

# BIOTECHNOLOGY : PRINCIPLE AND PROCESSES

↓  
Given by : Karl Ereky (1970)

→ For large scale production of pigs.

↳ It is the use of organism or their component to generate product and services useful to human beings.

Two types:

- ① Old biotechnology / traditional
- ② New ————— / modern.

(Bfr 1970)

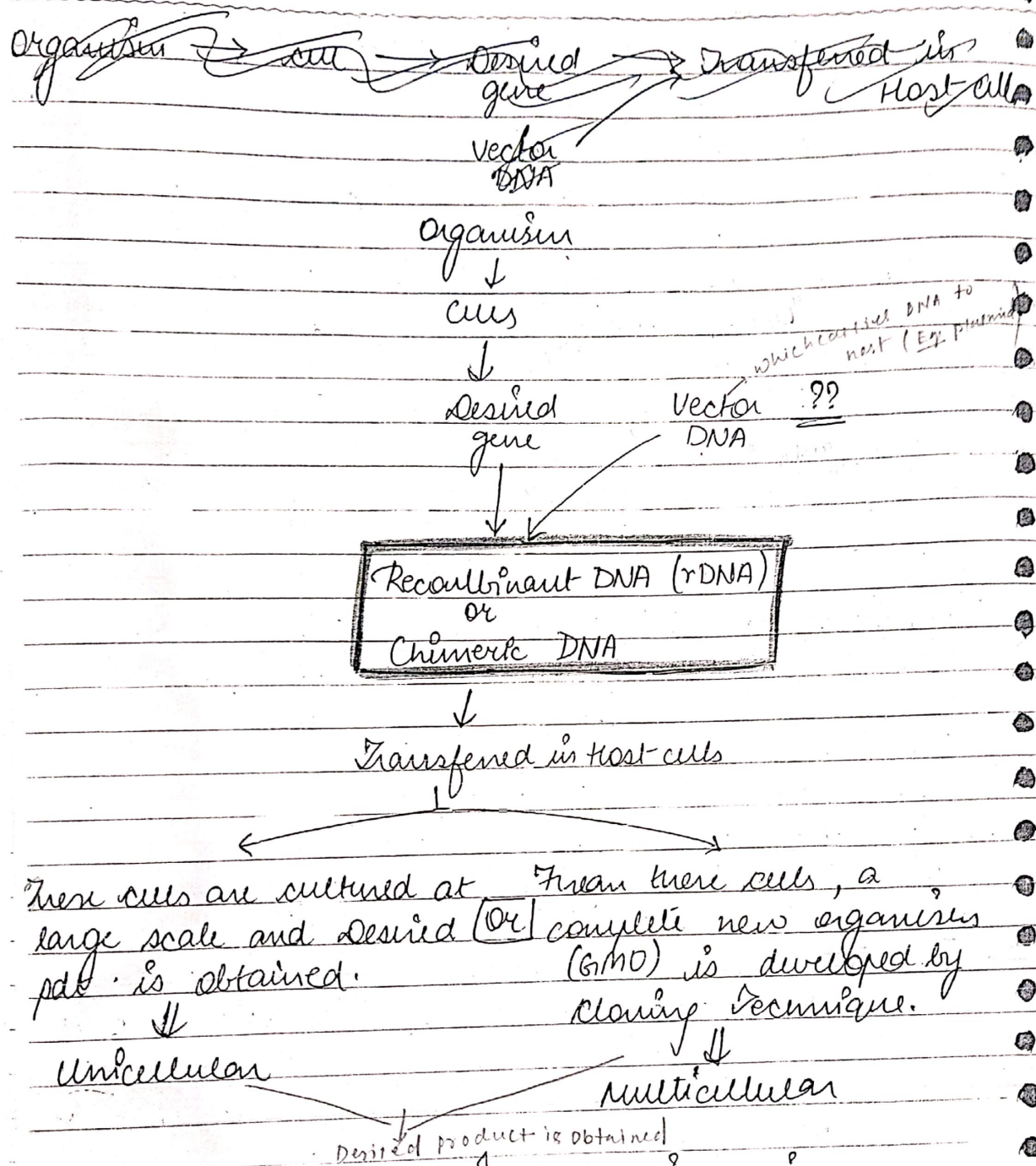
Old Biotechnology: In this natural efficiency of living org. are used to produce useful prod. for human beings.  
eg. curd, bread, wine.

New Biotechnology: after 1970

It is the production technology based on Genetic engineering.  
By this, the genome of org. is modified & then it is used for product.

It is based on two main principle:

- ① Formation of Transgenic organism / GMO by the techniques of genetic engineering. This involves Recombinant DNA Tech. and gene transfer technique.
- ② Maintenance of Transgenic org. / GMO: By large scale culture → Desired prod. is obtained.



\* There are two milestone discoveries:

- (1) Restriction endonuclease enz.
- (2) Discovery of plasmid.



Stanley Cohen & Herbert Boyer

↳ made 1<sup>st</sup> rDNA.

↳ They combined an antibiotic<sup>resistant</sup> gene of bacteria & the plasmid of *Salmonella Typhimurium* and thus forms 1<sup>st</sup> rDNA.

Paul Berg.

↳ 1<sup>st</sup> successful gene transfer.

SV-40 gene virus → In *E. coli*  
by  $\lambda$ -phage

rDNA formation

↓  
gene transfer technique

Two parts : [I] Tools Req.  
[II] Procedure.

Tools : 4 types

- ① Enzymes
- ② Vector DNA
- ③ passenger DNA → desired gene (or) alien DNA
- ④ host cells.

Enzymes. (5 types.)

- ① Lysing enz. → For lysis of cells  
eg. lysozyme, chitinase,  
cellulase, pectinase,  
mucase, lipase.

DNA ligase  
↓  
Molecular glue/gum

Restriction endonuclease  
↓  
Molecular scissors

Alkaline phosphatase  
↓  
prevents recirculation of circular DNA.

Lysosome for Bacteria  
cellulase for Fungi  
Cellulase } for Plants  
pectinase }  
protease }  
lipase } for animals.

2) cleaving enz: Cutting of DNA  
Types.

Exonuclease  
→ Cuts DNA at terminal ends.  
→ not so useful.

Endonuclease  
→ cuts DNA at intermediate places.  
→ special enz:  
Restriction endonuclease  
[Molecular scissors]

3) Synthesizing enz: For synthesis of DNA  
eg. DNA polymerase (DNA → DNA)  
Reverse transcriptase (RNA → DNA)

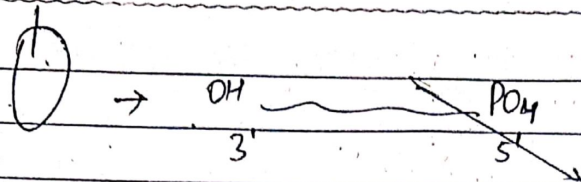
4) Joining enz: for joining of DNA segment.  
eg. DNA ligase  
(Molecular glue/gum)

5) Other enz: Alkaline phosphatase

It removes phosphate group from 5<sup>th</sup> end of DNA.  
It prevents recirculation of any circular DNA.



Imp RE  $\rightarrow$  only in Bacteria / prokaryotes  
(Microbial Varies  $\rightarrow$  Strains)



Restriction endonuclease enzyme :  
V. Imp. at intermediate places

$\rightarrow$  It cuts DNA either obliquely or straight  
at some specific site (Restriction site)

$\rightarrow$  Each Restriction enz. have its own special  
site.

1<sup>st</sup> discovered by : Smith  
1<sup>st</sup> R.E was : Hind II [from Haemophilus  
influenzae bacteria]

later on Smith, Nathans & Arber.

They discovered ECORI enz. from E. coli.

\* So far, 8900 different types of R.E. enz. have  
been discovered from 230 strains of Bacteria.

\* RE. enz. are found in prokaryotes (Bacteria) only  
not in Eukaryotes.

$\downarrow$   
Bact. R.E. enz. Virus  
KO DNA. KO cut V.K.K.K.  
K.H.A.M. K.H. delta K.H.

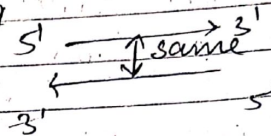
$\rightarrow$  to protect itself from  
virus (Bacteriophage).

\* These enz. are a part of defence system of  
bacteria & protect the bacteria from  
external viral DNA.



This enz. never cut its own DNA because bacteria modify the restriction sites by an enz. Methylase by adding methyl gp.

Restriction site : 4/6/8 bp long  
palindromic DNA seq.



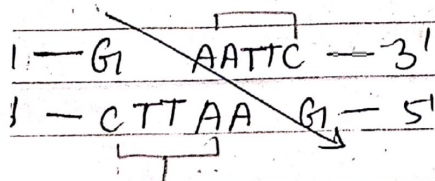
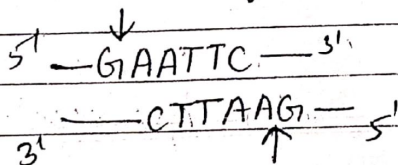
Mode of cutting

Oblique

Cuts DNA near to the centre of palindrome

It produces sticky ends.

ECORI enz.



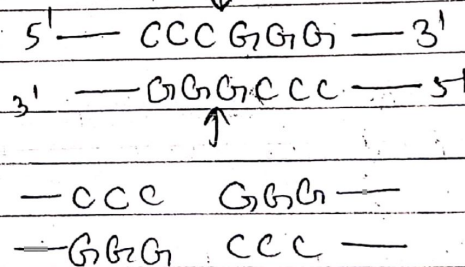
free end / sticky end

straight

Cuts DNA at the centre of palindrome.

It produces Blunt ends or flush ends.

eg. SmaI enz.





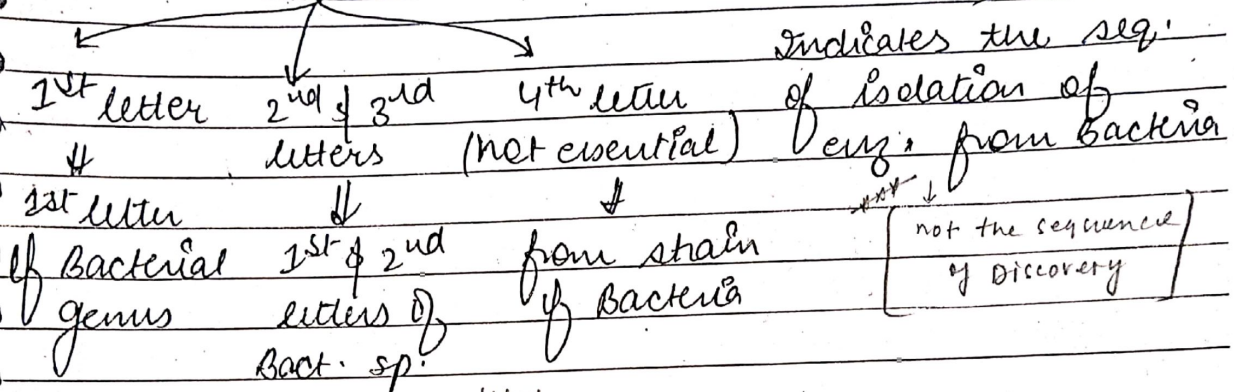
①

## Restriction endo. enz.

### Nomenclature :

↳ Name of R.E. enz. consist of  $\approx$  3/4 letters + Roman no.

3/4 letters + Roman no.



eg. ECORI enz. obtained from E. coli RY13 Bact.

Genus    Species    Strain

seq. no.

Vector DNA : special DNA which can carry / transfer desired gene in host cell.

↓  
It also have self replication ability.

↓  
cloning vectors

⇒ Selection of vector DNA depends on →

Size of desired gene

Type of host cell.



Vector DNA = self replicating ability

(2)

A vector DNA must have atleast 3 important properties:-

① presence of "ori" - It must have atleast one ori, so that it can replicate.

preferably → high copy no. type

② presence of Selectable marker gene:-

↳ special genes which helps in identification of vector DNA / rDNA in host cell.

eg. Antibiotic Resistant gene

Ampr gene / tetr gene

enz. gene → 'gal' gene (galactose)

③ presence of cloning / Restriction sites:-

A vector should have restriction site of several enz. i.e. it should be polylinker but only one site of each enz. should be present.

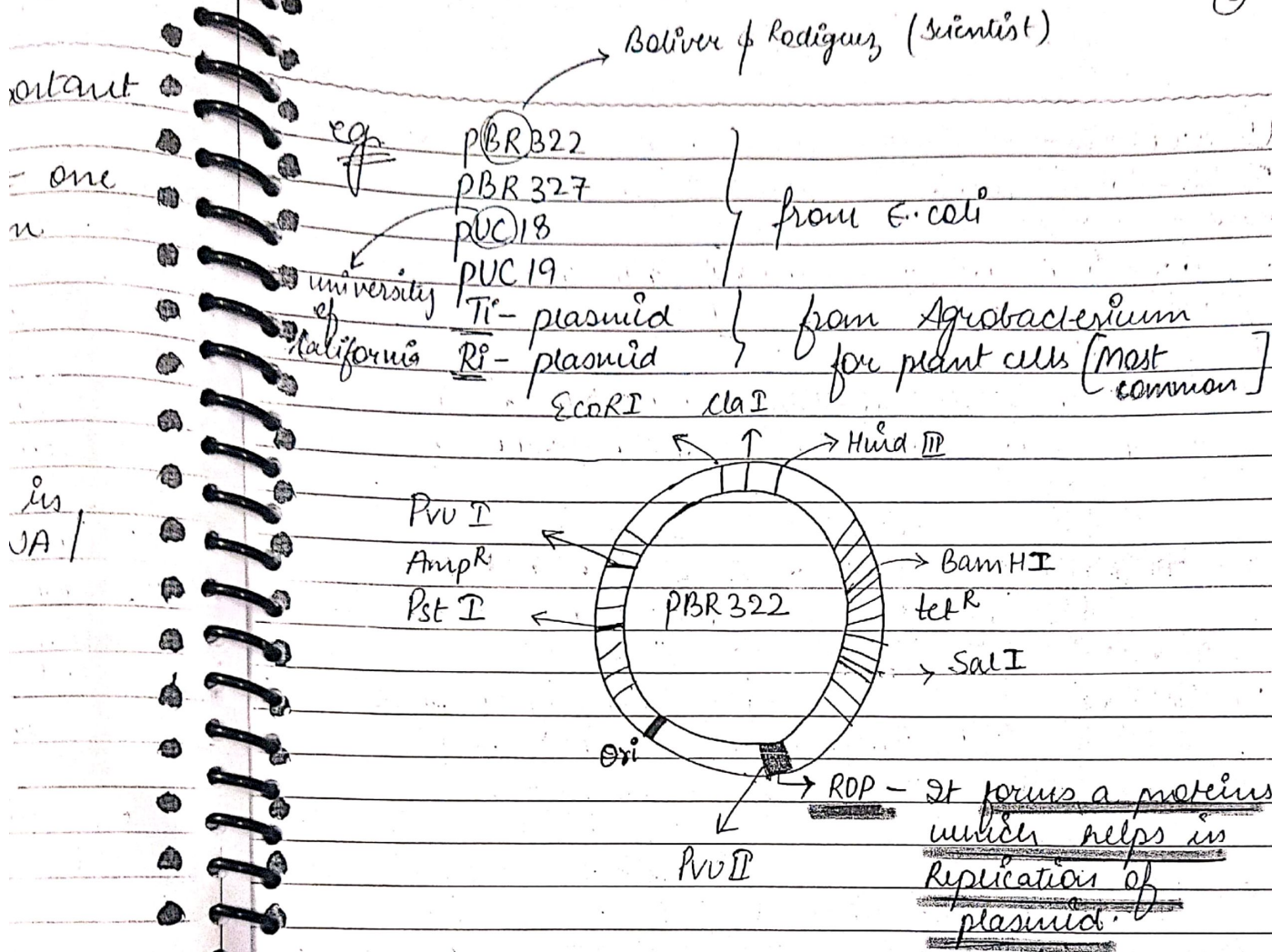
eg. ① Plasmid DNA

↳ V. Common

↳ Capacity → It can carry 0.5-8 kbp sized gene.



(3)



(2) Bacteriophage DNA → capacity - 9-23 Kbp

↳ virus that infects bacteria &

eg.  $\lambda$ -phage has the ability to replicate in bacterial cells.  
M-13 phage

(3) Plant and animal viruses

eg. CMV (for plant cell)

SV-40 (for mouse cell)

Adenovirus  
Herpes virus  
Retrovirus → Most common Vector for animal cell

#### ④ Cosmid

↳ Artificial vector

[ cos' site of Bacteriophage DNA + Plasmid ] Capacity = 35-40 kbp

→ 12bp long special sequence which help in Replication and Packaging of DNA.

#### ⑤ Phagemid

↳ Artificial vector

[ DNA of filamentous Bacteriophage + Plasmid ] Capacity ⇒ 10 kbp

#### ⑥ Artificial chromosome

↳ Large sized ~~artificial~~ artificial vector.

↳ Recombinant Artificial chromosome

BAC ⇒ 50 - 300 kbp

YAC ⇒ 1000 - 2500 kbp

MAC ⇒ > 2000 kbp

↳ Mutation # C

#### ⑦ Transposons (jumping genes)

↳ movable part of gene natural part of gene.

genes which can change their locus (move from one locus to other locus).

↳ Discovered by : B. McClintock.



(Lady Mendel)

(5)

Transposons discovered by Macclintok  
↓ (Lady Mendel)  
In maize

## Passenger DNA

↳ It is the desired gene which has to be transferred in host cells.

There are 3 methods to obtain desired gene:

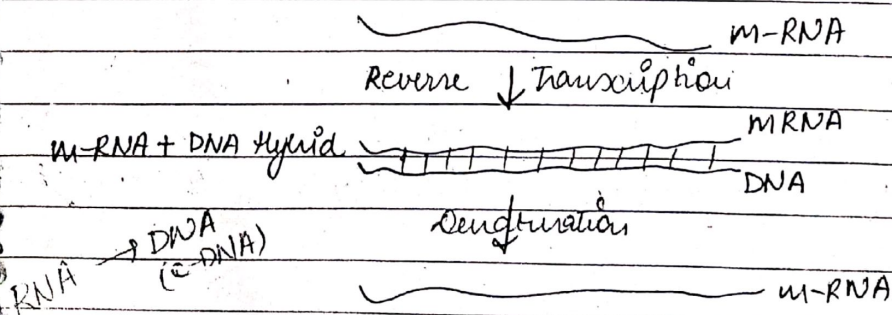
### (1) Random DNA method

In this desired gene is obtained directly from cells by the use of restriction enz. digestion, gel electrophoresis, Southern Blotting and autoradiography technique.

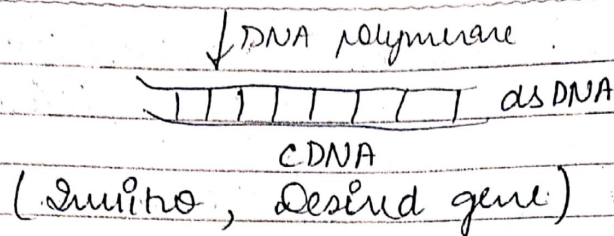
Entire process is just like DNA fingerprinting only RE and probe will change.

### (2) cDNA formation (Complementary / copy DNA)

↳ It is the DNA synthesized from RNA.



(6)

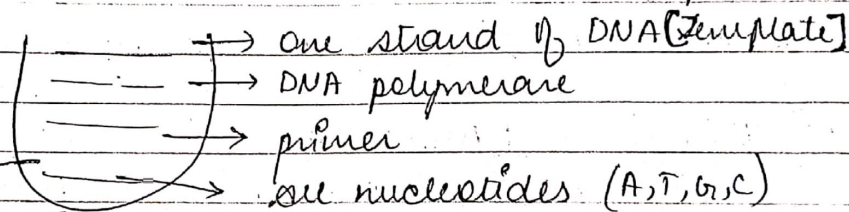


### ③ Synthetic DNA formation

- ↳ artificial synthesis of entire desired gene.
- ↳ For this, entire code seq. must be known.

1<sup>st</sup> done by ⇒ Kornberg (1961)

↳ In vitro DNA Replication



He obtained  
a ds DNA

\* H. G. Khorana : Complete artificial gene synthesis  
(without Template)

↓  
(1968) ⇒ He synthesized Alanine t-RNA gene of Yeast

77 bp long  
Non functional in cell.

Later (1979) ⇒ He syn. Tyrosine t-RNA gene of E. coli.

207 bp long  
Functional in cell.



(7)

### Host Cells.

cells in which desired gene is transferred.

→ Several types

(Depends on exp.)

→ Bacterial cell

→ yeast cell

→ Plant cell

→ Animal cell

### Limitations

① Pure culture X

They are separated and their pure culture is developed.

(II) Procedure of rDNA formation & Transfer of gene :

major steps :

### (1) Isolation of DNA

cells having  
desired gene  
are taken

Lysis of cells  
by Lysing  
enzymes

Protoplast  
obtained.

Finally purified  
DNA in ppt. ←  
by chilled ethanol.

↓  
From protoplast  
some macromolecules  
are removed by  
their digestive enz.  
like proteins by  
protease, RNA by  
RNase, Ribonuclease.

2) Cutting of DNA at specific sites to obtain desired genes.

From separated DNA, desired gene is obtained by Random DNA method.

→ Desired gene can also be obtained by cDNA formation or synthetic DNA formation.

→ At the same time a suitable vector is also selected and isolated.

3) ~~Amplification~~ Amplification of desired gene by PCR :

The obtained desired gene is very less in amount so its amount is ↑ by PCR.

PCR → polymerase chain reaction

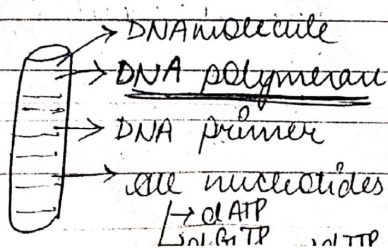
or people's choice reaction

Invented by ⇒ Kary Mullis (1983)

PCR ⇒ It is an Automated, Rapid Technique of in-vitro DNA amplification.

PCR based on → in vitro DNA replication  
↓  
test-tube

Denaturation - Renaturation properties of DNA





{ DNA primers used in test tube.  
RNA primer used by cell.

★ DNA polymerase used was → special type of  
Heat Resistant enzyme obtained from some  
Thermophilic Bacteria.

→ eg. Taq polymerase : from *Thermus aquaticus*  
(72°C → Best efficiency) Bacteria.

→ Pfu polymerase : from *Pyrococcus furiosus* Bact.

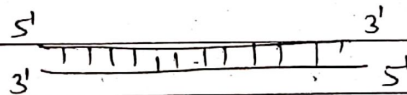
→ Vent polymerase : from *Thermococcus littoralis* Bact.

PCR is a (3) step Thermal cycle :

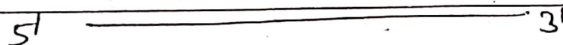
① Denaturation (92°C)

② Annealing (754°C)

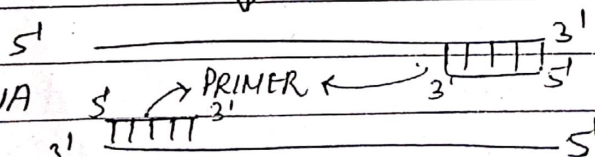
③ Extension (72°C)



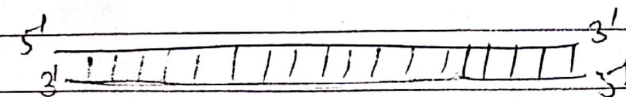
↓ Denaturation (92°C)



↓ Annealing (754°C)



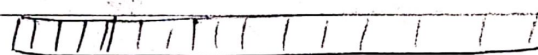
↓ Extension (72°C)



after the  
completion of  
each cycle  
the amt of DNA  
gets doubled.

no. of DNA  
after (n) cycles

↓  
 $2^n$

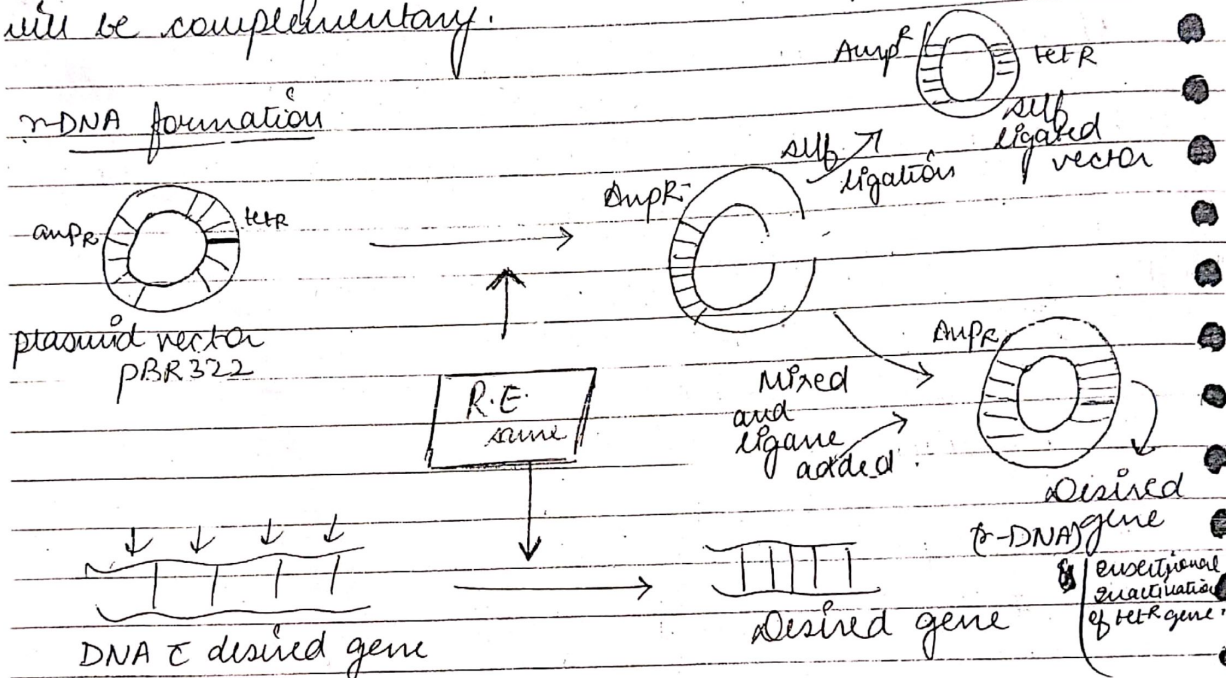


DNA primers attach in  $3' \rightarrow 5'$  dir<sup>n</sup>.

#### (4) Formation of Recombinant DNA (r-DNA)

Both desired gene and vector DNA are cut by same R.E. enz. so that their free ends will be complementary. Both are mixed and ligase enz. added. Ligase will join both of them and forms r-DNA.

#### r-DNA formation



R.E. used is same for both desired gene and vector but RE for or taken according to desired gene. only then r-DNA formed.

Self ligation of circular DNA also occurs which cannot be stopped by alkaline phosphatase as it would remove the  $\gamma$  phosphate from 5' end of all the vector DNA.

Insertional inactivation of *tetr<sup>R</sup>* gene.



⑤ Transfer of r-DNA in suitable host cells :

↳ v. complicated.

↳ done by several methods (Depends on type of host cells)

① Transfer of r-DNA in Bacteria :

Bacterial cells are treated w/ lysozyme and divalent ion ( $Ca^{+2}$ )

② creates pores in cell wall of bacteria so that its efficiency to take r-DNA increases.

↓  
r-DNA is forcefully inserted in bacterial cell by Heat/Thmp shock treatment.

Heat shock treatment.

r-DNA and bacterial cells are mixed and placed in ice ( $0^{\circ}C$ ) → suddenly there are shifted at high temp. ( $42^{\circ}C$ ) → immediately placed back in ice ( $0^{\circ}C$ ).

② Transfer of r-DNA in plant cells

↳ commonly done by Ti-plasmid / Ri-plasmid vector

natural genetic engineer

← from agrobacterium tumifaciens

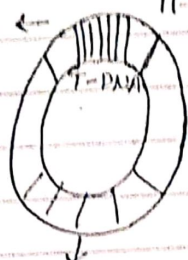
↓  
from A. Rhizogenes

causes crown gall disease in dicot plants.

↓  
Hairy root

Ti-plasmid → Tumor inducing plasmid  
Desired gene is inserted in Ti gene.

new plasmid is known as disarmed plasmid.



virulence region (vir) ↓  
consists of 6 operons. (A, B, C, D, E, G)

→ when this region gets activated it helps in transfer of T-DNA in plant genome.

activated by an inducer ⇒ acetosyringone

↓  
found in cells of dicot pl.  
(not in monocot)

T-DNA induces Tumor formation.

3) Transfer of T-DNA in animal cells →

↳ Very complicated

→ commonly done in Zygote / Embryo cells.

→ Commonly done by some vector like Retrovirus or by vectorless method.

♂ gametes      ♀ gametes

↓ Fertilization

Zygote

← Desired gene is inserted.

↓  
Embryo

↓  
Implanted in surrogate mother

↓  
Birth of transgenic animal



1<sup>st</sup> Transgenic animal: Mouse

↓  
in which growth hormone  
was transferred.

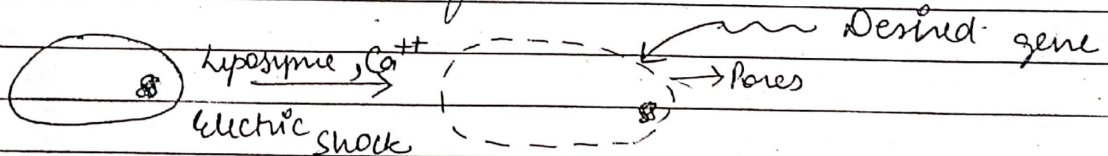
↓  
size ↑↑

↓  
kfr as Supermouse

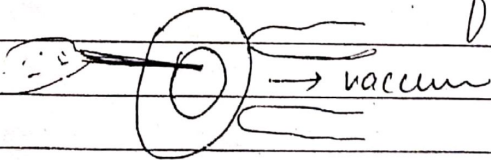
Later on 2002, a gene of jelly fish was  
transferred in Rhesus monkey. The monkey  
was named ANDI.

Vectorless gene transfer / Direct gene transfer  
↳ Low success rate.

① ELECTROPORATION: for Bacterial cell



② MICROINJECTION: for animal cell



③ PARTICLE GUN (gene gun / Biolistics) for plant cell.

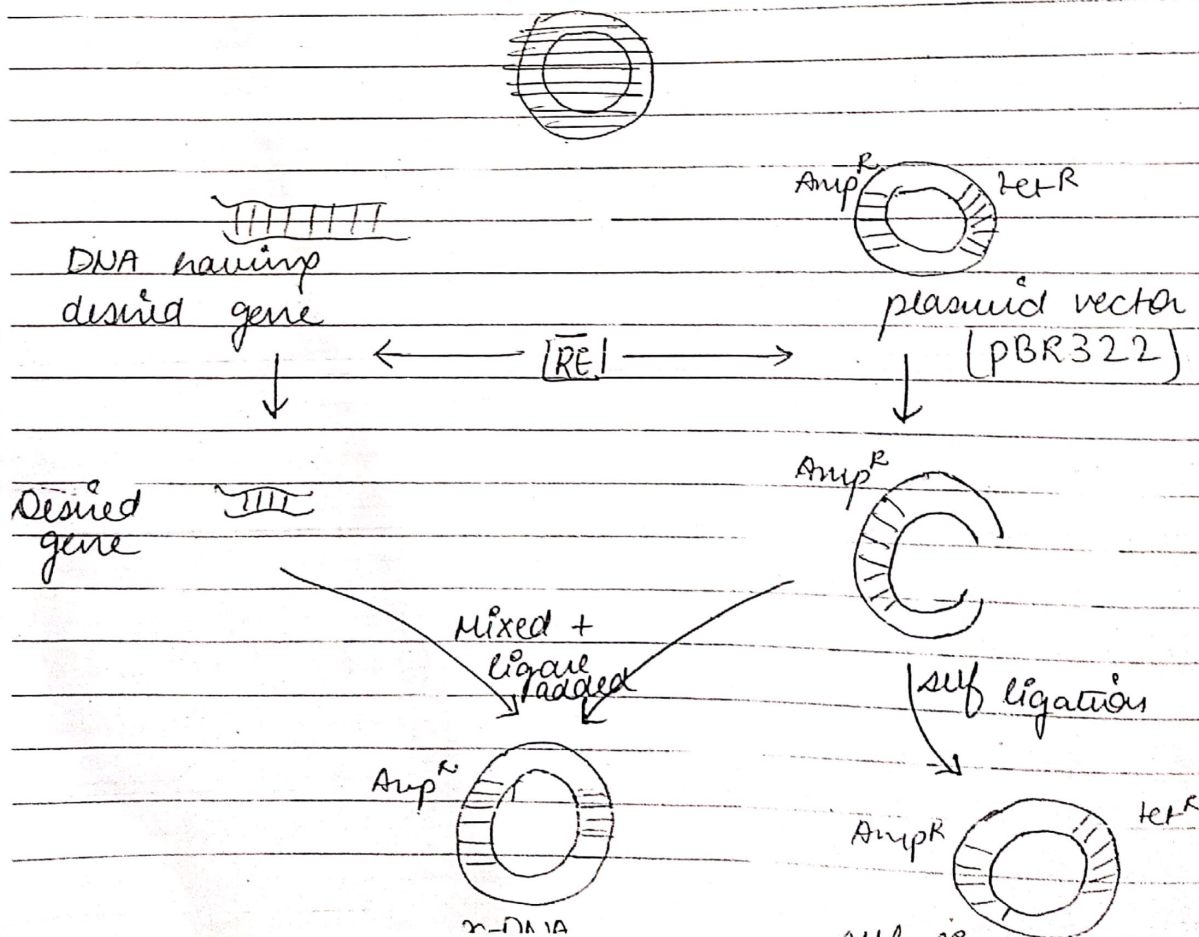
↳ gold / Tungsten coated  $\bar{c}$  DNA

④ Chemically mediated transfer  
eg. Polyethylene glycol (PEG)

⑤ Liposome mediated transfer  
↓  
lipid bags

⑥ Selection and screening of Recomb. Host cells =

r-DNA will transfer in all host cells  
so recombinant cell must be separated from  
non-recombinant cell and it is done by the  
help of selectable marker genes of vector DNA.  
↳ Reporter gene.

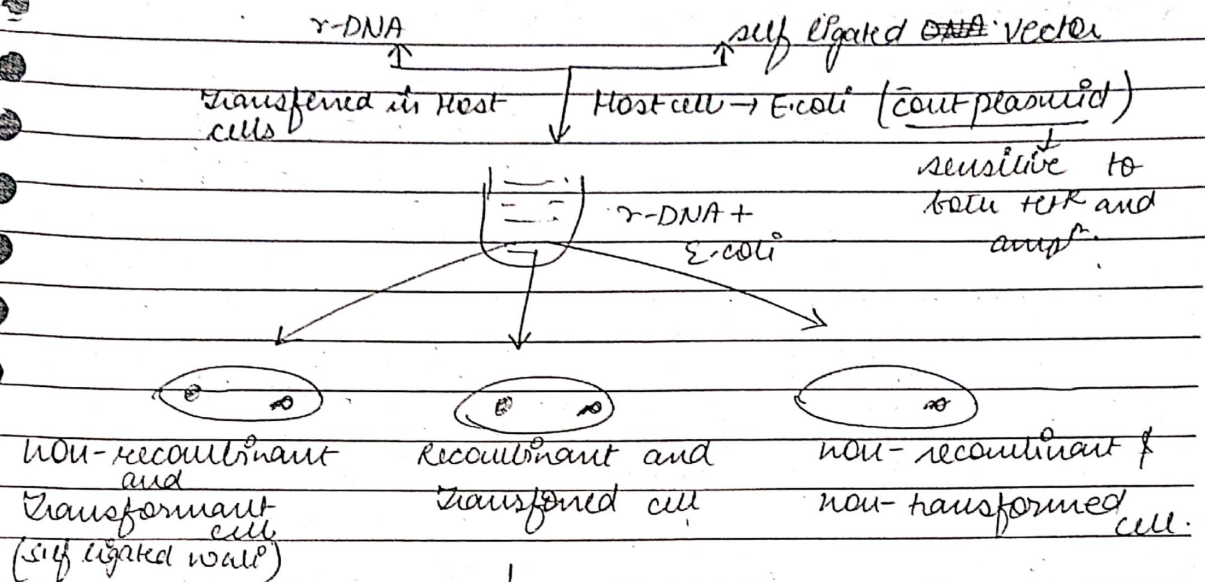




Prupli: culps in differentiate Transformants.

↳ jiska insertional inactivation nahi hoga.

Set (jiska insertional inactivation nahi hai vo help in recomb.)

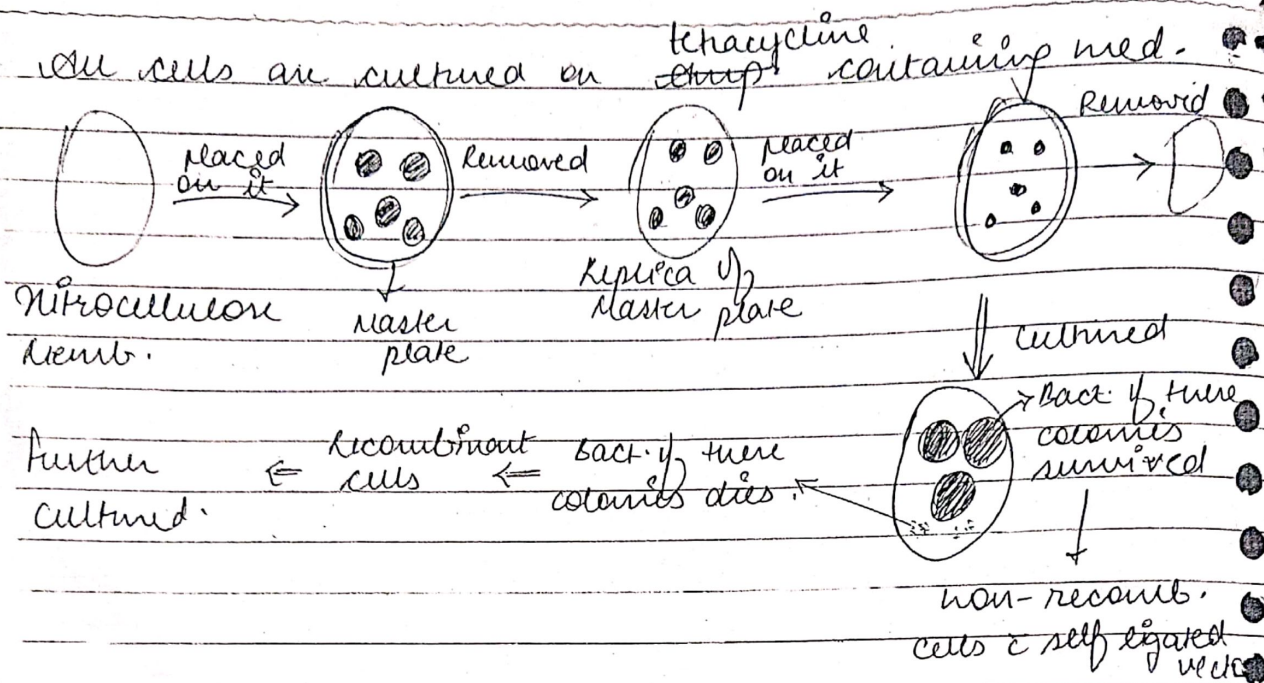


all cells are cultured on  
ampicillin containing med.

all transformants will survive and non-transformants will die.   
→  $\bar{c}$  r-DNA &  $\bar{c}$  self ligated vector.

From Transformant cells recombinant cells are separated from non-recombinant cells by Replica Plate Method or Nucleic Acid Hybridisation.





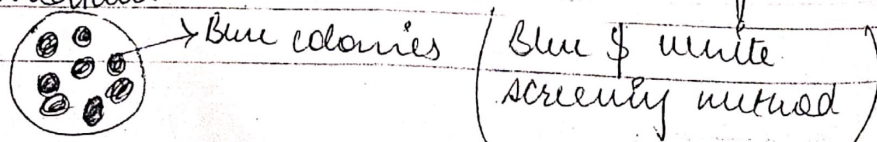
When some marker genes are antibiotic resistant gene in a vector then it is difficult to separate recombinant cells from non-recombinant cells having self ligated vector. Thus it is better to take one antibiotic resistant gene and one *lacZ'* gene as a marker gene in vector.

↓  
Same procedure till all transformants will survive and non-transf. cells will die.  
Instead of  $tet^R \rightarrow gal$  gene (*lacZ'*).

X-gal  $\rightarrow$  Blue colored

plasmid vector  $\Rightarrow$  pUC18/19

all Transf. cells are now cultured on X-gal containing medium.





- (7) Obtaining desired gene product at large scale:
- ↳ The desired gene will express itself in recombinant host cell (heterologous host) and produces recombinant protein.
  - The obtained recombinant host cells are very less in amount so its amount is increased by culturing them at large scale.

Production Tech. always develops at (3) levels :

- (1) Laboratory level
- (2) Pilot plant level
- (3) Industrial level.

Lab level : 1<sup>st</sup> / Primary level

At this level a fundamental technique is developed i.e. desired gene is transferred in host cell. All the growth conditions essential for the culture of cells are also determined.

Pilot Plant level :

Intermediate level

At this level cells are cultured in large amount of medium and glasswares are replaced by large metallic containers known as BIOREACTOR.

like pH, Temp, type of medium, O<sub>2</sub> availability etc.

At this level cost of the prod. and quality of the prod. is also determined.

Industrial level : At this level cells are cultured in large amount of med. in very large sized bioreactor and a permanent manufacturing unit is established.



## BIOREACTOR.

- Large, metallic container
- capacity ~ 100 - 1000 L or more
- can provide all growth conditions necessary for culture of cells.

→ It consists of two types of growth sys. :-

Support growth sys.

Suspended growth sys.

→ cells are cultured on solid med.

→ a continuous rotation for aeration is not required.

→ It is difficult to transport through pipeline.

→ cultured in liquid med.

→ continuous rotation for aeration is req. (O<sub>2</sub> availability)

→ easy to transport

→ It consists of two types of culture :

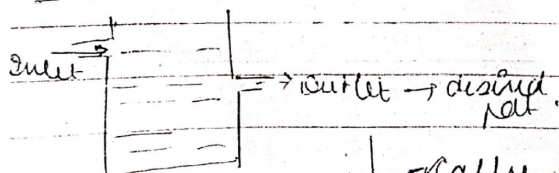
Batch Culture

Continuous Culture

→ In this cells are cultured in batches of med. and here both solid/ liq. medium can be used.

→ In this a continuous system is developed where fresh medium is added from one side and used med. is taken out from one side.

and in culture cells are maintained at their physiologi-



-ally most active log/exponential



→ commonly stirring type of bioreactors are used.

Two types.

simple stirred  
tank bioreactor

sparged stirred  
tank bioreactor

### DOWNSTREAM PROCESSING :-

usually starts after the completion of biosynthetic phase.

→ product obtained in Bioreactor

↓ undergoes  
several processes ⇒ Downstream processing

usable form of pdt.

Downstream processing :- They are different for diff. pdt.

- ↳ separation and purification of product.
- ↳ addition of preservatives, coloring agent, flavouring agents etc.
- ↳ quality control testing.
- clinical trials (if medicine)