Polymerase Chain Reaction (PCR)

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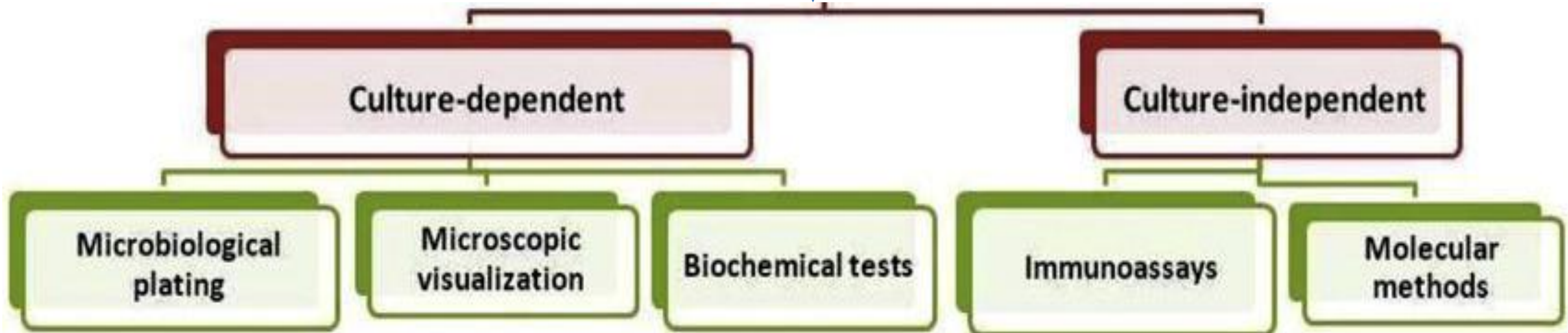
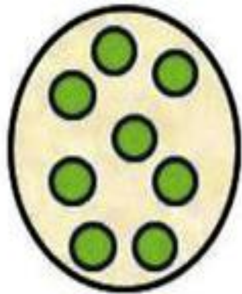
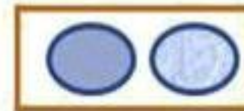
Polymerase Chain Reaction (PCR)

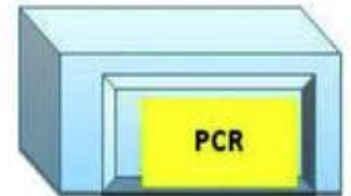
- Microbial analysis in foods is an integrated part of management of microbial safety in the food chain.
- Molecular techniques, especially the polymerase chain reaction (PCR), are one of the most important rapid methods for the sensitive and specific detection of pathogenic micro-organisms.
- PCR has evolved to a convenient and rapid method in recent years.
- Convenient PCR kit available in market.

Conventional Methods of Microbial Analysis





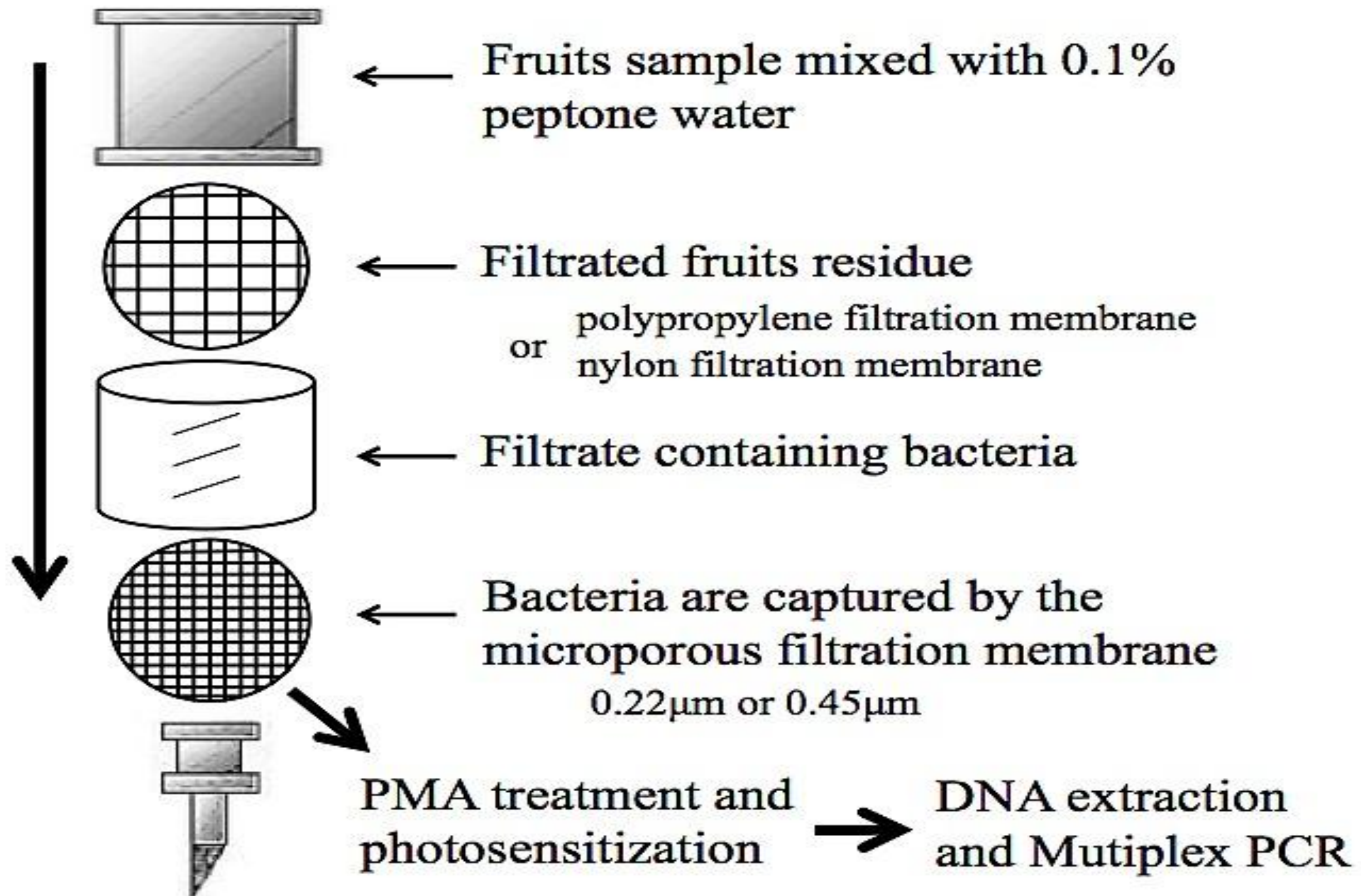




Polymerase Chain Reaction (PCR)

- Polymerase chain reaction (PCR) is most commonly used molecular-based method for the detection of micro-organisms in food.
- PCR is a method for synthesising multiple copies of (amplifying) a specific piece of DNA.
- PCR can use the smallest sample of the DNA to be cloned and amplify it to millions of copies in just a few hours.
- Discovered in 1985 by Kerry Mullis, PCR has become essential and routine tool in most biological laboratories.

PCR in Food Microbial Analysis



Principle of PCR

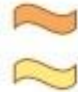
- The PCR involves the **primer mediated enzymatic amplification** of DNA.
- PCR is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand.
- Primer is needed because DNA polymerase can add a nucleotide only onto a pre-existing 3′-OH group to add the first nucleotide.
- DNA polymerase then elongate its 3 end by adding more nucleotides to generate an extended region of double stranded DNA.

For PCR four basic components are required:

- A **DNA template** containing the target sequence
- **Primers** – a pair of short single stranded DNA sections
- A **heat-stable DNA-polymerase enzyme**, usually *Taq* polymerase
- **Free nucleotides** as building blocks

PCR Components
PCR Process (ONE Cycle)

DNA Sample


Primers

C
G
A
T
Nucleotides

Taq polymerase

Mix Buffer

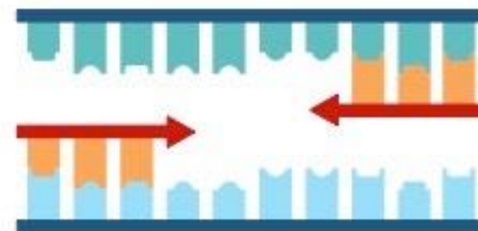
PCR Tube

°C
Thermal Cycler

PCR Cycle

95°C – Strands separate
1. Denaturing

55°C – Primers bind template
2. Annealing


72°C – Synthesise new strand
3. Extension

Typical Laboratory Work Flow

To perform microbial analysis in any food sample three steps are there:

Stage 1. Sample enrichment

Stage 2. Sample preparation

Stage 3. DNA amplification and analysis

Total time for assay normally 20-30 hours but can be as little as 12 hours depending on the assay.

Typical Laboratory Work Flow

Enrichment

Manual or Automated Sample Prep

PCR Analysis

PCR Technique in Food Microbiology

Stage 1. Sample enrichment 25g of sample added to 225ml of enrichment broth and incubated overnight. Enrichment times can be as little as 8 hours but 16-24 hours is more normal.

Stage 2. Sample preparation Collect enriched samples and lyse cells to extract target DNA. Add extracted DNA to PCR tubes/wells containing PCR assay reagents. Time 1-3 hours

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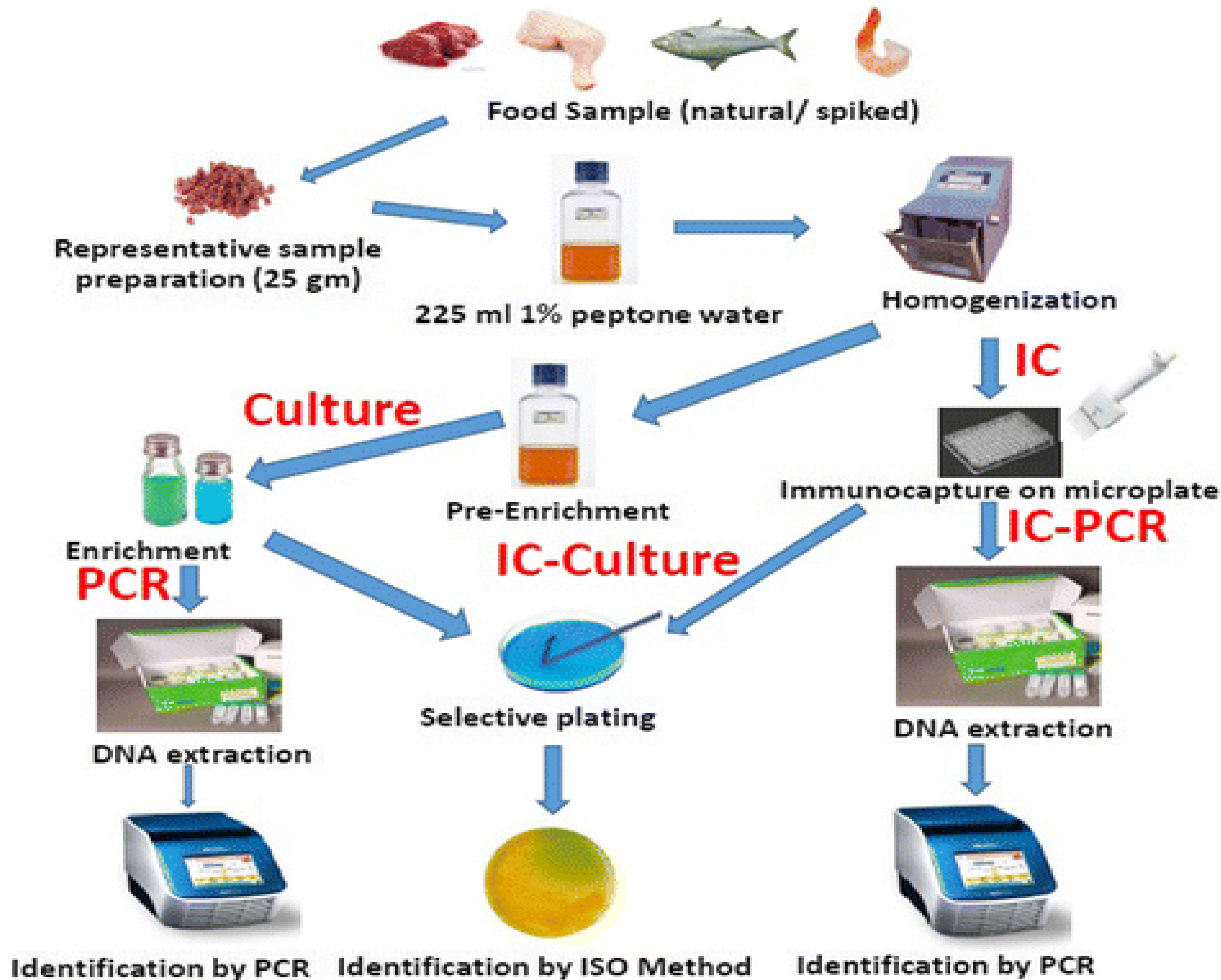
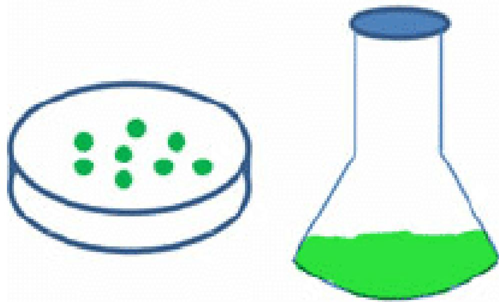

Food Sample (natural/ spiked)
Representative sample preparation (25 gm)
225 ml 1% peptone water
Homogenization
IC
Culture
Pre-Enrichment
Immunocapture on microplate
Enrichment
IC-PCR
IC-Culture
PCR
Selective plating
DNA extraction
DNA extraction
Identification by PCR
Identification by ISO Method
Identification by PCR

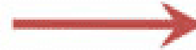
PCR Technique in Food Microbiology

Stage 3. DNA amplification and analysis Place samples in automated thermocycler/analyser and start PCR. Dedicated software manages cycling and detection and calculates results. Time 2-3 hours

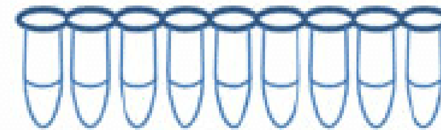
PCR Technique Outline

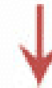
Cells on agar plates
or in flasks





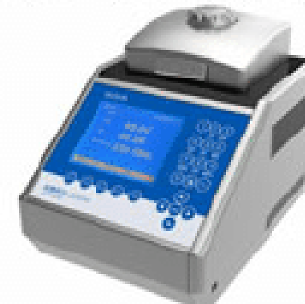
PCR tubes containing
10 ul of 2X PCR buffer

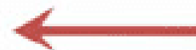




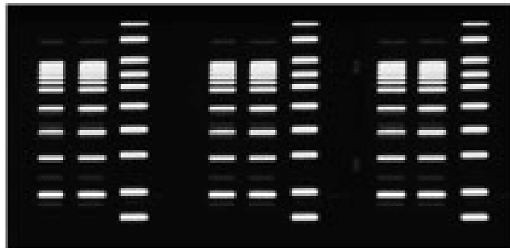
Boil for 5-20 min and cool dowr

Mixture* (10 ul) added for PCR





DNA gel analysis



Sample Preparation for PCR

1. Food sample grinded for homogenisation and The minimum quantity required is 1 g.
2. From the test sample, two analytical samples (100 mg) taken for DNA extraction. A quantity of 50 g is stored as a reference, if the analysis fails or if it needs to be repeated.
3. Different physical and/or chemical treatments to extract DNA and to eliminate of all other materials

CONTI------

SAMPLE PREPARATION

COLLECTION OF FOOD SAMPLE

PROCESS OF SAMPLE
(Homogenization, Washing)

CONCENTRATION OF PATHOGEN
(Enrichment, Immunocapture, Buyont densitycentrifucation)

TEMPLATE EXTRACTION
(Heat, Detergent, Chemical, Solvent)

CONCENTRATE TEMPLATE
(Alcohol Precipitation, Binding Matrices)

Sample Preparation for PCR

- Step of thermal lysis done in the presence of cetyltrimethylammonium bromide (CTAB), followed by several extraction steps for the removal of protein or polysaccharide compounds for protein rich food sample.

- Samples containing high quantity of starch have to be treated with enzymes like α-amylase; for the meat-derived products, a longer treatment (up to 3 h) with proteinase-K to remove protein

CONTI------

Sample Preparation For PCR

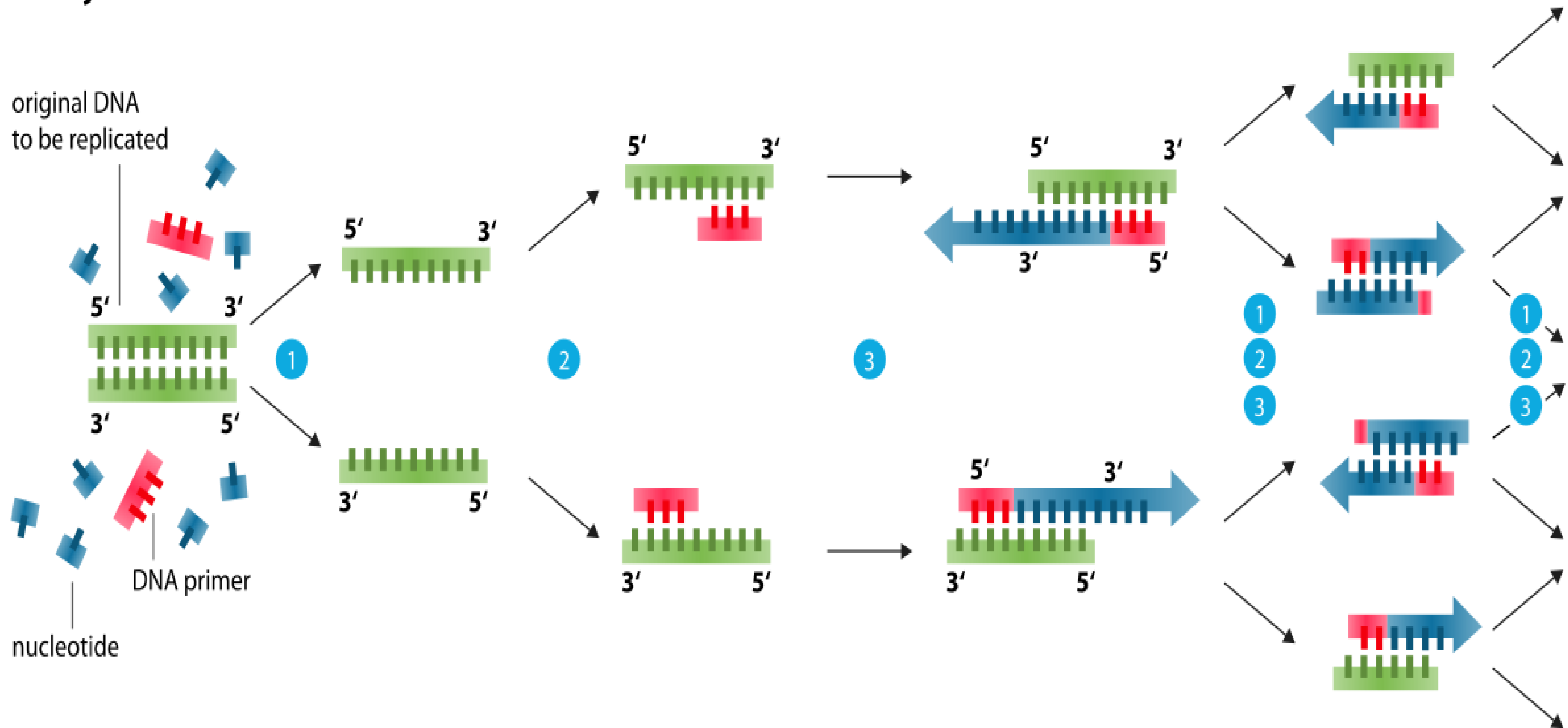
- To evaluate the DNA yield and purity, the absorbance method was applied. Therefore a spectrophotometer is used to measure the absorbance at 260 nm for DNA concentration.
- The concentration of the DNA samples originated from unprocessed food products as seeds or meats are usually higher and their quality meets the purity requirements.
- But the DNA quality is not optimal if highly processed materials are analyzed, mainly due to the DNA degradation during the mechanical and thermal treatments.

Procedure of PCR

A series of 3 major cyclic reactions conducted in an automated, self-contained thermocycler machine. They are:-

1. **Denaturation**
2. **Annealing**
3. **Elongation/Extension**

Polymerase chain reaction - PCR



1 **Denaturation** at 94-96°C

2 **Annealing** at ~68°C

3 **Elongation** at ca. 72 °C

1. Denaturation

Denaturation :
This step involves heating the reaction mixture to 94°C for 15-30 seconds. During this, the double stranded DNA is denatured to single strands due to breakage in weak hydrogen bonds.

2. **Annealing**

Annealing : The reaction temperature is rapidly lowered to 54-60°C for 20-40 seconds. This allows the primers to bind (anneal) to their complementary sequence in the template DNA.

3. **Extension**

Elongation : Also known at extension, this step usually occurs at 72-80°C (most commonly 72°C). In this step, the polymerase enzyme sequentially adds bases to the 3′ each primer, extending the DNA sequence in the 5′ to 3′ direction. Under optimal conditions, DNA polymerase will add about 1,000 bp/minute.

Types of PCR

1. **Multiplex PCR:** offers a more rapid detection as compared to simple PCR through the simultaneous amplification of multiple gene targets. Here several sets of specific primers are used in mPCR assay while in conventional PCR only one specific set of primer is required.

2. **Real-Time or Quantitative PCR (qPCR):** It does not require agarose gel electrophoresis for detection of PCR products unlike conventional PCR. The fluorescence intensity is proportional to the amount of PCR amplicons

Benefits of PCR

- Time saving over other methods.
- Very high specificity and generally requires fewer repeat tests than most other rapid methods.
- Flexible, allowing for several pathogens to be assayed in a single run (multiplex PCR).

Thank You!