Advanced lab techniques
Lecture 4

Polymerase Chain Reaction (PCR)

By

Assist. Lec. Hawraa Rahman Younis

DNA amplification

In a crime scene, a sample of DNA was found, however **amount of DNA was not enough to** be analyzed.

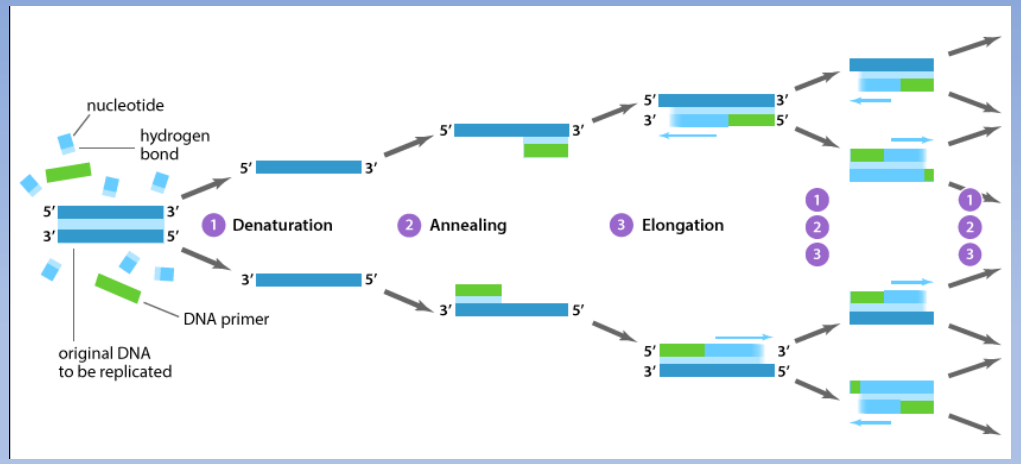
After DNA extraction, the scientist want to study a specific **part of a gene** to do sequencing.

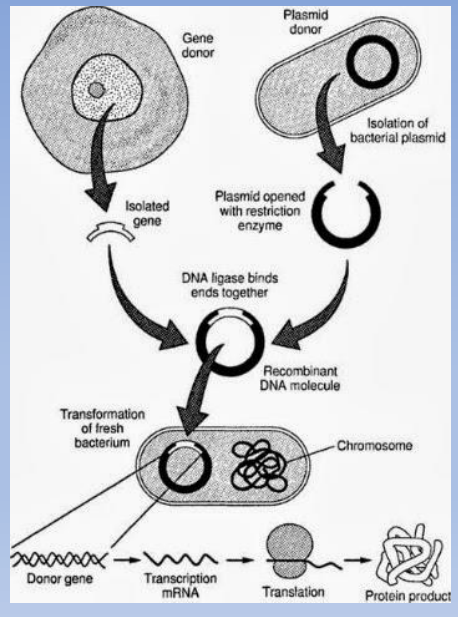
How scientist solve these problem ?

Mainly there are two methods: for amplification

Amplifying segment of DNA

- Polymerase chain reaction
- Cloning





PCR is a means to amplify a particular piece of DNA

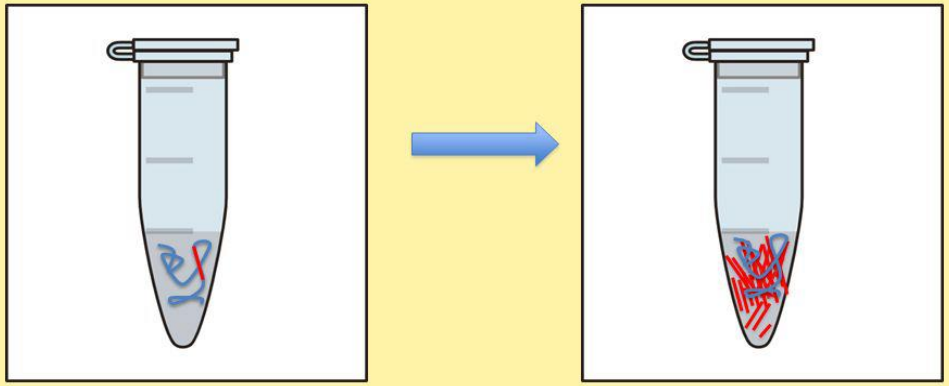
Amplify= making numerous copies of a segment of DNA.

• PCR can make billions of copies of a **target sequence of DNA** in short time.

• It is a laboratory version of DNA Replication in cells.

The laboratory version is commonly called “in vitro” since it occurs in a test tube while “in vivo” signifies occurring in a living cell.

• PCR is a method of amplifying (=copy) a target sequence of DNA.



- So…

➔How the amplification will be done?

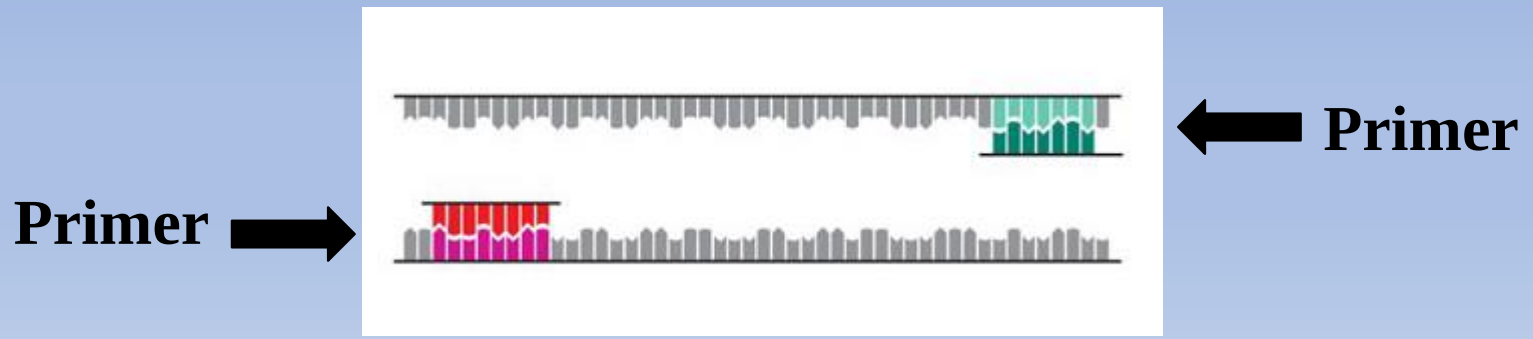
➔How you will determine your target sequence?

➔How the amplification will be specific for certain segment?

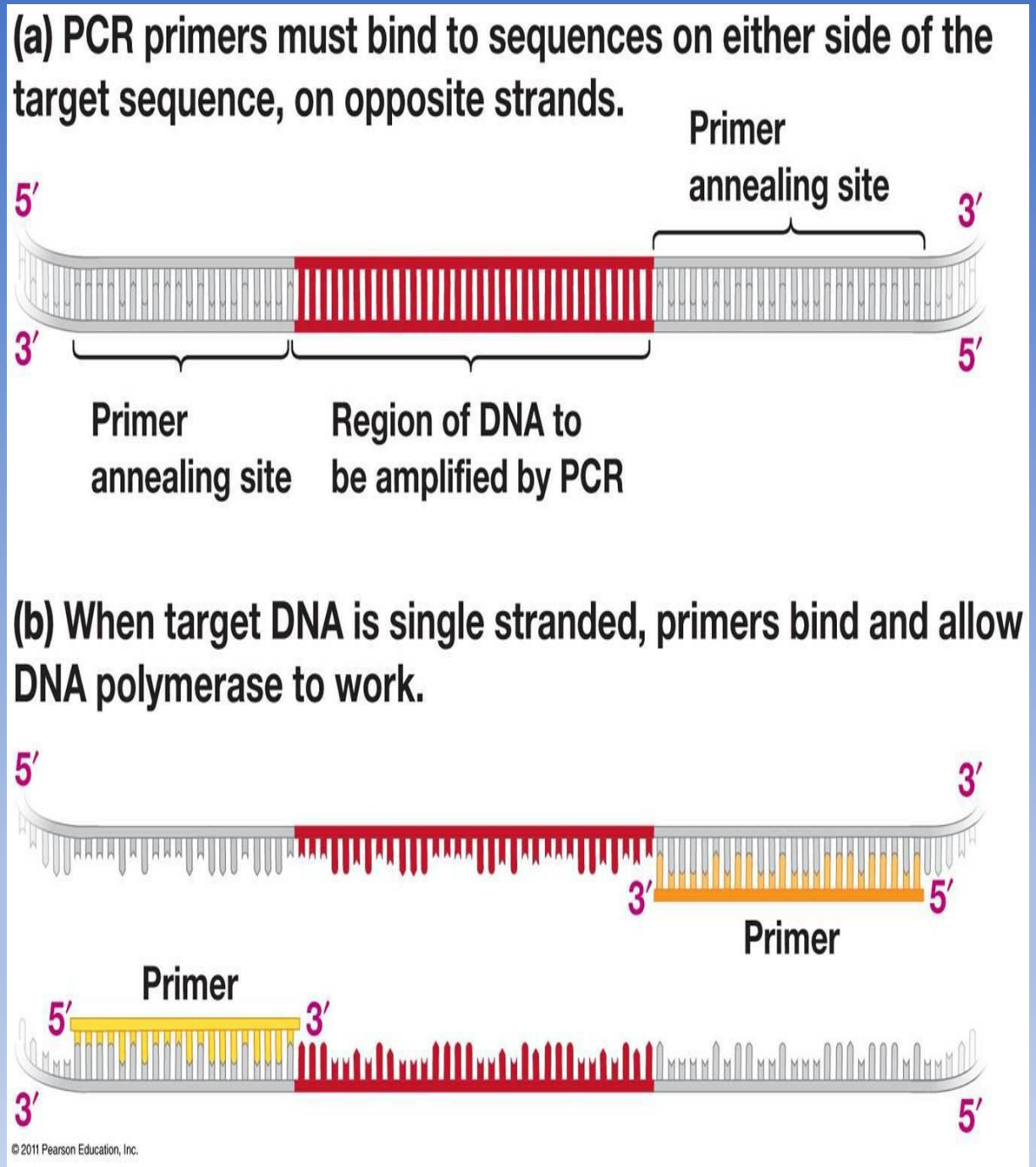
- **You must to understand these questions**

Amplification of specific target sequences

- PCR **does not copy all of the DNA** in the sample. It copies only a very specific sequence of genetic code from a template DNA, targeted by PCR primers.
- It does require the knowledge of some DNA sequence information which flanks the fragment of DNA to be amplified (**target DNA**).



- From this information two synthetic oligonucleotide primers may be chemically synthesized each complementary to a stretch of DNA to the 3’ side of the target DNA, one oligonucleotide for each of the two DNA strands (DNA polymerase can add a nucleotide only onto a **preexisting 3’-OH group**).



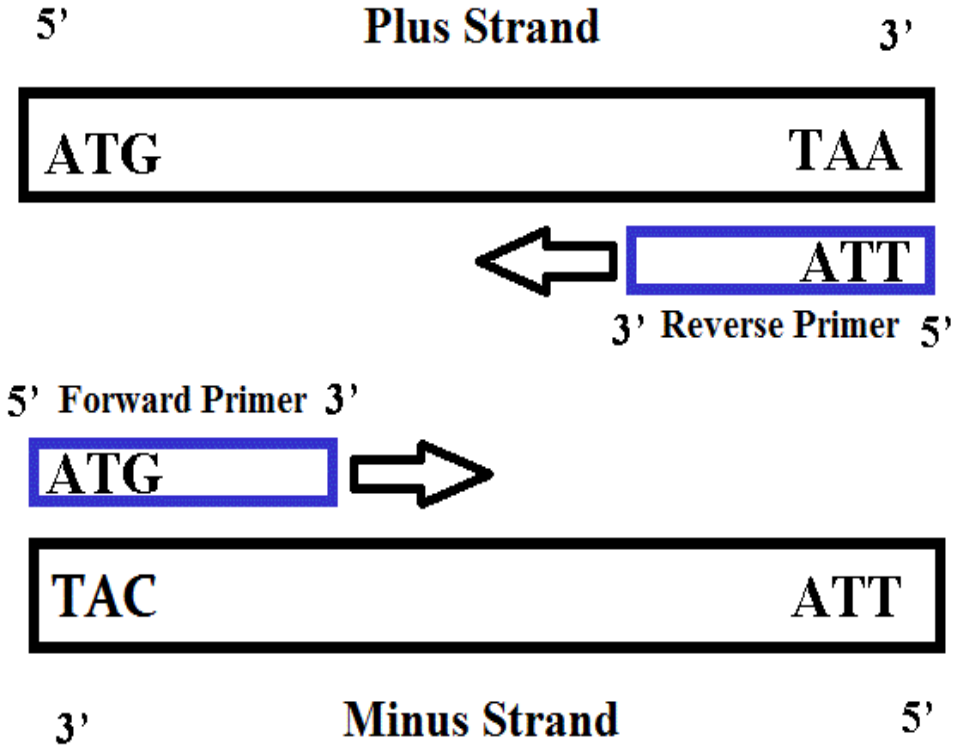
Why we need two primer

- In a PCR reaction you need **two primers** to amplify the target sequence:

➔**One called:** Forward primer, which have the same sequence of forward DNA strand and bind to the complementary reverse strand.

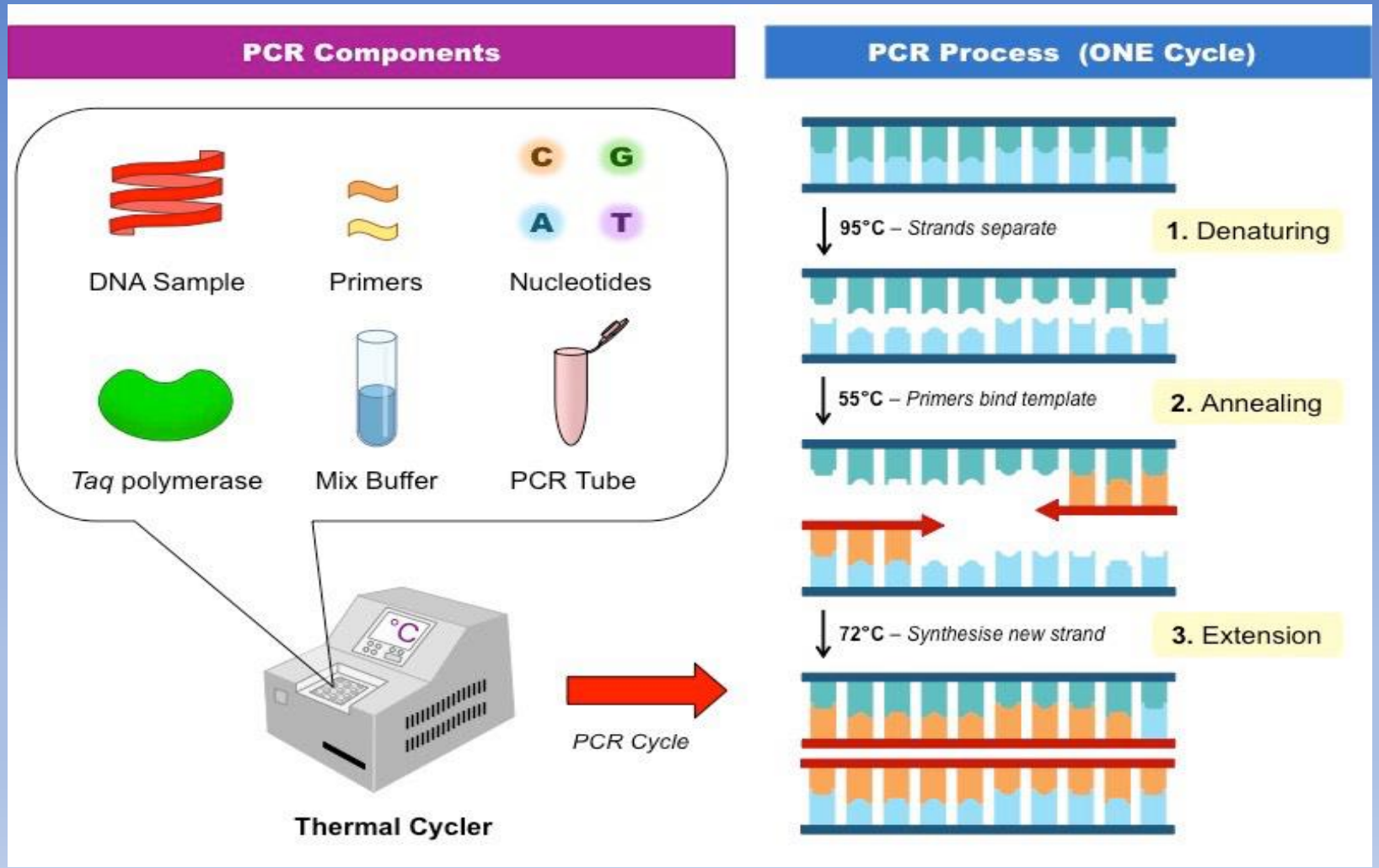
➔**The second called:** Reverse primer, which have the same sequence of reverse DNA strand and bind to the complementary forward strand.

*If there is only one primer, only one strand of the double stranded DNA will be amplified in the PCR reaction.



Components of PCR

Additional reagents may included



PCR cycle

- PCR proceeds in **THREE** distinct steps Governed by **Temperature:**

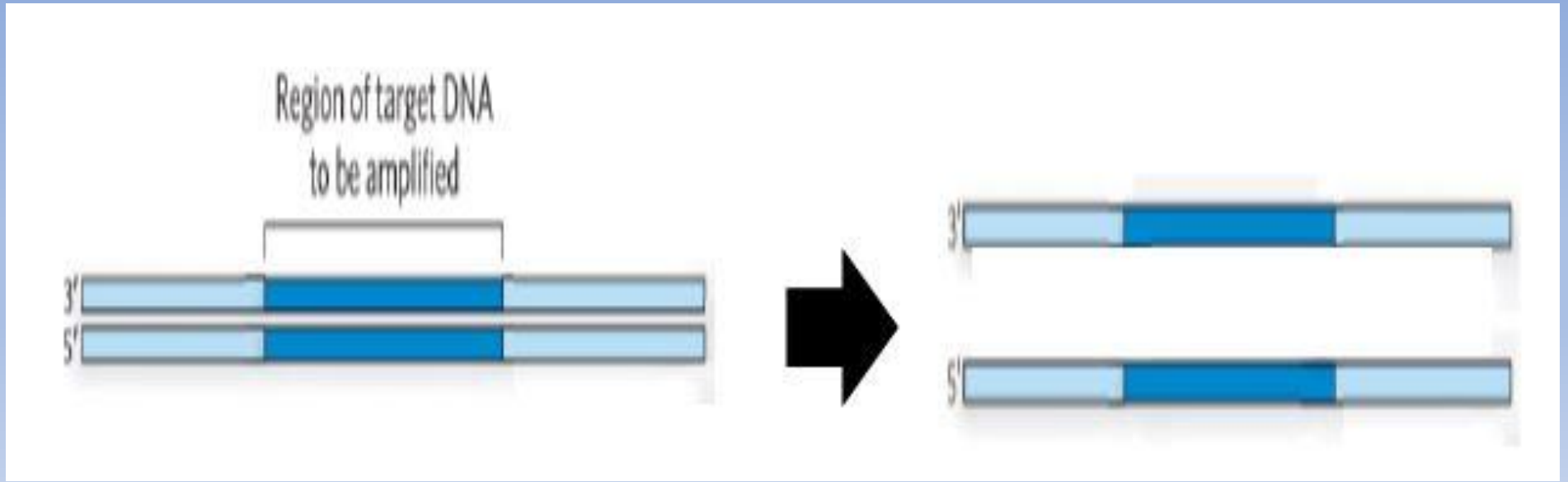
The double-stranded template DNA is denatured by heating, typically to 95°C, to separate the double stranded DNA.	The reaction is rapidly cooled to an annealing temperature to allow the oligonucleotide primers to hybridize to the template.	The reaction is heated to a temperature, typically 72°C for efficient DNA synthesis by the thermostable DNA polymerase.
Denaturation: (95°C)	***Annealing: (50-65°C)***	**Extension:** ***(72°C)***

1. ***Denaturation***

The double-stranded template DNA is denatured by heating, typically to **95°C**, to separate the double stranded DNA **(why?).**

- Breaking the **……?.....** bonds.

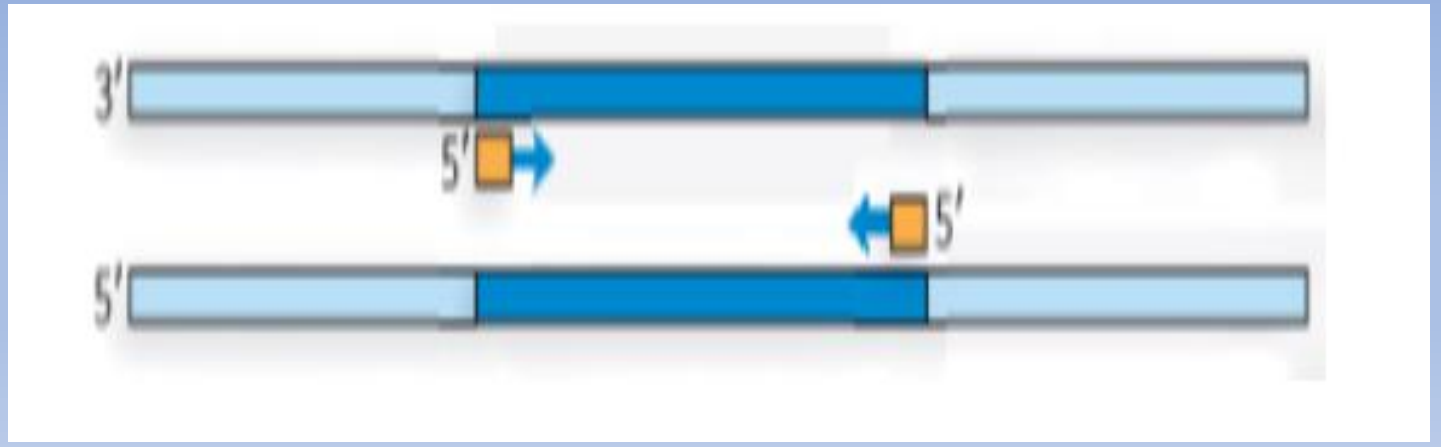
Step 1
(94–97 °C)



2. Annealing:

- The reaction is rapidly cooled to the primer annealing temperature (50-65 °C) to allow the oligonucleotide primers to hybridize to single stranded template.
- Primer will anneal only to sequences that are complementary to them (target sequence).

Step 2
(50–65 °C)

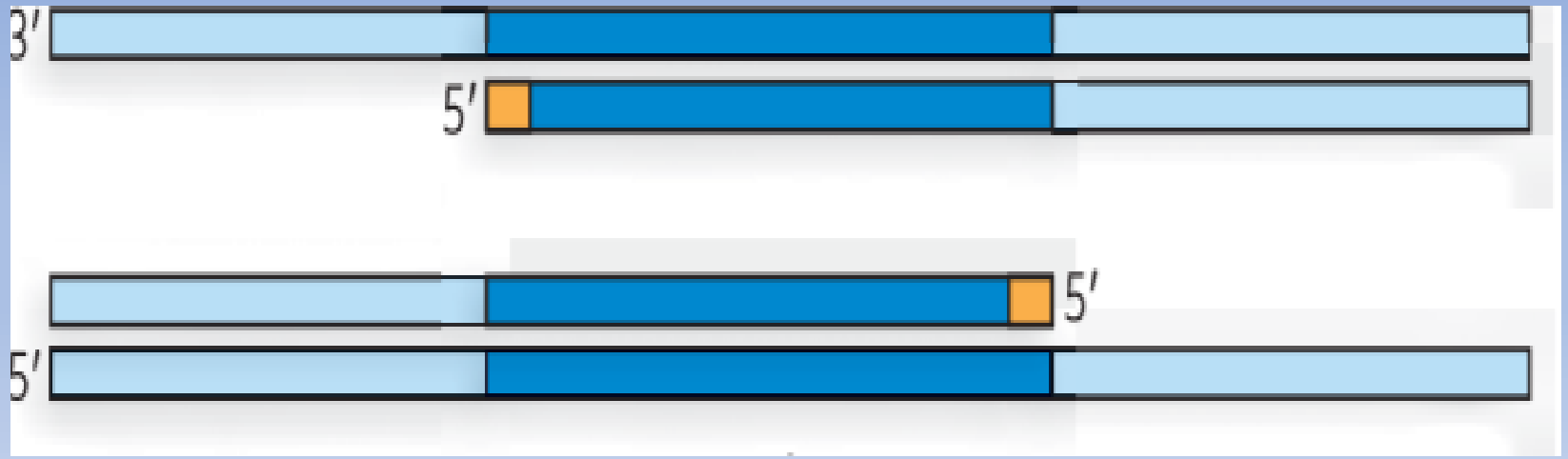


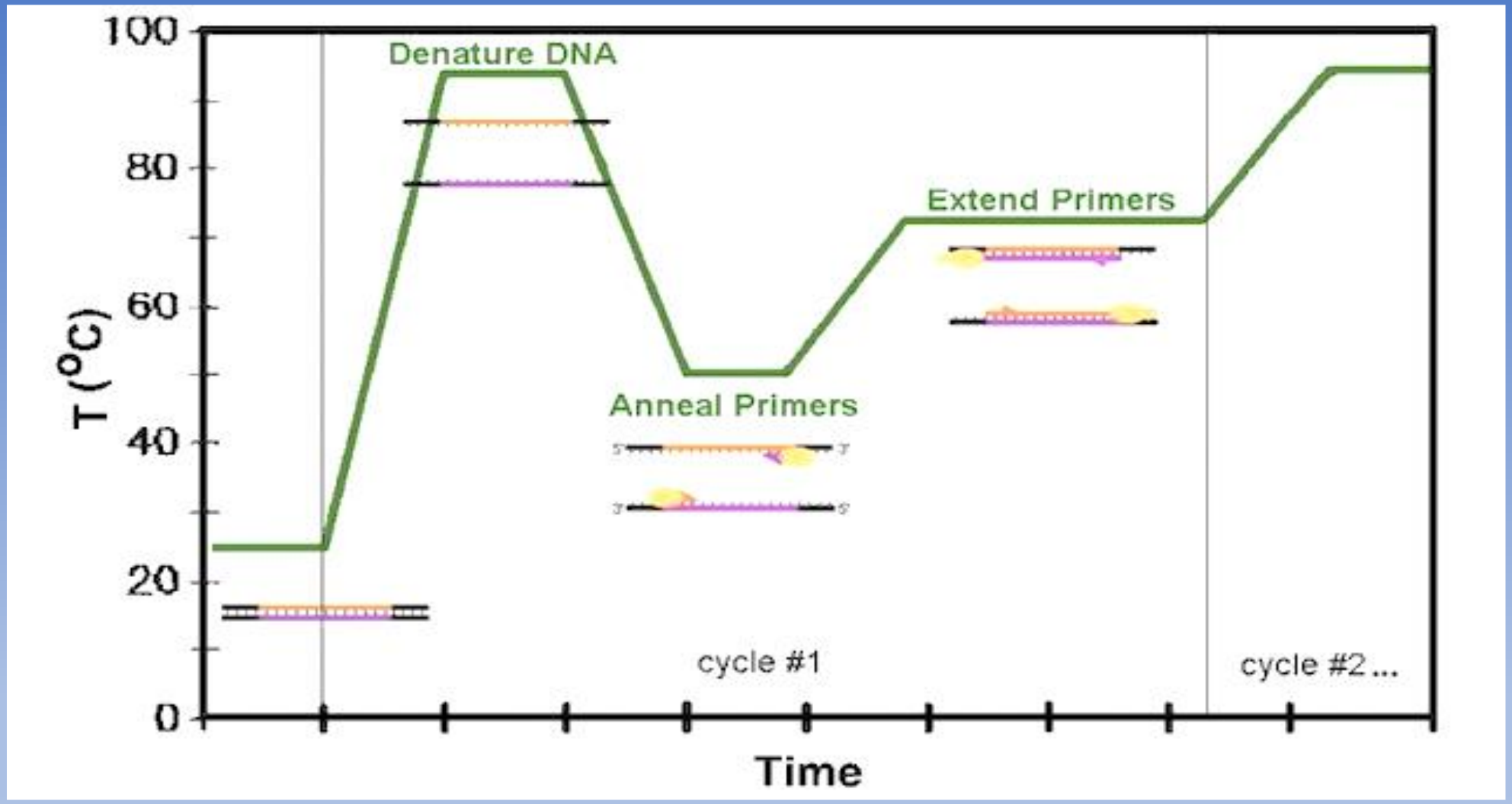
3. Extension

The reaction is heated to a temperature depends on the DNA polymerase used.

- **Commonly** a temperature of 72°C is used with this enzyme.
- This means that 72°C is the optimum …… of DNA polymerase.
- At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template

Step 3
(72 °C)

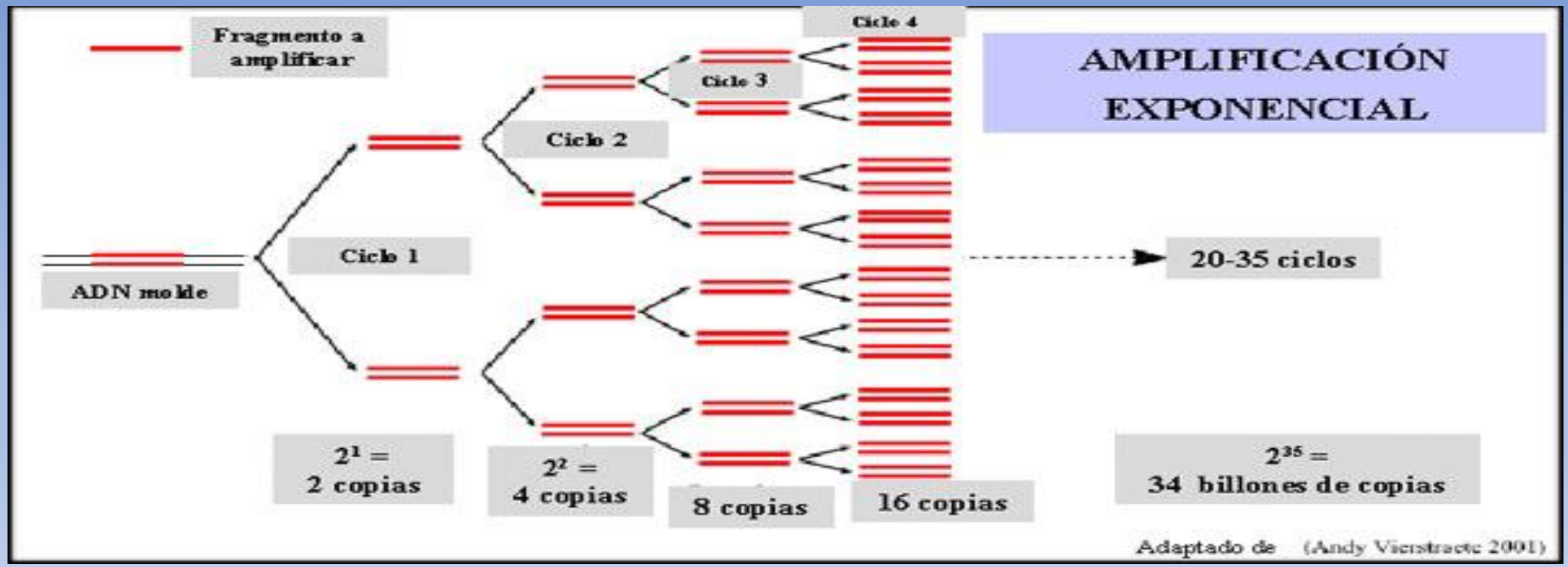




One cycle x25

At the end of the PCR reaction, the **specific sequence** will be accumulated in billions of copies.

In only 20 cycles, PCR can product about a million (220) copies of the target.



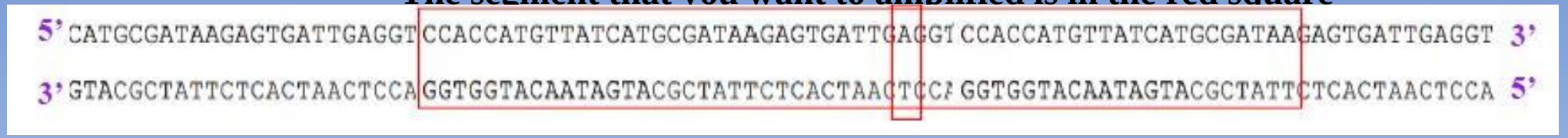
Performing PCR steps

Example

You want to study a mutation in a DLG3 gene and how it relate to memory:

1. Find the sequence of the gene from any website, eg.Ensebmle.
2. Determine your **target region**.

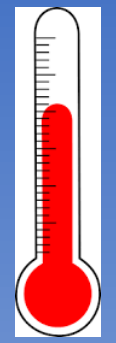
The segment that you want to amplified is in the red square



3. Design the primers using primer design tool, eg.Primer3, then send them to any company who will synthesize them.
4. Make sure that the area that you want to study is **between the primers (**the region to be studied should be between the forward and reverse primer**)**.
5. Check primer specificity by BLAST.
6. Optimize your PCR and trouble shooting.
7. Start PCR.

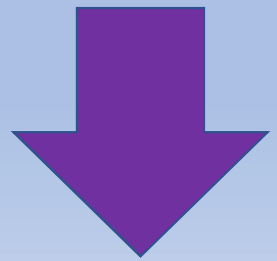
Starts your PCR
1. denaturation

95°C



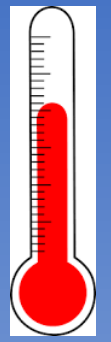
5’ CATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAA GAGTGATTGAGGT 3’

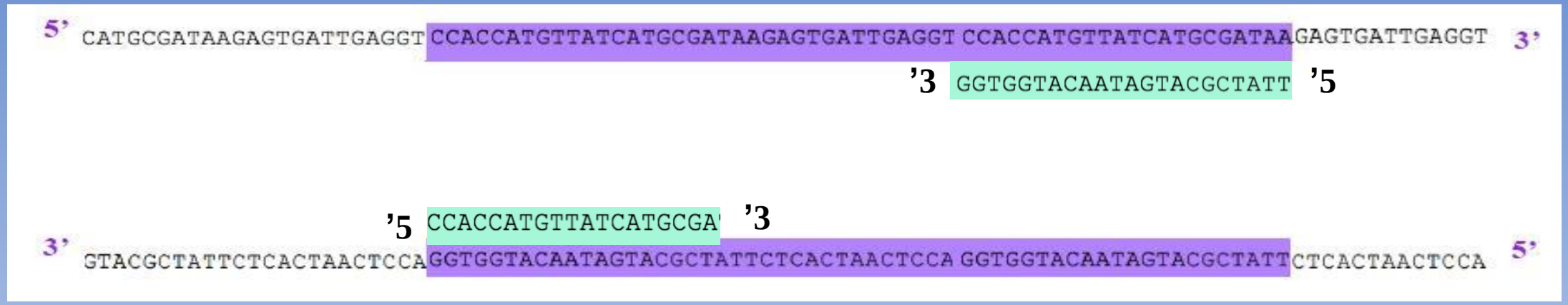
3’ GTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATT CTCACTAACTCCA 5’



2. Annealing:

58 °C



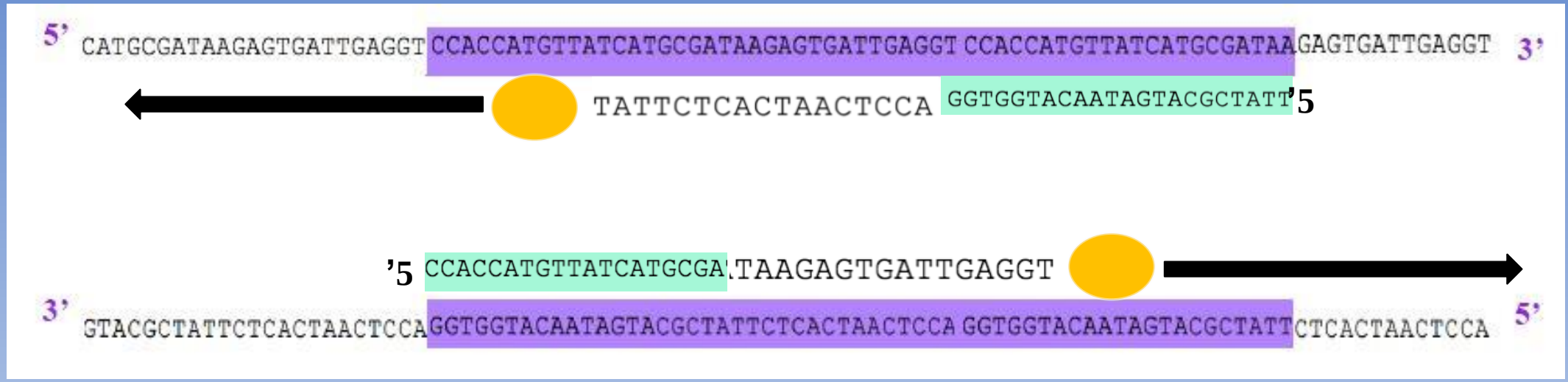


Forward primer: CCACCATGTTATCATGCGA

Reverse primer: GGTGGTACAATAGTACGCTATT

3. Extension:

72 °C



Taq DNA polymerase

AGE results

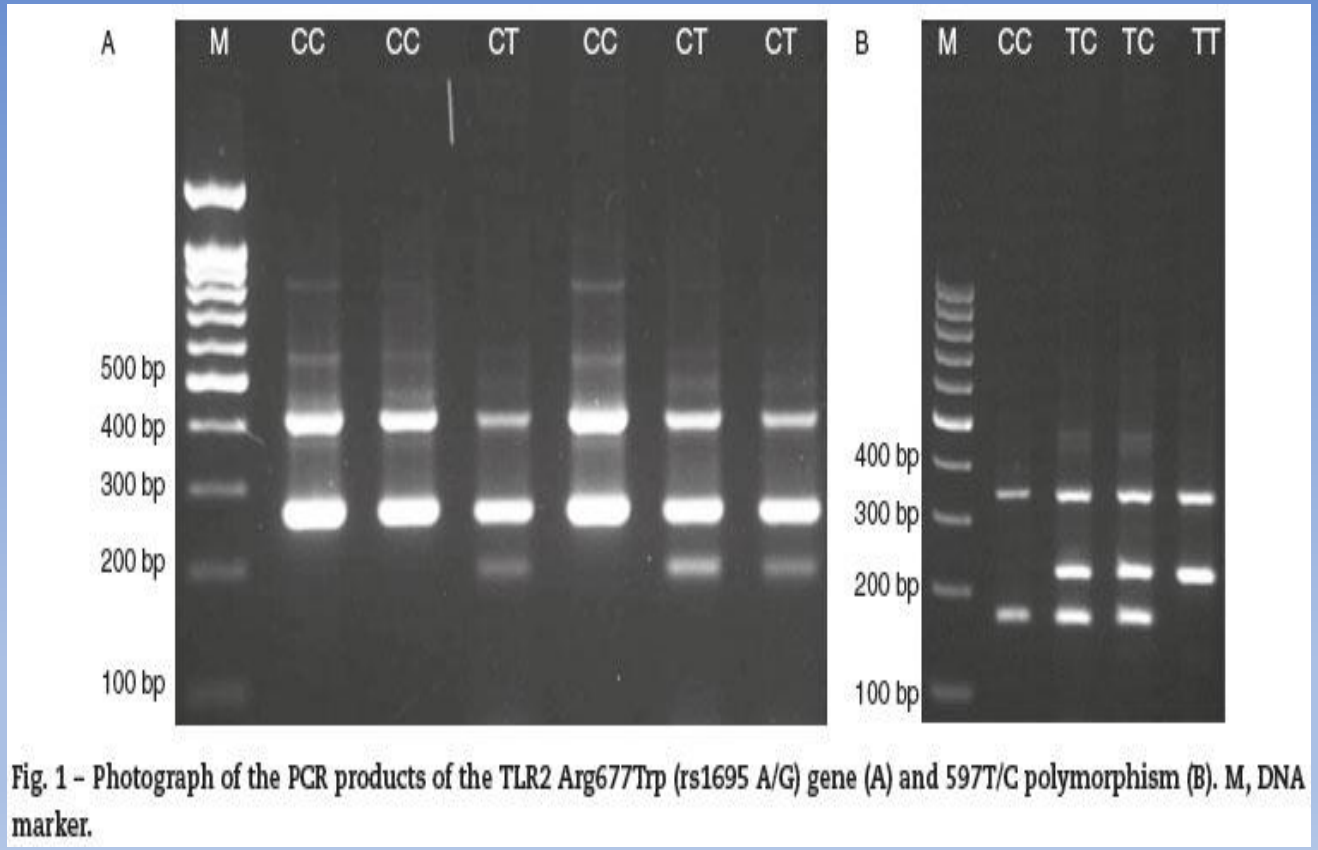


Fig. 1 – Photograph of the PCR products of the TLR2 Arg677Trp (rs1695 A/G) gene (A) and 597T/C polymorphism (B). M, DNA marker.

Target sequence

PCR advantages

- ✓Simplicity,
- ✓easier methodology,
- ✓ sensitive,
- ✓extensively validated standard operating procedure
- ✓availability of reagents and equipment

PCR applications

- ✓Genotyping.
- ✓RT-PCR.
- ✓Cloning.
- ✓Mutation detection.
- ✓Sequencing.
- ✓Microarrays.
- ✓Forensics.
- ✓Paternity testing.

Home work

- Suppose you perform a PCR that begins with one double-strand of the following DNA template:

A. Draw one cycle of PCR reaction below the following diagram.

B. Label the template DNA, the primers, and what is happening at each step.