PCR

Study Material for

B.Sc. Part III

Botany Hons.

Paper V

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PCR

polymerase chain reaction (PCR):

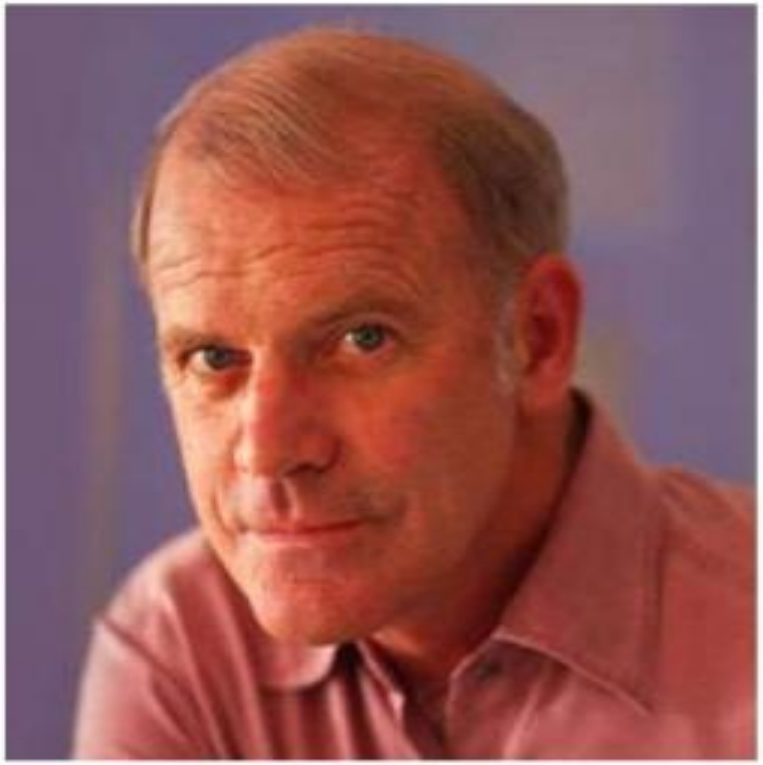
- It is a molecular technology aim to amplify a single or few copies of the DNA to thousands or millions of copies.
- Developed in 1983 by Kary Mullis, PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications. These include diagnosis of infectious diseases, DNA sequencing and DNA-based phylogeny.
- In 1993, Mullis was awarded the Nobel prize in Chemistry along with Michael Smith for his work on PCR.

PCR Requires:
DNA TEMPLATE
*PRIMERS
ENZYMES

HISTORY OF PCR

PCR

- Kary B. Mullis, developed PCR in 1985 and was awarded the Nobel Prize for Chemistry in 1993.
- PCR machine =thermocycler



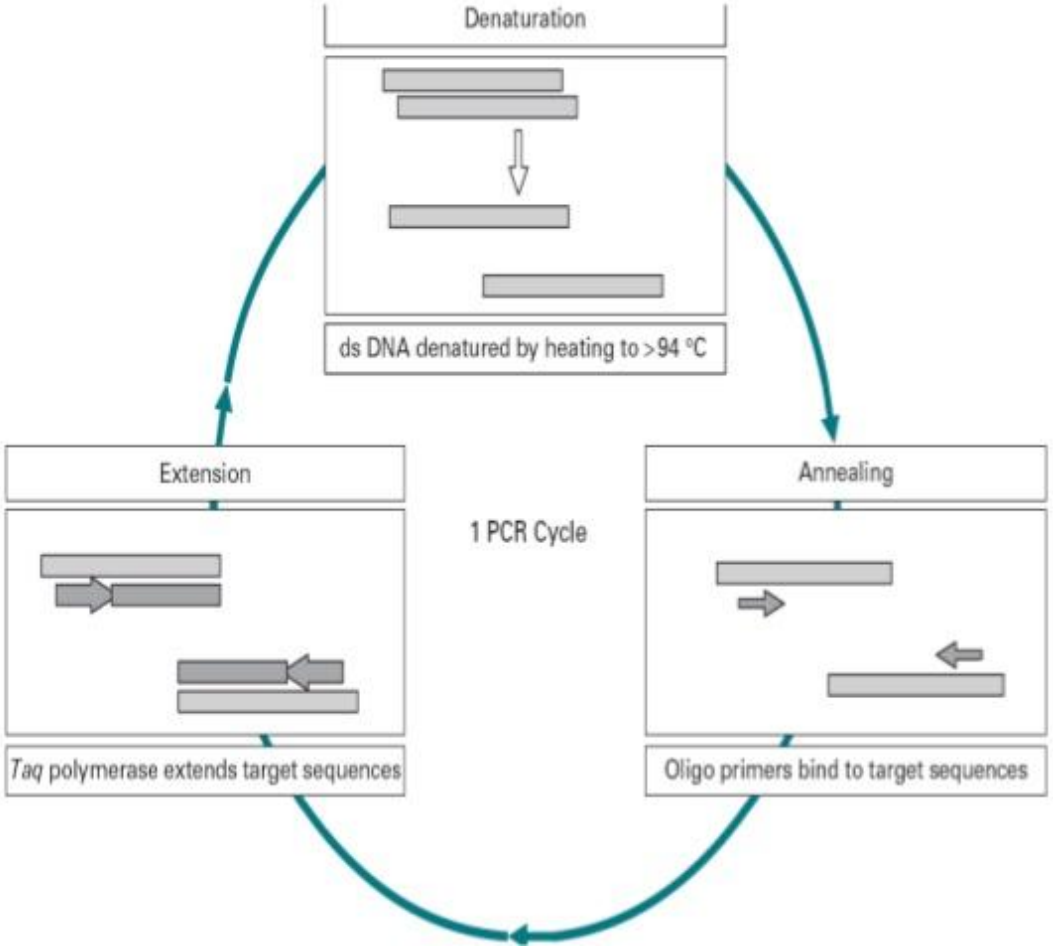
THERMOCYCLER

WORKING MECHANISM OF PCR

- **PCR is a cyclic reaction which completes in three steps:**

- **DENATURATION**
- **ANNEALING**
- **POLYMERZATION / EXTENSION**

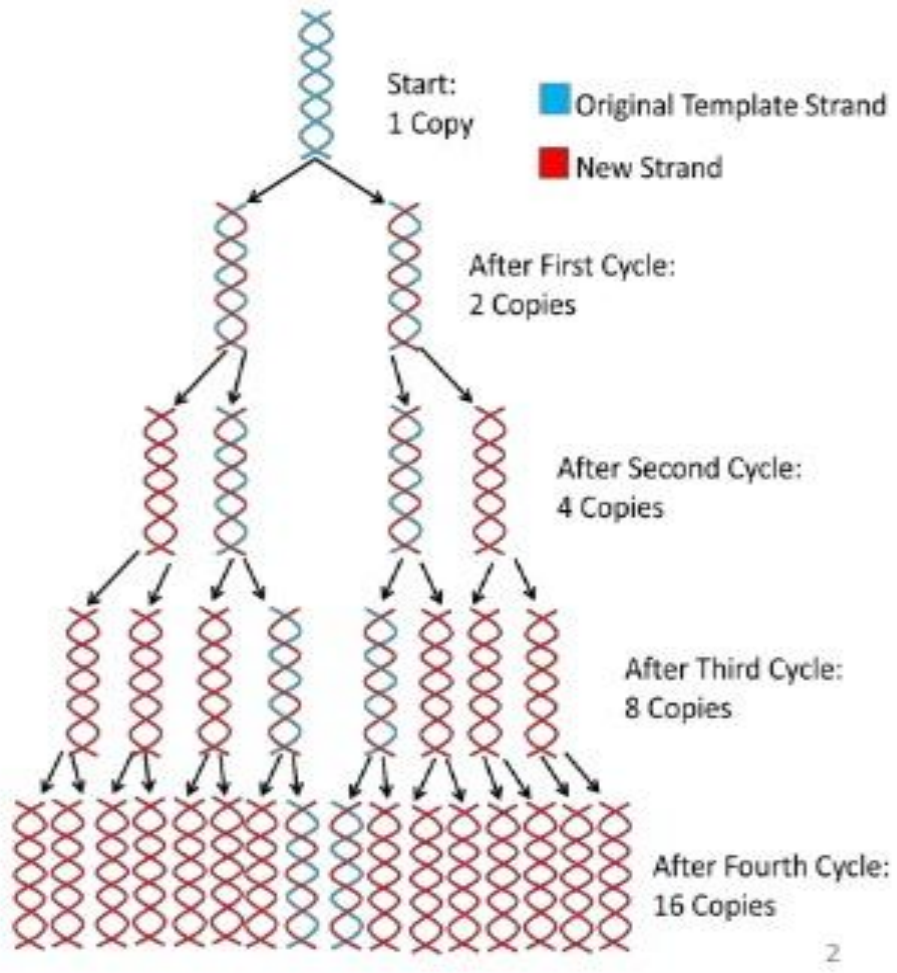
PCR CYCLE


Denaturation
ds DNA denatured by heating to >94 °C
Annealing
Oligo primers bind to target sequences
Extension
Taq polymerase extends target sequences
1 PCR Cycle

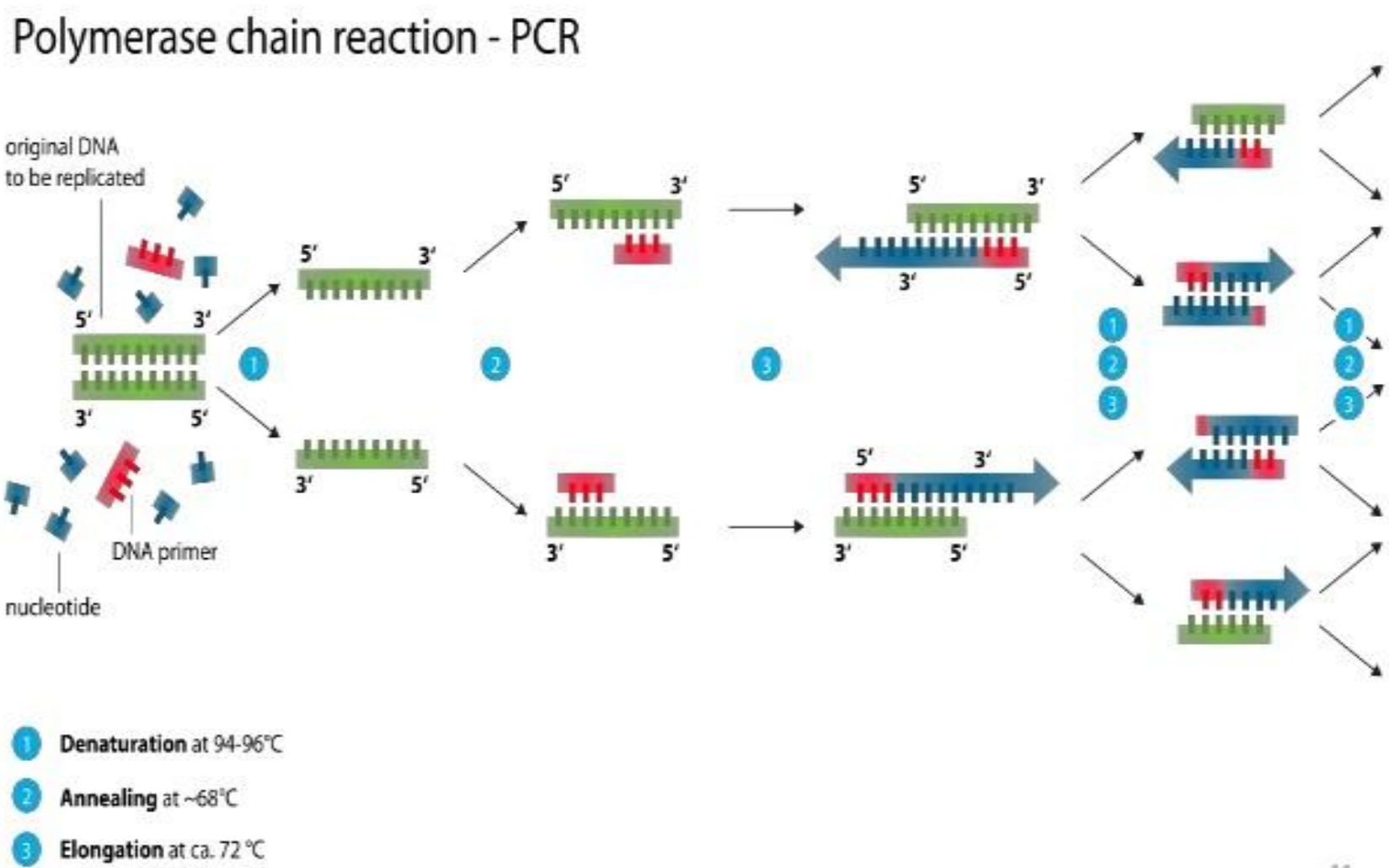
PCR

WORK-MECHANISM

- PCR targets and amplifies a specific region of a DNA strand.
- It is an in-vitro technique to generate large quantities of a specified DNA.
- PCR is 'photocopier.'



WORKING MECHANISM OF PCR



DENATURATION:

- The reaction mixture is heated to a temperature between 90-98° C so that the ds DNA is denatured into single strands by disrupting the hydrogen bonds between complementary bases.
- Duration of this step is 1-2 mins.

ANNEALING

- The annealing step allows the hybridisation of the two oligonucleotide primers, which are present in excess, to bind to their complementary sites that flank the target DNA.
- The annealed oligonucleotides act as primers for DNA synthesis, since they provide a free 3’ hydroxyl group for DNA polymerase.
- TEMPERATURE :45-60°C

ENZYME USED IN POLYMERIZATION

Taq DNA polymerase

- The availability of a thermostable DNA polymerase enzyme isolated from the thermophilic bacterium *Thermus aquaticus* found in hot springs provided the means to automate the reaction.
- Taq DNA polymerase has a temperature optimum of 72°C and survives prolonged exposure to temperatures as high as 96°C and so is still active after each of the denaturation steps.

EXTENSION OR POLYMERIZATION

In this step,

- DNA polymerase synthesizes a new DNA strand by extending the 3' end of the primers.
- Time of the elongation depends on the length of the sequence to be amplified. Since Taq polymerase can add 60-100 bases per second under optimal conditions, synthesis of a 1Kbp fragment should require a little less than 20 seconds.
- most protocols recommend 60 seconds per 1 Kbp DNA to account for time needed to reach the correct temperature and to compensate for other unknown factors that can affect reaction rate.
- The shortest possible time should be used to preserve polymerase activity.

TYPES OF PCR

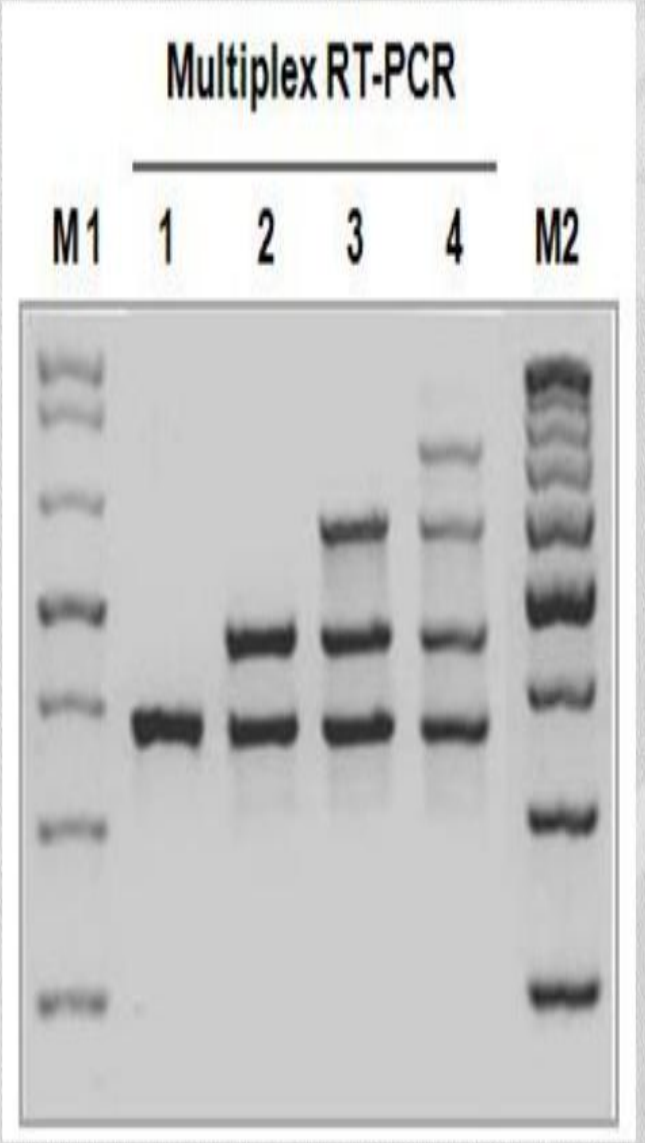
- Conventional (Qualitative)PCR.
- Multiplex PCR.
- Nested PCR.
- RT-PCR and qRT-PCR.
- Quantitative PCR.
- Hot-start PCR.
- Touchdown PCR.
- Assembly PCR.
- Colony PCR.
- Methylation-specific PCR.
- LAMP assay.

Multiplex-PCR:

It is a special type of the PCR used for detection of multiple pathogens by using Multiple primers sets each one targets a particular pathogen.

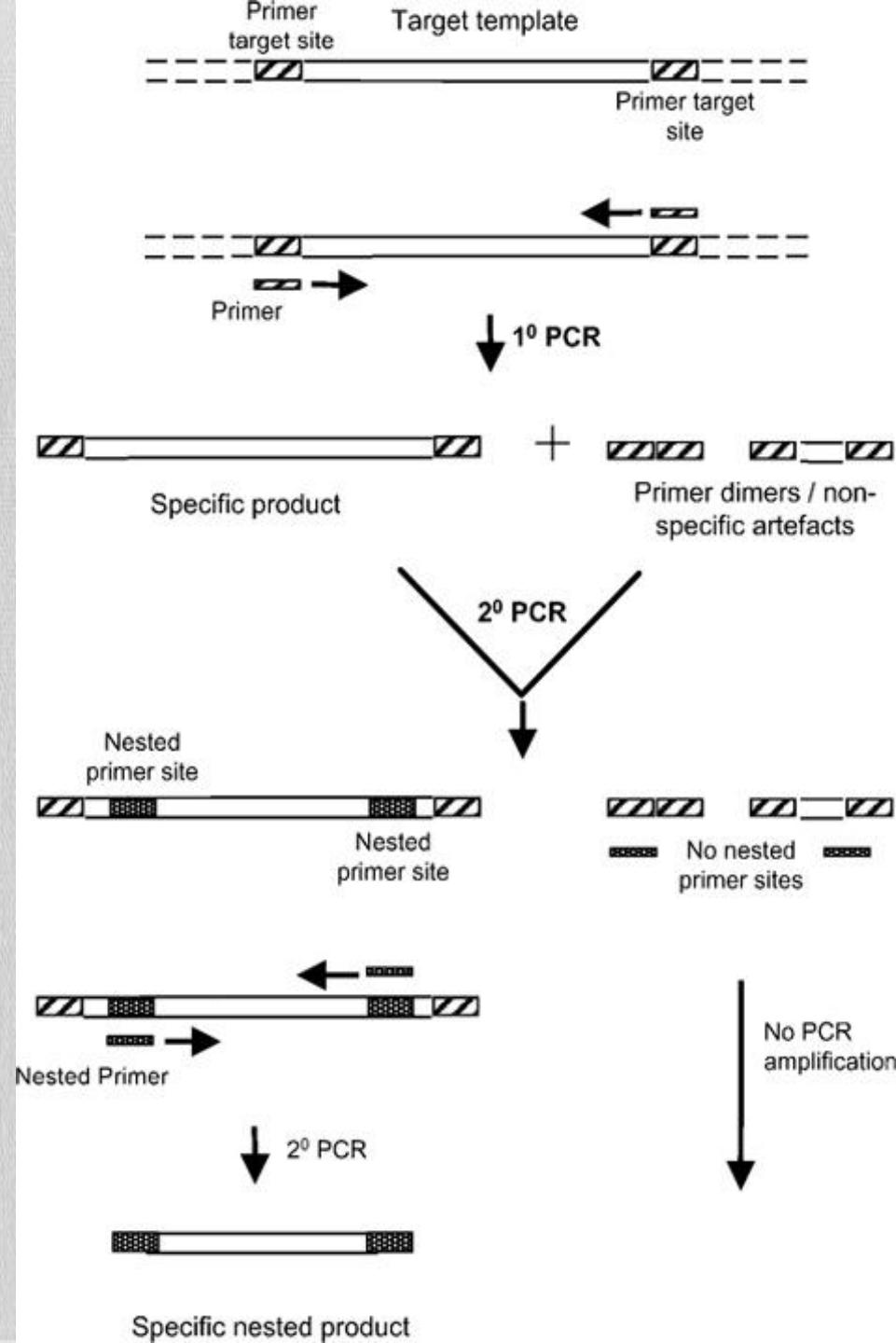
Uses:

This permits the simultaneous analysis of multiple targets in a single sample.



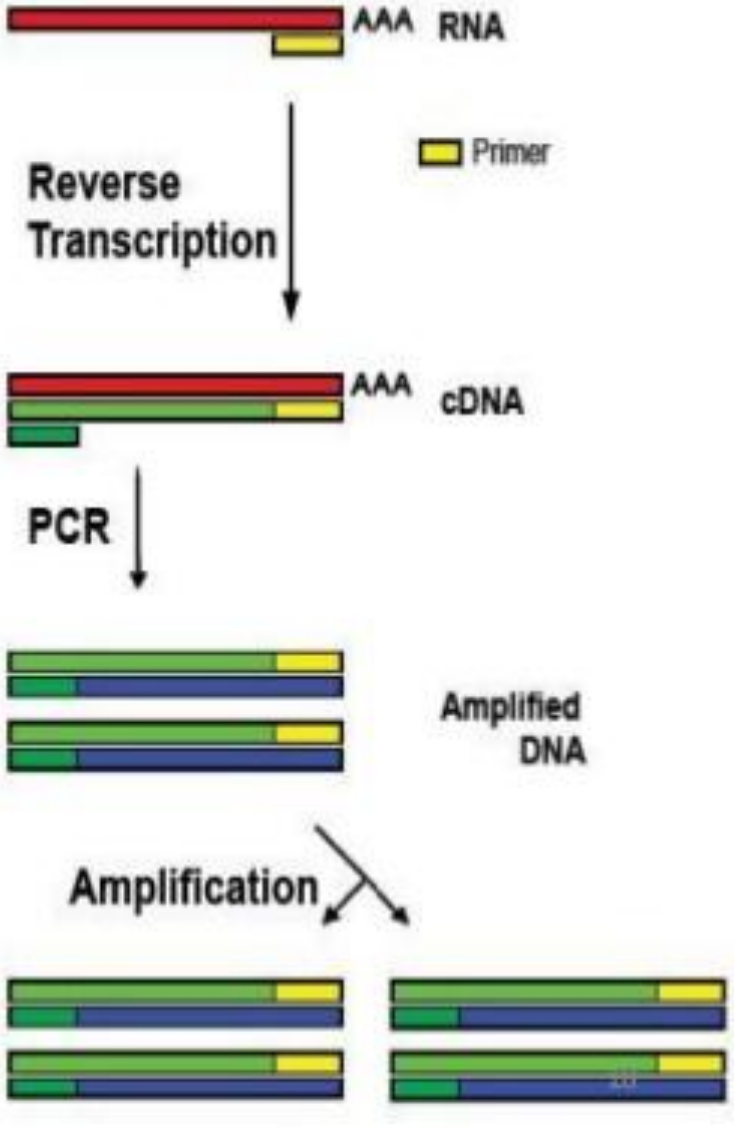
Nested-PCR:

- Used to increase the specificity of DNA amplification.
- Two sets of primers are used in two successive reactions.
- In the first PCR, one pair of primers is used to generate DNA products, which will be the target for the second reaction.



REVERSE TRANSCRIPTION PCR (RT-PCR)

- for amplifying DNA from RNA.
- Reverse transcriptase reverse transcribes RNA into cDNA, which is then amplified by PCR.
- Some thermostable DNA polymerases used in the PCR such as Tth have a reverse transcriptase activity under certain buffer conditions.



RT-PCR (Reverse Transcription PCR, Real Time - PCR)

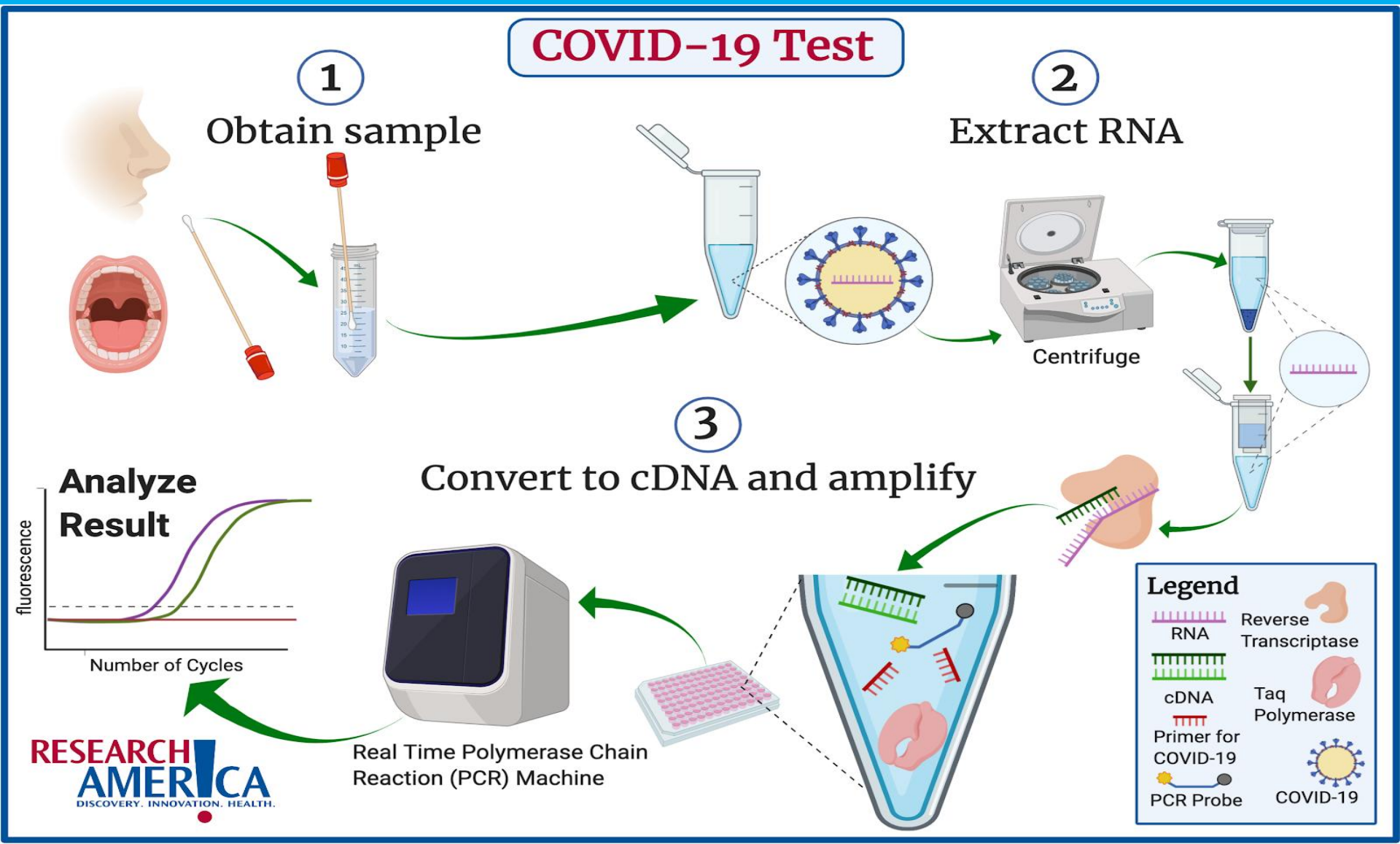
- Used to reverse-transcribe and amplify RNA to cDNA.
- PCR is preceded by a reaction using reverse transcriptase, an enzyme that converts RNA into cDNA.
- The two reactions may be combined in a tube.

Uses:

1-Detection of RNA virus like (HCV).

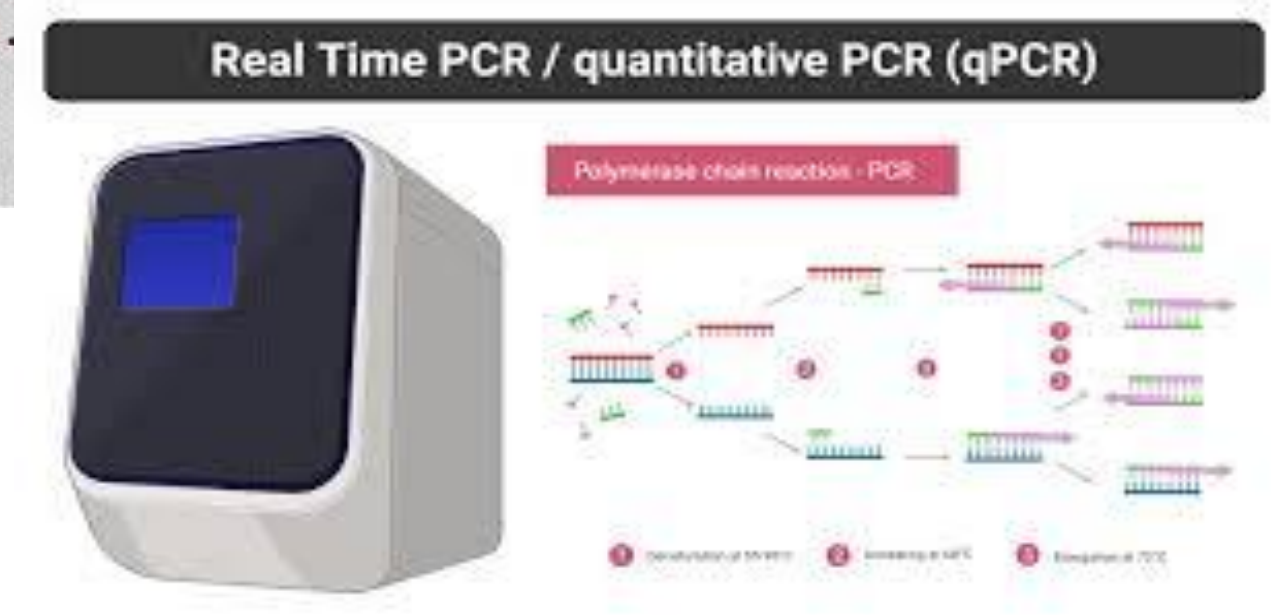
2-Detection of other M.O. through targeting of their Ribosomal RNA.

USE OF RT-PCR IN DETECECTION OF COVID-19



Quantitative – PCR:

- Used to measure the specific amount of target DNA (or RNA) in a sample.
- By measuring amplification only within the phase of true exponential increase, the amount of measured product more accurately reflects the initial amount of target.
- Special thermal cyclers are used that monitor the amount of product during the amplification.

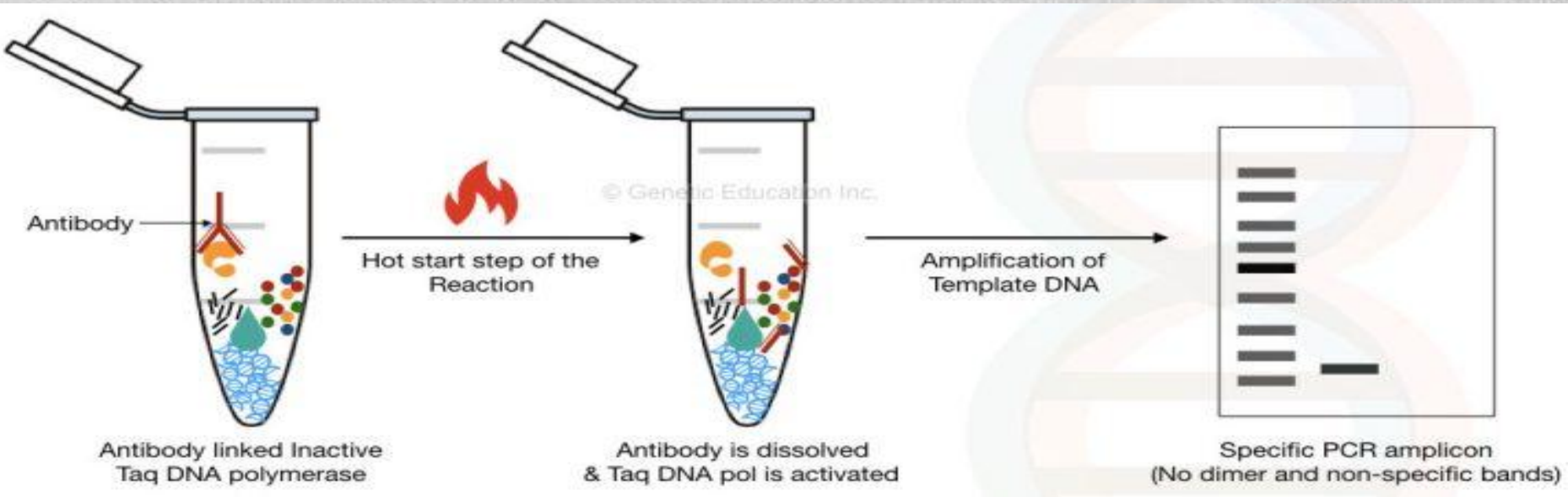


Quantitative Real-Time PCR (qRT-PCR)

- Method use fluorescent dyes, such as **Sybr Green**, or fluorescence-containing DNA probes, such as **TaqMan**, to measure the amount of amplified product as the amplification progresses.

Hot-start PCR:

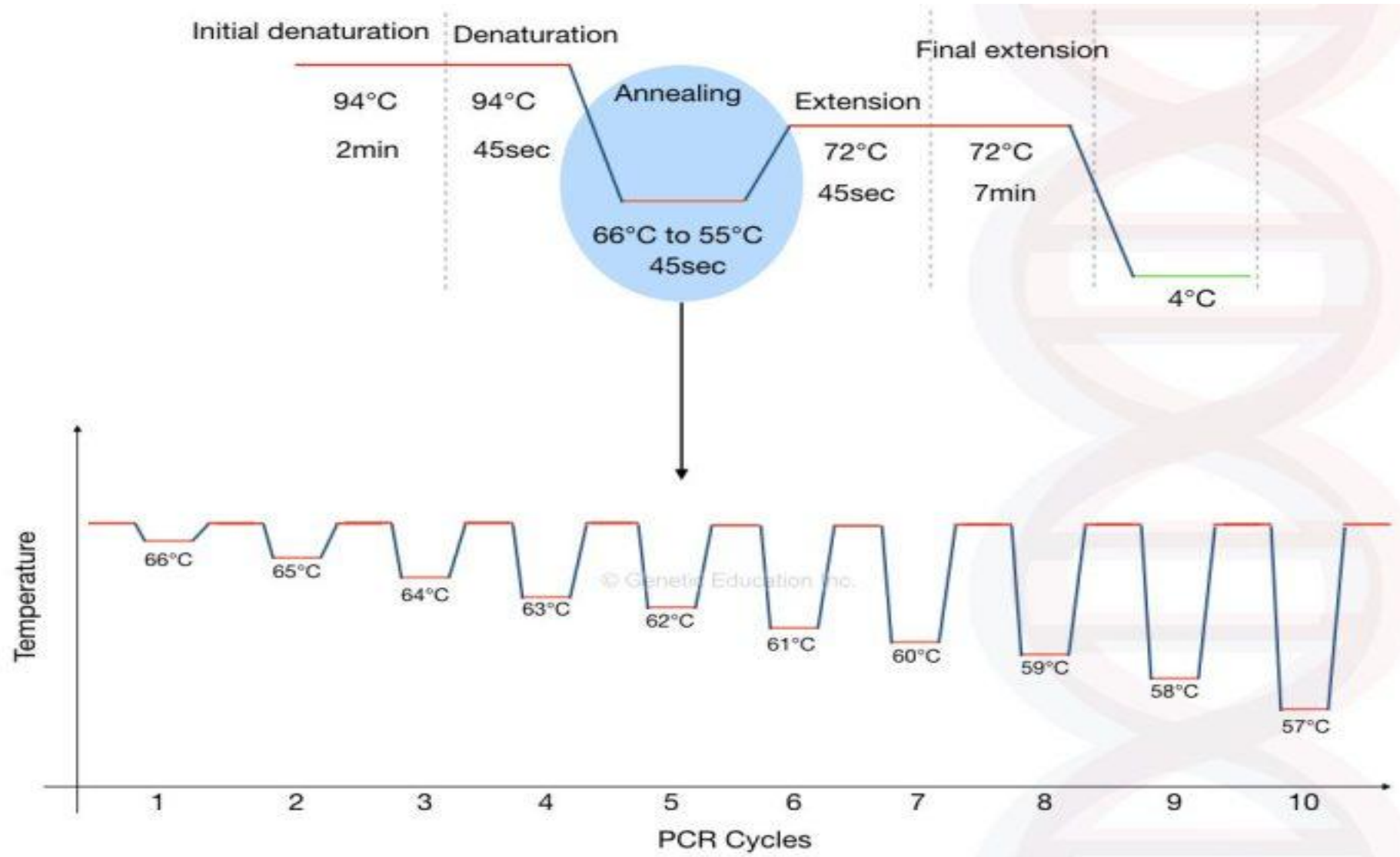
- It is a technique performed manually by heating the reaction components to the DNA melting temperature (e.g. 95°C) before adding the polymerase.



Touchdown PCR:

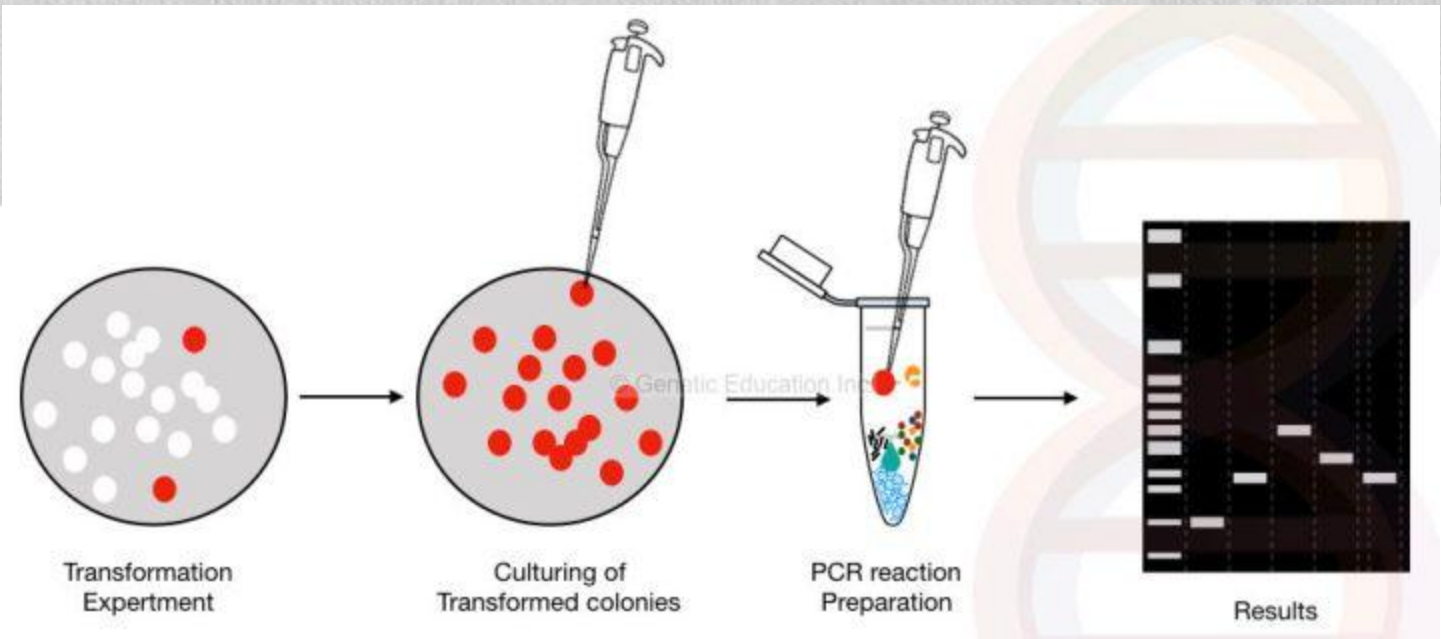
- In this type the annealing temperature is gradually decreased in later cycles.
- The annealing temperature in the early cycles is usually 3-5°C **above** the standard T_m of the primers used, while in the later cycles it is a similar amount **below** the T_m.
- The initial higher annealing temperature leads to greater specificity for primer binding, while the lower temperatures permit more efficient amplification at the end of the reaction.

TOUCHDOWN PCR



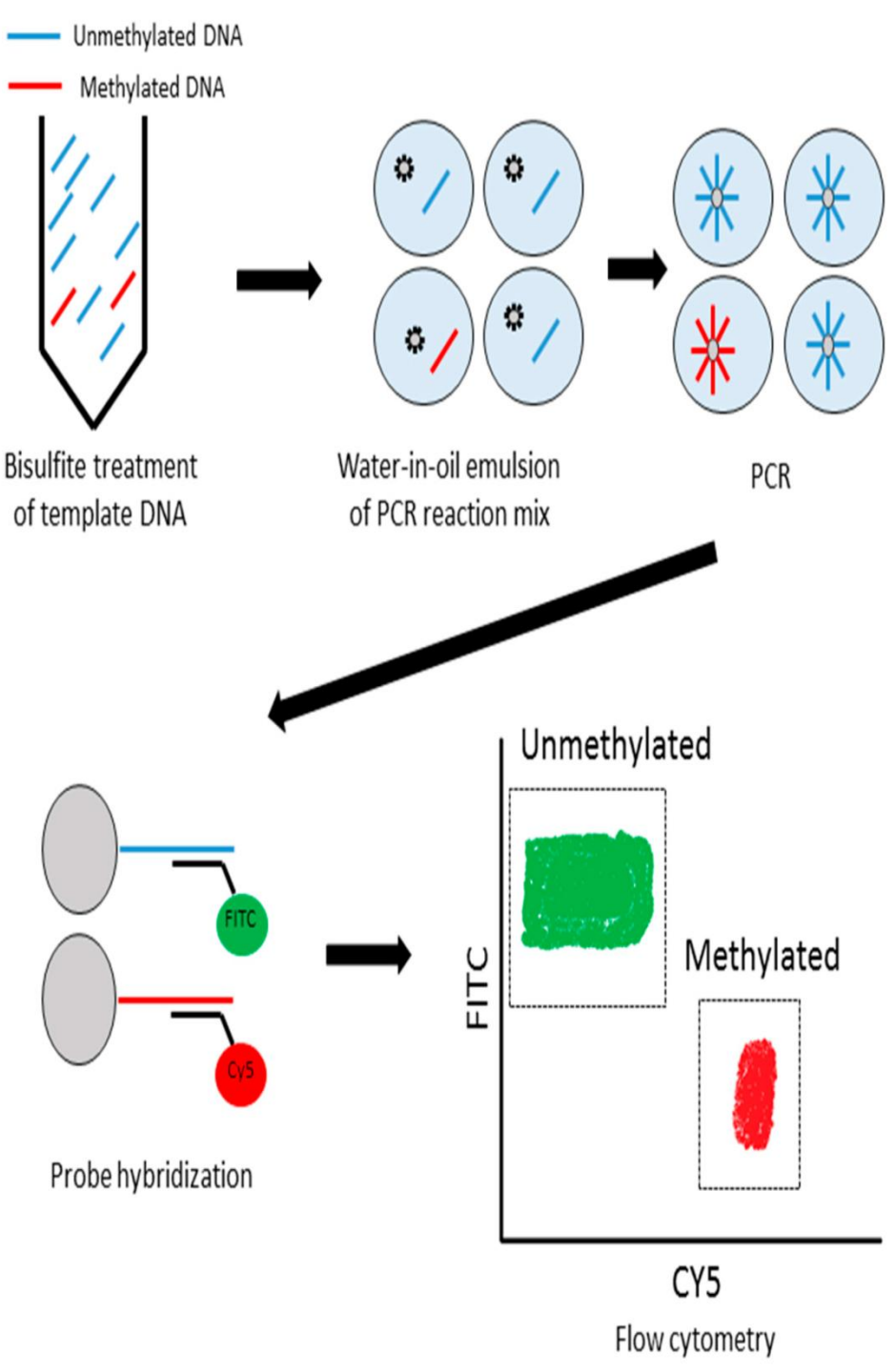
Colony PCR

- Bacterial colonies are screened directly by PCR, for example, the screen for correct DNA-vector constructs.
- Colonies are sampled with a sterile pipette tip and a small quantity of cells transferred into a PCR mix.



Methylation-specific PCR (MSP)

- Used to identify patterns of DNA methylation at cytosine guanine islands (C&G islands) in genomic DNA. CpG islands, are concerned in regulation of gene expression in mammalian cells.
- Target DNA is first treated with sodium bisulfite, which converts unmethylated cytosine bases to uracil, which is complementary to adenosine in PCR primers.
- Two amplifications are then carried out on the bisulfite-treated DNA:



IMPORTANCE OF PCR

- Creates more DNA
  - More tests run on specific DNA
  - More DNA can be tested by different people
- Forensics
  - Allows small amount of DNA to be made into a lot
- Research
  - Mutated DNA can be reproduced and be tested different ways

PCR

- **Students are requested to share their queries on Whatsapp group: MMC Botany (D3)**

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