# Polymerase chain reaction (PCR)

# Polymerase Chain Reaction (PCR)=DNA Photocopier

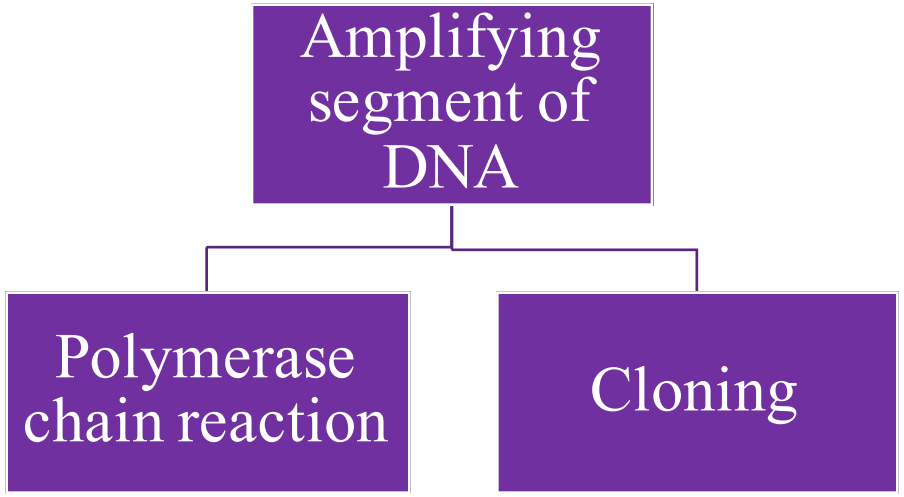
# DNA amplification:

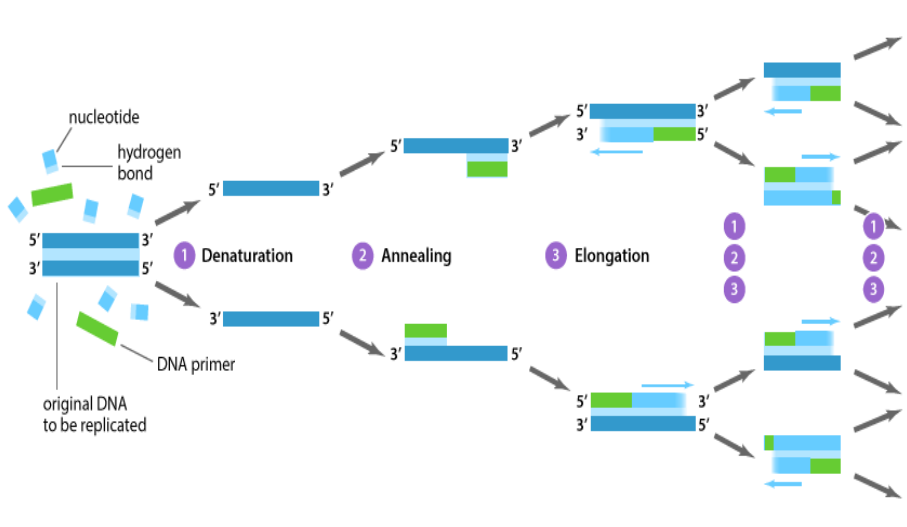
- In a crime scene, a sample of DNA was found, however **amount of DNA was not enough to** be analyzed.

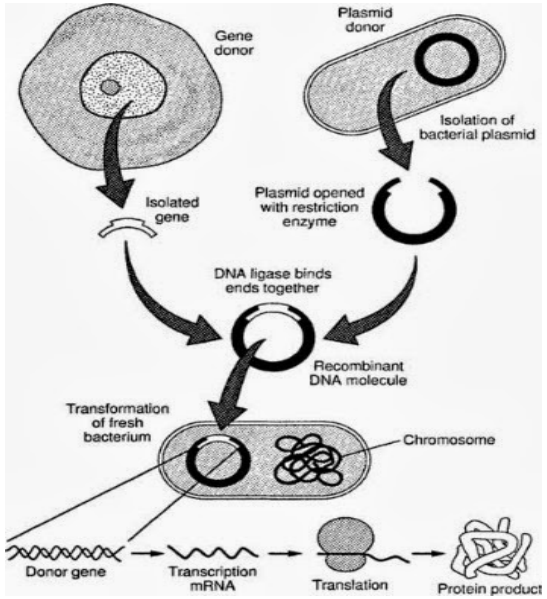
- After DNA extraction, the scientist want to study a specific **part of a gene** to do sequencing.

- How scientist solve these problem ?

- The solution is to do **amplification of parts of DNA**!!
- Mainly there are two methods:



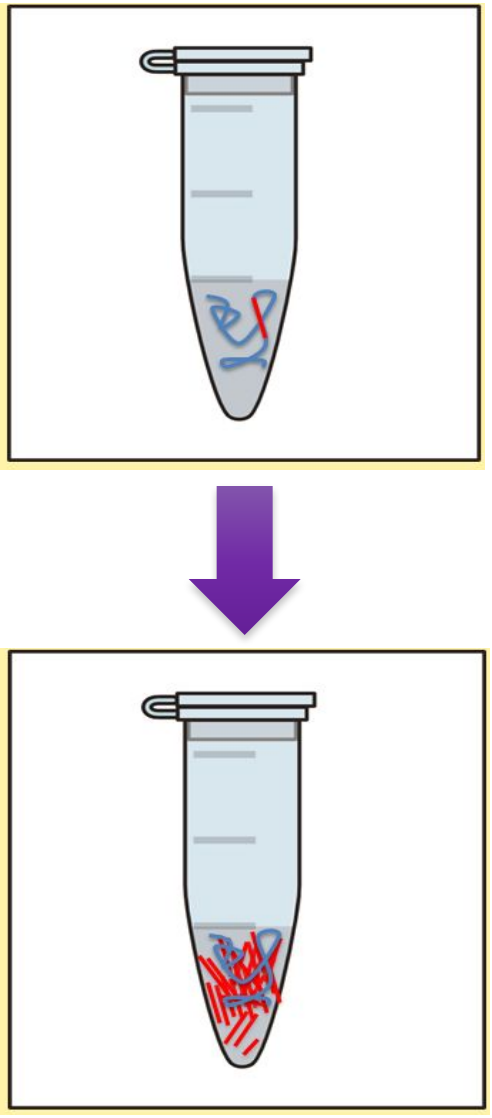



# PCR-Polymerase Chain Reaction:

- 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.

- So…

➔ How the amplification will be done?

➔ How you will determine your target sequence?

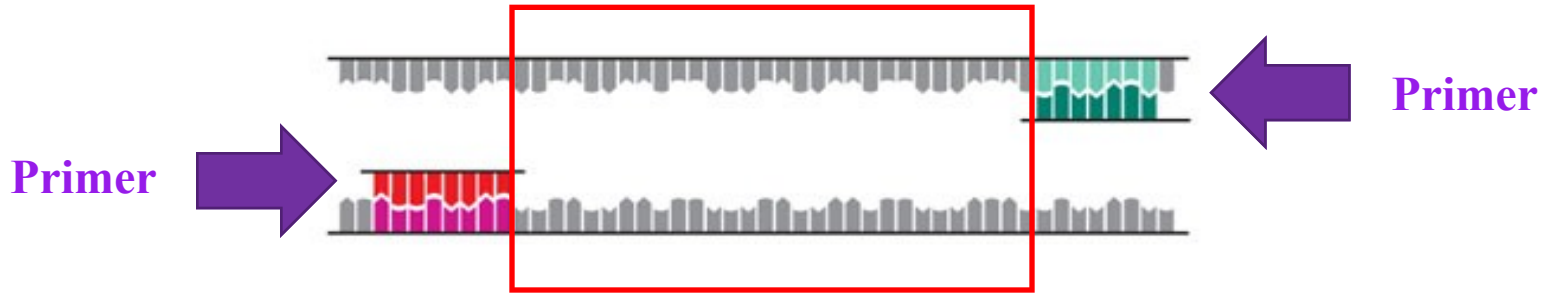
➔ How the amplification will be specific for certain segment?

**You must to understand these questions**

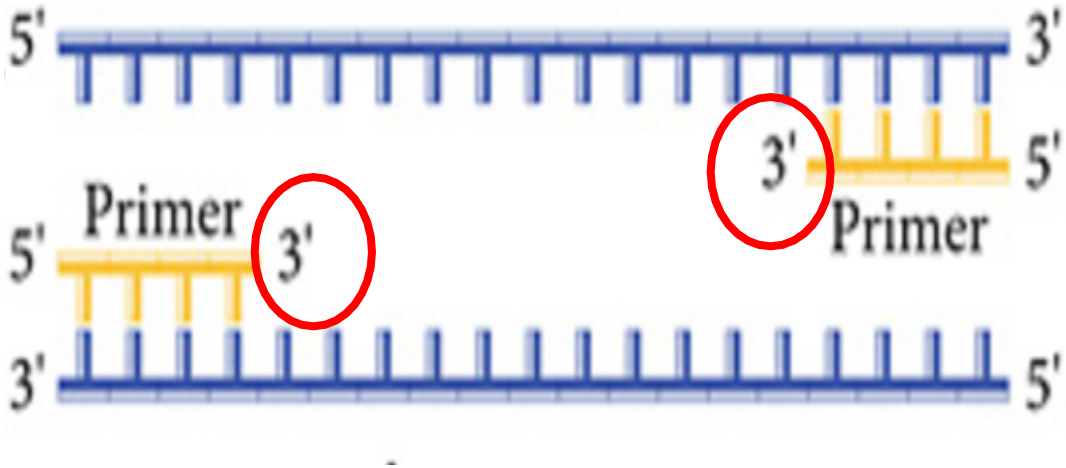
# Amplification of a specific target sequence:

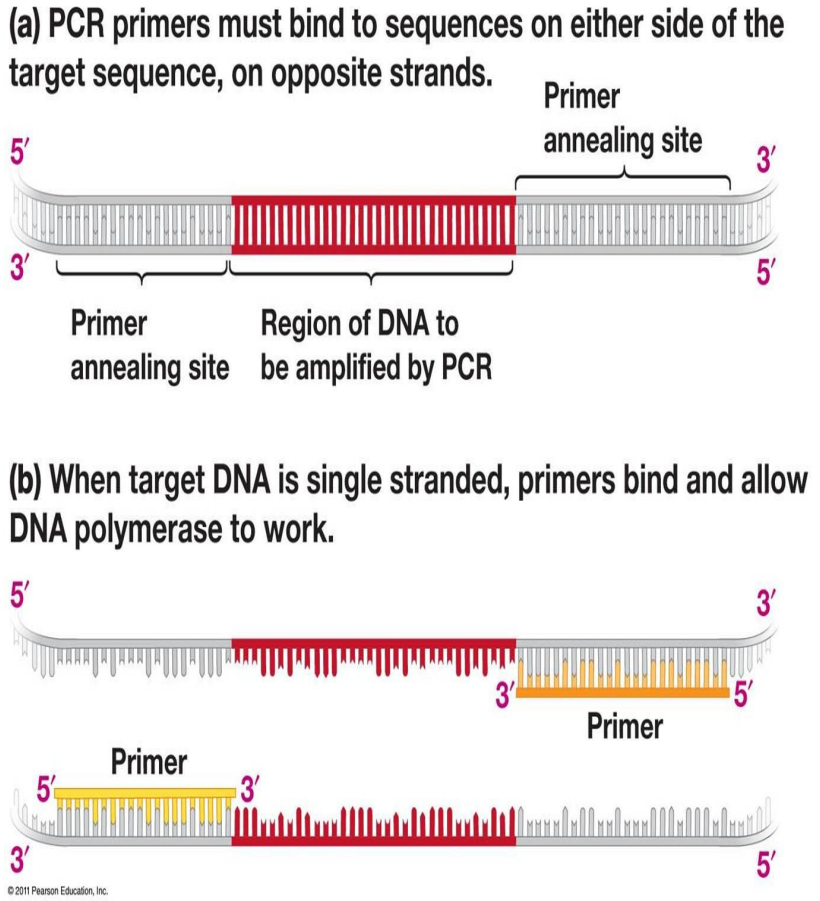
- PCR **does not copy all of the DNA** in the sample. It copies only a very specific sequence of genetic code from a template DNA, targeted by PCR primers.

- It does require the knowledge of some DNA sequence information which flanks the fragment of DNA to be amplified (**target DNA**).

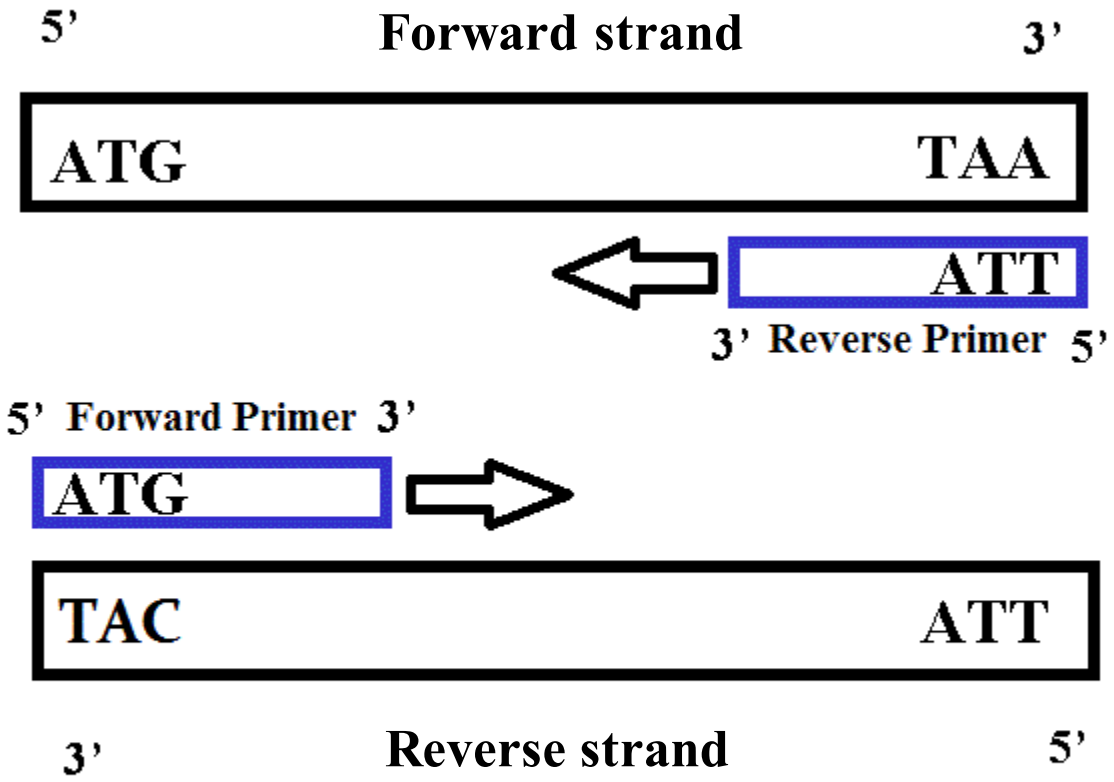


- From this information two synthetic oligonucleotide primers may be chemically synthesised each complementary to a stretch of DNA to the 3' side of the target DNA, one oligonucleotide for each of the two DNA strands (DNA polymerase can add a nucleotide only onto a **preexisting 3'-OH group**).



# Why we need two primers ?



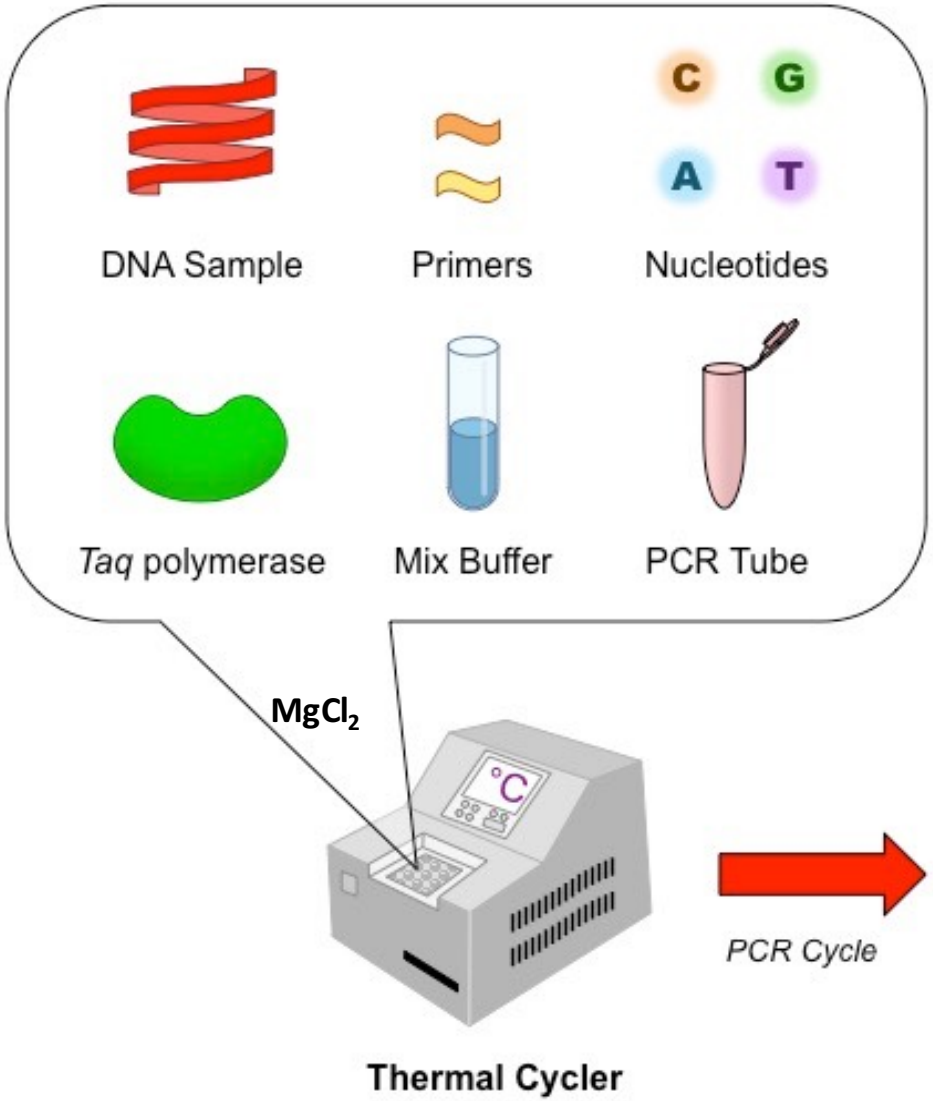

- In a PCR reaction you need **two primers** to amplify the target sequence:

➔**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



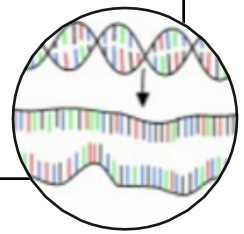

Additional reagents may included

# The PCR Cycle :

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

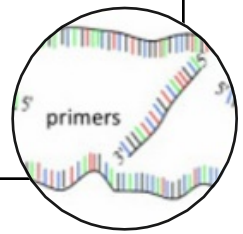
- The double-stranded template DNA is denatured by heating, typically to 95°C, to separate the double stranded DNA.

**Denaturation:**
***(95°C)***



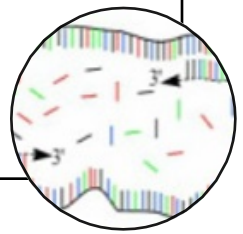
- The reaction is rapidly cooled to an annealing temperature to allow the oligonucleotide primers to hybridize to the template.

****Annealing**:
***(50-65°C)***




- The reaction is heated to a temperature, typically 72°C for efficient DNA synthesis by the thermostable DNA polymerase.

**Extension:**
***(72°C)***



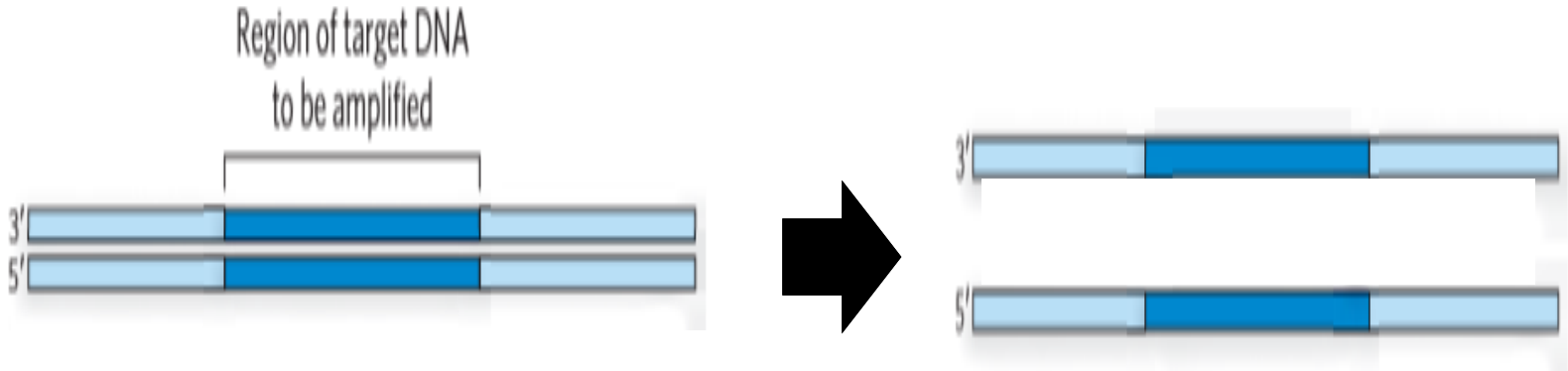
# 1. Denaturation:

- 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).
- What is the type of the bond?

**Step 2**

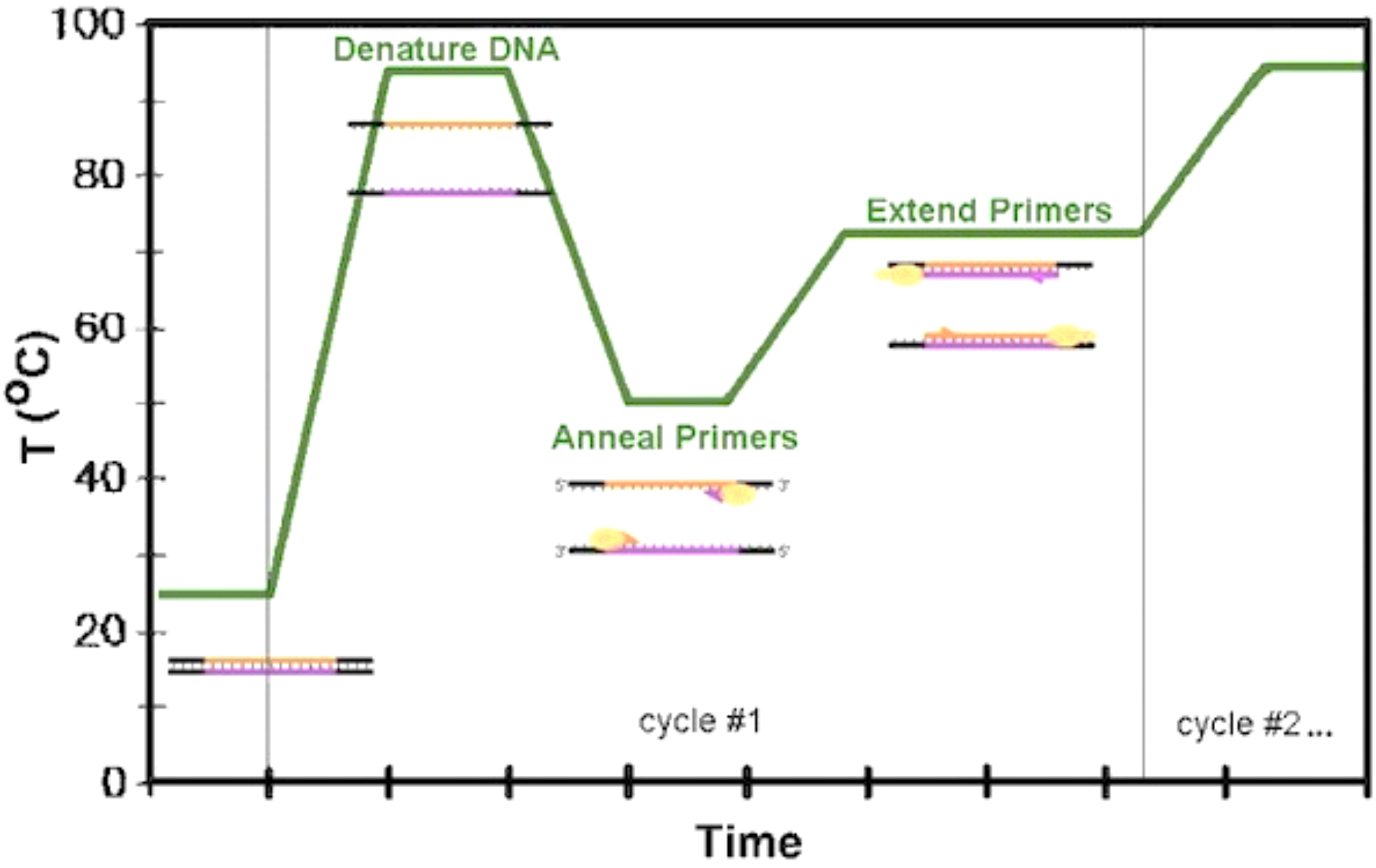
**(**50–65 °C **)**

# 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

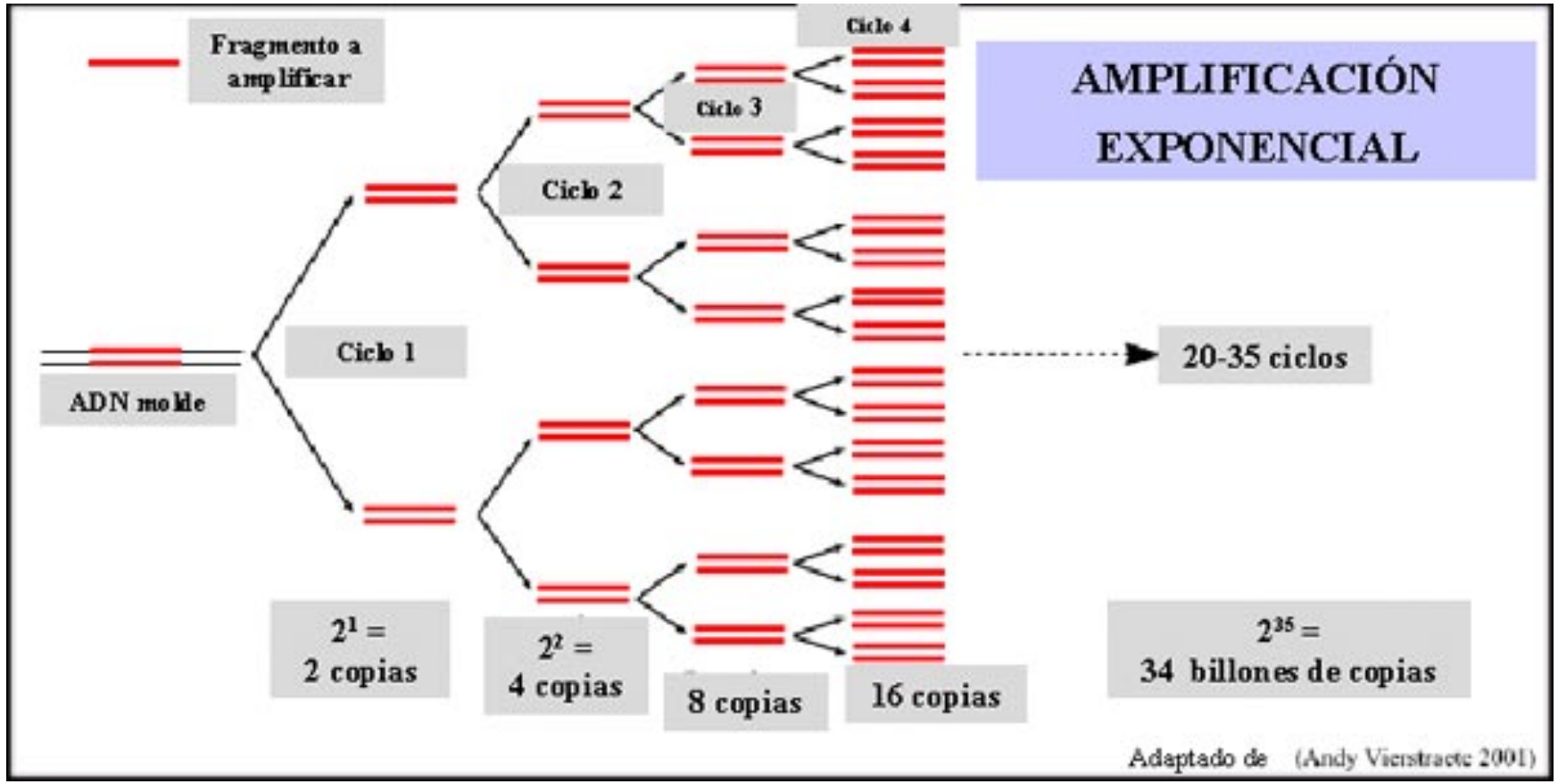
**Step 3**

(72 °C )

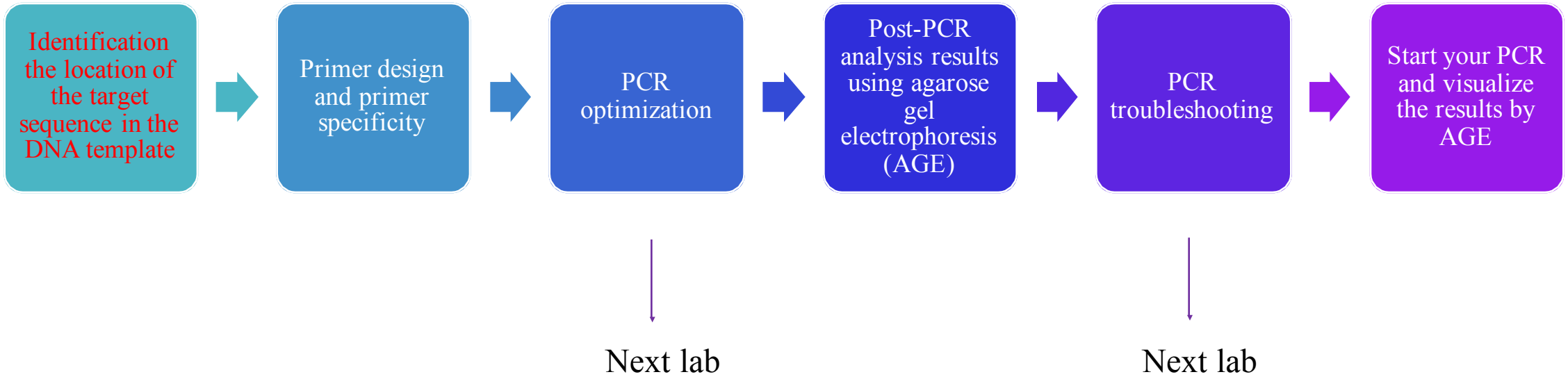



One cycle X 25

- At the end of the PCR reaction, the **specific sequence** will be accumulated in billions of copies (amplicons).
- In only 20 cycles, PCR can product about a million ($2^{20}$) copies of the target.



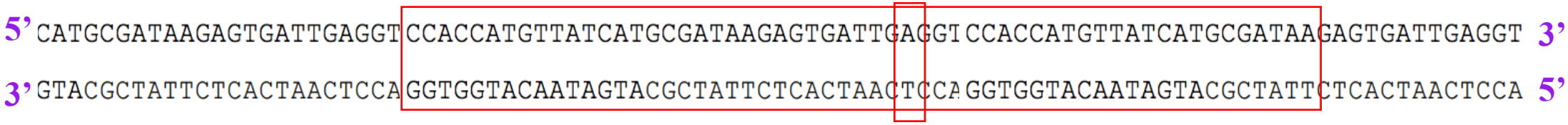
# Performing PCR steps :



# 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**

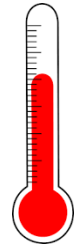



  3. Design the primers using primer design tool, eg.Primer3, then send them to any company who will synthesize them.
  4. Make sure that the area that you want to study is **between the primers (**the region to be studied should be between the forward and reverse primer**)**.
  5. Check primer specificity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

# Start your PCR !

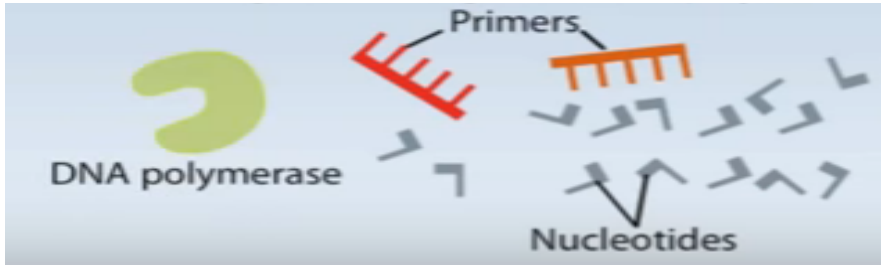
## 1. Denaturation:

**95 °C**



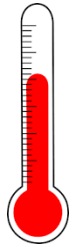
5’ CATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAA GAGTGATTGAGGT 3’

3’ GTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATT CTCACTAACTCCA 5’



# Start your PCR !

## 2. Annealing:

58 °C 

5’ CATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAA GAGTGATTGAGGT 3’

3’ GGTGGTACAATAGTACGCTATT 5’

5’ CCACCATGTTATCATGCGA 3’

3’ GTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATT CTCACTAACTCCA 5’

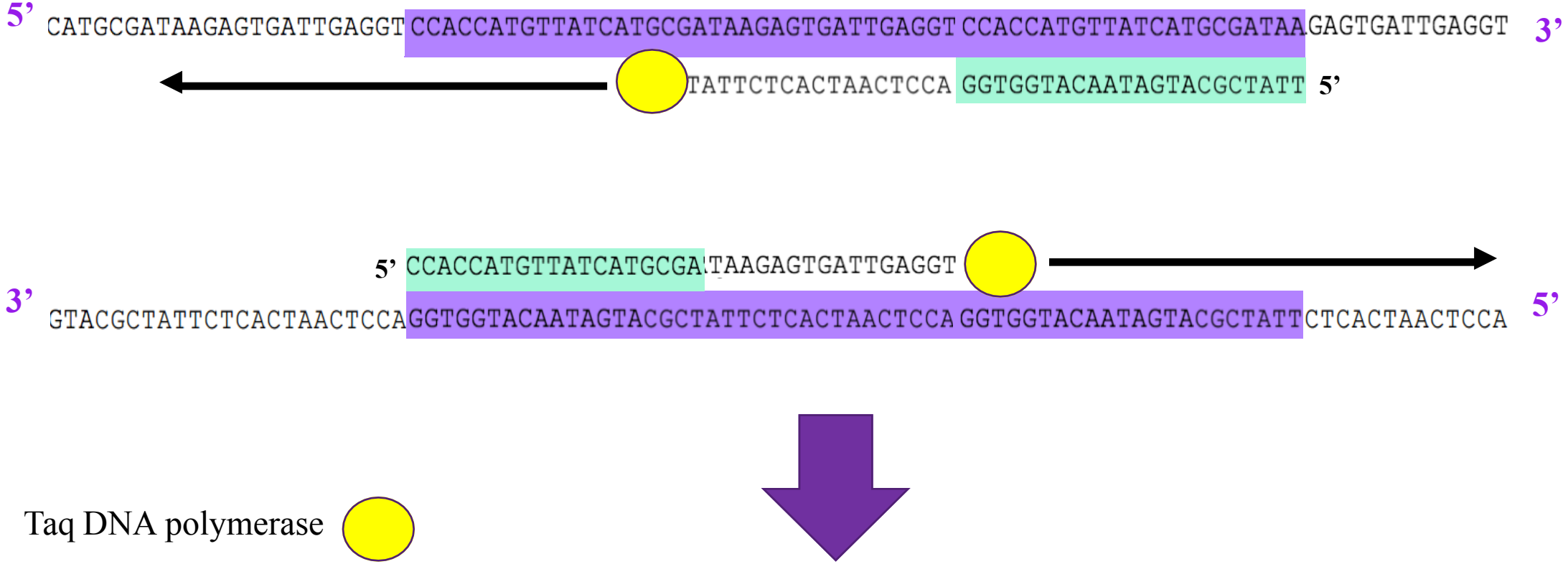
Forward primer: 5’ CCACCATGTTATCATGCGA 3’

Reverse primer: 3’ GGTGGTACAATAGTACGCTATT 5’

# Start your PCR !

## 3. Extension:

72 °C




Taq DNA polymerase

# Start your PCR !

## 3. Extension:

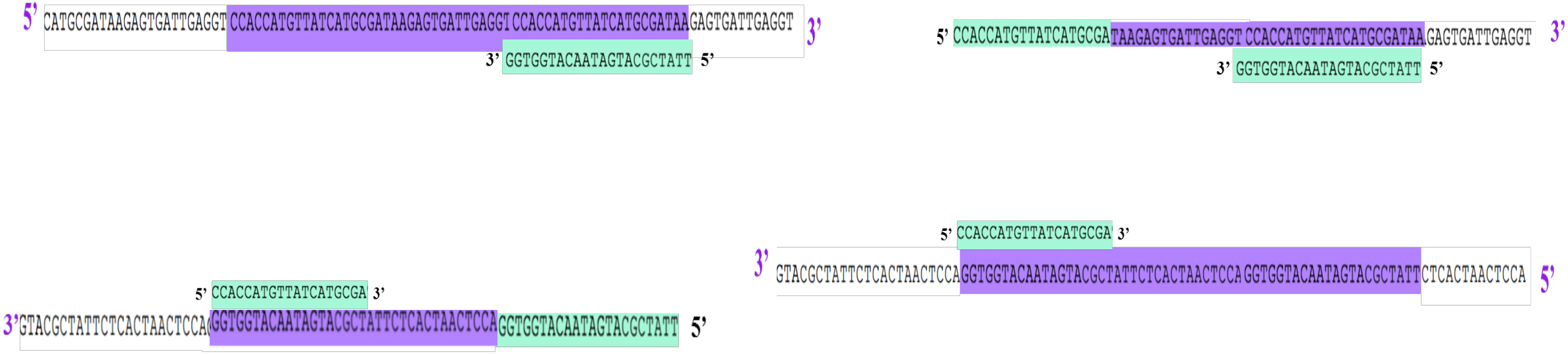
72 °C


Cycle # 1:
1 DNA amplified to 2 DNA

# Cycle 2

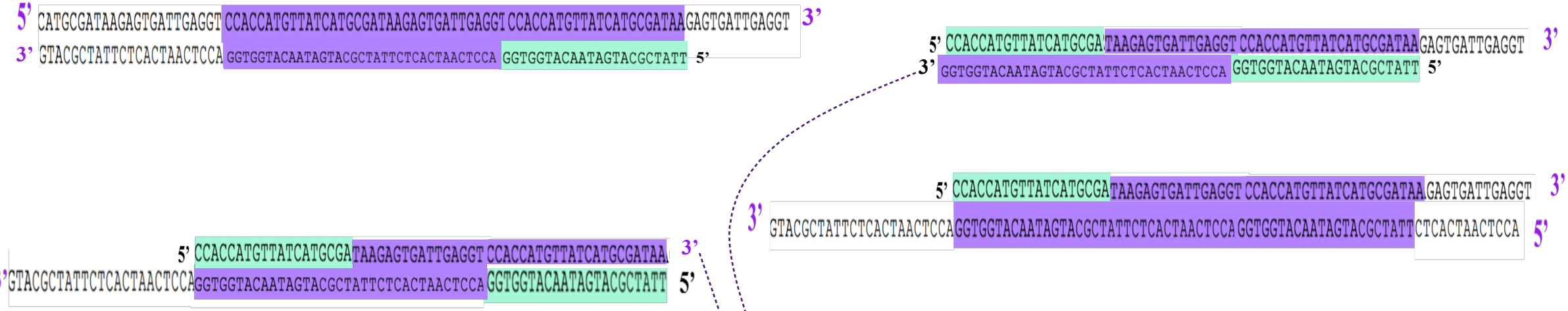
## 1. Denaturation


## 2. Annealing



# Cycle 2

**3. Extension**




# Cycle 3


**Target sequence**

Appeared after three cycles and start to accumulate

**After 30 cycles:**

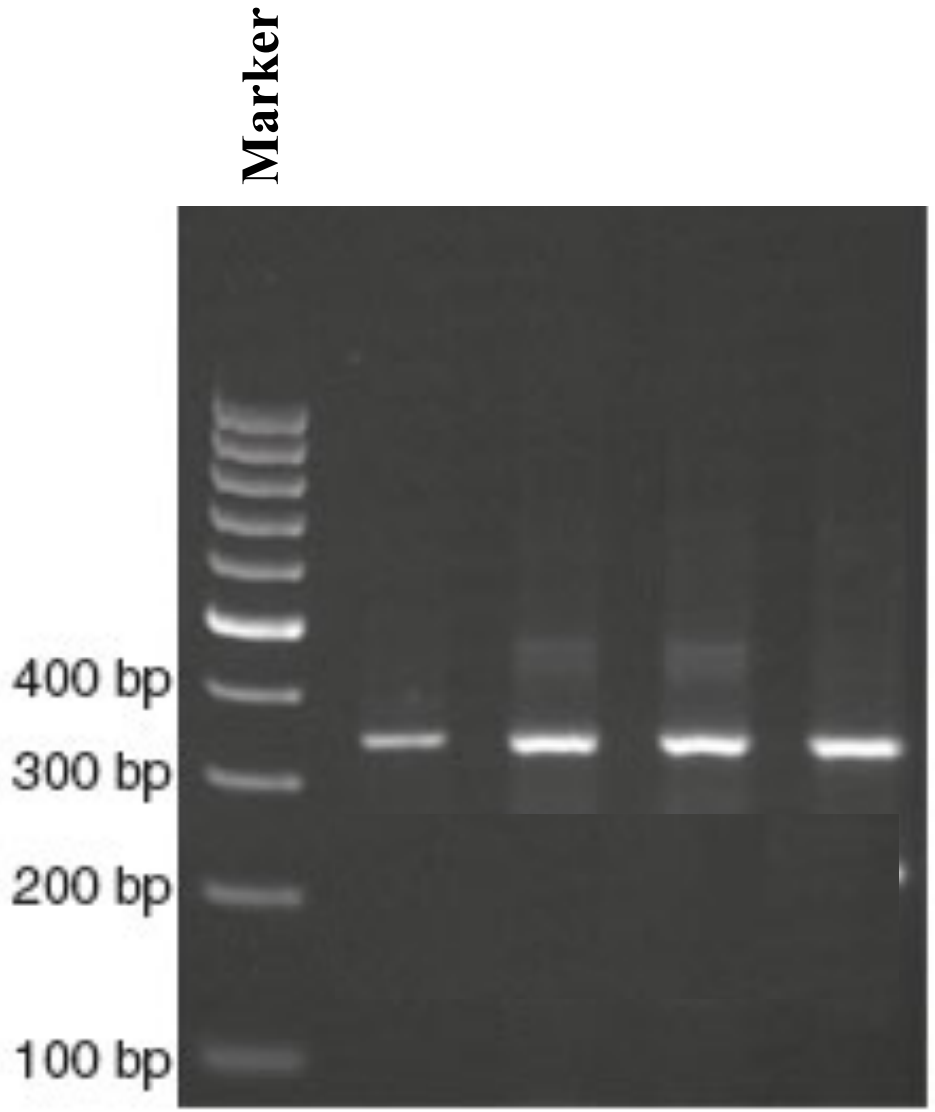
$2^{30}$ copy of target DNA !!

**How you will make sure that you target sequence is amplified?**

**It is very important to know your product size, why?**

➔ **Our target sequence size is 350 bp**

# AGE results




**Target sequence**

# PCR advantages:

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

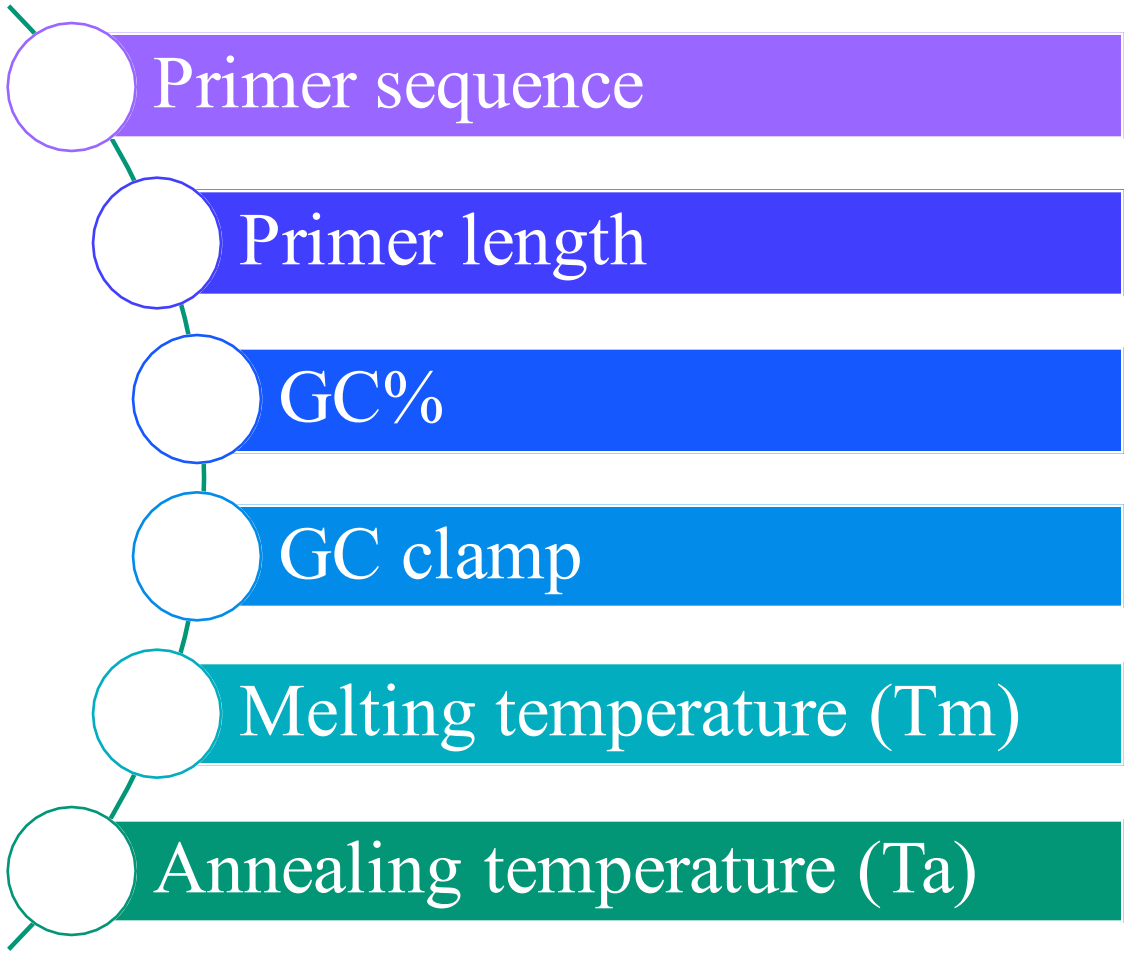
# PCR application:

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

# PCR Optimization:

- There is no single set of conditions that is optimal for all PCR reactions.
- Next Lab.

# Primer Design Guidelines:



# Primer Design Guidelines:

## 1. Primer sequence:

- Must be complementary to flanking sequences of target region.
- Avoid:

➔Complementary sequences between primers.

➔Repeat (ex: ATATATAT) ➔ misprime.

➔Runs (ex: AGCGGGGGAT) ➔ misprime.

➔Mismatch at 3' end.

➔Cross Homology.

## 2. Primer length:

- It is generally accepted that the optimal length of primers is **18-25 bp.**
- Not too long nor too short

# Primer Design Guidelines:

## 3. GC content:

- **GC% =** Number of G's and C's in the primer as a percentage of the total bases.
- Should be 40-60%.

## 4. GC clamp:

- Presence of G or C bases within the last five bases from the 3' end of primers.
- Not more than 2  G's or C's .

5'-CAACATAATAGCGACAACA**CTAGA**-3'

# Primer Design Guidelines:

## 5. Melting temperature (Tm):

- What is Tm?
- Melting temperatures in the range of **50-60 °C** generally produce the best results.
- Maximum difference between primer pairs is 5°C.
- The Tm of the primer can be calculated by the following formula:

$$Tm = [(G + C) \times 4] + [(A + T) \times 2]$$

## 6. Annealing Temperature (Ta):

- The primer melting temperature is the estimate of the DNA-DNA hybrid stability and critical in **determining the annealing temperature.**
- Depends directly on **length** and **GC composition** of the primers.
- Too high Ta ➔ produce insufficient primer-template hybridization.
- Too low Ta ➔ lead to non-specific products caused by a high number of base pair mismatches.

# Now… you should be able to answer the following questions:

- How the amplification will be done?
- How you will determine your target sequence?
- How the amplification will be specific for certain segment?
- What are the requirements to carry PCR?

# Home Work:

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

```
→5′-CTACCTGCGGGTTGACTGCTACCTTCCCGGGATGCCCAAAATTCTCGAG-3→
:::::::::::::::::::::::::::::::::::::::::::::::::::::
←3′-GATGGACGCCCAACTGACGATGGAAGGGCCCTACGGGTTTTAAGAGCTC-5′←
```

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.