Polymerase Chain Reaction(PCR)

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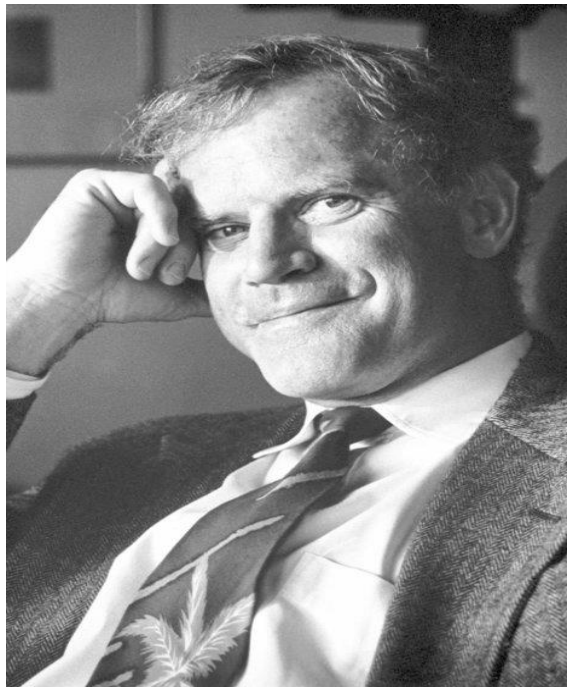
Introduction

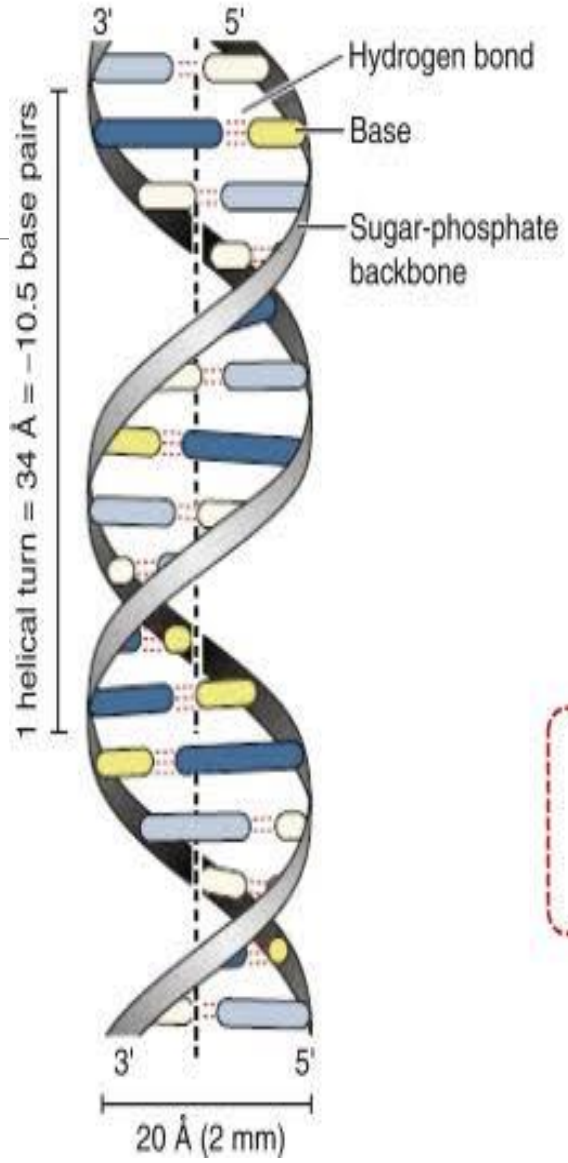
- This is a rapid automated method for the amplification of specific DNA sequences(or genes).
- This technique was invented by Kary B. Mullis, in 1983, for which he won the Nobel Prize in chemistry in the year 1993
- PCR consists of several cycles of sequential DNA Replication where the products of first cycle become the template for the next cycle.
- PCR has made it possible to generate millions of copies of a small segment of DNA.
- This tool is commonly used in the molecular biology and biotechnology labs.

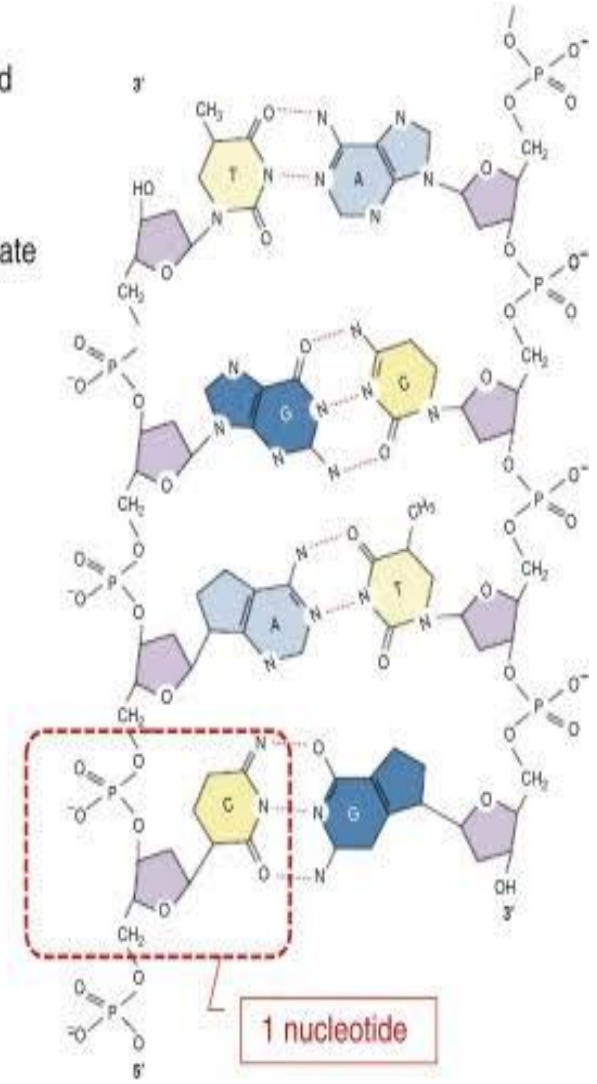


Kary B. Mullis
The Nobel Prize in Chemistry 1993
Prize motivation: "for his invention of the polymerase chain reaction (PCR) method."

Principle

- The PCR technique is based on the enzymatic replication of DNA.
- In PCR, a short segment of DNA is amplified using primer mediated enzymes.
- DNA Polymerase synthesises new strands of DNA complementary to the template DNA.
- The DNA polymerase can add a nucleotide to the pre-existing 3’-OH group only. Therefore, a primer is required.
- Thus, more nucleotides are added to the 3’ prime end of the DNA polymerase.





DNA Polymerase

Sources of DNA polymerase

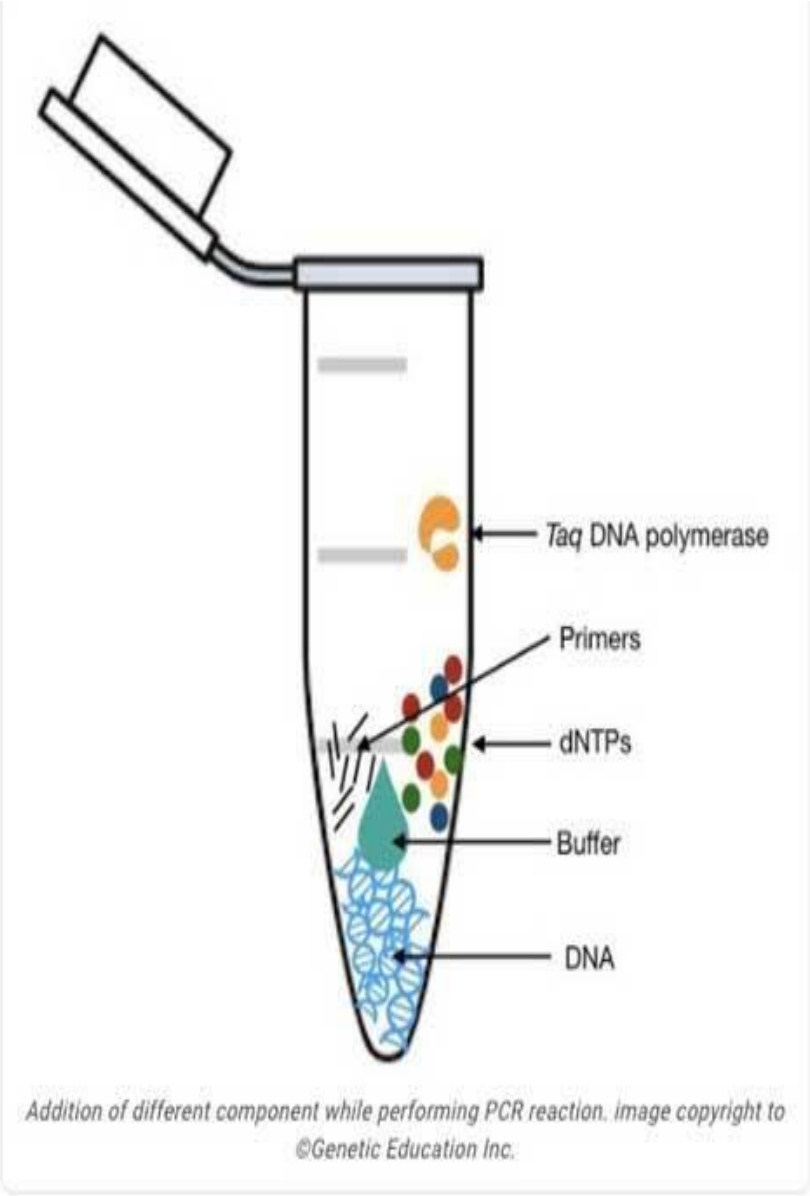
In the original technique of PCR, Klenow fragment of E. coli DNA polymerase was used.

This enzyme, gets denatured at higher temperature, therefore, fresh enzyme had to be added for each cycle.

A breakthrough occurred (Lawyer 1989) with the introduction of Taq DNA polymerase from thermophilic bacterium, Thermus aquaticus. The **Taq DNA polymerase is heat resistant, hence it is not necessary to freshly add this enzyme for each cycle of PCR.**

Components of PCR

1. DNA template – This is the DNA of interest from the sample.
2. Heat resistant DNA polymerase – Taq Polymerase(THERMUS AQUATICUS) is usually used as it is thermostable and does not denature at very high temperatures.
3. Deoxyribonucleotide triphosphate – These are single units of bases that provide energy for polymerization and are the building blocks for DNA synthesis.
4. Oligonucleotide Primers – These are short stretches of single-stranded DNA that are complementary to the 3’ ends of sense and antisense strands.
5. Buffer System – This is composed of magnesium and potassium, which create optimum conditions for denaturation and renaturation of DNA. In addition, the buffer system is also crucial for polymerase activity and stability.

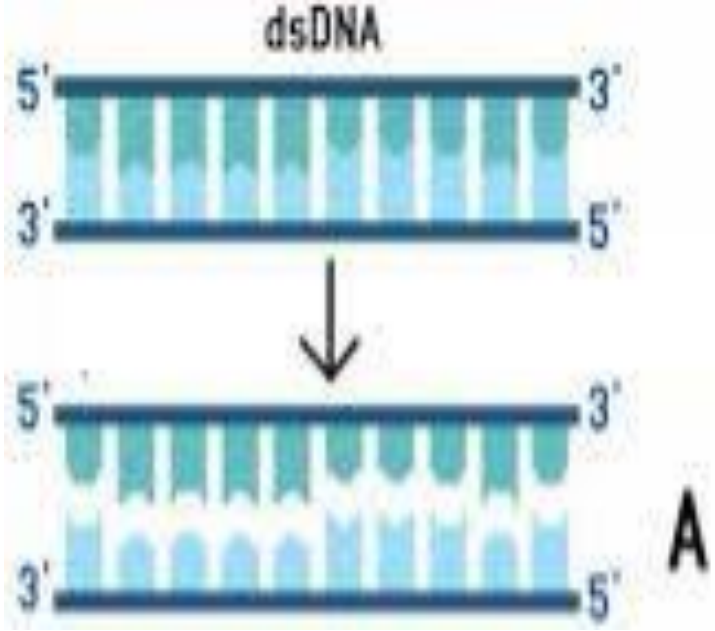


Addition of different component while performing PCR reaction. image copyright to ©Genetic Education Inc.

Steps of PCR

The PCR process involves a series of temperature-dependent steps and utilizes a DNA polymerase enzyme to catalyze the synthesis of new DNA strands.

1-Denaturation
2-Annealing or Renaturation
3-Extension or Synthesis



A

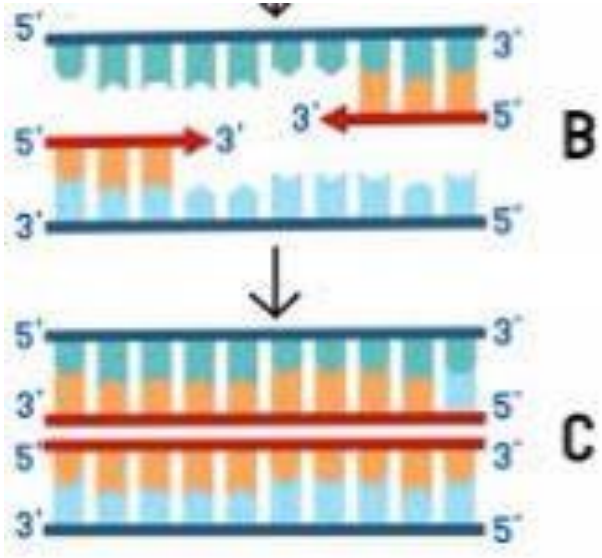
1. Denaturation (94-98°C):

The double-stranded DNA template is heated to a high temperature, usually around 94-98°C. This causes the hydrogen bonds between the complementary DNA strands to break, resulting in the separation of the two strands. This step is often referred to as “melting.”

Steps of PCR

2. Annealing (50-65°C):

The temperature is lowered to a specific range (typically 50-65°C), allowing short DNA primers to bind to the complementary sequences at the beginning of the target DNA region. These primers serve as starting points for DNA synthesis.

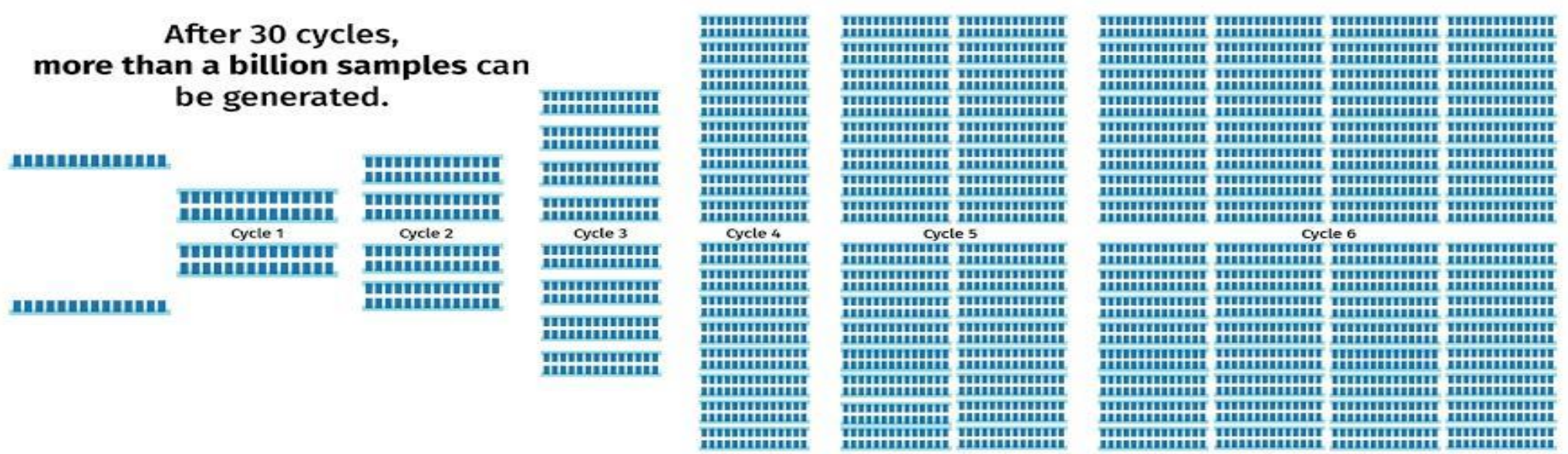


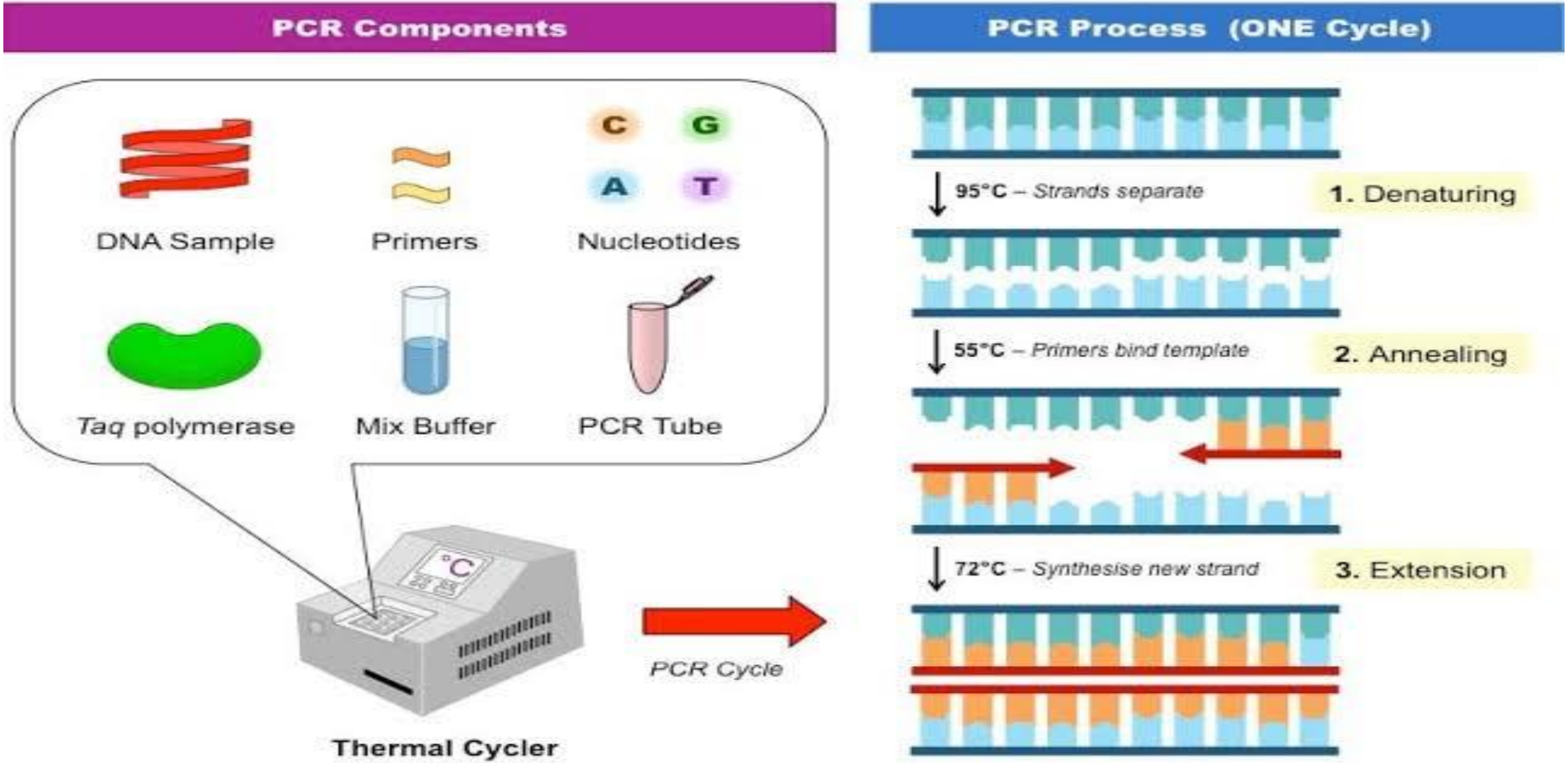
3. Extension (72°C):

The temperature is raised to around 72°C, which is the optimal temperature for the DNA polymerase enzyme (usually Taq polymerase) to synthesize a new DNA strand by adding nucleotides complementary to the template strand. The primers dictate the sequence of the newly synthesized DNA strand.

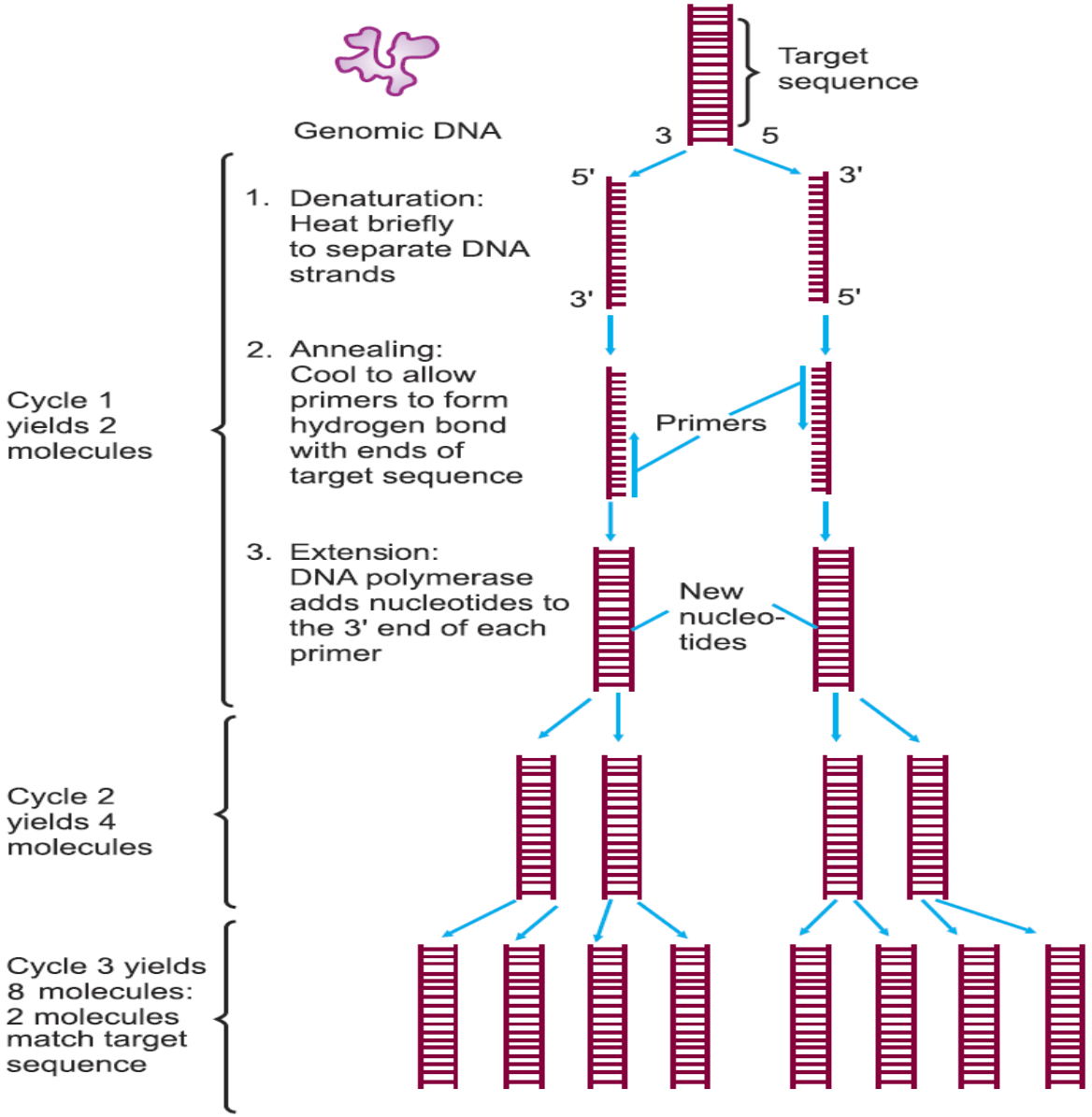
Steps of PCR

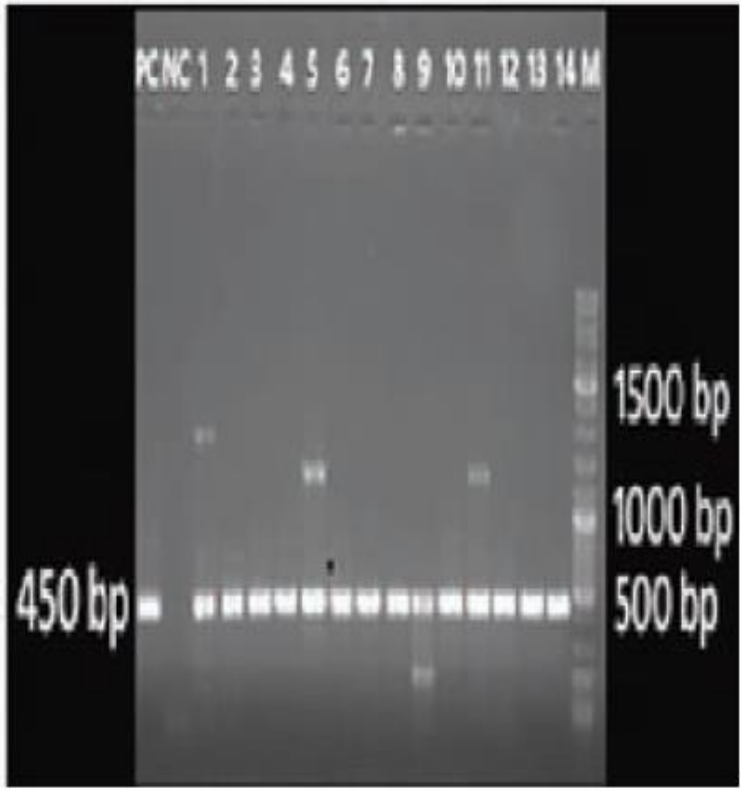
These three steps—denaturation, annealing, and extension—are typically repeated for a specific number of cycles, usually around 20 to 40 cycles. Each cycle doubles the amount of DNA, resulting in an exponential increase in the target DNA segment.




PCR Components
DNA Sample
Primers
C
G
A
T
Nucleotides
Taq polymerase
Mix Buffer
PCR Tube
°C
Thermal Cycler
PCR Cycle
PCR Process (ONE Cycle)
95°C – Strands separate
1. Denaturing
55°C – Primers bind template
2. Annealing
72°C – Synthesise new strand
3. Extension

Amplification of genomic DNA by PCR





Thermocycler (conventional PCR) to amplify the desired DNA and gel documentation of amplified DNA bands of specific base pairs compared to a molecular weight ladder (*Source:* Dept of Microbiology, PIMS)

Conventional RT-PCR

DNA amplification technique

Reverse transcriptase PCR — RNA viruses

Real-time PCR (quantitative) — Viral load

Nested PCR — Two primers used

Multiplex PCR — More than one primer used

Types of PCR

Reverse-Transcriptase (RT–PCR)

- The conventional PCR technique can only amplify the dsDNA sequences. Therefore, the RNA viruses cannot be amplified by this method directly. In this modification with the help of the reverse transcriptase (RT) enzyme, a complementary copy of DNA is made from the RNA and this cDNA is then used a template for PCR.

Types of PCR

qPCR or Real-Time PCR or quantitative PCR

- DNA molecules are tagged using a fluorescent dye, which is used to monitor and quantify PCR products in real time.
- In this method, the advantage over the conventional method is that it quantifies the PCR by monitoring the amplification process while the PCR is ongoing. Hence, it is called real time. This gives the estimate of the pathogen DNA load in the test specimen
- The detection is done using :

- **Non-specific fluorescent dyes** like SYBR green that intercalate with dsDNA s it gets synthesised, the fluorescent being proportional to the DNA synthesised.
- Specific set of DNA probes which are labelled with fluorescent reporters that get detected only after the probe hybridizes with the complementary DNA sequence.

Quantitative PCR (qPCR)

Types of PCR

Nested PCR

- In conventional PCR, sometimes, the primers bind to non-specific regions. In order to take care of this non-specificity, a second set of primers are used in the successive cycles to amplify a secondary target within the first target.
- After the initial 25-35 PCR cycles, an additional PCR is conducted using new primers "nested" within the original primers, which reduces the risk of unwanted products.

Multiplex PCR

- Primers specific to more than one pathogen are used simultaneously in the same reaction to give multiple PCR products.

Types of PCR

AB StepOnePlus
Fast Real-Time PCR System

7900HT Fast Real-Time PCR System
(Sol Efroni's lab)

Qiagen's Rotor-gen
(Oren Levy's lab)

Bio-Rad CFX-96

Thermo PikoReal
(Bachelet Lab)

Types of PCR

Hot Start PCR – in which heat is used to denaturate antibodies that are used to inactivate Taq polymerase

Long-range PCR – longer ranges of DNA are formed by using a mixture of polymerases

Assembly PCR – longer DNA fragments are amplified by using overlapping primers

Asymmetric PCR – only one strand of the target DNA is amplified

In situ PCR – PCR that takes place in cells, or in fixed tissue on a slide

Advantages of PCR technology

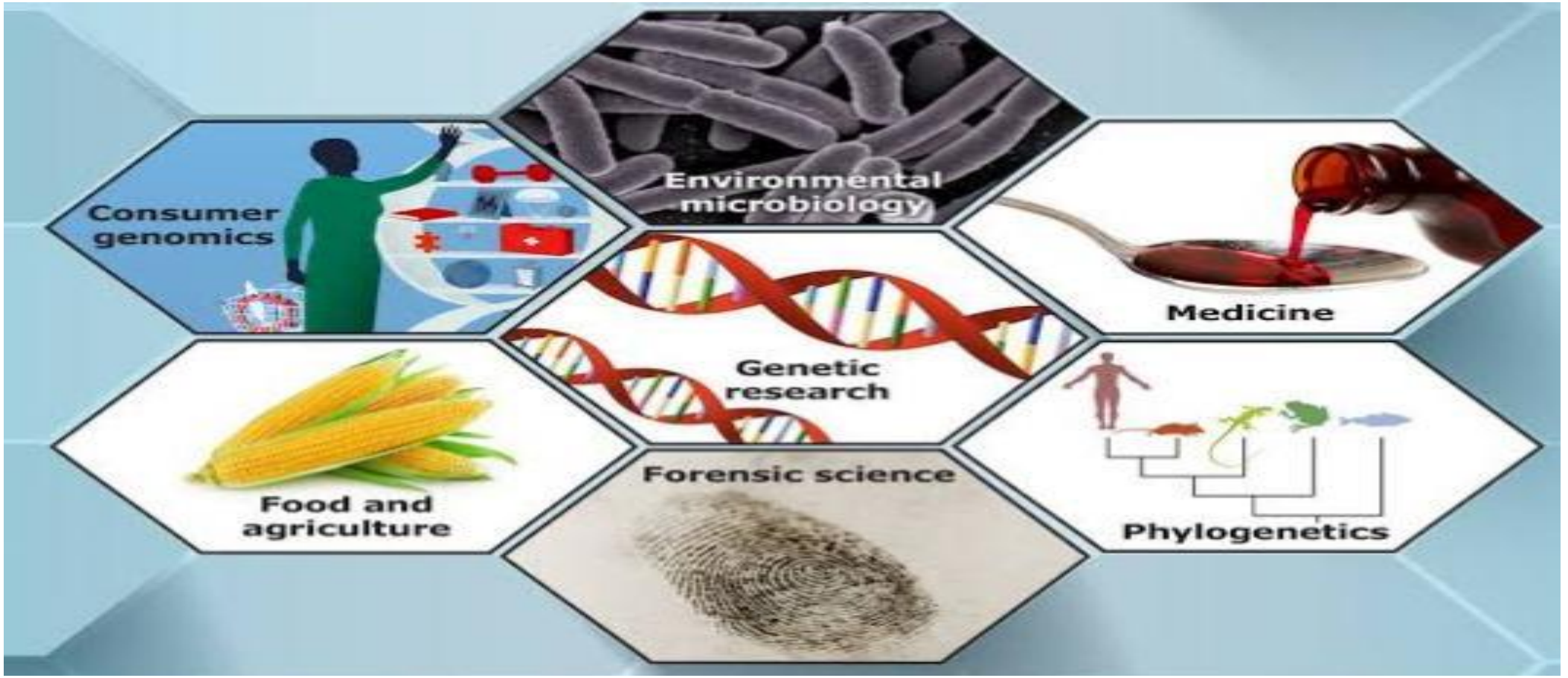
- ***Highly specific:*** PCR can distinguish DNA sequences by just one nucleotide, making it a very accurate technique.
- ***Sensitive:*** PCR is a very useful technique when the amount of DNA sample is limited because it allows the detection of even a single copy of a specific DNA template.
- ***Versatile:*** The PCR technique can be used for various applications like genetic testing, criminal investigations, and paternity tests.
- ***Rapid and efficient***: PCR can efficiently and rapidly amplify a small amount of DNA sample to million copies in just a few hours.

Disadvantages of PCR technology

- ***Contamination***: The PCR technique is very susceptible to contamination from other sources of DNA or RNA or the environment. This can mislead data interpretation.
- ***Cost and complexity***: PCR can be expensive and requires expert knowledge for high-throughput projects.
- ***Lack of novel information***: Since PCR can only amplify and target specific DNA sequences targeted by the primers, PCR provides limited information and cannot detect novel DNA sequences.
- ***Inhibition from sample content***: The whole PCR cycle can be disrupted by inhibitors that co-purify with DNA, such as heam from blood samples, reducing the sensitivity of the process.
- ***Errors in amplification***: Base substitutions, indels, and other alterations in DNA sequences can lead to inaccurate amplification and hence, false results.

Overall, PCR significantly impacts many research areas but careful quality measures should be performed while designing and interpreting PCR experiments.

Applications of PCR Technology



Applications of PCR Technology

Clinical Diagnosis/Medicine

Prenatal diagnosis of inherited diseases :
PCR is employed in the prenatal diagnosis of inherited diseases by using chorionic villus samples or cells from amniocentesis. Thus, diseases like sickle-cell anemia, □-thalassemia and phenylketonuria can be detected by PCR in these samples.

Diagnosis of retroviral infections :
PCR from cDNA is a valuable tool for diagnosis and monitoring of retroviral infections, e.g., HIV infection.

Applications of PCR Technology

Clinical Diagnosis/Medicine

Diagnosis of bacterial infections :
PCR is used for the detection of bacterial infections e.g., tuberculosis by Mycobacterium tuberculosis.

Diagnosis of cancers :
Several virally-induced cancers (e.g., cervical cancer caused by human papilloma virus) can be detected by PCR.
Further, some cancers which occur due to chromosomal translocation (chromosome 14 and 18 in follicular lymphoma) involving known genes are identified by PCR.

PCR in sex determination of embryos :
Sex of human and live stock embryos fertilized in vitro, can be determined by PCR, by using primers and DNA probes specific for sex chromosomes.
Further, this technique is also useful to detect sex —linked disorders in fertilized embryos.

Applications of PCR Technology

Forensic Science

1)Used as a tool in genetic fingerprinting.

2)Identifying the criminal from millions of people:
A single molecule of DNA from any source (blood strains, hair, semen etc.) of an individual is adequate for amplification by PCR. Thus, PCR is very important for the identification of criminals.

3**) Paternity tests**

Applications of PCR Technology

Research and Genetics

Compare the genome of two organisms in genomic studies:

The differences in the genomes of two organisms can be measured by PCR with random primers. The products are separated by electrophoresis for comparative identification. Two genomes from closely related organisms are expected to yield more similar bands.

In the phylogenetic analysis of DNA from any source such as fossils:

PCR is very important in the study of **evolutionary biology**, more specifically referred to as **phylogenetics**. As a technique which can amplify even minute quantities of DNA from any source (hair, mummified tissues, bone, or any fossilized material), PCR has revolutionized the studies in palaentology and archaelogy. The movie 'Jurassic Park', has created public awareness of the potential applications of PCR!

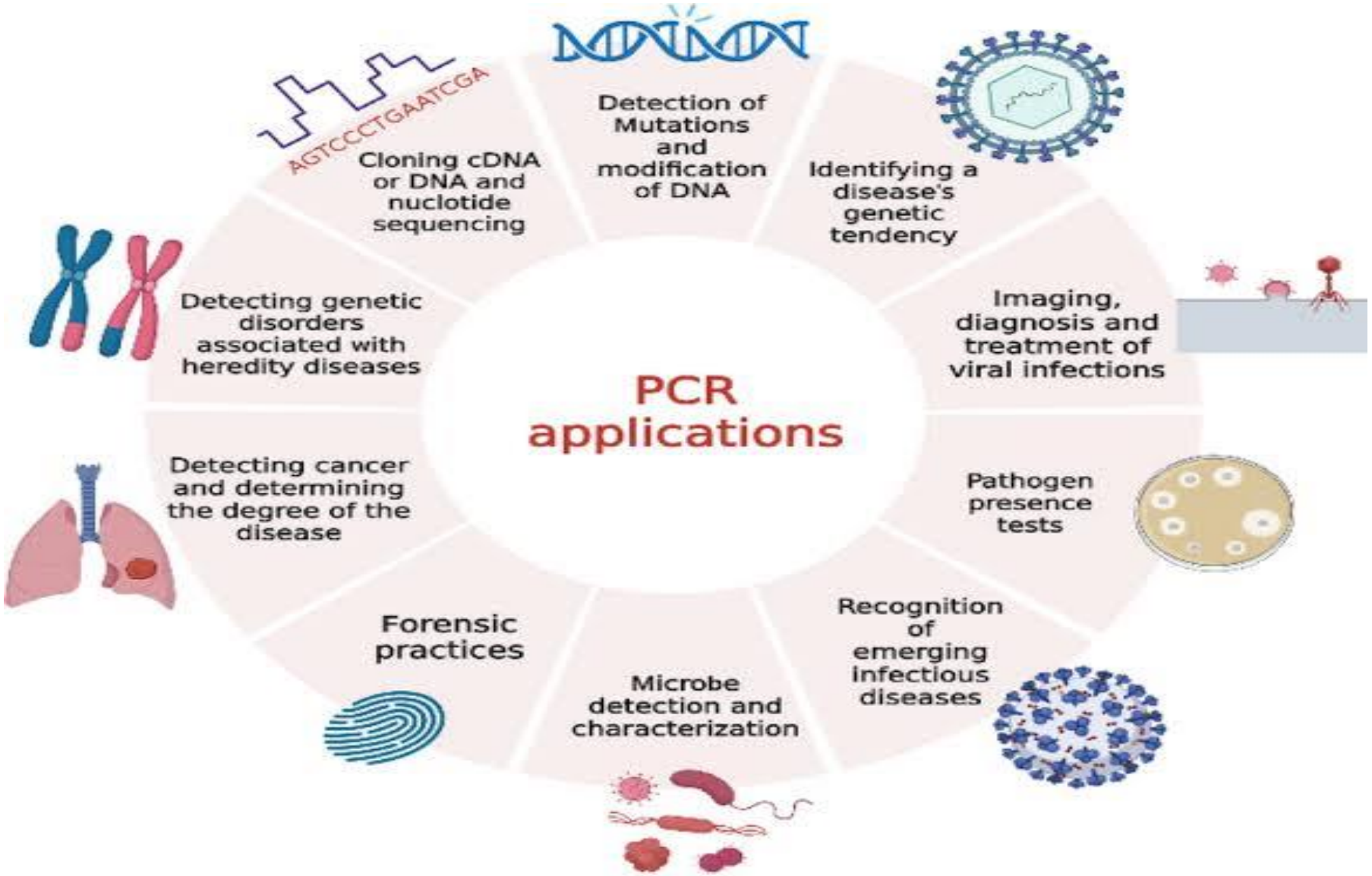
Applications of PCR Technology

Research and Genetics

DNA sequencing:
As the PCR technique is much simpler and quicker to amplify the DNA, it is conveniently used for sequencing. For this purpose, single strands of DNA are required.

Analysis of gene expression

Gene Mapping


PCR applications
Detection of Mutations and modification of DNA
Identifying a disease's genetic tendency
Imaging, diagnosis and treatment of viral infections
Pathogen presence tests
Recognition of emerging infectious diseases
Microbe detection and characterization
Forensic practices
Detecting cancer and determining the degree of the disease
Detecting genetic disorders associated with heredity diseases
Cloning cDNA or DNA and nuclotide sequencing
AGTCCCTGAATCGA


THANK YOU!