MODULE - 02

• CLONING VECTOR

Vector - A DNA molecule that carry foreign/designed DNA to form rDNA, then transferred to host cell
(Gene - Part (Fragment) of DNA)

★ rDNA = Vector + Desired gene
↓
Delivered to host cell
↓
rDNA replicates and form desired DNA/element.

★ When rDNA form, then it have gain properties to self replication.

Characteristics

→ Size of Vector is Small
→ Should have capacity to Self Replicate in host cell.
→ Should have Restriction site (RE)
(Restriction Site - It make hollow space for insert Desired gene)
→ have multiple Cloning sites.
→ Have Selective marker gene (For Identification)

→ having Ori (Origin of Replication)

★ Types of Cloning Vectors

- HAC (Human Artificial Chromosomes)
- YAC (yeast Artificial Chromosomes)
- BAC (Bacterial Artificial Chromosomes)
- Cosmid
- Bacteriophage
- Plasmid
- Retroviral Vector

↳ **Plasmid** :- Extrachromosomal DNA Present in the Bacteria
- → mostly vectors formed by Plasmid combination.
- → More capacity of Self Replication.
- → Different Plasmids having different Properties.
- → Example_ PBR (Plasmid Bolivar Rodriguez) - PBR 318 & PBR 320

↳ **Bacteriophage** :- These organisms engulf (eat) harmful foreign Bacterial organisms.

→ enter in organism → ↑s in Number → engulf harmful Bacterias

Bacteriophage

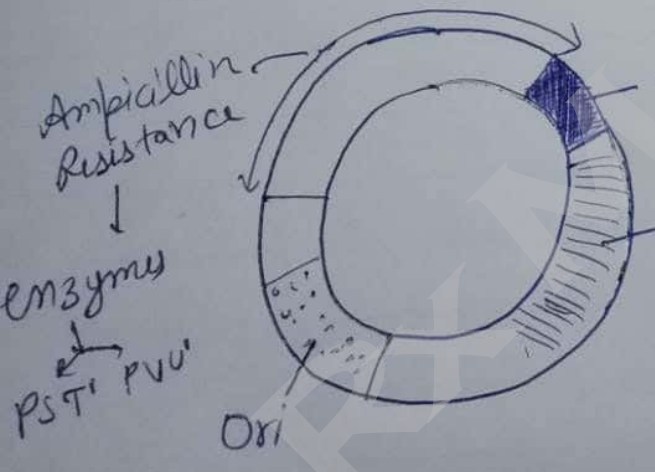
↳ **Cosmid** :- Plasmid or Bacteriophage without DNA

PBR 322 - An Artificial (first) vector develop by Bolivar and Rodriguez in 1977 from E. coli.

- It contain genes for origin of replication, resistance to Ampicillin and Tetracycline with unique ~~Rec~~ Recognition for 20 Restriction Endonucleases.

Gene markers - Ampicillin, tetracycline Restriction site.

Cloning site - Bam HI, , Sal I, SPh I, ECo R I.



enzymes - Cla I, Bam HI, Hind III, Sal I et

PBR 322 plasmid

• RESTRICTION ENDONUCLEASE (RE)

Restriction Endonuclease is an Enzyme used in cutting of Vector.

→ It is a Proteinous substance.

→ A Bactria contain Restriction Endonuclease, it cut the Bacteriophage when attack on Bacteria.

→ Bactria contain RE cannot harm or cut itself Because it add methyl group on adenine and cytonine Base with help of methylase on DNA, that Protect it from RE.

→ RE are Important tool for genetic Engineering.

→ Isolated from Bacteria and used in labs.

→ RE Recognise short and specific nucleotide sequence in DNA known as Recognition Sequence

↓

hydrolyse the Bond between adjacent Nucleotide and cut the DNA molecule of sequence

Types of Restriction Endonuclease

- Type I
- Type II
- Type III

Type I - In this type, DNA cut far from Recognition Sequence

Type II - Cut the DNA at Specific Position.

Type III - Multifunctional Protein (contain Sub units)

Applications :-

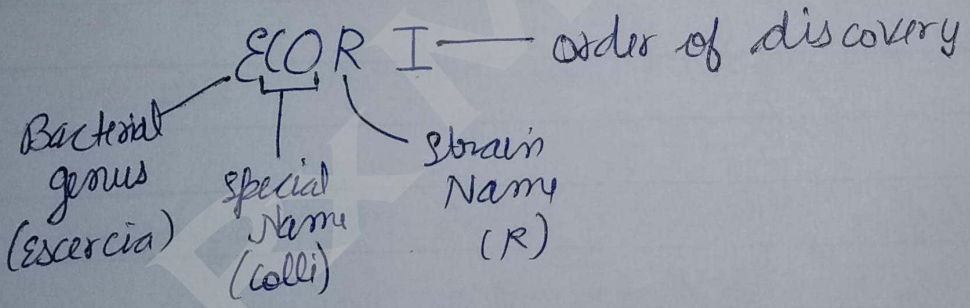
→ used in RELP Technique (Restriction Fragment Length Polymorphism)

→ helpful in gene cloning

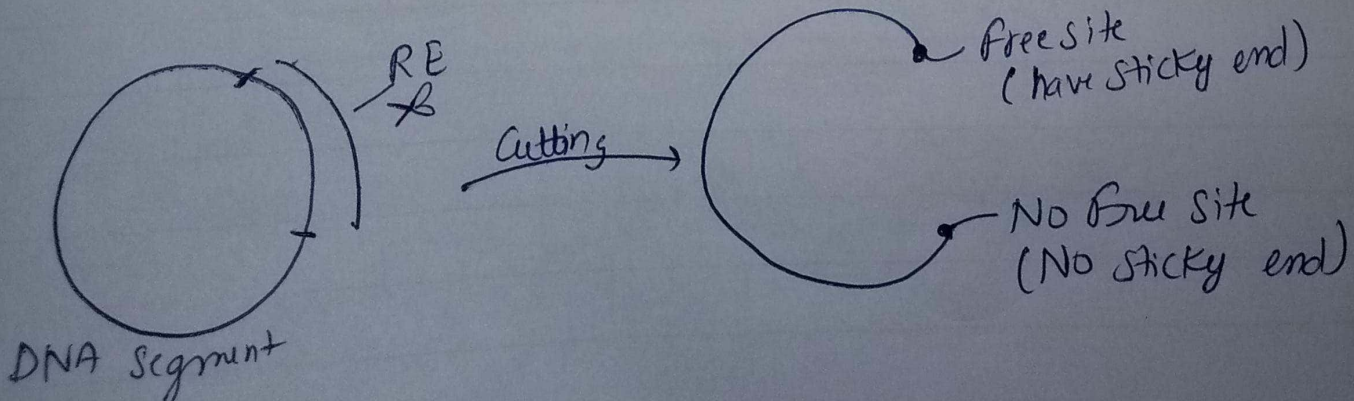
↓

To cut Vector.

Nomenclature :-



→ RE cut DNA by Palindromic Sequence (Same sequence at Both sides)
(exam - CUUC, AUUA)



• DNA LIGASE

Enzyme used in association of DNA Parts

↓

Connect 2 Strands of DNA together

↓

the association between Phosphate group of 1 Strand and deoxyribose sugar on another group.

→ DNA ligase is functional in Joining of okazaki Fragment (form during Replication - Strands)

→ used for introduction of gene (Desired) into Plasmid Vector.

→ used to ligate (stick) 2 DNA Strands.

→ used in vitro & in vivo.

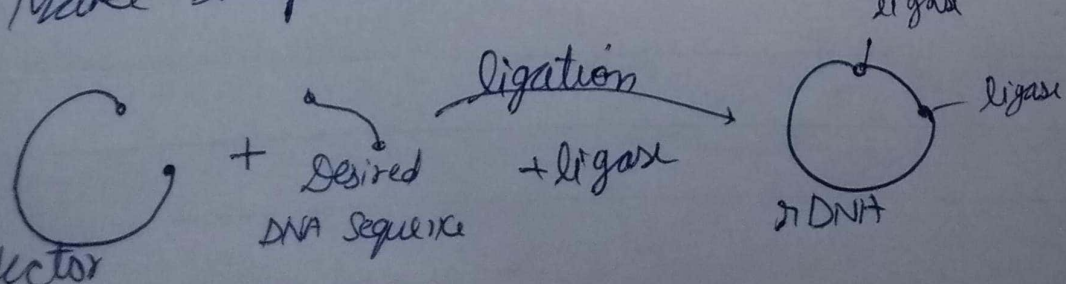
→ found in Virus and Some Bacterias

→ Required
- ATP
- NADP
- Cofactor

} To stick / form / Joint the Segments.

→ DNA Sequence Joined to other DNA molecule Covalently.

→ Make Phosphodiesterase Bond.

→ 

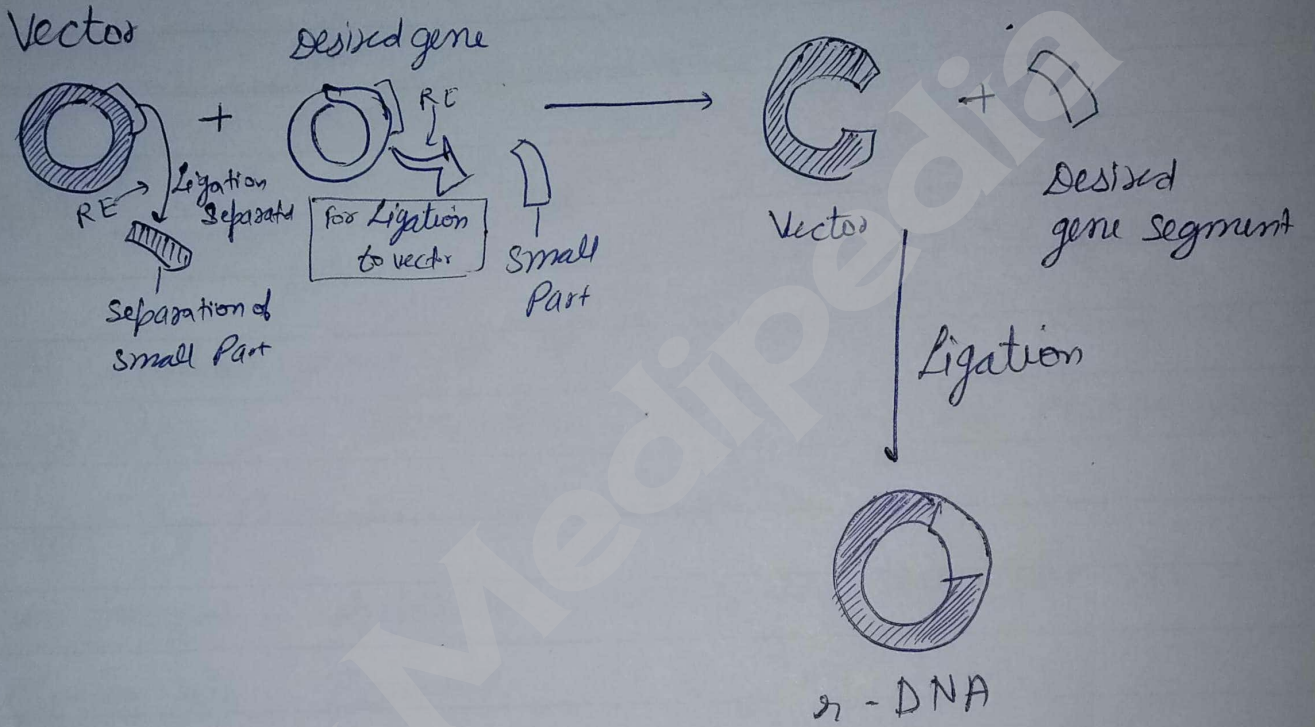
Applications

→ Role in DNA Technology

→ used in cloning experiments
(DNA ligase I, II, IV)

• RECOMBINANT DNA TECHNOLOGY (rDNA)

The Process of formation of Required DNA, Enzyme, Substance by transferring recombination of Vector and Required DNA Part to the host Cell.



PROCEDURE

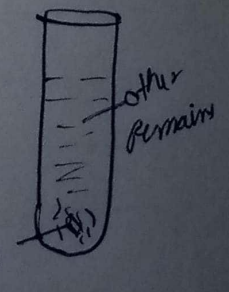
① Isolation of DNA :- Lysis of Cell (Breakdown/cutting) from which Desired/Required DNA can be obtained.

↓

In the Sample prepared or obtained By Cell Breakdown

↓

DNA can obtained in precipitated form by using chilled Ethanol



↓

Removal of DNA from Test tube (Spooling)

② Cutting of DNA :-

The DNA obtained by Separation or Spooling process.

↓

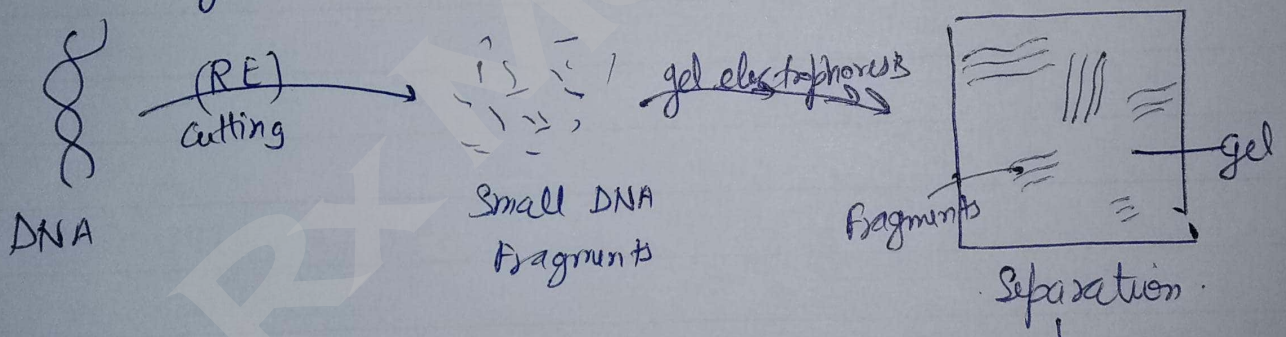
The DNA can cut with Restriction Endonuclease (RE) Enzyme in Small fragments (Parts)

↓

The Small Parts also Separate according to their Sizes (Small Fragment / Long Fragment) by Process of gel electrophoresis.

↓

The DNA Fragments (Size wise) can be Removed from gel.



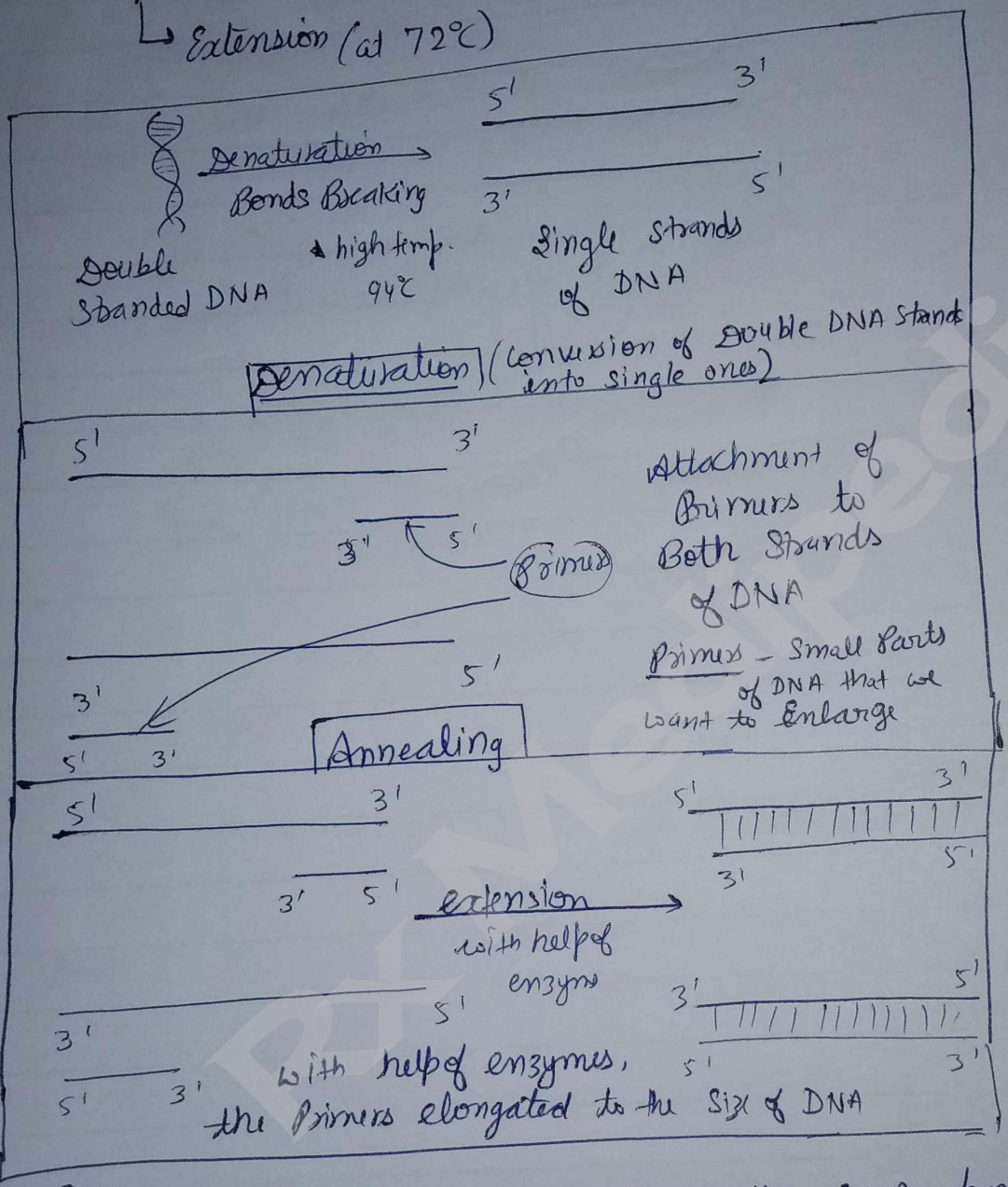
↓

Elusion
(Removal of DNA Fragment from gel)

③ Amplification of DNA :- with the Help of PCR (Polymerase Chain Reactions)

DNA Fragments can be elongated having 3 Steps.

* Steps of Amplification (PCR)
  - Denaturation (at 94°C)
  - Annealing (at 54°C)
  - Extension (at 72°C)



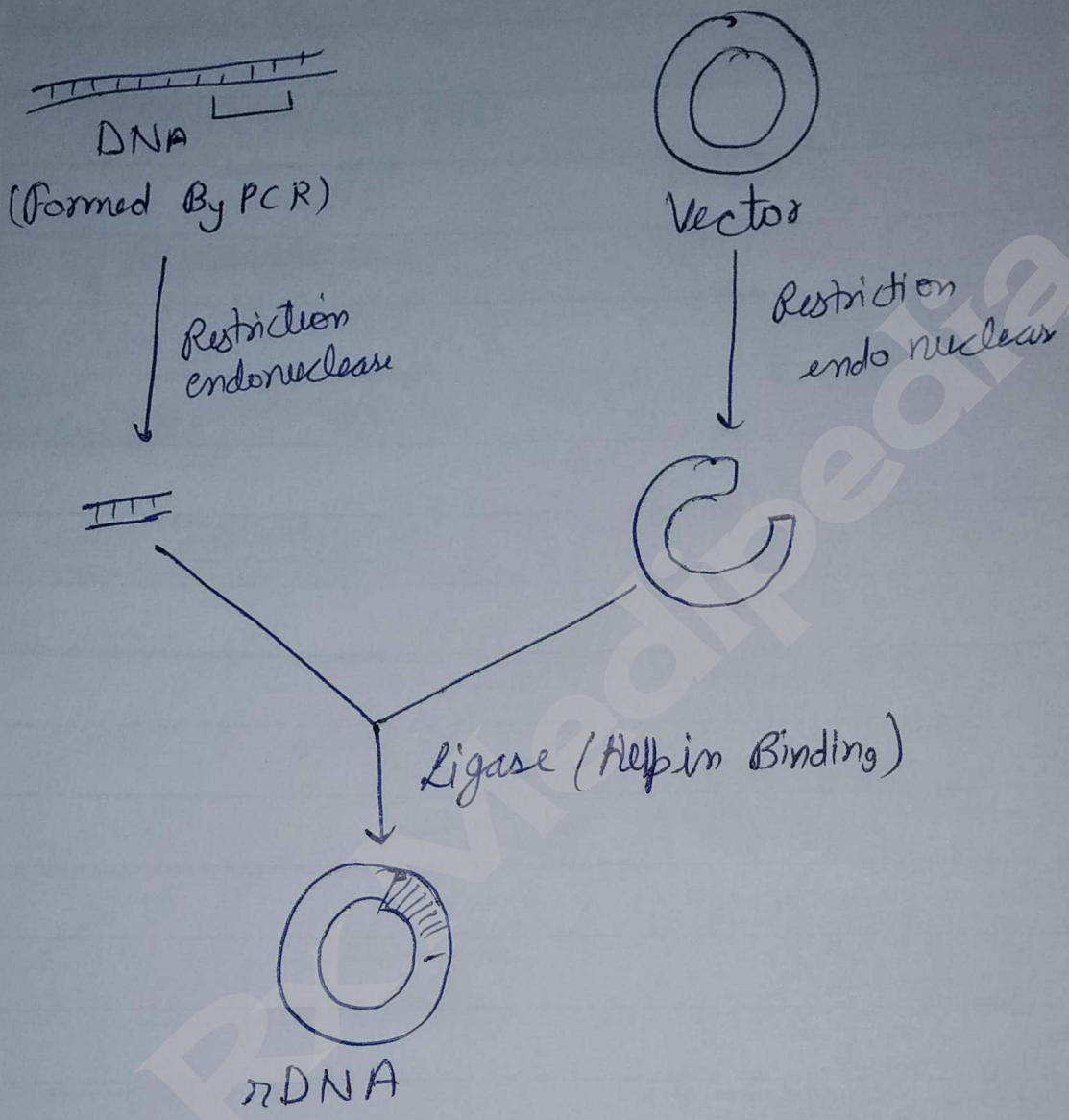
(4) Formation of rDNA: The DNA formed by amplification can be cut by Restriction endonuclease enzyme. and attached to the vector by Ligase.

↓

The rDNA formed by attachment can Placed in the host Cells (Animals, Bacterias etc)

↓

the rDNA can copy the DNA inside host Body.



↓

Transferred into host cell (E.Coli)

↓

Known as Recombinant Cell.

• APPLICATION OF GENETIC ENGINEERING IN MEDICINE

- Gene Maping
- Genetic disorder
- Gene therapy
- DNA Finger printing
- Vaccines
- Pharma Products

★ Gene Maping:

→ Technique used to Identify a gene location and the distance between 2 genes.

→ used to Identify Absence or Presence of gene.

→ unit of Gene Maping - Centimorgan

↓

used to measure distance between 2 gene.

★ Gene Therapy: Therapy in when genes can be replaced with other gene or transfer the gene if their is gene deficiency.

exam - SCID (Severe Combine immuno Deficiency)

ADA deficiency → with help of rDNA Technology

(Adenosine deaminase)

↓

Responsible for immunity

↓

ADA transfer to Body with the help of Vector in Bone Marrow

↓

Increase Immunity.

* DNA fingerprinting :- By using hair, Blood or other Body tissue

↓

the identification of organism, Person by DNA fingerprinting.

→ widely used in crime Identifications by using clues (hair etc)

→ develop by sir Alec Jaffery.

* Vaccines :- dead / Inactive or weak virus Antibodies used to make vaccines and introduce inside the Body.

↓

By introduction in Body, Body make antibodies to same virus and able to fight with that type of original virus.

* Pharma Products :

→ used in Production of Insulin, GH (Growth hormone), Treat fertility.

→ In Production of human Albumin (Protein in Blood), Vaccines, Anti-haemophilic factor

• APPLICATIONS OF r-DNA TECHNOLOGY AND GENETIC ENGINEERING IN PRODUCTION OF INTERFERONS, VACCINES - HEPATITIS, HORMONES - INSULIN

1. INTERFERONS

Interferons are natural occuring substance release from Body to Fight with viral Infection and Boost Immunity.

→ It can interfere with virus, effective in treatment of Infection.

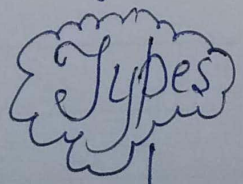
→ Effective in treatment of Influenza and hepatitis

→ Large number of Interferons produced by gene Clonning.

↓

Introduce r-DNA in E. Coli (host Cell)

→ Interferon having Antiviral Activity & anti proliferative activity (cancer Fight)



- Interferon α (Leucocyte)
- Interferon β (Fibroblast)
- Interferon γ (Lymphocyte)

Production of Interferons :-

When Virus Enters into Cell and Produce Viruses

↓

In this Case, Cell activates interferon gene

↓

Interferon gene release & Stick on receptor called Ganglioside Receptor or Interferon Receptor with neighbour Cells.

↓

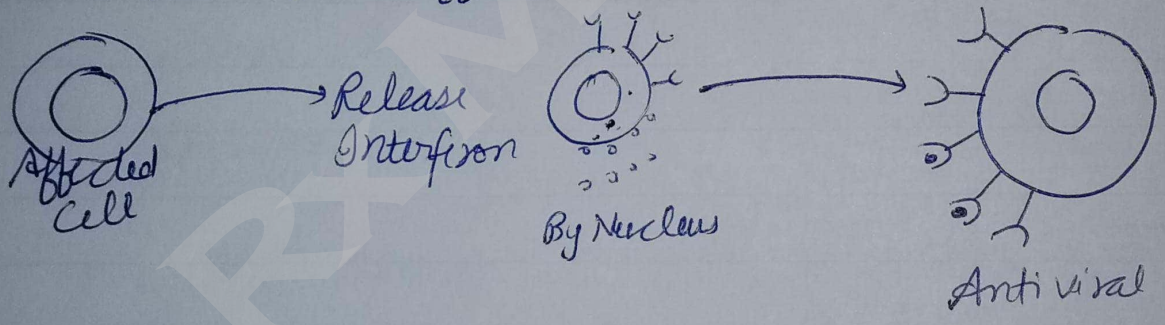
Signal Produced to the Nucleus.

↓

Nucleus produce Antiviral Protein (like Protein Kinase R)

↓

No Virus affect other Cells.



→ Interferon α - used to treat hepatitis B & C

→ Interferon β - used to treat Sclerosis & Autoimmune disorder

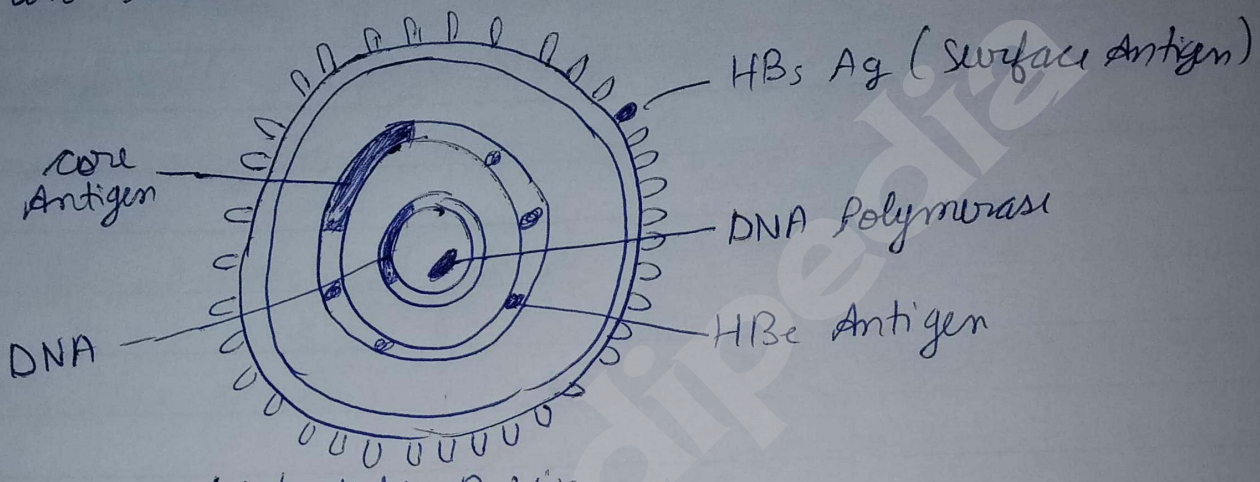
→ have antitumor activity.

2. VACCINE- HEPATITIS

Hepatitis is 50-100 times more Infectious that AIDS.

→ Most Common B-Virus

→ hepatitis B-Virus Produce irritation, pain, Swelling in liver.



Hepatitis B Virus

→ Hepatitis B-Vaccines was 1st Vaccine By using r-DNA Technology

Step Involve in Production of Hepatitis B Vaccine

① Hepatitis B Virus → lysis of Cell → Protein Denaturation
↓
Precipitation of DNA
↓
By help of Centrifugation
↓
Drying
↓
gene obtained

Preparation of Vector

(2.) E. coli (Plasmid) ⟶ DNA Extracted ⟶ Cut with Restriction Endonuclease

↓

Formation of Plasmid Vector.

(3.) Gene Isolated (Required) + Plasmid Vector ⟶ rDNA Formed

↓

Introduction to host cell (yeast cell)

↓

Called Recombinant Cell.

(4) Recombinant Cell ⟶ Isolated in Fermentation Tank. (Tsc No-)

↓

Production of HBs Antigen

3. HORMONE - INSULIN

→ Insulin is a Simple, Small Protein composed of 51 Amino acids and molecular weight is about 5808 Dalton.

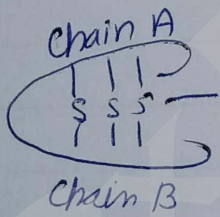
→ Insulin - A hormone secreted by β-cells (in Pancreas) that help in Balancing Blood Sugar level.

→ Insulin hormone structure is a dimer of chain A and chain B linked together by disulphide Bond called mature Insulin.

→ Mature Insulin made up of Pro-Insulin.

→ Pro Insulin is consist of 3 chains. A, B and C

→ when Pro Insulin modified into mature Insulin, Chain C degenerate or Removes.

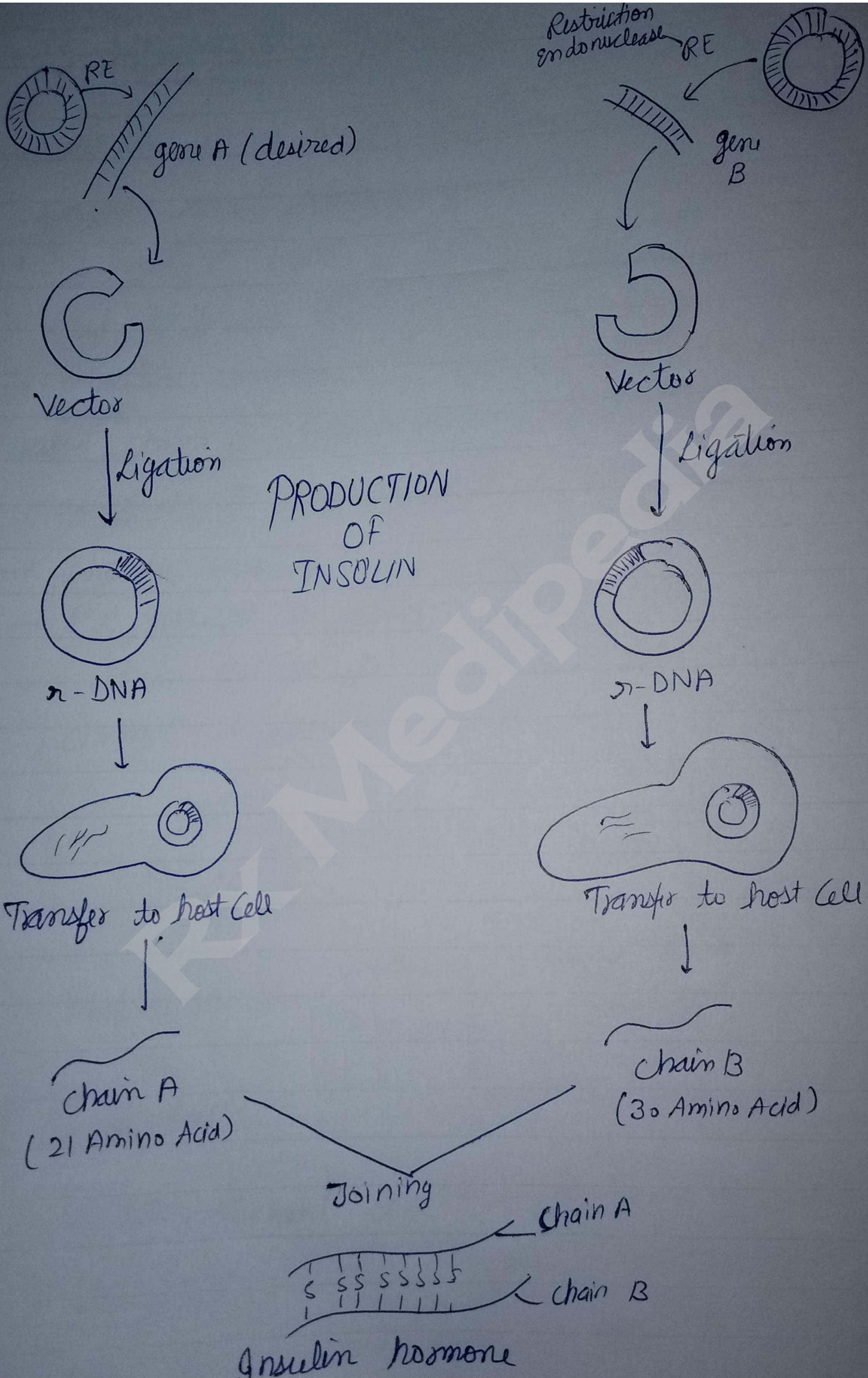


disulphide Bond

→ Mature Insulin → Chain A – Contain 21 Amino Acid
→ Chain B – Contain 30 Amino Acid
} 51 Amino Acids

Production

First recombinant Insulin Prepared by company Eli Lily 1983


PRODUCTION
OF
INSOLIN
RE
gene A (desired)
Vector
Ligation
r-DNA
Transfer to host cell
Chain A
(21 Amino Acid)
Restriction endonuclease
RE
gene B
Vector
Ligation
r-DNA
Transfer to host cell
Chain B
(30 Amino Acid)
Joining
Chain A
Chain B
Insulin hormone

#PCR- Polymerase Chain Reaction

A Technique used to make several copies of small fragments of DNA or RNA.

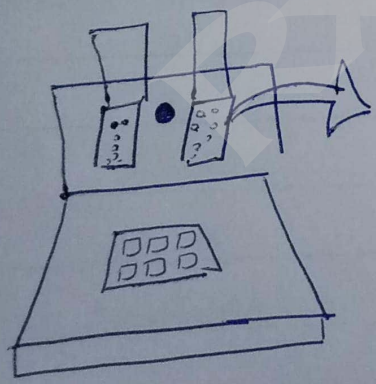
Single DNA Fragments → PCR → Billions of Copies of Fragments of DNA.

★ Objectives

→ To diagnose disease.
→ Crime Investigation
→ Genetic Research
→ Molecular Biology
→ Revolution in field of Diagnose & Research

★ Requirements to Perform PCR:

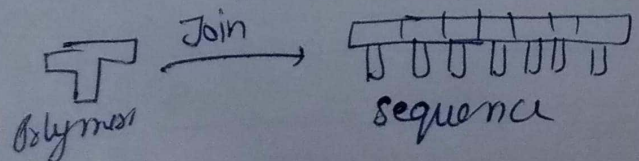
→ Thermal cycler or PCR Machine



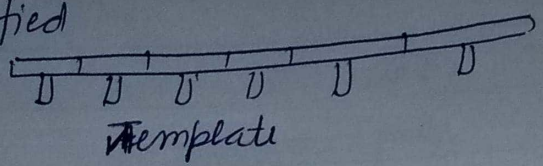
Contain Tubes

PCR Reaction Key Ingredients
- TAQ Polymerase
- Primers
- DNA Templates
- Nucleotides.

→ Taq Polymerase - Enzyme used in making new Strands.
→ Primers - DNA Polymerase Requires Primers to start Reactions



→ DNA Template - original DNA segment which we want to Amplified



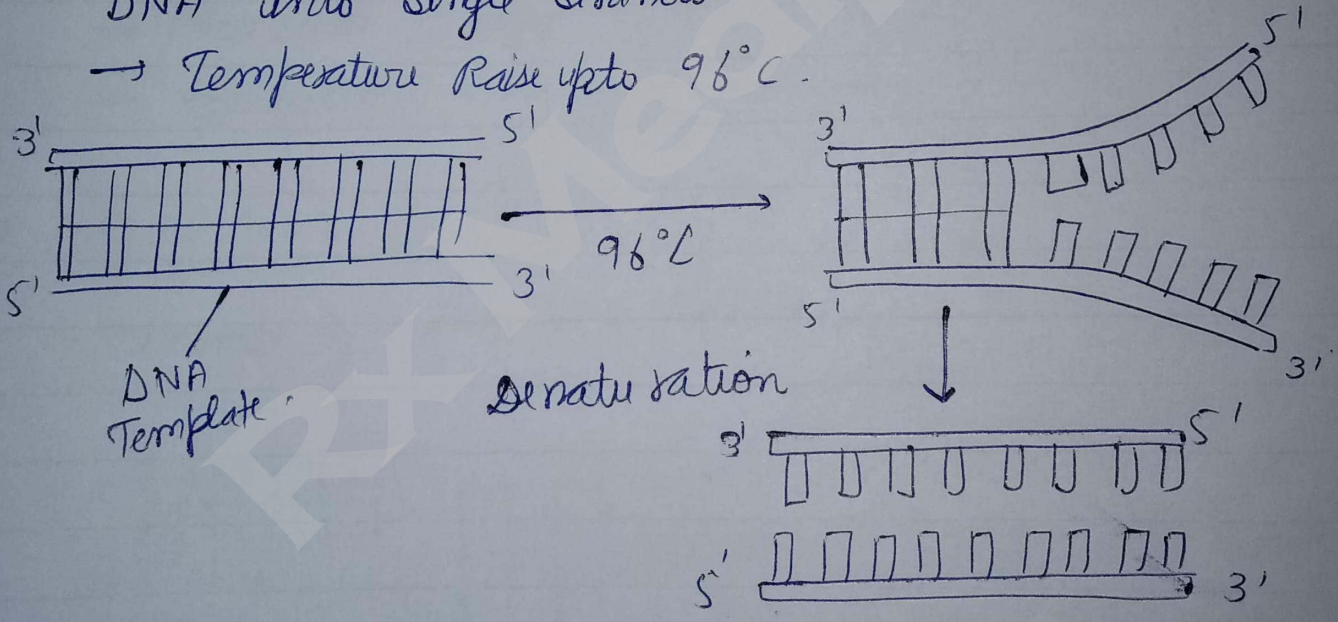
→ Nucleotides - Basic units used for DNA Synthesis

Steps of PCR :-

- Denaturation
- Annealing
- Extension

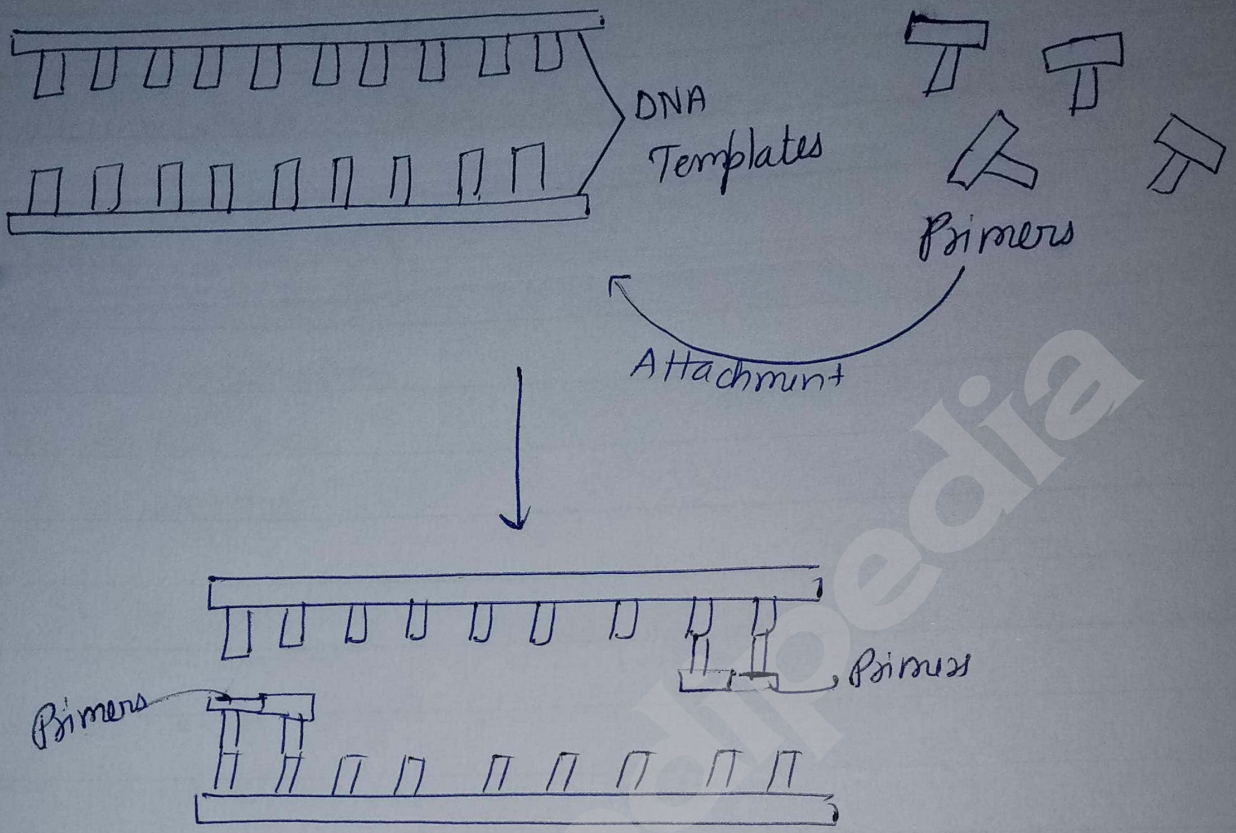
(1) Denaturation - Process of Separation of Double Stranded DNA into Single Strands.

→ Temperature Raise upto 96°C.



(2) Annealing :- word used to increase the temperature from 72°C then, cool to temperature in 55°C. So that Primers in PCR Machine Binds to target sequence of Single Stranded DNA

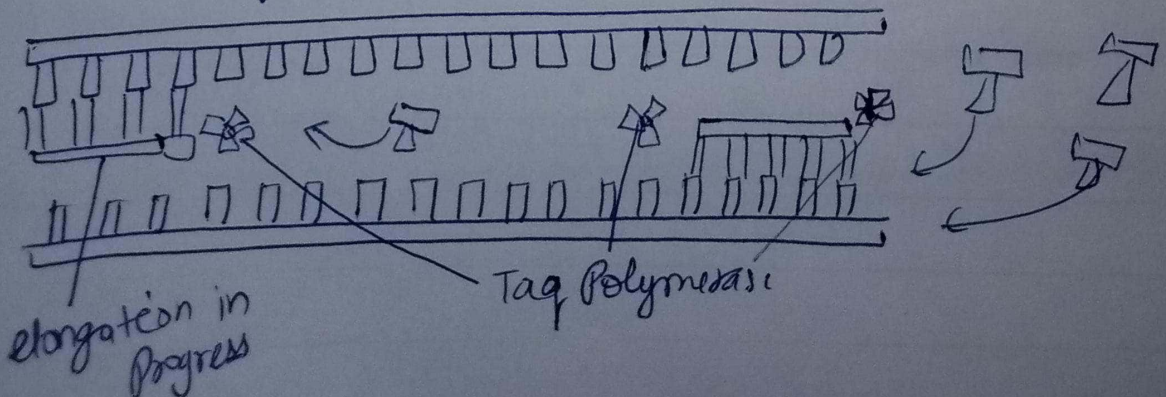
→ Primers bind to template DNA by complimentary Base Pairing (Matching Sequence).

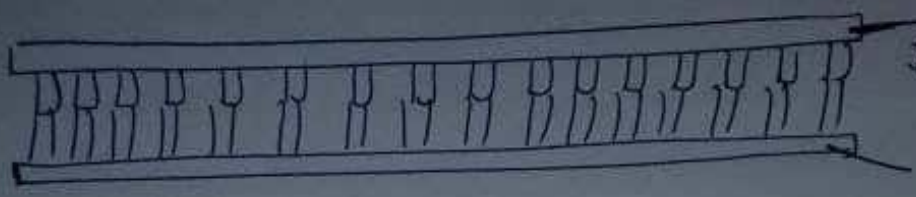


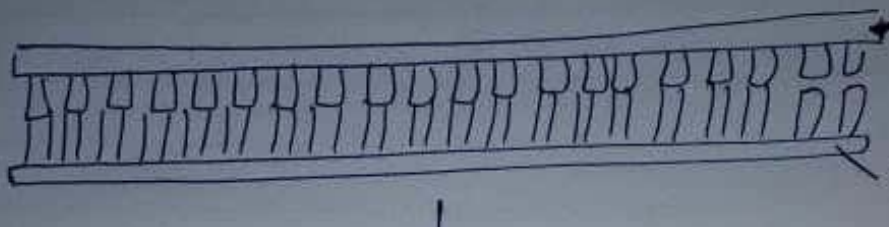
(3) Extension: Free nucleotides can attached to the Primer Sequences to form New DNA Strands which is complimentary to original Strand.

→ Free Nucleotides can attached with the help of Taq Polymerase

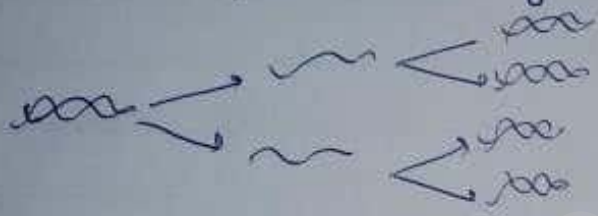
→ Temperature increase to 72°.







Repetation of Steps Result in millions of Copies Formed.



→ Take 2-4 hours or Depends upon Length of DNA Strands.

→ After Reaction, temperature decreases to 15 °C to Store to Product of Reaction.