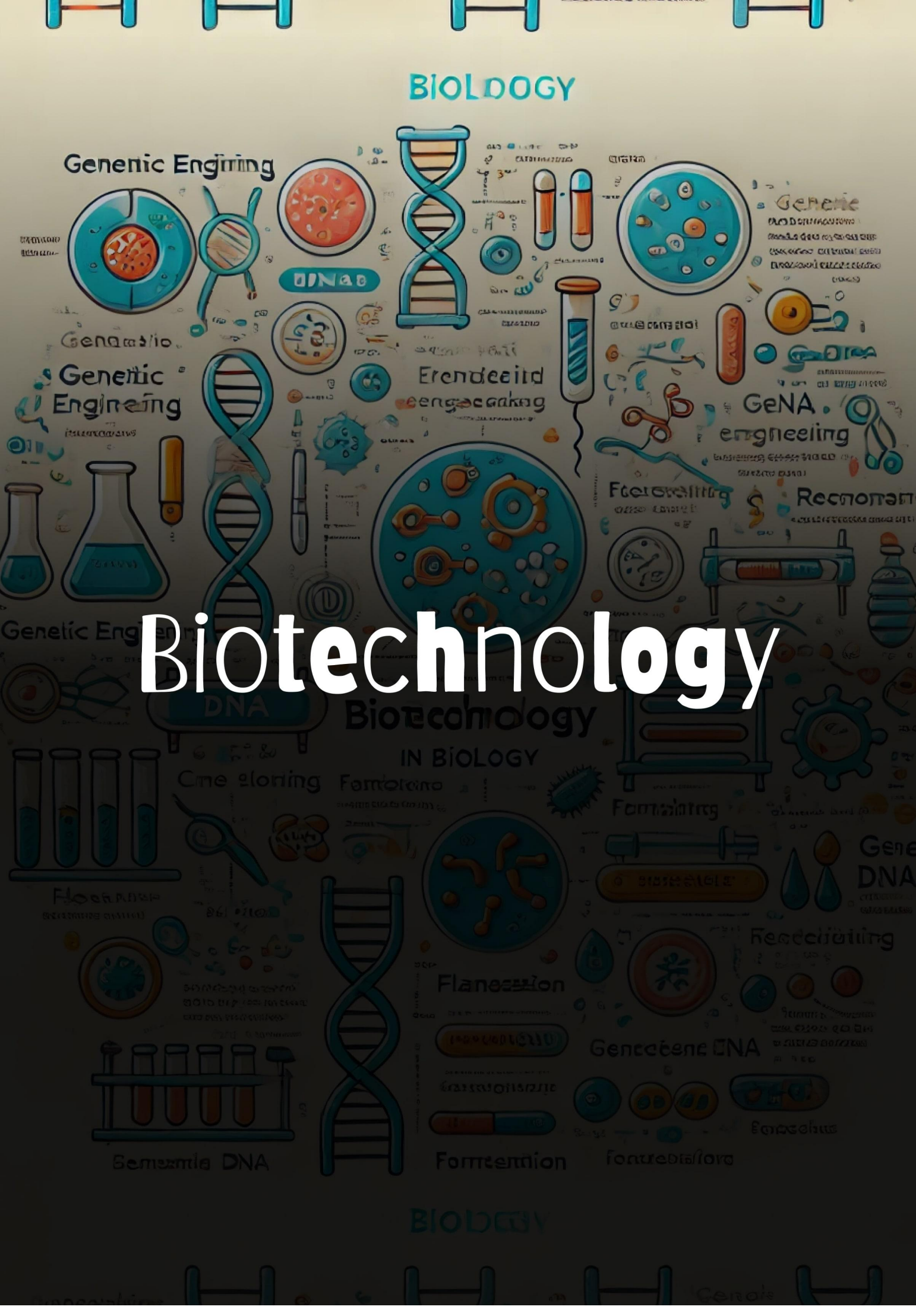


Biotechnology

BIOTECHNOLOGY ☆

- use of natural science, microorganism, animal cells, plant cell their product for human welfare.

- According to European Federation of Biotechnology (EFB) "The integration of natural science microorganism, their products, cells, parts, there of, molecular analogue for product and services.

OLD BIOTECHNOLOGY

Before 1970, related to milk and dairy products.

NEW BIOTECHNOLOGY

After 1970, Genetic Engineering.

The term was given by Karl Ereky for pig production.

- Two milestone discoveries of Biotechnology were
  - Discovery of plasmid (vector)
  - Discovery of Restriction Endonuclease (Molecular Scissors.

- Cohen and Boyer made first recombinant DNA by combining antibiotech resistance gene with the plasmid of Salmonella typhimurium.

Paul - berg ⇒ father of Genetic Engineering.

Simian Virus - 40 (S. V - 40) (Passenger DNA) → Vector → E. Coli (Host)

Tool of Biotechnology

1. Enzymes
2. Vector
3. Passenger
4. Host cell

Lysing Enzymes

They will cause cell lysis.

EXAMPLE:→
- (a) For a bacterial cell → lysozymes.
- (b) For fungal cell → chitinases.
- (c) For plant cell → Cellulases and pectinases.
- (d) For animal cell → Lipases and Proteases.

Cleaving Enzymes

These enzymes will cut the DNA.
They are of two types :→

(a) Exonucleases → They will cut the DNA nucleolite from terminal end.

(b) Endonucleases → They will cut the DNA from inner sides.

Restriction Endonucleases

- These enzymes are produced by bacteria.
- Purpose:→ For self-defence against bacteriophages.
- They will cut the DNA at specific site, these are known as palandromic sequence.
- These sequences are 4|6|8 nucleotide long sequence.
- Bacterial genome undergoes methylation process, so, these R.Es will not recognise the palandromic sequences.
- More than 900 R.Es have been isolated from 230 bacterial strain.
- First R.Es discovered was Hind-II by Smith.
- Arbour, Nathan, Smith discovered E.CO.R. I. (A,N,S) → Nobel Laurels.

Naming of Restriction endonuclease

E Co R.I → Roman Number → order of Isoln

- E → Genus → Escheria
- Co → 2nd 3rd → Species → coli
- R → 4th (not essential) → Strain → Ry
- I → Roman Number → order of Isoln

Cut ends

Oblique

G↓A A T T C
C T T A A↑G

G A A T T C
C T T A A G

Straight

G G↓C C
C C↑G G

G G | C C
C C | G G

TYPES OF RESTRICTION ENDONUCLEASE

I

- Specific
- It requires Mg^{+2} ATP S. Adenosyl methionine
- Bifuctional (cutting & methylation

II

- Very specific
- It requires only Mg^{+2}
- only cutting

III

- Very specific
- It requires Mg^{+2} ATP, S. Adenosyl methionine.
- cutting and methylation - bi functional.

Type II R.Es are used in Biotechnology.

E.Co.K.I →	5' G↓A A TT C 3' 3' C T T A A↑C 5'	Escheria coli
Hind - III →	A↓A G C TT T T C G A↑A	H. influenzea
Bam - I →	G↓G A T C C C C T A G↑G	Bacillus amyldiquifacience
Hae - III →	G G C C C C G G	H. aegypticus
Sma - I →	CC C G G G G G G C C C	Serratia Marcescens
E.Co.K.V →	5' G A T↓A T C 3' 3' C T A ⋮ T A G 5' ↑	

Synthesizing Enzyme

- DNA Polymerase
- Reverse transcriptase

Joining enzymes — T_4 - DNA ligase

Other enzymes — Alkaline phosphatase

It cause removal of phosphate from 5'end so circular DNA will convert in linear DNA.

VECTOR

A good vector must have following three properties :→

ORI site

→ origion of replication initiation site is essential feature.

→ If vector have high copy number then it is perfomation.

Selectable Markers

- Selectable markers are useful for differentiating transformants from non - transformants.
- Antibiotic resistance gene are used as selectable markers.
- Transformant :→ Host with vector.
  Non - transformant :→ Host without vector.

E·coli ← HOST + Vector → Plasmid

Host without vector → Non - transformant

Host with vector → Transformant

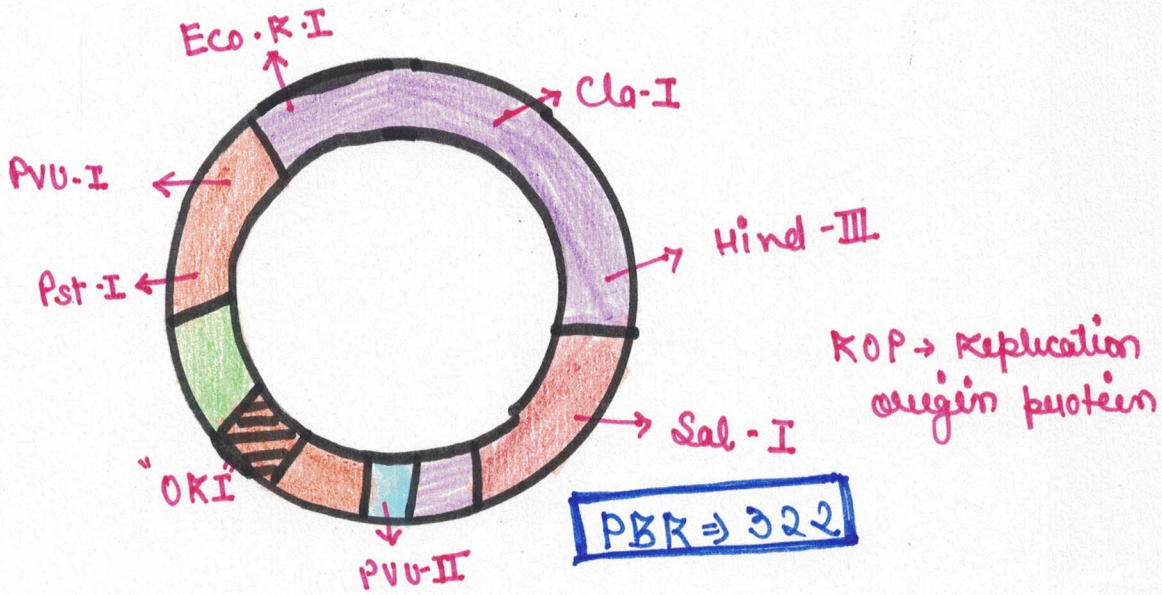
CLONING SITE / RESTRICTION ENDONUCLEASE Site

- Same Restriction endonuclease must be used for vector different R·Es.
- A good vector must have R·Es sites for many different R·Es

1 vector ⇒ Site for many different R·Es

- A vector must have only one writing site for particular R·Es.

Only 1 Site on Vector → for particular R·Es.



TYPES OF VECTOR

PLASMID

- Circular double stranded extra chromosomal.
- Independent replication

PBR - 322

PBR - 327

P ⇒ Plasmid

BR ⇒ Bolivar & Rodriguez lab

PUC - 18

UC ⇒ University of California lab

PUC - 19

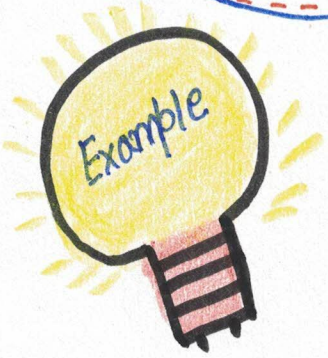
For PLANT CELL

- Ti-Plasmid
- Ri-Plasmid

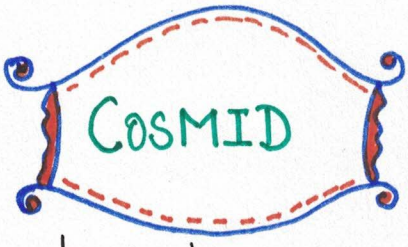
- they can carry gene from 0.5 to 8kb.

BACTERIOPHAGE

- These are viruses.



λ-phage, m-phase
(9kb to 28kb gene size)



- Cos site + Plasmid
- cos site is present in bacteriophages and it is 12 base pair long sequence and causes package of DNA. (30 kb to 45 kb)

- For plant ⇒ Tobacco Mosaic virus cauliflower Mosaic Virus
- For Animals ⇒ → Retro Virus (mostly commonly used)
  → Adeno virus

- They have filamentous DNA of bacteriophages attached with plasmid.

BAC → 50 - 300 kb
YAC → 1000 - 2500 kb
MAC → > 2000 kb

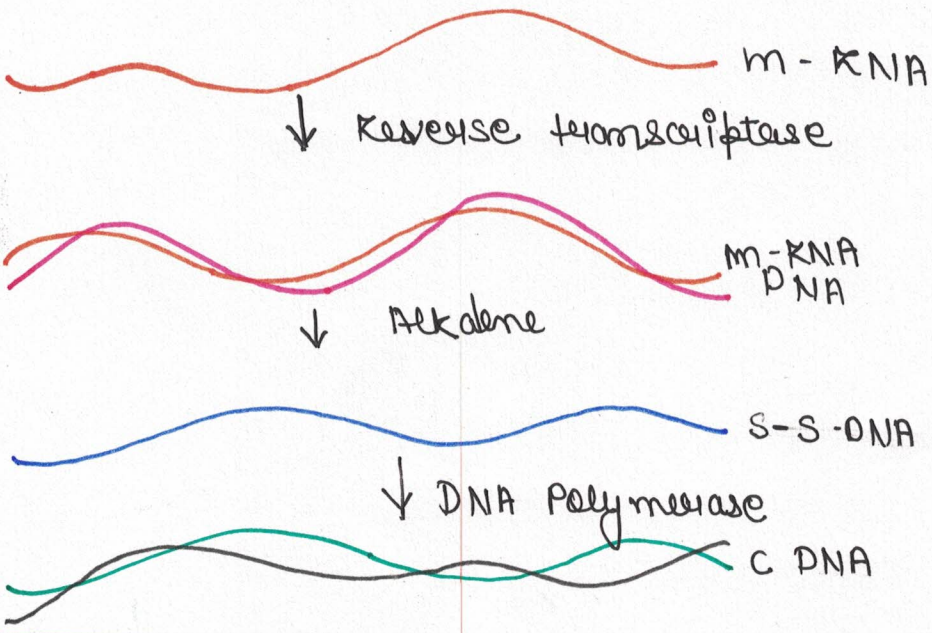
- They can be used your plant and animals both.

PASSENGER DNA

Random Fragmented DNA

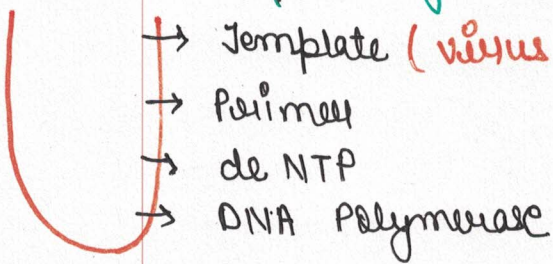
- DNA is exposed to R.Es so random fragments of various length will results.

Complementary DNA



Synthetic Gene

- Kornenberg 1961 → First synthetic gene were formed over template strand (Non-functional)



Khorana → without template

1968 → Yeast → Alanine t-RNA Gene → 77 bp long → Non-functional

1979 → E. Coli → Tyrosine - t-RNA → 207bp → Functional

Khorana discovered Ty - DNA - ligase

HOST CELL

- Bacterial cell
- Yeast cell
- Plant cell
- Animal cell

Technique of Recombinant DNA Technology

Transfer of desired gene in a host cell take place in following steps :-

Step-1 LYSIS OF CELL

* For bacterial cell → Lysozymes
* For fungal cell → Chitinase
* For plant cell → Cellulase + pectinase
* For animal cell → lipases + proteases

Step - 2 PRECIPITATION OF DNA AND EXPOSURE TO RESTRICTION ENDONCLEASES

- chilled enthanol is used to precipitation DNA after cell lysis.

Step - 3 AMPLIFICATION OF PASSENGER DNA

- If sample DNA is less in amount then polymerase chain rxn is performed.

POLYMERASE CHAIN REACTION

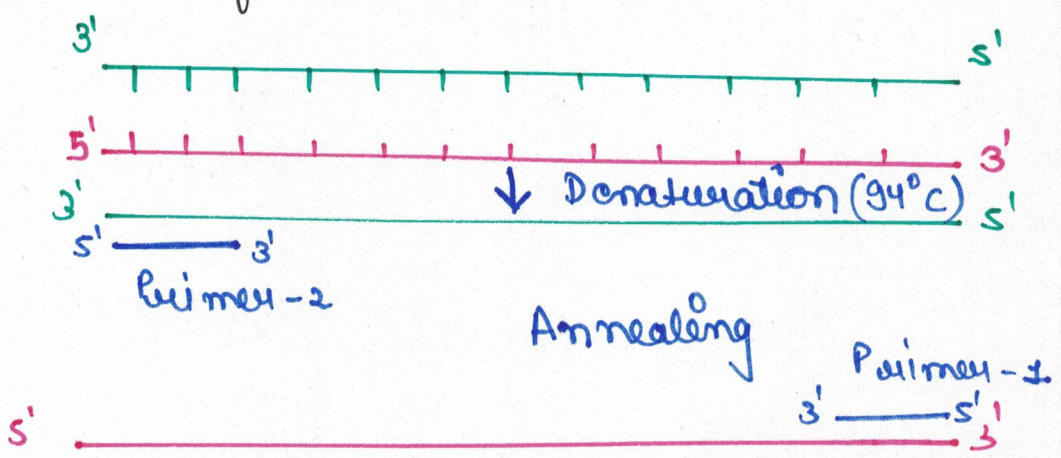
- Also known as People's choice Reaction
- Kary - Mullis developed PCR and got Nobel Prize in 1993
- Specialised tube → This is used because it can tolerate high temperature.

Taq - polymerase ⟶ Thermus aquiticus

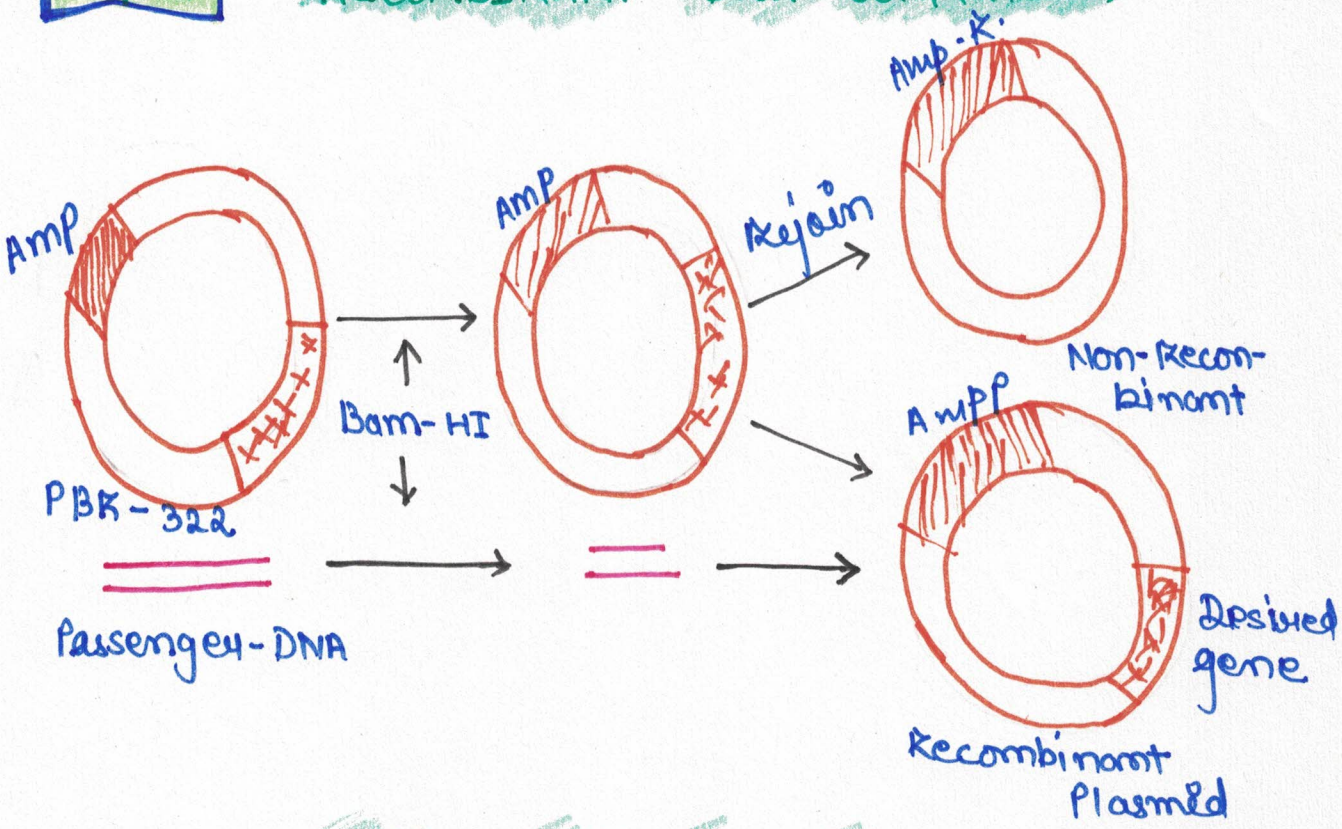
Pfu ⟶ Pyrococcus furiosus

Vent ⟶ Thermococcus litoralis

- In around 30 cycles 1 billion copies are found of a sample DNA.



Step-4 RECOMBINANT DNA FORMATION



Step-5 ENTRY INTO HOST CELL

ENTRY INTO BECTERIAL CELL →

Bacterial cell is treated with lysozyme and calcium so it becomes porous for some time. These cells are transformed to ice temperature then they are shifted to 42°C temp. and again shifted back to ice temperature.

ENTRY INTO PLANT CELL →

Ti - Plasmid and Ri plasmid are used for entry into plant cell.

Lysome mediated - these are vacuoles of lipid membrane.

Step-6 PLATING METHOD :→

Principle :→ E.coli, PBR-322, Passenger DNA
Bam H-I

Non-Transformant
Non-recombinant
↓
Amp-R (absent)
Tet-R (absent)

Transformate
Plasmid present

Recombinant
Amp·R·(+)nt
tet·R (-)nt
Desired gene (+)nt

Non-Recombinant
Amp·R·(+)nt
tet R (+)nt

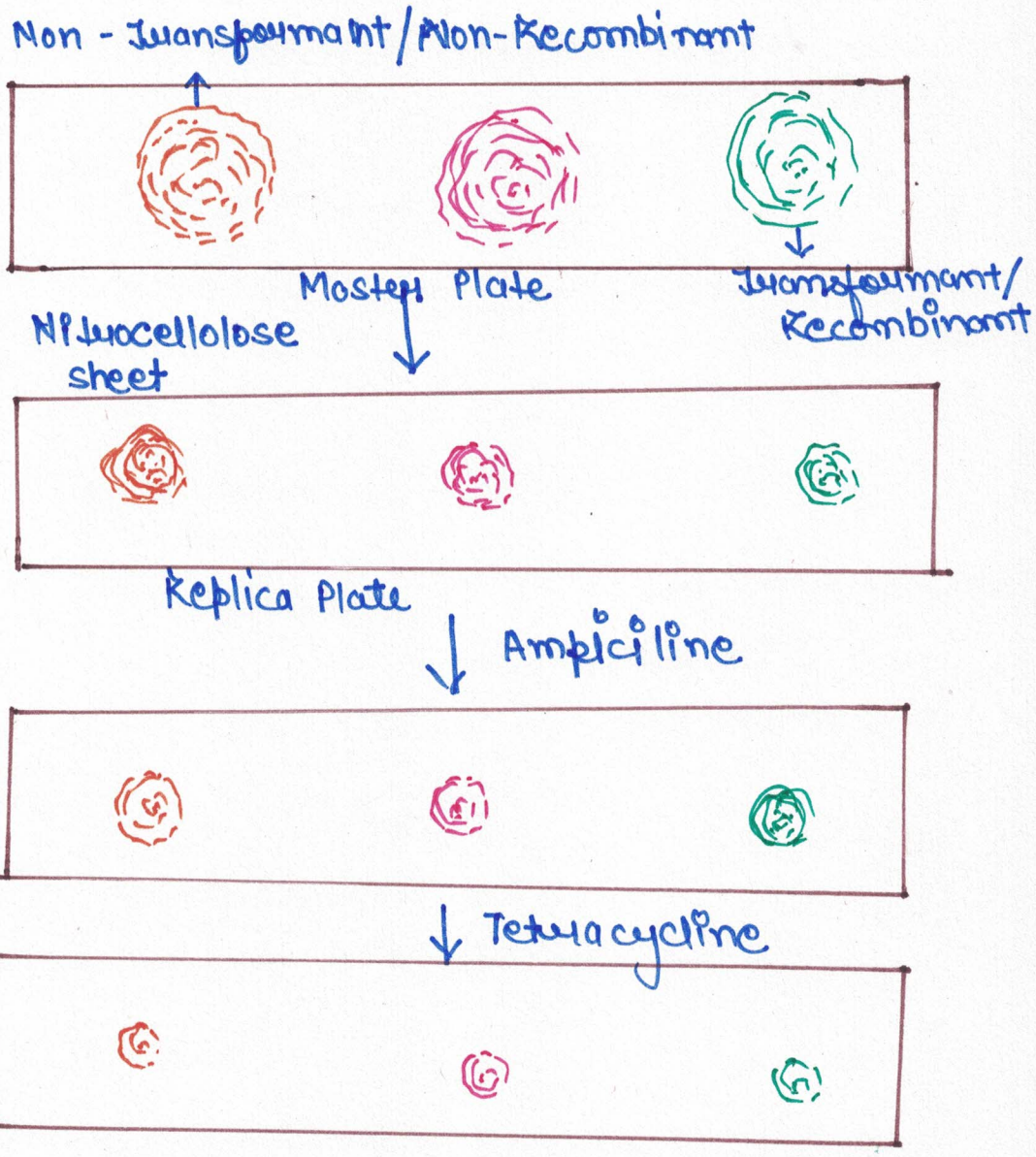
Growth PLATE
↓ Ampicilline
→ Non-Transformant - Death

→ Transformant

Non-Recombinant
↓
Growth

Recombinant
↓
Death.

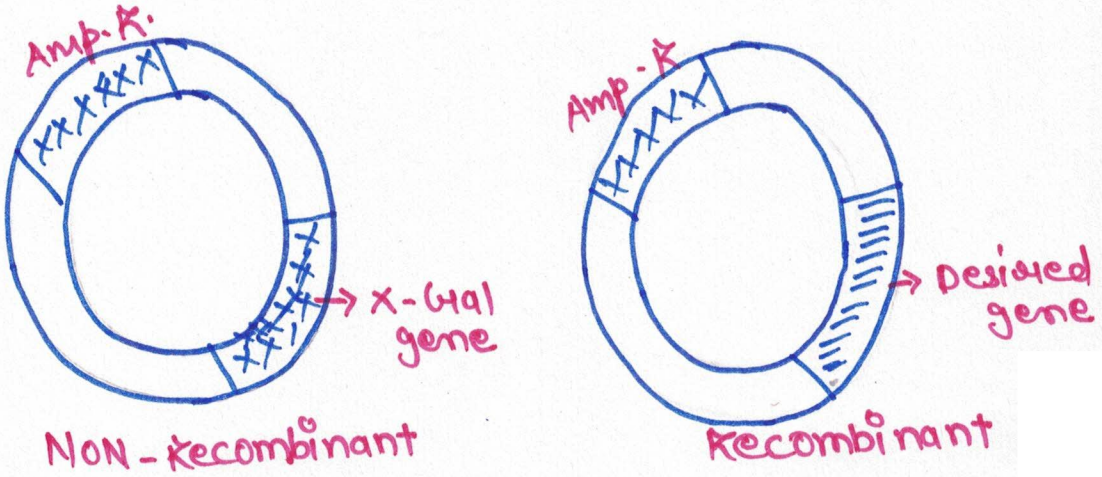
Replication Plate Method



Blue - white Screening Method

Replica - plate method is cumbersome procedure because simultaneously plating on two plates requires (Master plate and Replica Plate)

So, Blue - white screening method was discovered



- It is one of the best example of natural genetic engineering.
- This bacteria has a Ti - plasmid this plasmid has two parts.

Ti-plasmid ⇒ (1) T-DNA (2) Vir - region

1. T-DNA → this region has Ti- gene it is tumor inducer gene which causes crown gall tumor.
2. Vir - region → this region has 6 operon system i.e. A, B, C, D, E, G.

- In dicot plant acetyl syringosine is present this act as inducer so Ti plasmid causes crown gall disease in dicots not in monocots.

DISARMD PLASMID

When Ti-gene is removed from Ti plasmid and desired gene is inserted such plasmid is known as Disarmd plasmid.

Agrobacterium Khizogenus :-

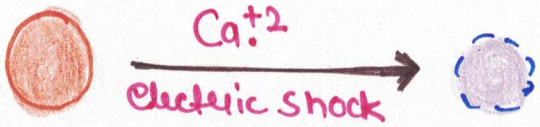
Ki-plasmid causes hairy root disease.

ENTRY INTO ANIMAL CELL

Entry into animal cell is generally performed during early part of life / zygote or embryo). because in these stage there are totipotent cell and they are capable of giving rise to a whole organism.

VECTORLESS GENE TRANSFER

Method-I ELECTROPORATION :→ Cell is exposed to minute electric shock for gene entry.

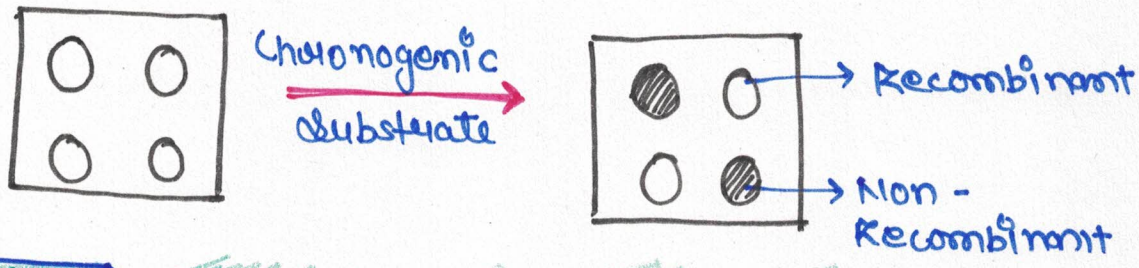


Method-II BIOLISTIC GUN / PARTICLE GUN / GENE GUN :→ Gold or tungsten particle coated with DNA are bombarded over cell for gene entry.

Method - III Chemical Method - Application of polyethylene glycol (PEG) over cell will help in gene entry.

Chromogenic substrate → (β galactosidase) Blue colour

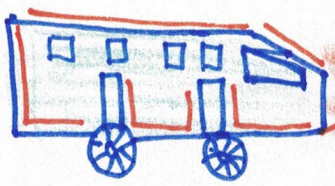
transformant Plate



Step - 7 OBTAINING GENE PRODUCT FROM FOREIGN GENE

→ At three different level gene product can be obtain :-

- At laboratory level → At this level ideal growth conditions are determined.
- Pilot Project level → At this level cost effectiveness is determined.
- At Industry level quality control is maintained.



BIOREACTORS

- These are steel containers having volume of 100 to 1000 litres.

PARTS OF BIOREACTORS :→

- Temperature control unit
- PH control unit
- Foam control unit
- oxygen delivery system
- Agitator system
- Support part (sampling part)

Two type of culture in Bioreactor

- Continus culture → suspended growth system
- Batch Culture → support growth system

DOWN STREAM PROCESSING

After completion of biosynthetic stage the product undergoes series of process to improve quality, they are collectively known as **Down - stream processing.**

- Speration and purification
- Addition of flavouring agent
- Addition of preservative
- Clinical trials of medicine before launch