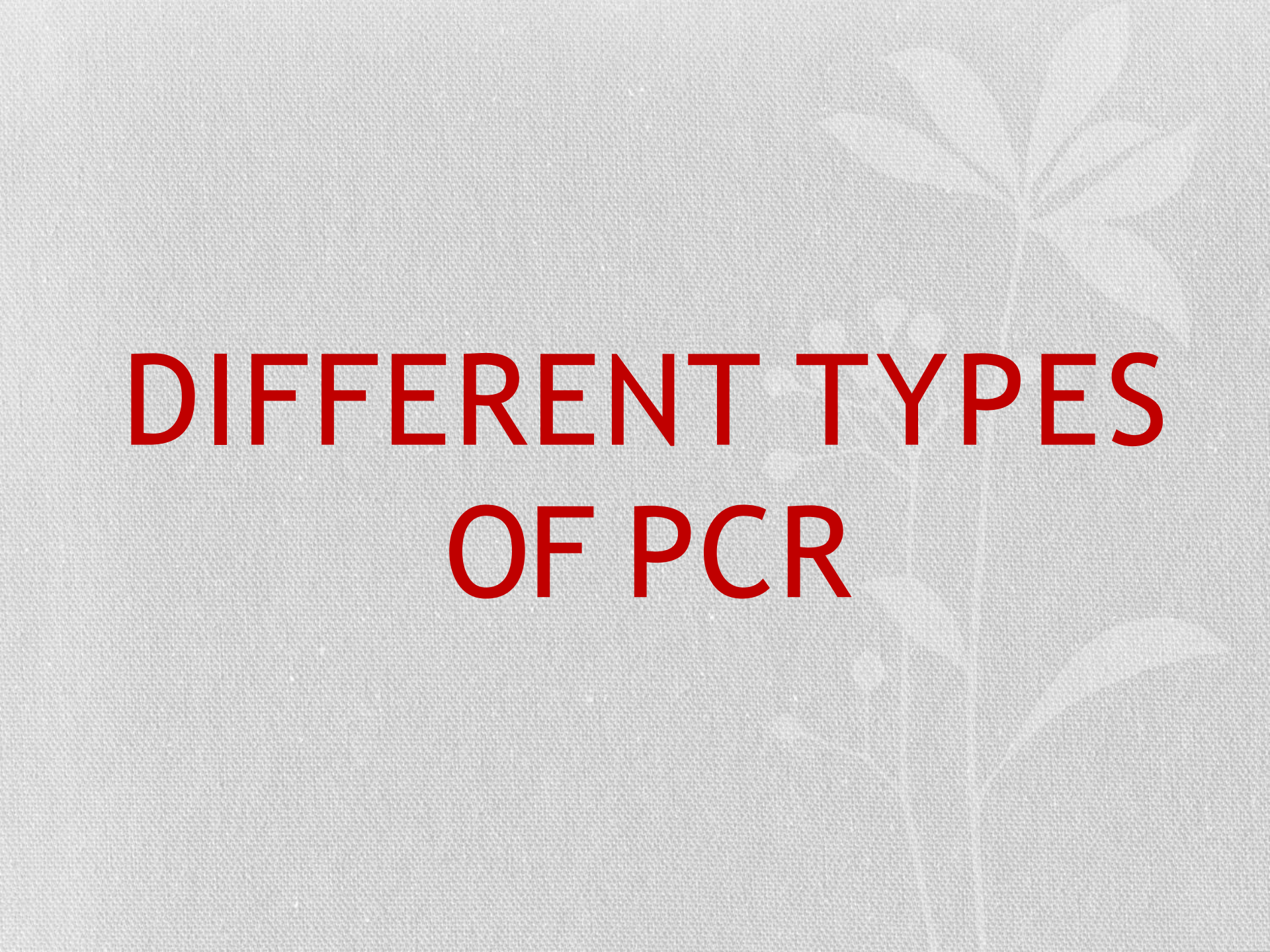


DIFFERENT TYPES OF PCR



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.

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.**



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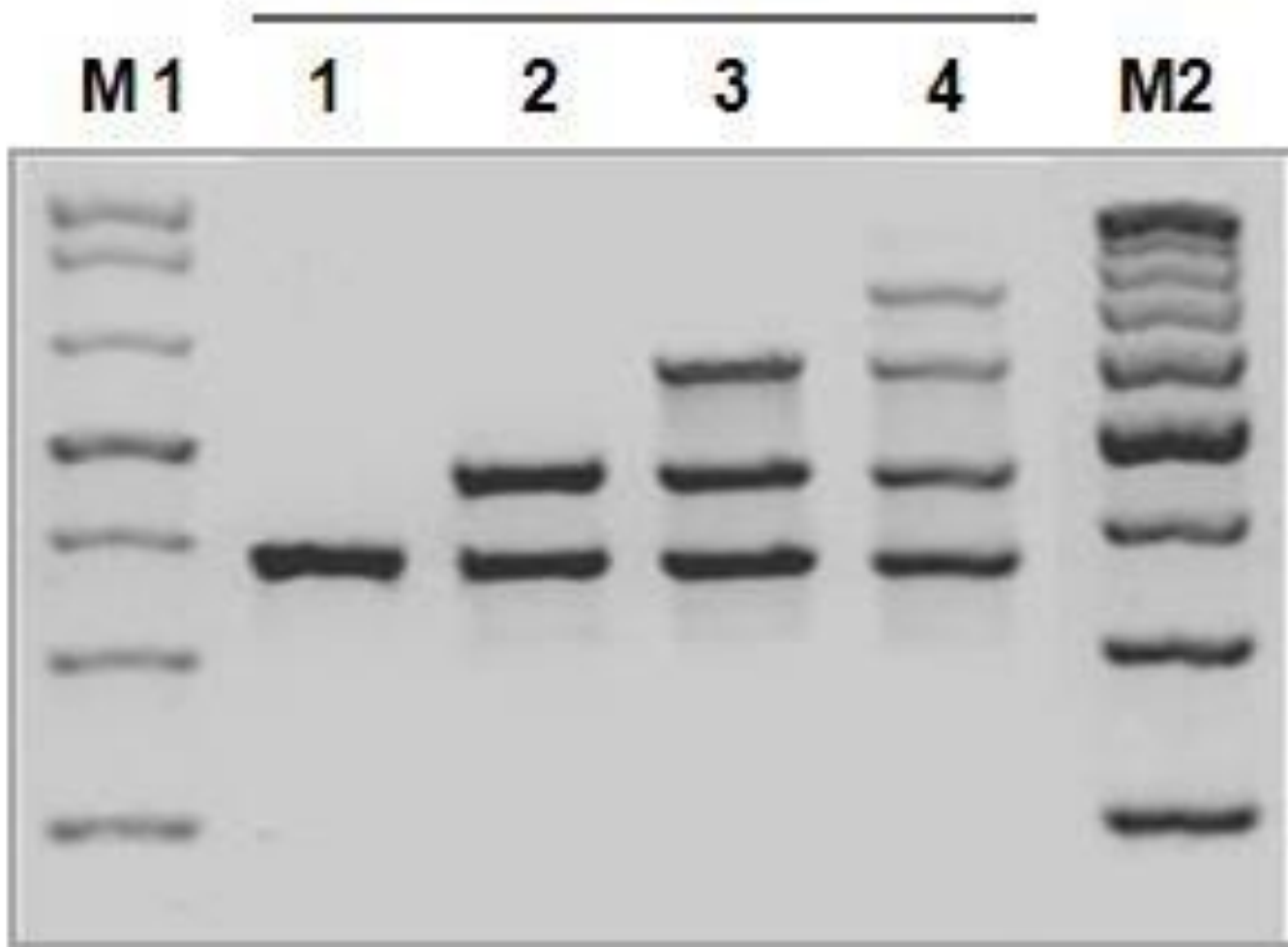
Multiplex-PCR:

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.

Multiplex RT-PCR



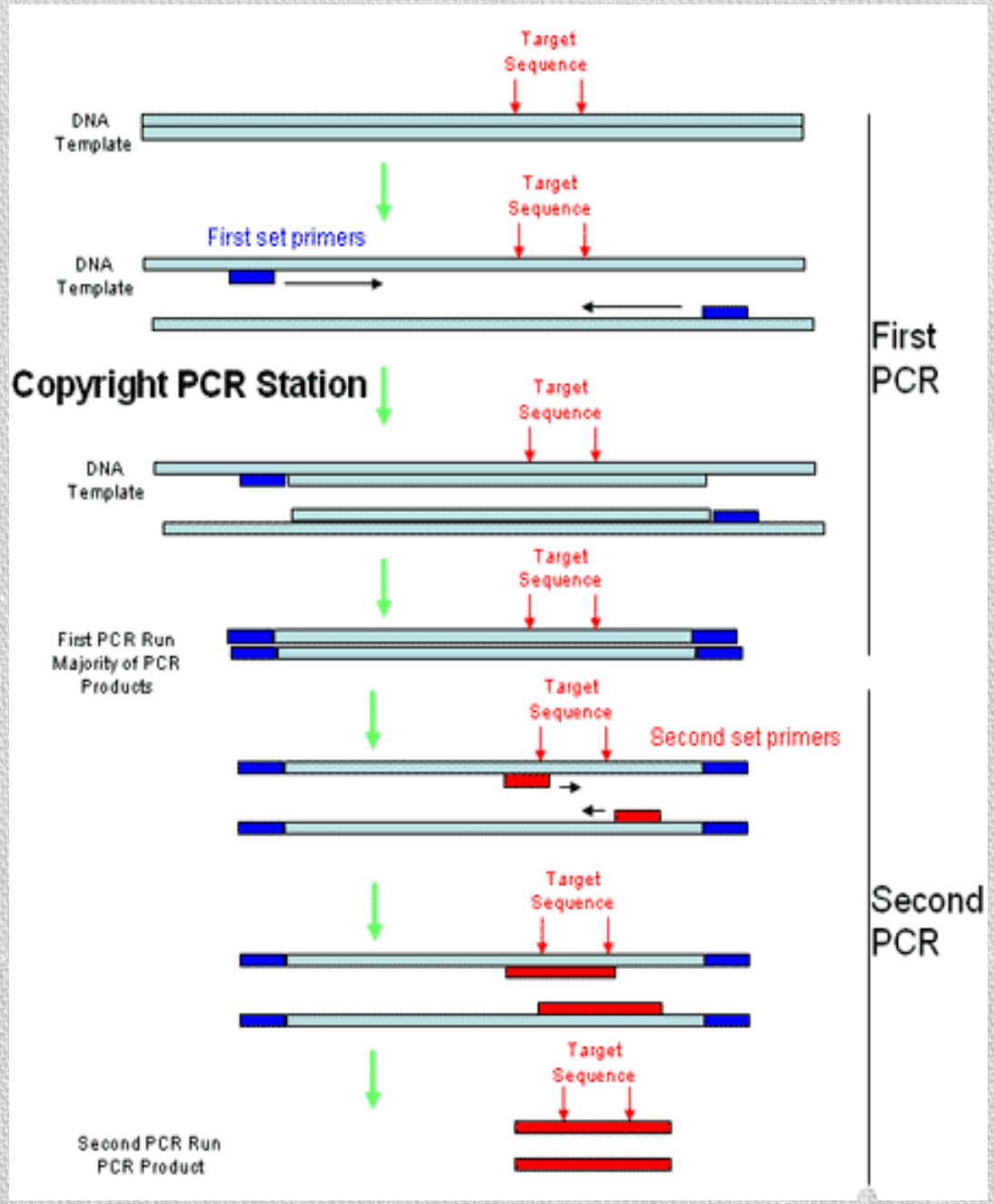
Nested-PCR:

- 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.

- Using one ('hemi-nesting') or two different primers whose binding sites are located (nested) within the first set, thus increasing specificity.

Uses:

Detection of pathogens that occur with very few amount.



Copyright PCR Station

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

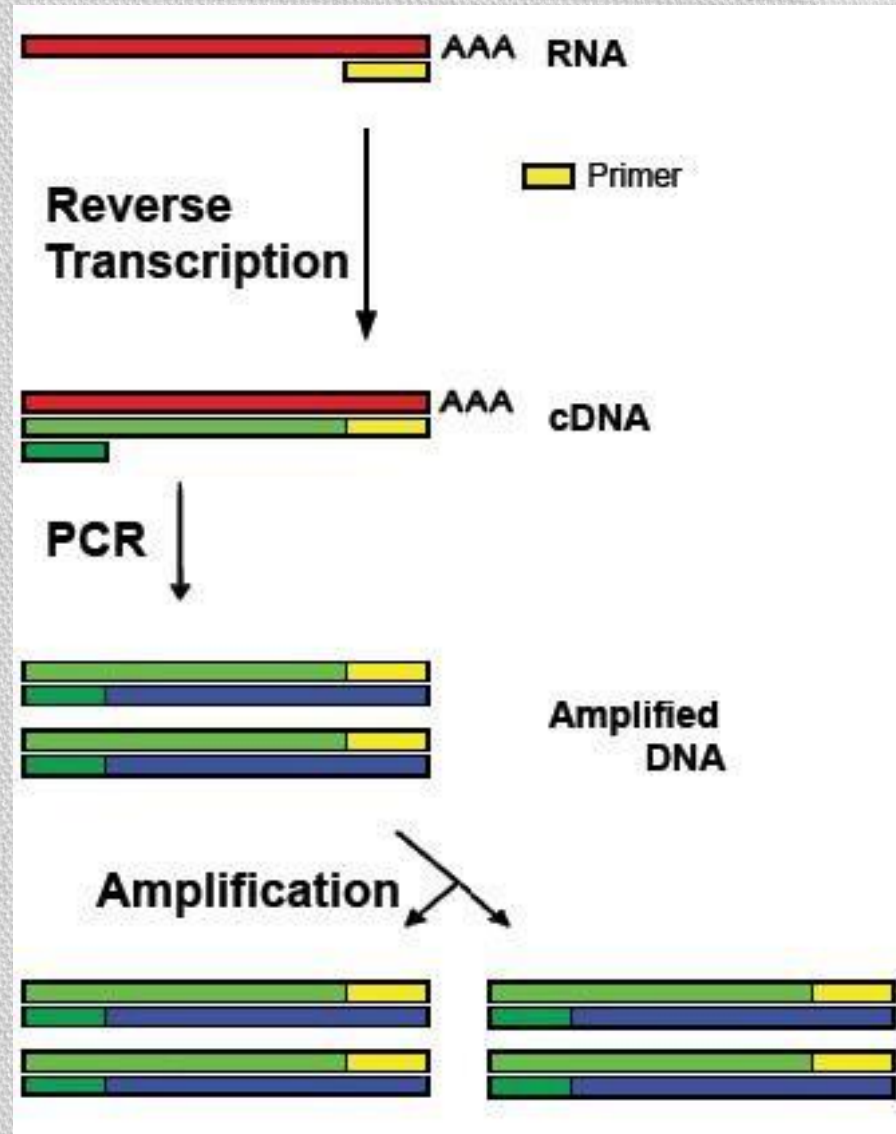
- Used to reverse-transcribe and amplify RNA to cDNA.
- PCR is preceded by a reaction using **reverse transcriptase**, an enzyme that converts RNA into cDNA.
- The two reactions may be combined in a tube.

Uses:

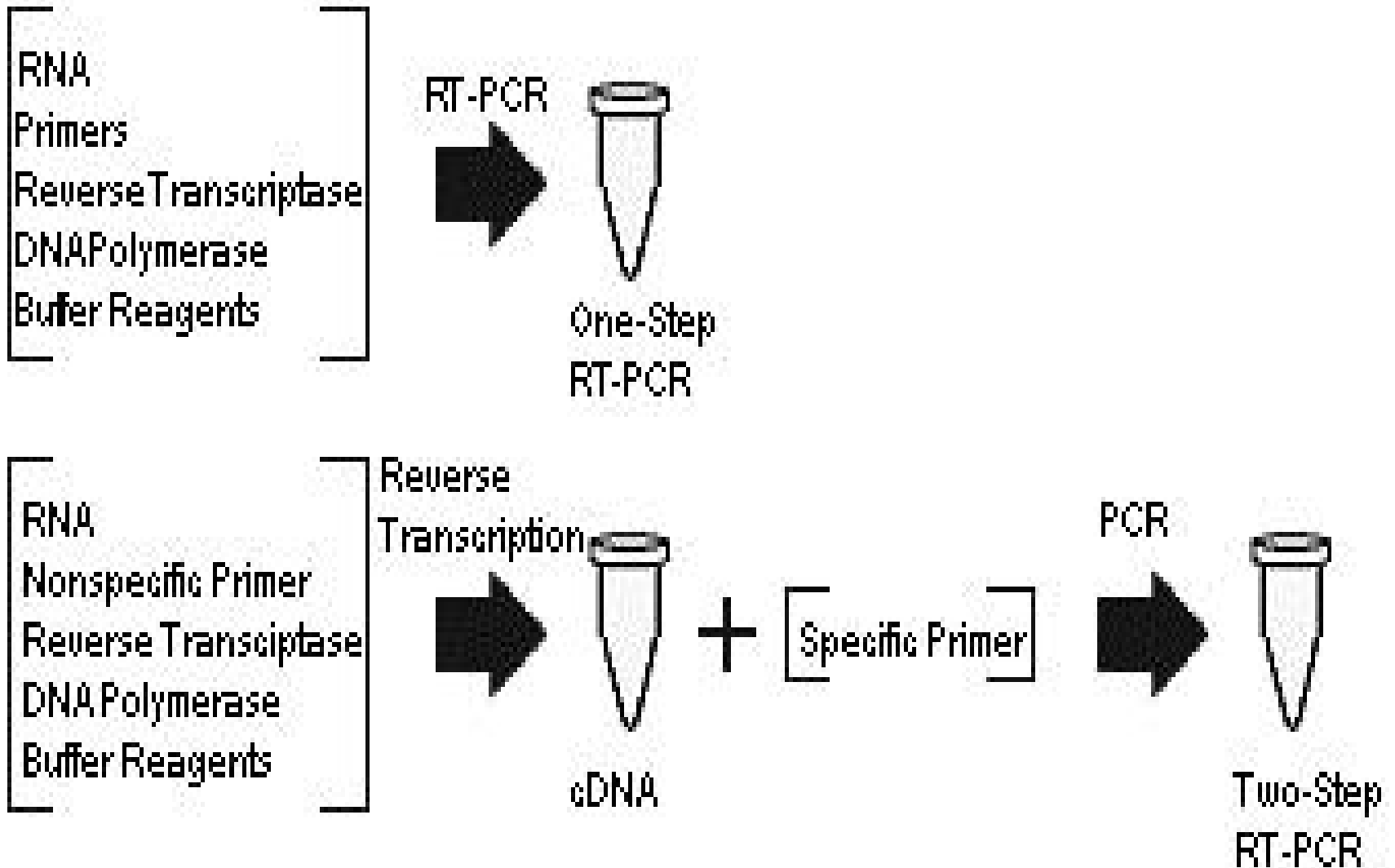
1 Detection of RNA virus like (HCV).

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

Reverse Transcription *PCR*, Real Time - *PCR*



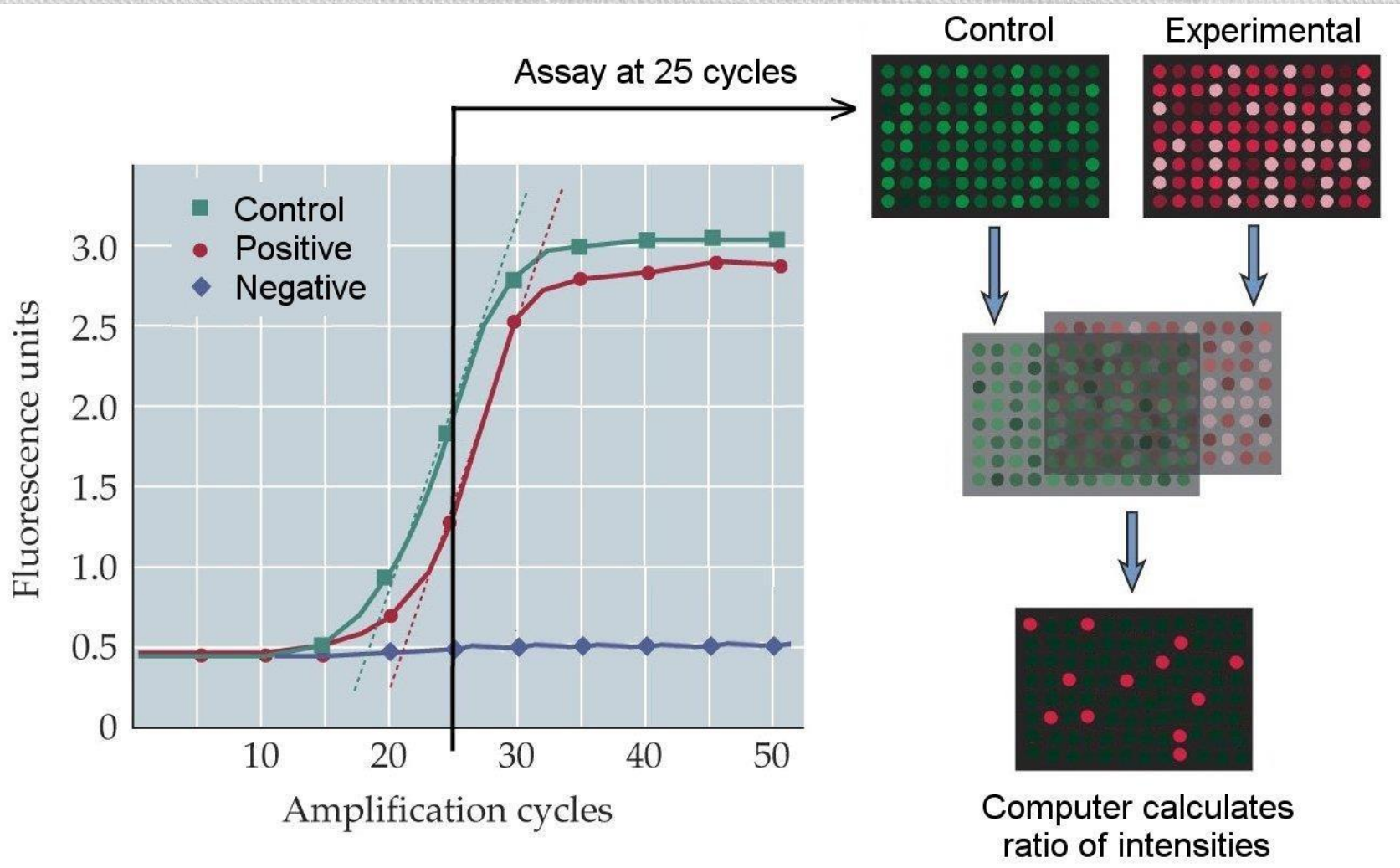
Reverse Transcription *PCR*, Real Time - *PCR*



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.

Progress of **DNA** amplification during *real time* (**RT-PCR**) by measuring the release of fluorescent "flashes" during amplification. A computer measures the rate of "flashing" in 96 simultaneous **experimental PCR** reactions relative to a **control** reaction



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.

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:

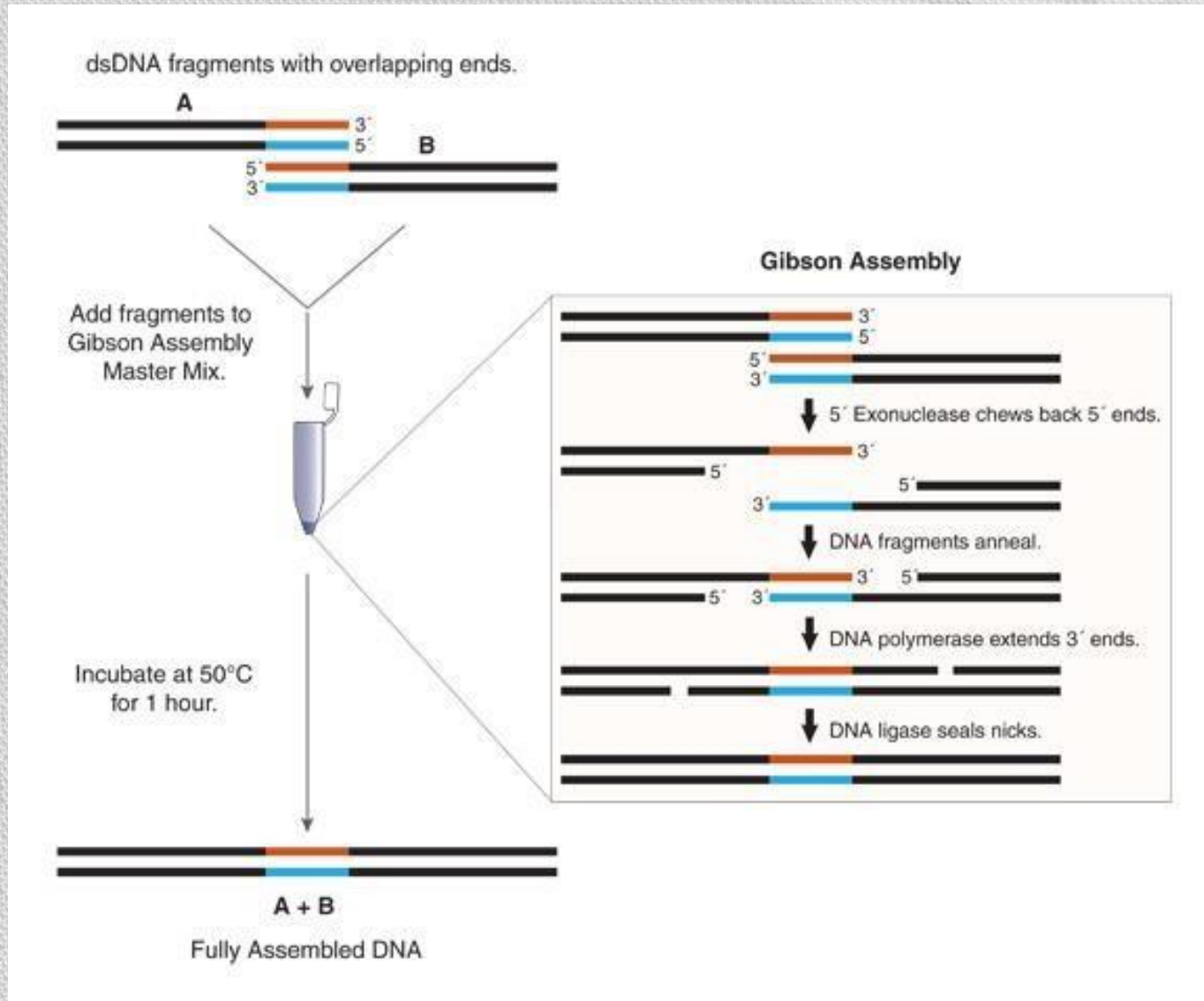
- In this type the annealing temperature is gradually decreased in later cycles.
- The annealing temperature in the early cycles is usually 3-5°C **above** the standard T_m of the primers used, while in the later cycles it is a similar amount **below** the T_m .
- The initial higher annealing temperature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

Assembly-PCR

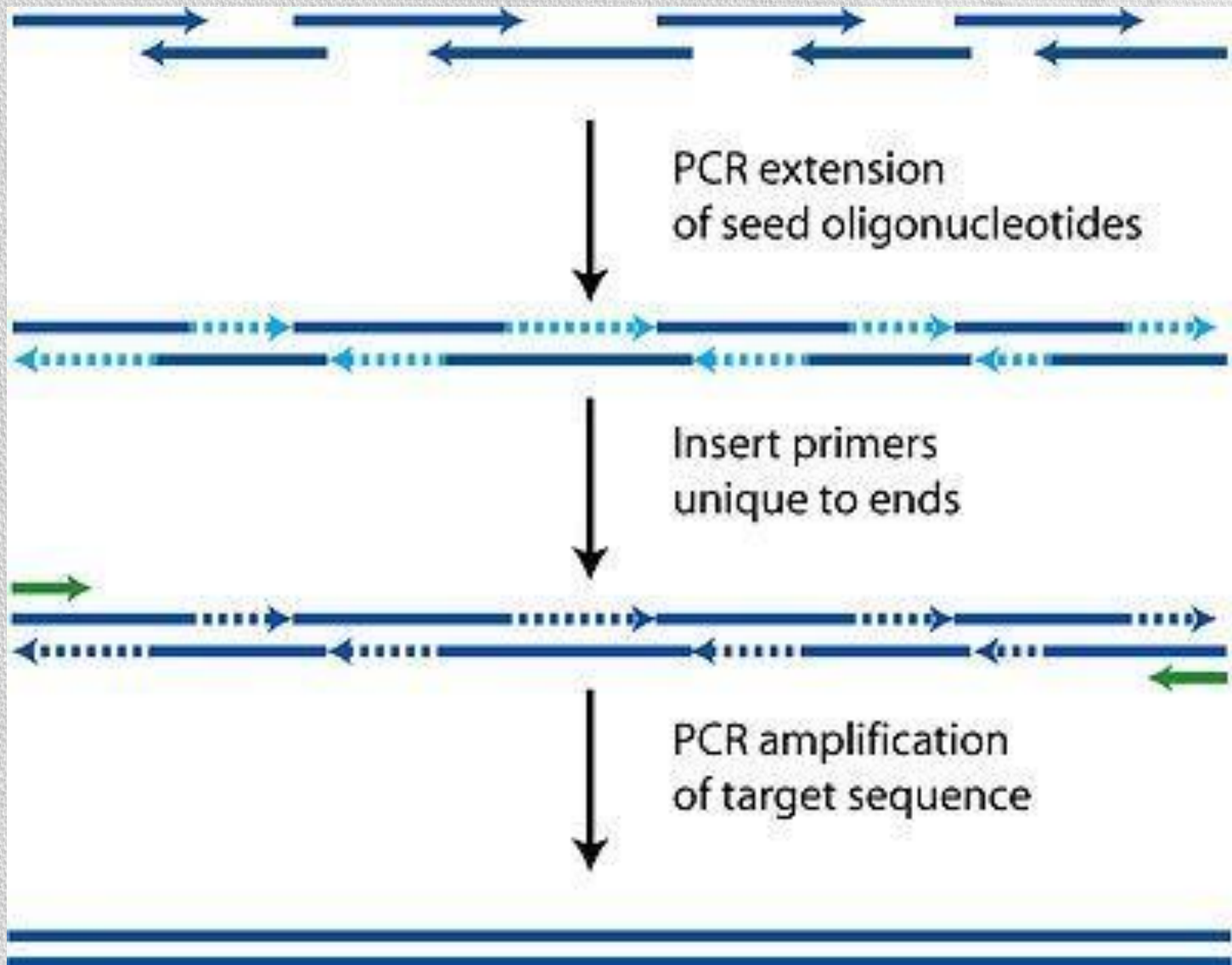
(also known as *Polymerase Cycling Assembly* or *PCA*)

- In this type synthesis of long DNA structures by performing PCR on a pool of long oligonucleotides with short overlapping segments, to assemble two or more pieces of DNA into one piece.
- It involves an **initial PCR** with primers that have an overlap and a **second PCR** using the products as the template that generates the final full-length product.
- This technique may substitute for Ligation-based assembly

Assembly PCR:



Assembly 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.

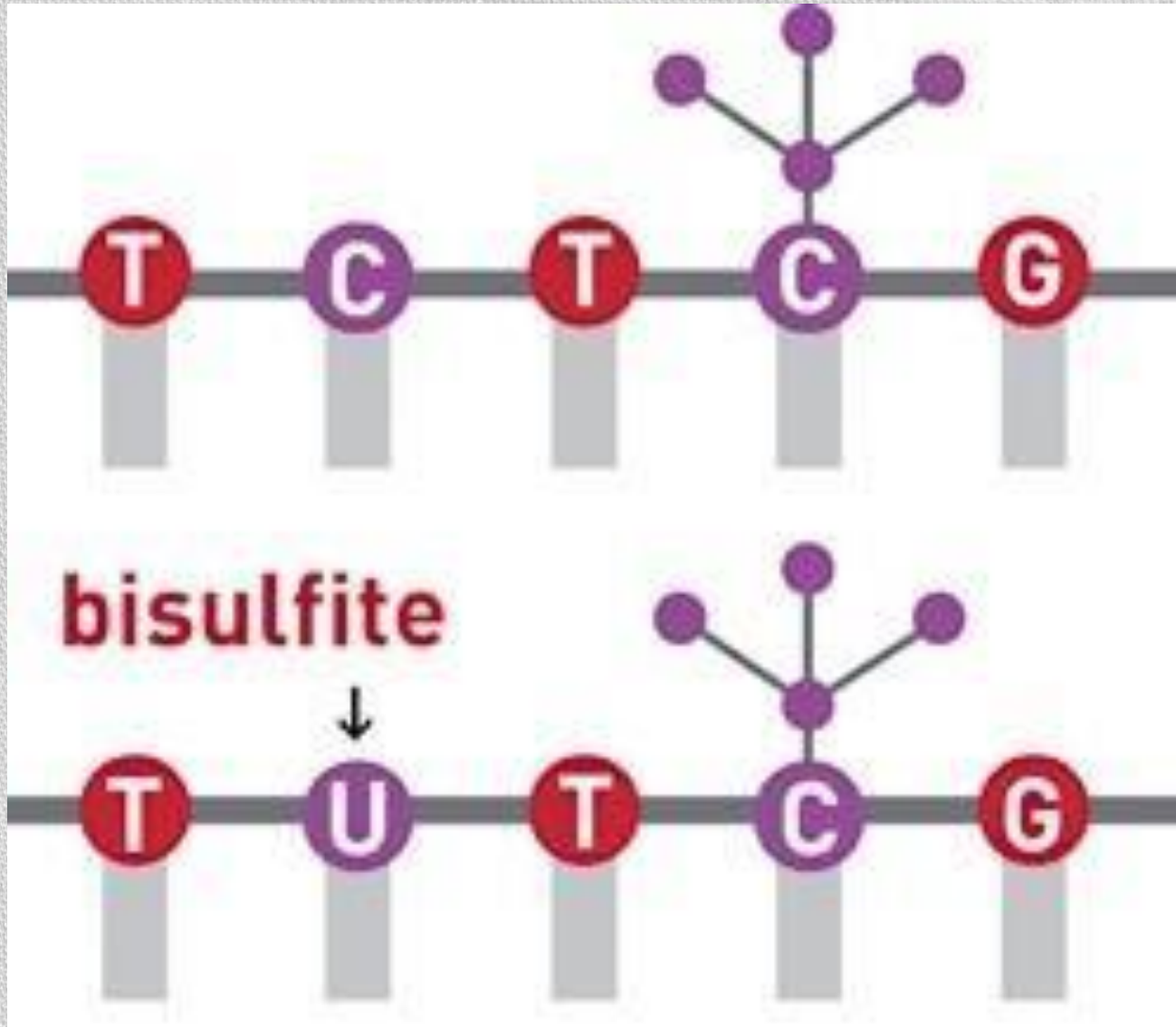
Methylation-specific PCR (MSP)

- 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:

Methylation-specific PCR (MSP)

- One primer set anneals to DNA with cytosine (corresponding to methylated cytosine),
- The other set anneals to DNA with uracil (corresponding to unmethylated cytosine).
- MSP used in quantitative PCR provides quantitative information about the methylation state of a given CpG island.

Methylation-specific PCR (MSP)



LAMP assay:

(Loop-mediated isothermal amplification)

- It is a Modified type of the PCR using 3:6 primers sets one of them is loop like primer.
- This test use Bst- polymerase enzyme

(Bacillus stearothermophilus DNA Polymerase).

- Using only two temperatures (63°C for 45 min. then 85°C for 5 min.), may be carry out in water bath.

Before you speak:

THINK

T: is it True?

H: is it Helpfull?

I: is it Inspiring?

N: is it Necessary

K: is it Kind?