GENE CLONING

FACULTY:-

PROFESSOR

DR. MUSHTAK T. SALIH AL-NEDA

The unite of heredity is the **gene.** The gene is a segment of DNA that carries in it nucleotide sequence information for a specific biochemical or physiological property. In all organisms the genetic material is DNA, except some RNA bacteriophages (e,g., Qβ-MS2) and some RNA viruses (e.g., influenza and reovirus), in which genetic information is stored in ribonucleic acid (RNA).

- Gene: The basic biological unit of heredity. A segment of deoxyribonucleic acid (DNA) needed to contribute to a function.

- An official definition: According to the official Guidelines for Human Gene Nomenclature, a gene is defined as "a DNA segment that contributes to phenotype/function.

- DNA: Genes are composed of DNA, a molecule in the memorable shape of a double helix, a spiral ladder. Each rung of the spiral ladder consists of two paired chemicals called bases. There are four types of bases. They are adenine (A), thymine (T), cytosine (C), and guanine (G). As indicated, each base is symbolized by the first letter of its name: A, T, C, and G. Certain bases always pair together (AT and GC). Different sequences of base pairs form coded messages

- The gene: A gene is a sequence (a string) of bases. It is made up of combinations of A, T, C, and G. These unique combinations determine the gene's function, much as letters join together to form words. Each person has thousands of genes -- billions of base pairs of DNA or bits of information repeated in the nuclei of human cells --which determine individual characteristics (genetic traits).

- The chromosome: Genes are arranged in precise arrays all along the length of 23 pairs of much larger structures: the chromosomes. One chromosome in each pair comes from the mother and the other one from the father. The chromosomes in any particular pair look like each other, except in a boy. There is one pair of chromosomes, which usually settles the sex of the individual. This pair has two X chromosomes in females and one X and one Y chromosome in males

- The X and Y chromosomes: These chromosomes -- the X and Y are always capitalized -- are the sex chromosomes. All the other chromosomes in the human chromosome complement are numbered from 1 to 22 and are called the autosomes (literally, the other chromosomes).

Plasmids are play the role in the following:-

1- The spread of multiple antibiotic and drug resistance in pathogenic bacteria.

2- The instability of industrially important microorganism.

Types of plasmids

he plasmids are classified functionally into:

1- F and F^- plasmid: fertility factors.

2- R plasmids: carrying genes coding for antimicrobial resistance phenomenon.

3- Col plasmids: coding for colicins , the proteins that kill sensitive *Escherichia coli* cells to this amino acid. There are another type of plasmid code for vibriocin that kills sensitive *Vibrio cholera*.

4- Virulence plasmids which are responsible for virulency of microorganisms.

5- Degradative plasmids.

All conjugative plasmids have 2 resistance transfer factor (RTF) components

1-One segment carrying a set of genes involved in conjugative DNA transfer (RTF components)

2-Second segment carrying the antibiotic and /or drug resistance genes (R- determinants).

The plasmid have transposons elements (Tn- elements) which pass from bacterial strains to another and are the source of a suddenly acquired resistance to one or more antibiotics by a bacterium.

The characters of T_n elements (transposons):-

1- They are mobile segments of DNA.

2- They contain genes coding for their own re- arrangement.

3- They contain genes that specify resistance to various antibiotics.

DNA recombination:

Once the DNA is transferred from the donor to the recipient cell by one of the three processes previously described, it can integrate into the host cell chromosome by recombination. There are two types of recombination:-

1-Homologous recombination:- The two pieces of DNA that have extensive homologous regions pair up and exchange pieces by the processes of breakage and reunion.

2-Non-homologous recombination:- In which little, if any, homology is necessary.

DNA cloning

DNA cloning is a technique for reproducing DNA fragments. It can be achieved by two different approaches (1) **cell based approach**, and (2) **polymerase chain reaction (PCR).**

A-Cell based approach:-

In the cell-based approach, a vector is required to carry the DNA fragment of interest into the host cell. The following figure shows the typical procedure by using plasmids as the cloning vector.

The essential steps in DNA cloning using plasmids as vector:

A- DNA recombination: The DNA fragment to be cloned is inserted into a vector. The recombination vector must also contain an antibiotic-resistance gene. See figure1,2

B- Transformation: the recombination DNA enters into the host cell and proliferates. It is called "transformation" because the function of the host cell may be altered. Normal *E. coli* cells are difficult to take up plasmid DNA from the medium. If they are treated with $CaCl_2$, the transformation efficiency can be significantly enhanced. Even so, only one cell in about 10.000 cells may take up a plasmid DNA molecule. See figure 3.

C- Selective amplification: A specific antibiotic is added to kill *E.coli* without any protection. The transformed *E.coli* is protected by the antibiotic-resistance gene whose product can inactivate the specific antibiotic. The numbers of vectors in each *E.coli* cell are not the same, because they may also reproduce independently. See figure 3.

D- Isolation of desired DNA clones.

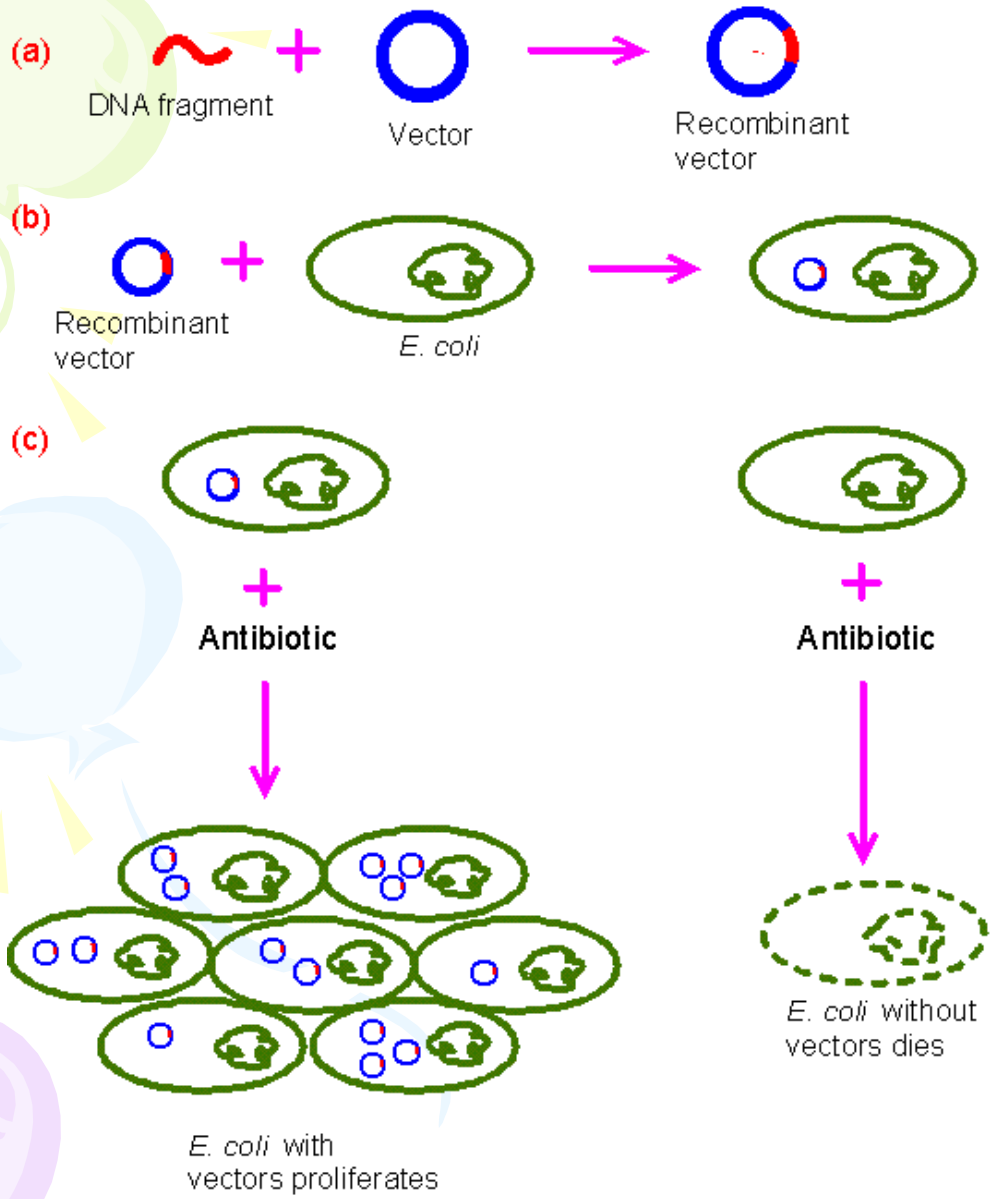


Figure 4. The essential steps in DNA cloning using plasmids as vectors

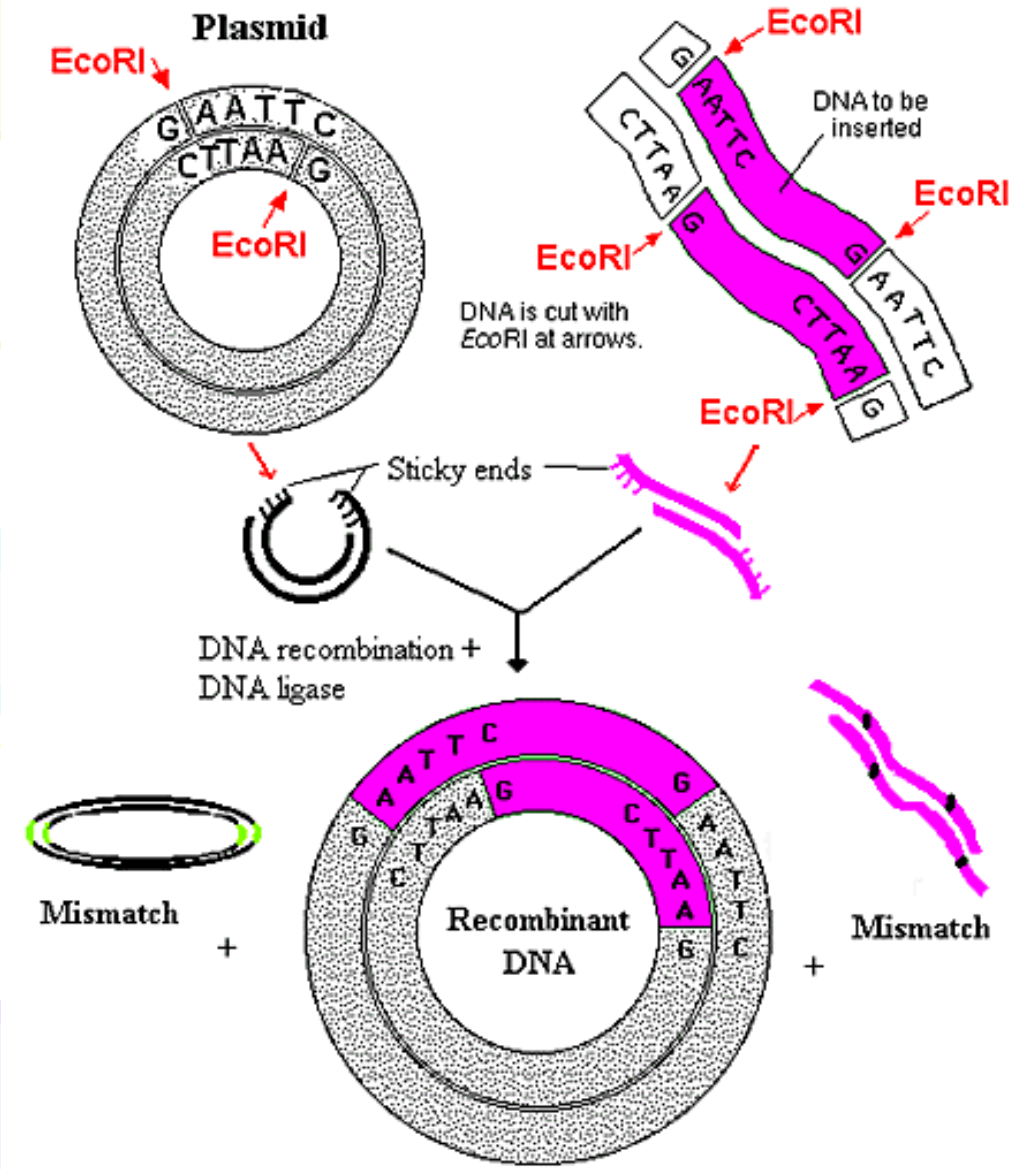


Figure 5. The inserting of DNA sample into sample

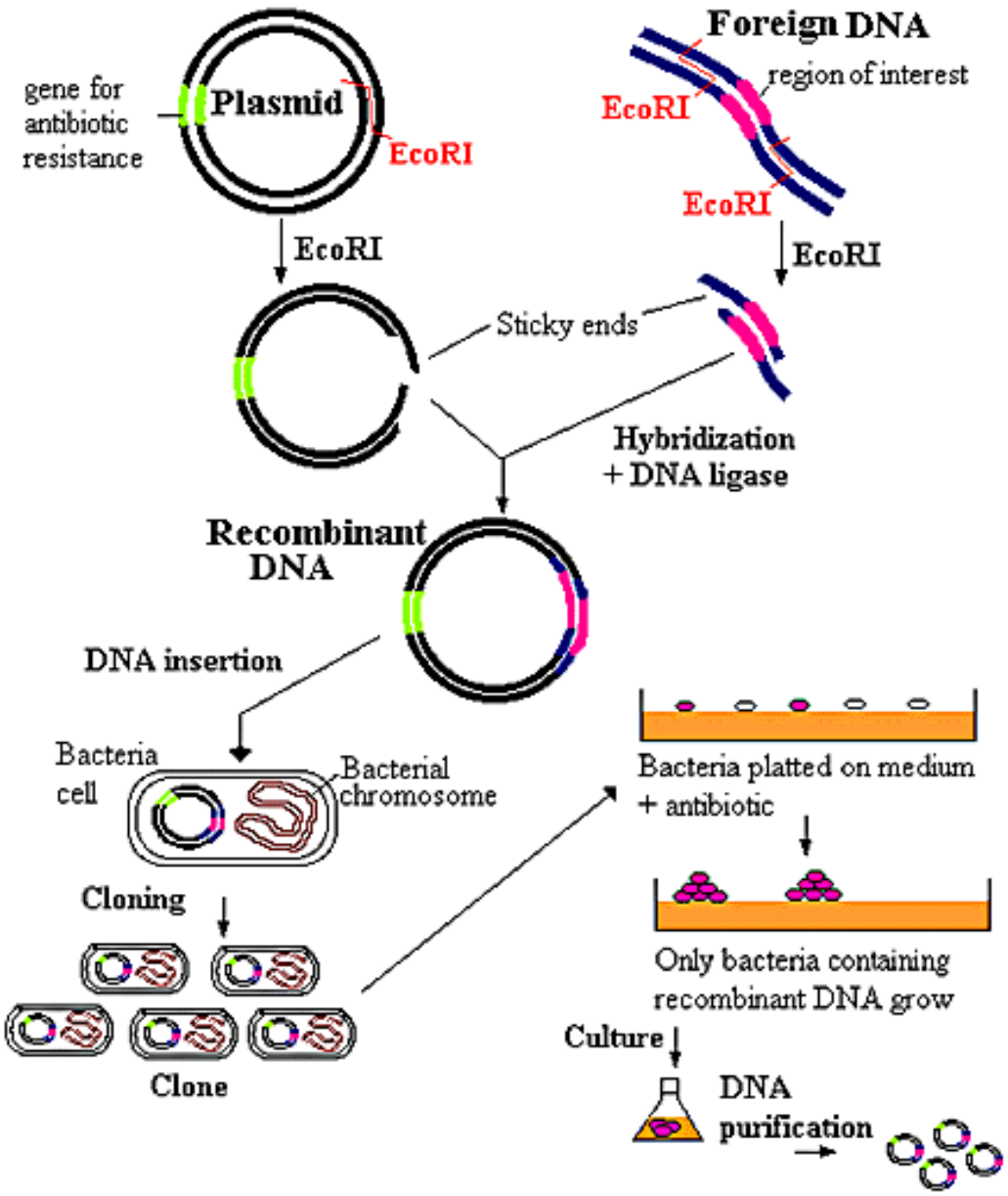


Figure 6. The process by which a plasmid is used to import recombinant DNA into a host cell for cloning.

C- Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) is an *in vitro* technique which allows the amplification of a specific deoxyribonucleic acid (DNA) region that lies between two regions of Know DNA sequence.

PCR amplification of DNA is achieved by using oligonucleotide primers, also known as amplimers. These are short, single-stranded DNA molecules which are complementary to the ends of a defined sequence of DNA template. The primers are extended on single stranded denatured DNA (template) by a DNA polymerase, in the presence of Deoxynucleoside triphosphates (dNTPs) under suitable reaction conditions. This result in the synthesis of new DNA strands complementary to the temperature strands. These strands exist at this stage as double–stranded DNA molecules. Strand synthesis can be repeated by heat denaturation of the double stranded DNA, annealing of primers by cooling the mixture and primer extension by DNA polymerase at a temperature suitable for the enzyme reaction. Each repetition of strand synthesis becomes a template for any further cycle of amplification and so the amplified target DNA sequence is selectively amplified cycle after cycle.

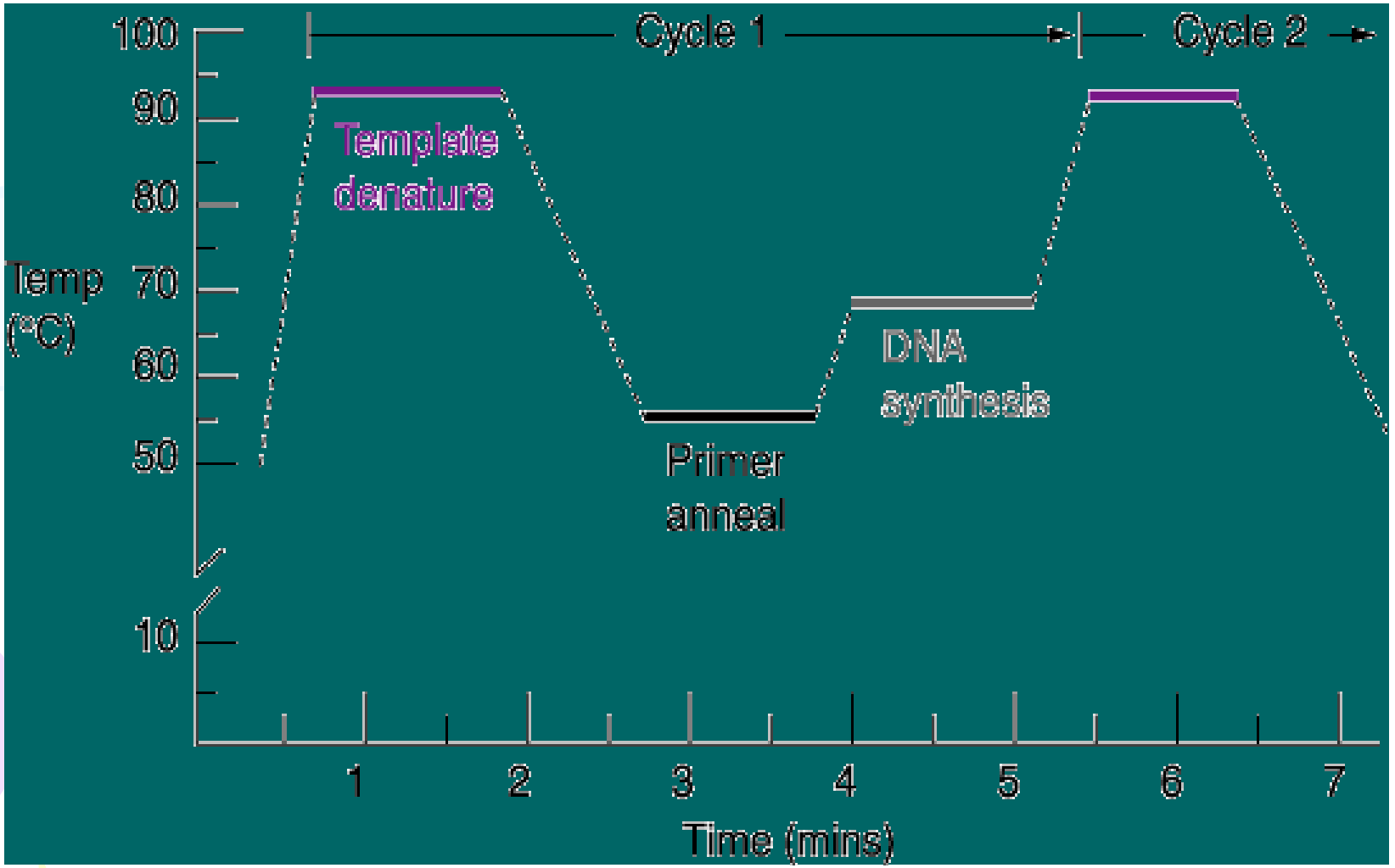
Temperatures

- **Denaturing:**
- 94 – 98 c depending on GC contains
- and the long of DNA
- **Annealing:**
- **55 – 65 c depending on** *Tm*
- Annealing = ***Tm*** – (1- 5)
- **Extension:**
- **72 c** Optimums Temperature for Taq polymerase

Typical Thermal Cycler Conditions

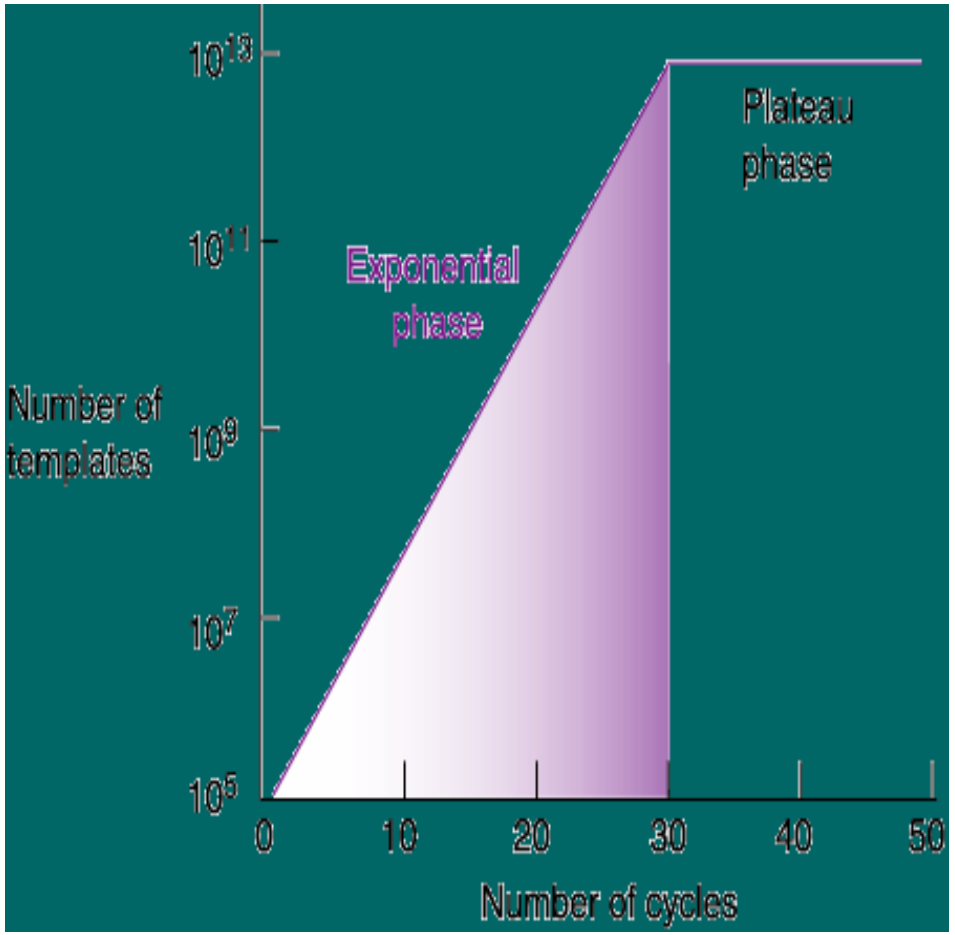
1.	Initial Denaturation	95c	4 min
2.	DNA Denaturation	95c	1 min
3.	Primer Annealing	65c	1 min
4.	Primer Extension	72c	1 min
5.	Repeat 29 more times		
6.	Final Extension	72c	5 min
7.	Hold at 4 C		

The temperature profile

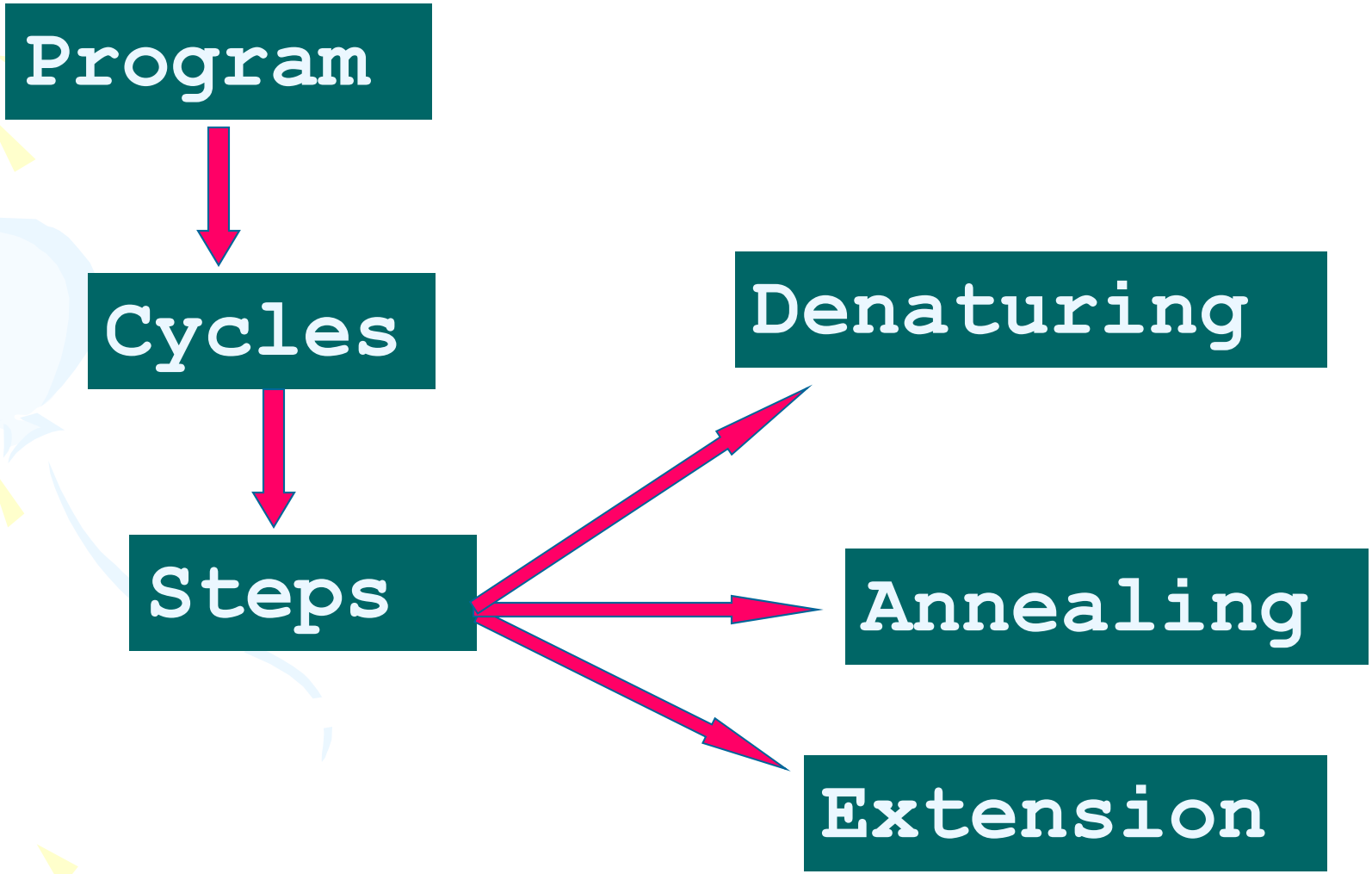


Amplification phase

- **Exponential phase:** lasts for about 30 cycles under standard reactions conditions.
-
- **Plateau phase:** results from limiting amounts of enzyme and reduced enzyme activity



PCR Programs



Genetic engineering and its application in medicine:

-To produce large amounts of biologically useful proteins. These include:

A-recombinant human proteins used as drugs or hormones like the following:

Proteins	**Their importance**
Chorionic gonadotropin	Treatment of inferility
Insulin	Treatment of Diabetes mellitus
Interleukins	Treatment of Cancer
Interferons (β,γ)	Treatment of Viral infection, cancer
Growth hormones	Treatment of Growth retardation

B-Recombinant enzymes with industrial uses:

Proteins	Industrial use
Rennin	Cheese makingff Cheese making
Protease	Detergents
Lipase	Cheese making
Catalase	Antioxidants in food

2- To create organisms (plant, animal, and microorganisms) with altered characteristics, for example, plants are resistance to disease.

3- In the diagnosis of most medically important bacteria e.g., tubercle bacilli (*Mycobacterium tuberculosis)* is diagnosed with the technique of polymerase chain reaction which is highly sensitive and specific .

4-For understanding the molecular basis of a number of disease e.g., familial hypercholesterolemia, sickle cell disease, thalassemias, cystic fibrosis.

5- For production of proteins for vaccination e.g., vaccine of hepatitis B surface antigen (HBsAg),and for diagnostic test (e.g., AIDS test)

6- In the gene therapy of sickle cell disease, thalassemias and other diseases