(Chapter#10)

Biotechnology

Introduction of Biotechnology:

Definition:

The use of living organisms in processes for the manufacture of useful products and for the wellfair of main kind.

Genetic Engineering:

Genetic engineering is basically called moldern biotechnology.
The artifical synthesis modification removal, addition & repair genetic material (DNA)

History:

- In **1944** working started on "Genetic Engineering" and it proves that DNA carries the genetic information.
- Scientist Isolate the enzyme of DNA synthesis. and then prepared the DNA outside the cell

- In 1970 they were able to cut and paste paste the DNA organisms.
- In 1978 scientist prepared Insulin by inserting the Insulin gene in bacteria.
- In 1990 "Human Genomes Project" was launched to map all the genes in human cell.
- In 2002 complete map of "Human Genome Project" was launched.

Recombinant DNA Technology:

Genetic engineering is also called "Recombinant DNA Technology".
It is involves in artifical synthesis modification removal addition and prepare genetic material.
We can obtain million of copies of specific DNA inside a bacterial cell.
(Recombinant DNA Technology)

Objectives of Recombinant DNA:

(1) Isolation of particular gene for various porpose such as gene therapy.
(2) Production of particular RNA and protein molecules.
(3) Production of enzyme & Drugs.
(4) Use for the production of varieties of plants.

Components of Recombinant DNA Technology: /Steps:

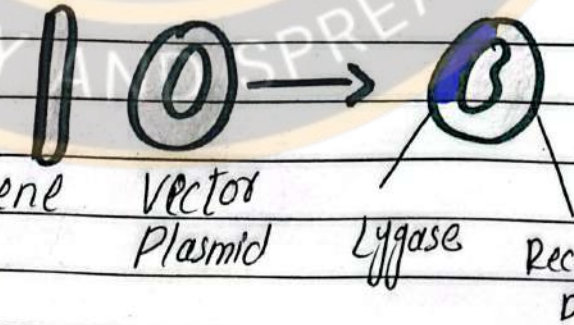
1) Isolation of Gene of Interest:

Genetic engineering help to identified the gene of interest of donor organisms.

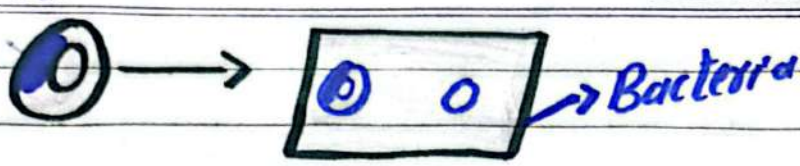
Restriction Endonucleases:

Special enzymes are used to cut the identified genes from the total DNA of donor organs.

(2) Insertion of Gene into Vector:

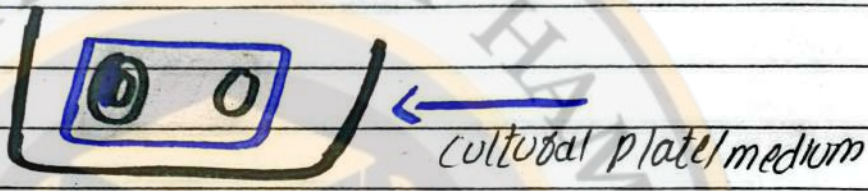


- When vector DNA with gene of interest combine it to form recombinant DNA.
- Tranfers of Recombinant DNA into host organisms.
- Recombinant DNA is transferred into the target host.



(3) Grow the GMO:

GMO are provided to stable culture medium for their growth to give as much copies of the gene of interest as needed.



(4) Expression of Gene:

GMO contains gene of interest and used it to manufacture the desired products which is isolated from the culture medium.

Molecular Scissors: (Restriction Endonuclease)

- It is the natural enzyme of bacteria used for protecting agaist virus.
- It cuts down the viral DNA it restricts the growth of virus.

The name "**Restriction Endonuclease**" is given because they are naturally found inside bacterial cell for "**restrict**" the growth of infecting virus.

Discovery:

In **1970** **Hamiltin O smith** first Isolated in restriction enzyme.

Restriction Site:

The specific site of DNA on which restriction enzymes cut the DNA

There are two types of cutting:

(1) **Staggered cut** → Used in Recombinant DNA.
(2) **blunt cut** → Used in polymerase chain Rxn.

Palindromic Sequence:

Sequence of four to six pairs in DNA In which nucleotide is arrange symmetrically in reverse order.

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

e.g:

TT GC AA
AA CG TT

Example: Ecoli
E restriction Enzyme

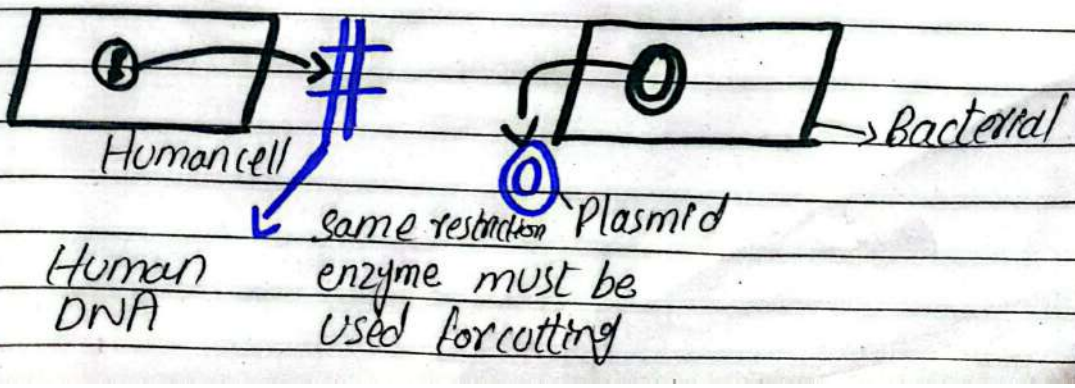
- Cuts by restriction enzyme produce singal standard but complementory ends of two DNA chains called **"sticky ends"**
- It can bind by complementory base pairing.

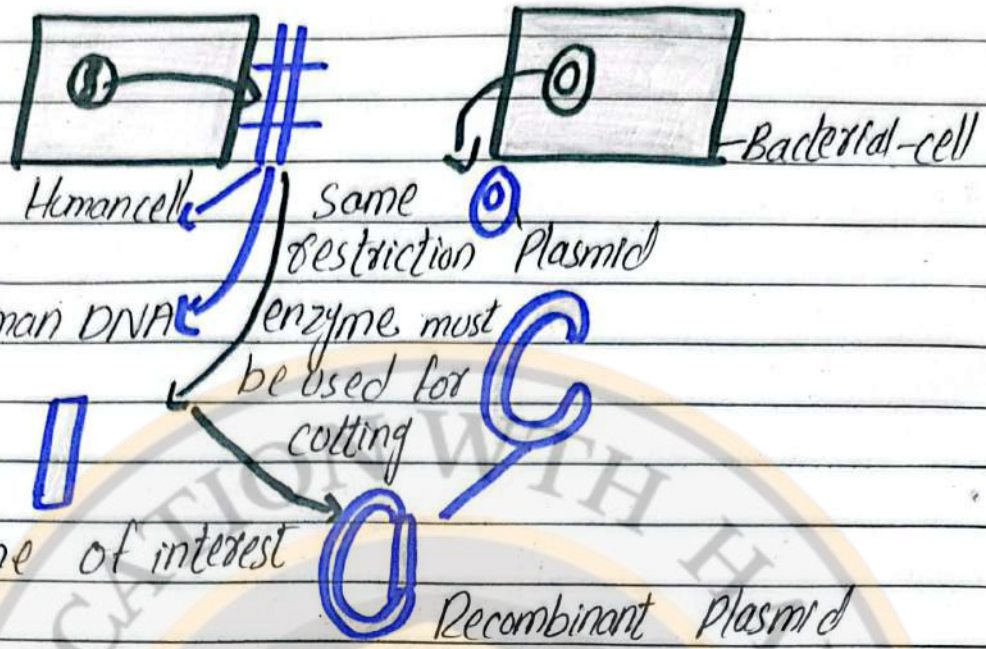
Molecular Carriers OR Vectors:

Vector are molecular carrier which are used to introduce recombinant DNA into the host cell.

Example: Plasmid.

Plasmid are natural extra chromosomal circular DNA in bacteria hit carry genes from antibiodies resistance and fertility etc.





DNA Lygase seals through sticky ends
Host cell take up recombinant plasmid.

Cloned Insulin gene can be Isolated.

Discovery (Plasmid)

It was discovered by the study of intestinal bacteria **(Escherichia coli)**

PSC 101 → Tetracycline.

Plasmid Steanly cohon

PBR 322 → Tetracycline and Ampicilline.

We can also used bacteriophage as a vector.

Characteristics of Plasmid:

(1) Origin of replication site
(2) Antibiotics Resistant gene
(3) Restriction site of different enzymes
(4) Small size than chromosomal DNA
(5) High copy numbers

PBR322 Cloning Vector:

It is plasmid or cloning vector.
It is a foundational synthetic plasmid cloning vector using in molecular binding **P for** Plasmid **'BR' for 'Bolivar Rodriguez'** and 322 is a number.

Size: It size is about (4,3,6,2) base pairs.
Origin: From PMB2 allowing self replication.

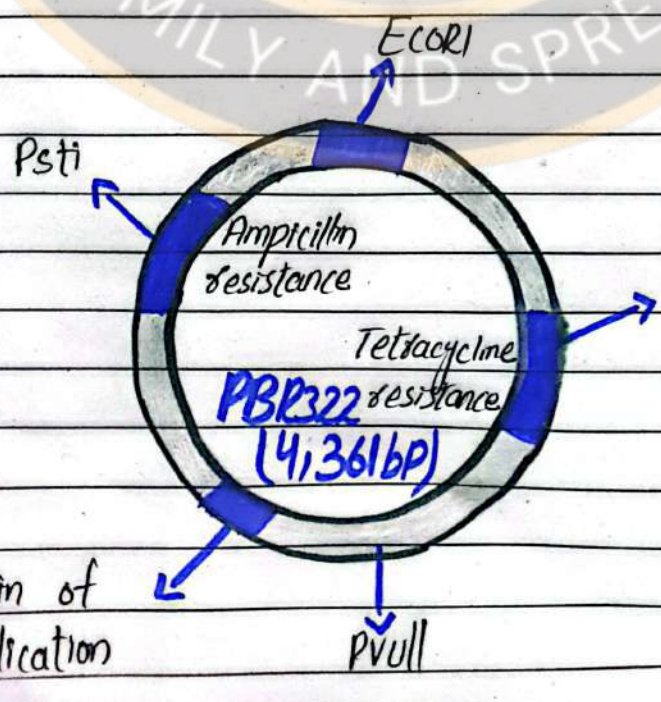
There are two antibiotics resistance genes:
1. Ampicillin resistance
2. Tetracycline resistance

It has some restriction side (**EcoRI, BamHI, PstI**) for cutting DNA may located in the restriction genes.

Function:

Insertion of DNA into a restriction site within a tetracycline and ampicillin inactive that allows identification of recombinant bacteria.

Significance: one of the first widely used vectors makes gene cloning acessible.



Types of Plasmid as Vector:

(1) Cloning Vector:

To insert replicate and amplify DNA fragments.
Example: PBR322

(2) Expression Vector:

To express the clone gene as a protein in the host cell.

(3) Shuttle Vector:

To replicate and function two or more different host.
Example: Bacteria and some yeast.

(4) Repoter Vector:

To monitor gene expression.

(5) Viral Vectors:

To deliver gene into eukaryotic cells.

Application:

It is used for:

(1) Gene therapy
(2) Vaccine development
(3) Synthetic biology
(4) Genetic Engineering
(5) Agriculture research
(6) Drug discovery

Mechanisms of Formation of Recombinant DNA:

(1) For longer quality of gene we use recombinant DNA technology.

(2) For less quality of gene we use polymera chan reaction.

(1) Isolation of Gene of Interest of plasmid:

Obtain the specific DNA (gene) you want to clone and a suitable plasmid a

DATE: _____ DAY: _____

circular DNA molecule from bacteria.

(2) Cutting with Restriction Enzyme:

Use the same restriction enzymes to cut both the DNA and plasmids this creates complementory sticky ends on both DNA and plasmid.

(3) Ligation (Joining):

Mix the cut gene fragments and the cut plasmid DNA ligase them forms permanent phosphodiester bond joining the DNA into the plasmid.

(4) Formation of Recombinant Plasmid:

The resulting recombinant DNA molecule now containing the foreign gene in the recombinant plasmid.

(5) The recombinant plasmid is introduce into the host cell for cloning.

DATE: _____ DAY: _____

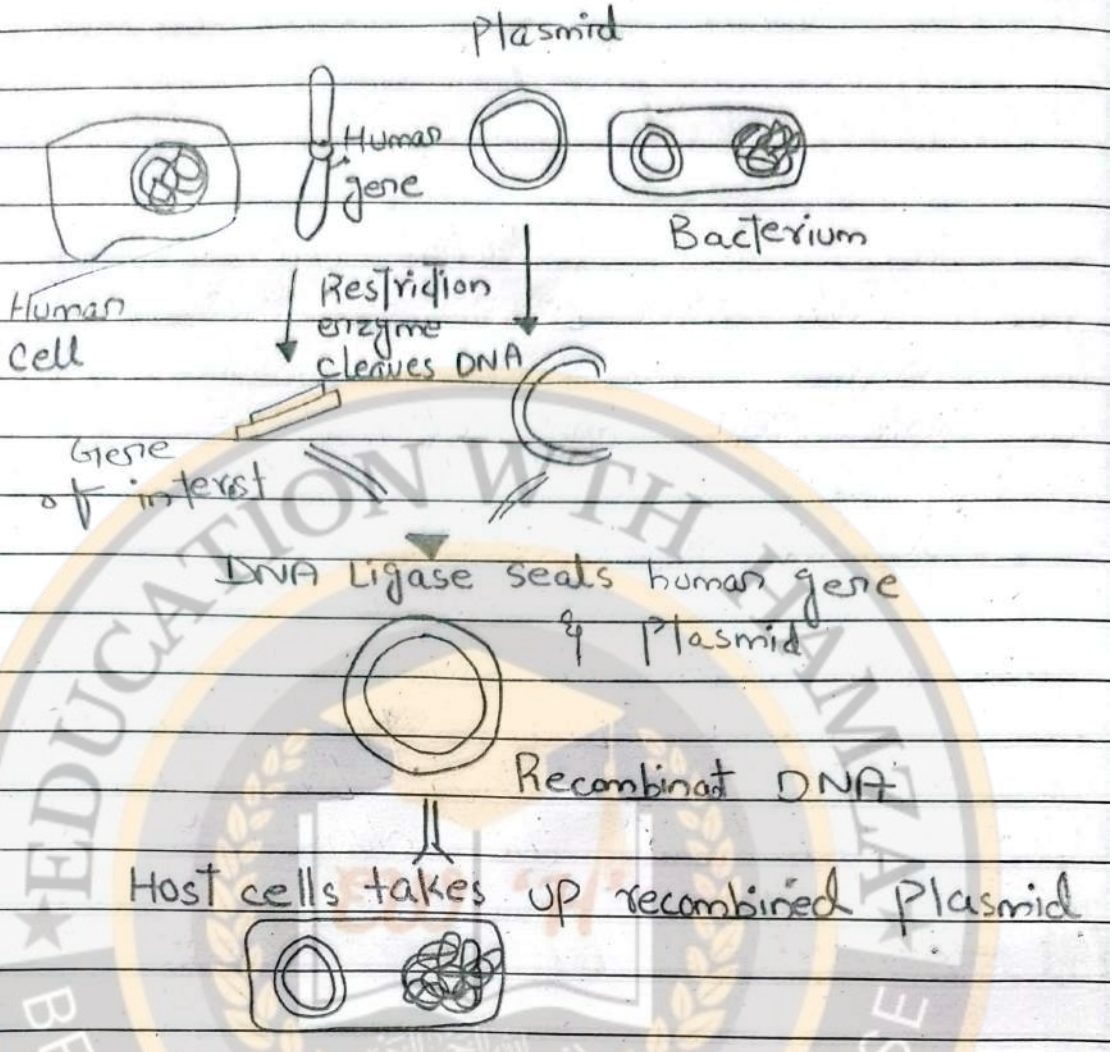


Fig: Steps for Formation of recombinant Plasmid

DATE: _____ DAY: _____

Production of Human Insulin:

(1) Identification & Isolation of Gene:

Insulin gene produce human Insulin two polypeptide chains alpha and beta.

Alpha contain 21 amino acids and beta contain 30 amino acids linked by disulphide bridge.

Genes for Insulin is called **INS**. It is derived from latin word "Insula" and "Island" is located on chromosomes 11 at position 11P and bone number 15.

(2) Selection of Suitable Plasmid:

(For Insertion of Gene)

Two commonly used plasmid for Insulin.

- PBR322
- PUC18 /PUC19

DATE: DAY:

(3) Creation of Recombinant DNA:

Bacteria E.coli cannot produce directly eukaryotic protein because two seperate synthesis for alpha and beta chains. Genes are inserted separately into two plasmid under PBR322 plasmid is engineered with the help of promoter. After a transformation bacteria become transformed bacteria.

(4) Insertion of Recombinant Plasmid Into Host:

Recombinant plasmid are then separately inserted into E.coli through a process called transformation.

(5) Production of Insulin Chain:

Bacteria are allowed to grow in for large fermentors it is grone with suitable culture medium then Insulin gene multiplication start.

DATE: _____ DAY: _____

(6) Extraction and Purification:

Cynogen bromide for the extraction and purification.

(7) Folding & Disulphide bridging of both Insulin Chains:

Treated with sodium disulphade and sodium sulphide for active protein.

(8) Purification & Testing:

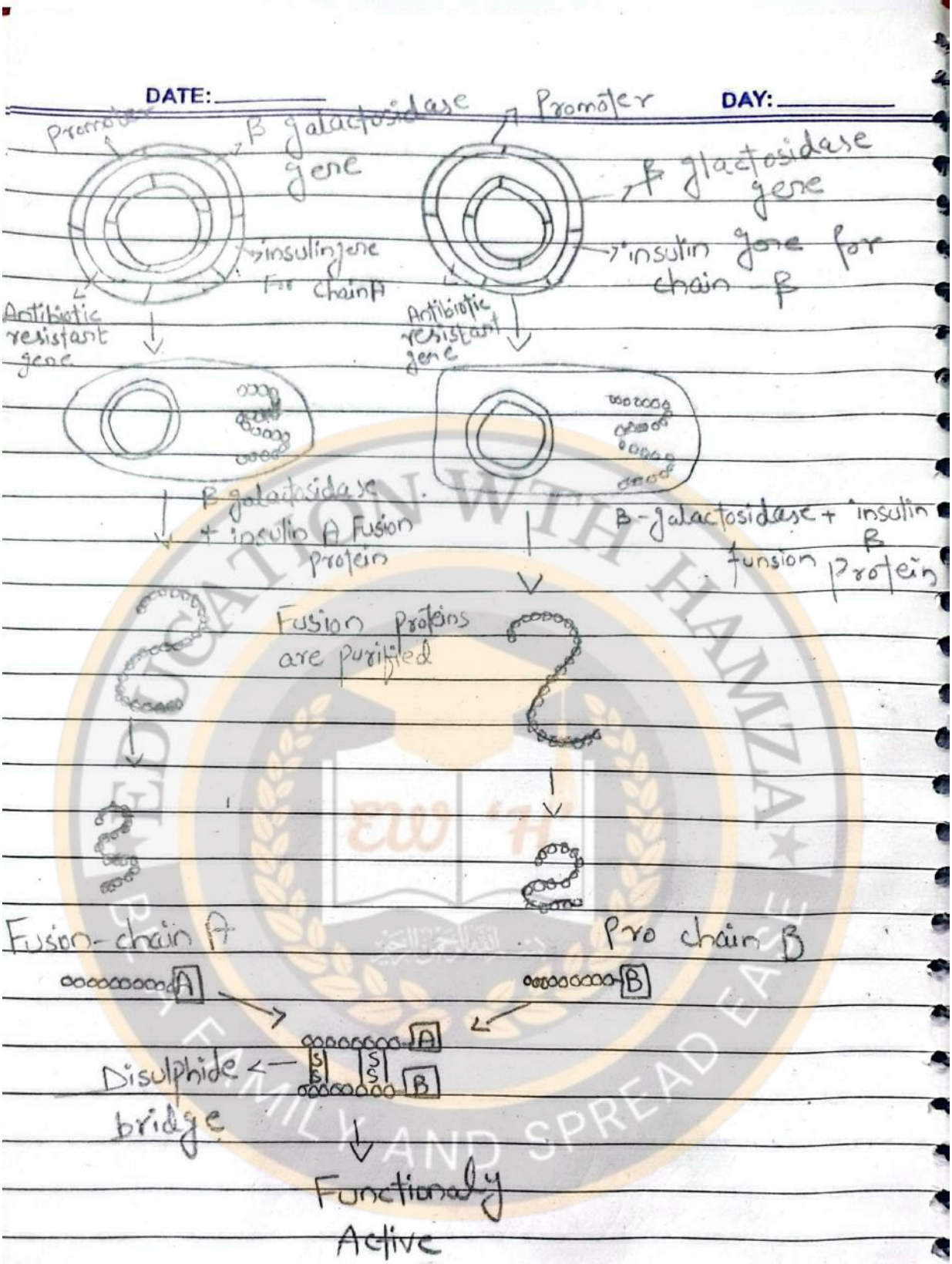
Final active Insulin is [illegible] further purified to remove impurities.

Testing would be done for check quality and effectiveness.

(9) Packaging For Storage & Usage:

After comformation of best quality Insulin is packaged into vials or Insulin pens.

DATE: _____ DAY: _____



DATE: _____ DAY: _____

Polymerase Chain Reaction:

Kary B. Mullis in 1983 it was discovered. An enzyme DNA polymerase is used to copy given peice of DNA again and again so multiple copies are used. This technique is called PCR.

It is very quick process.
Single gene of DNA can be copied.

Mechanism:

(1) Denaturation:

DNA is heated for one mint at 94°c.
Two DNA stands get separated now double standard DNA changes due to single strand DNA and now it work as a temperate.

(2) Primer Annealing:

Primer DNA strands for two mnute upto 54°c.
Primer are the sequence of about 20 base that are complementory to base on either side of target DNA.

DATE: DAY:

Extension & Polymerization:

After primer bind DNA polymerase copies the target DNA. At 3' prime end by using dNTPs.

72°C → optimum Temperature

DNA polymerase is temperature in sensitive (thermostable) It is expected from a bacterium which lives in hot spring. Enzyme known as Taq polymerase.

Uses:

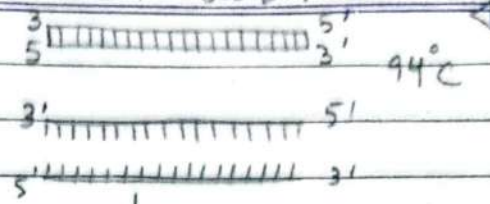
- Diagnosis of diseases
- Evolutionary history of human population.

Storage:

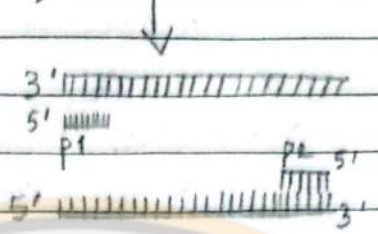
At the end extension temperature is set 4°C for a longer time to safety store the PCR products until it is not shifted into the same place.

DATE: _____ Parental ds DNA DAY: _____

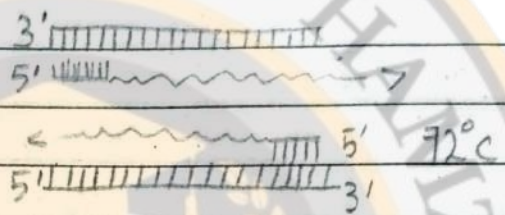
(a) Denaturation (1 Minute)



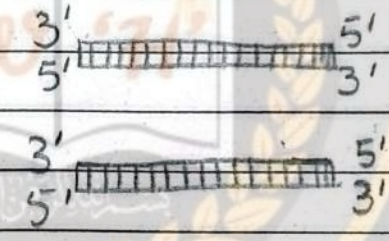
(b) Annealing (2 Minutes)



c) Extension (1-2 Minutes)



→ Two Daughter ds DNA



PCR cycl

Genetically Modified Organism

It is also known as transgenic organisms. It may be plants, animals or microorganisms.

Definition: A genetic material has been altered by adenine removing gene or modifying gene.

DATE: DAY:

(1) Transgenic Bacteria:

1970 E.coli to Introduce human Insulin.
E.coli producing human growth hormones.
By litering transgenic bacteria. (Bioremidiatio
Plasting ealing bacteria.

(2) Transgenic Plants:

First GM crop approve in 1994 one month die. Beti cotton gene produce and insectidal protein that killed warms.
It is disease resistance.
Uses: Improve yeild, Gold rice in rich with vitamin A.

(3) Transgenic Animals:

Drug toxicity and pharmacological testing.
For production of monochorldal anti-bodies. To serve as a model for human diseases.

Concern Related to GMO:

How to dispose of microbial waste.
How to deal antibiotic resistance.
Toxicity and alergy associated.