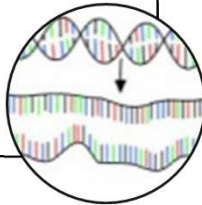


The PCR Cycle :

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

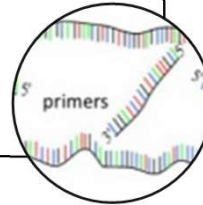
- The double-stranded template DNA is denatured by heating, typically to 95°C, to separate the double stranded DNA.

Denaturation:
(95°C)



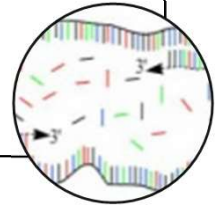
- The reaction is rapidly cooled to an annealing temperature to allow the oligonucleotide primers to hybridize to the template.

**** 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)

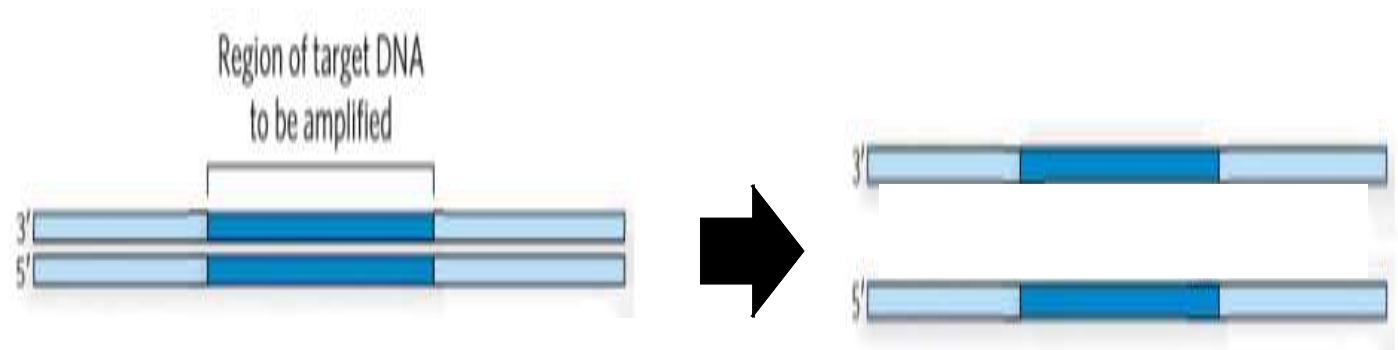


1. Denaturation:

- 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**)

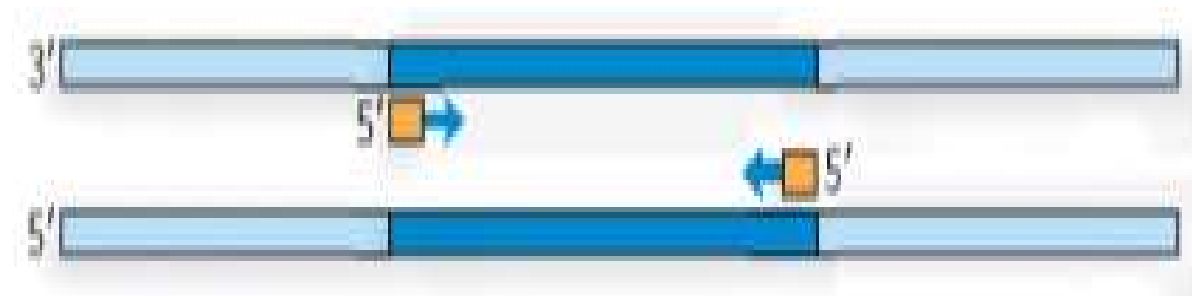


2. Annealing:

- 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)

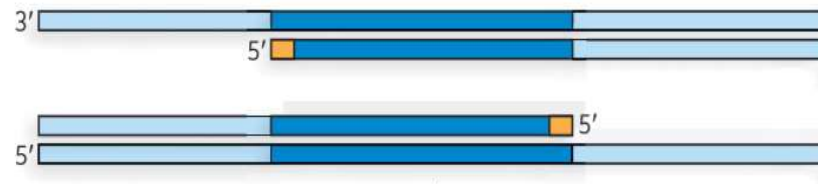


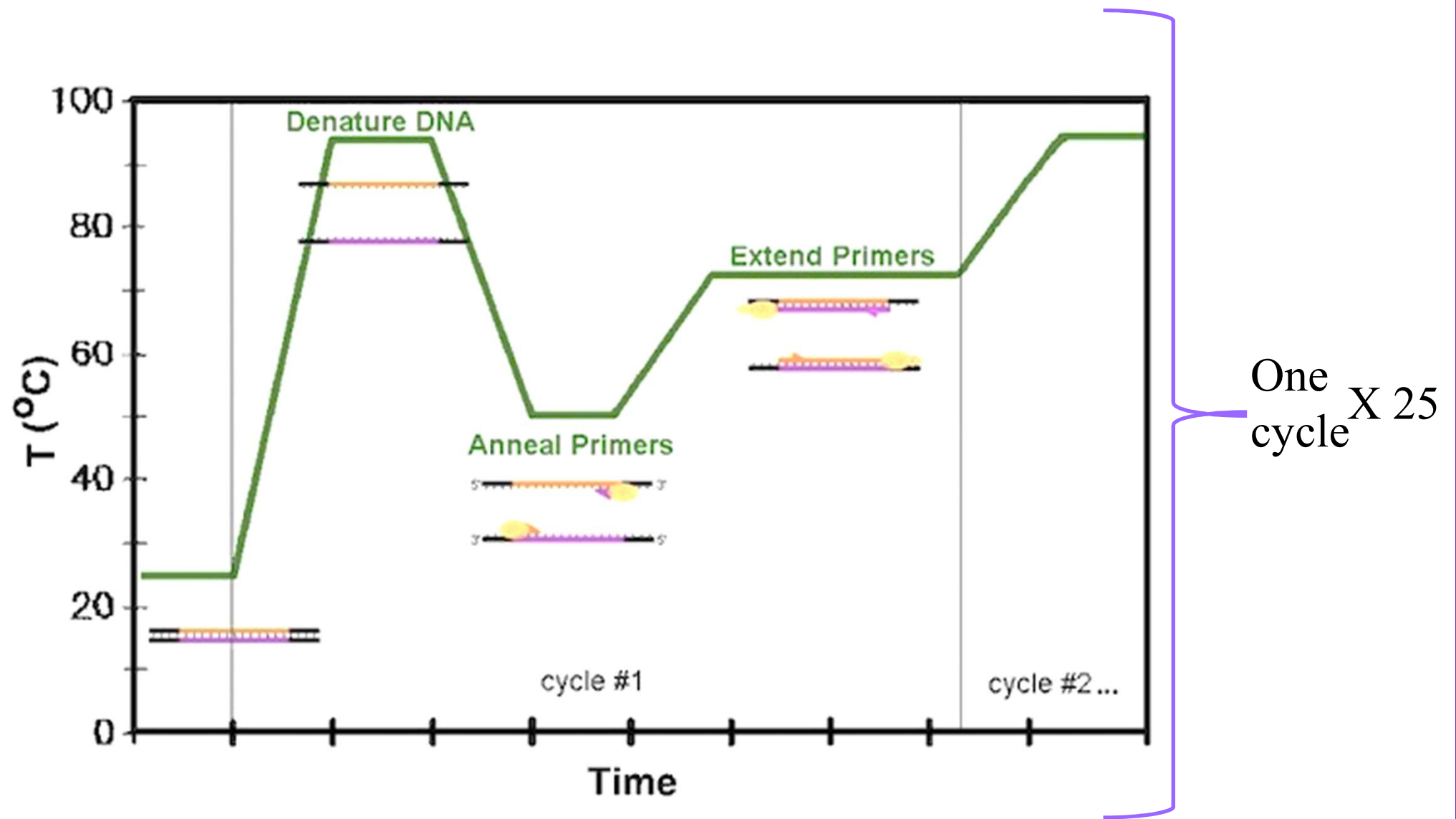
3. Extension:

- 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

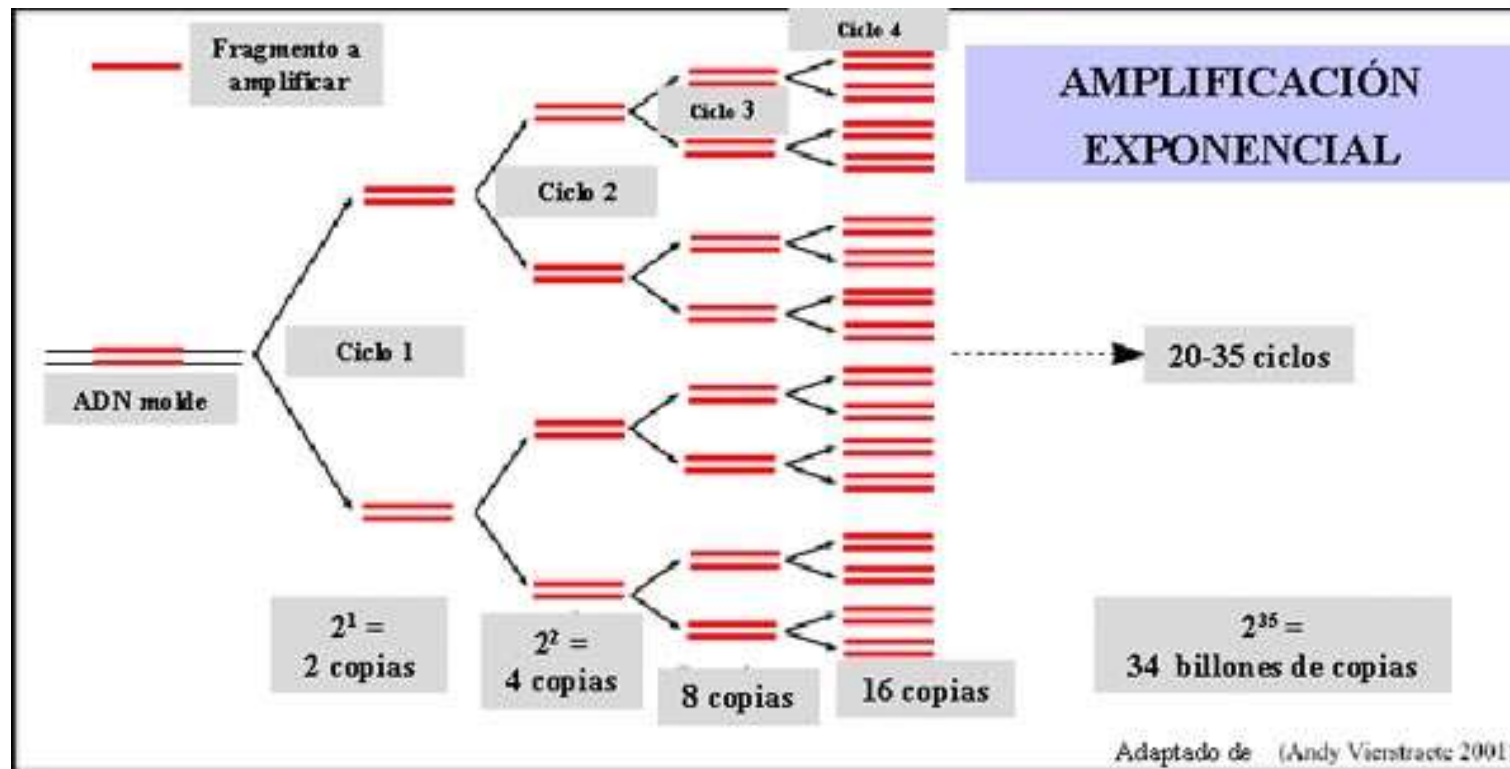
Step 3

(72 °C)

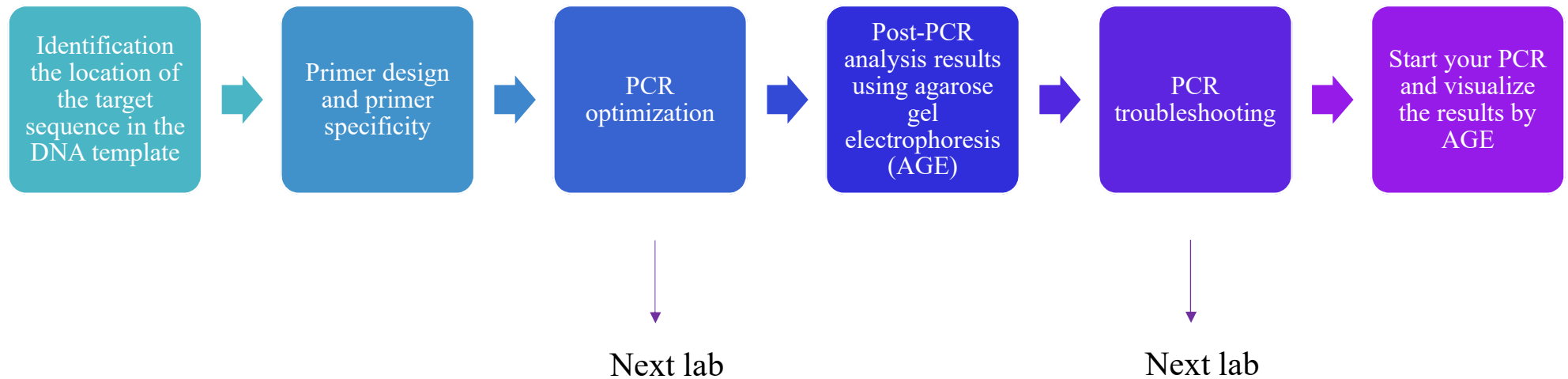




- At the end of the PCR reaction, the **specific sequence** will be accumulated in billions of copies (**amplicons**).
- In only 20 cycles, PCR can product about a million (2^{20}) copies of the target.



Performing PCR steps :





Example:

- You want to study a **mutation in a DLG3 gene** and how it relate to memory:
 - Find the sequence of the gene from any website, eg. Ensembl.
 - Determine your **target region**.

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

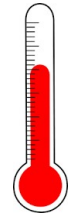
5' CATGCGATAAGAGTGATTGAGGT **CCACCATGTTATCATGCGATAAGAGTGATTGAGGT** CCACCATGTTATCATGCGATAAGAGTGATTGAGGT 3'
3' GTACGCTATTCTCACTAACTCCA **GGTGGTACAATAGTACGCTATTCTCACTAACTCCA** GGTGGTACAATAGTACGCTATTCTCACTAACTCCA 5'

- Design the primers using primer design tool, eg. Primer3, then send them to any company who will synthesize them.
- 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).
- Check primer specificity by BLAST.
- Optimize your PCR and trouble shooting.
- Start PCR.

Start your PCR !

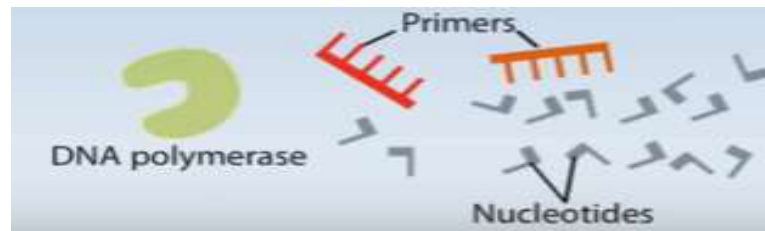
1. Denaturation:

95 °C



5' CATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT 3'

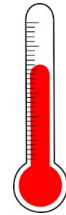
3' GTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA 5'



Start your PCR !

2. Annealing:

58 °C



5' CATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT 3'
3' GGTGGTACAATAGTACGCTATT 5'

5' CCACCATGTTATCATGCGA 3'
3' GTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA 5'

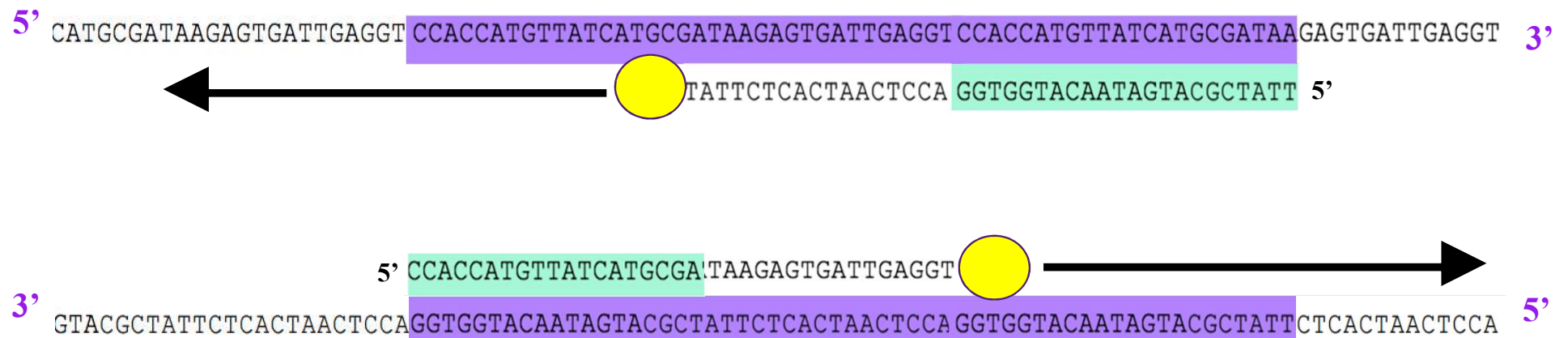
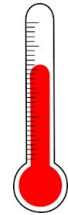
Forward primer: 5' CCACCATGTTATCATGCGA 3'

Reverse primer: 3' GGTGGTACAATAGTACGCTATT 5'

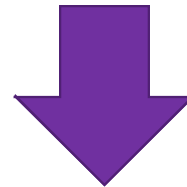
Start your PCR !

3. Extension:

72 °C



Taq DNA polymerase

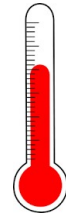


Start your PCR !

3. Extension:



72 °C



5' CATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT 3'
3' GTACGCTATTCTCACTAACTCCAGGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATT 5'

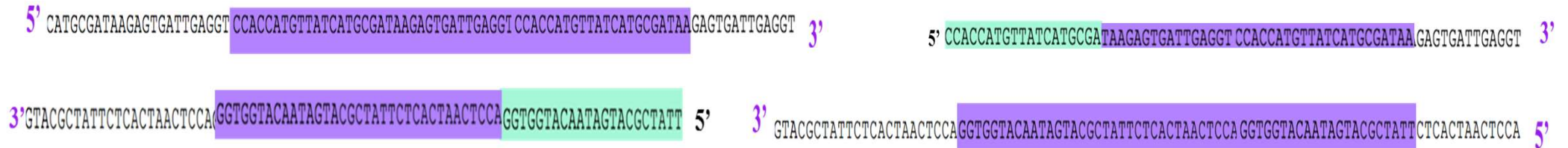
5' CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT 3'
3' GTACGCTATTCTCACTAACTCCAGGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA 5'

Cycle # 1:

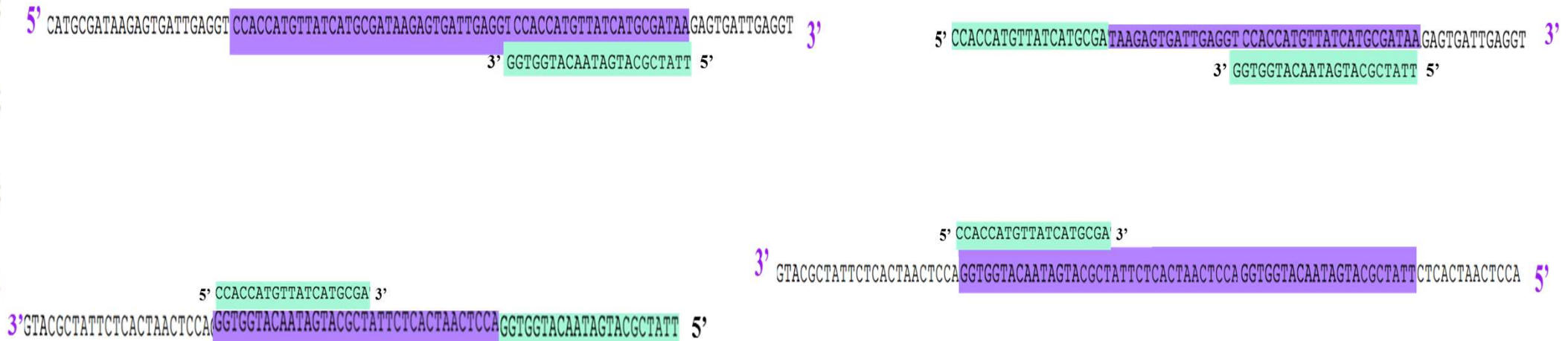
1 DNA amplified to 2 DNA

Cycle 2

1. Denaturation

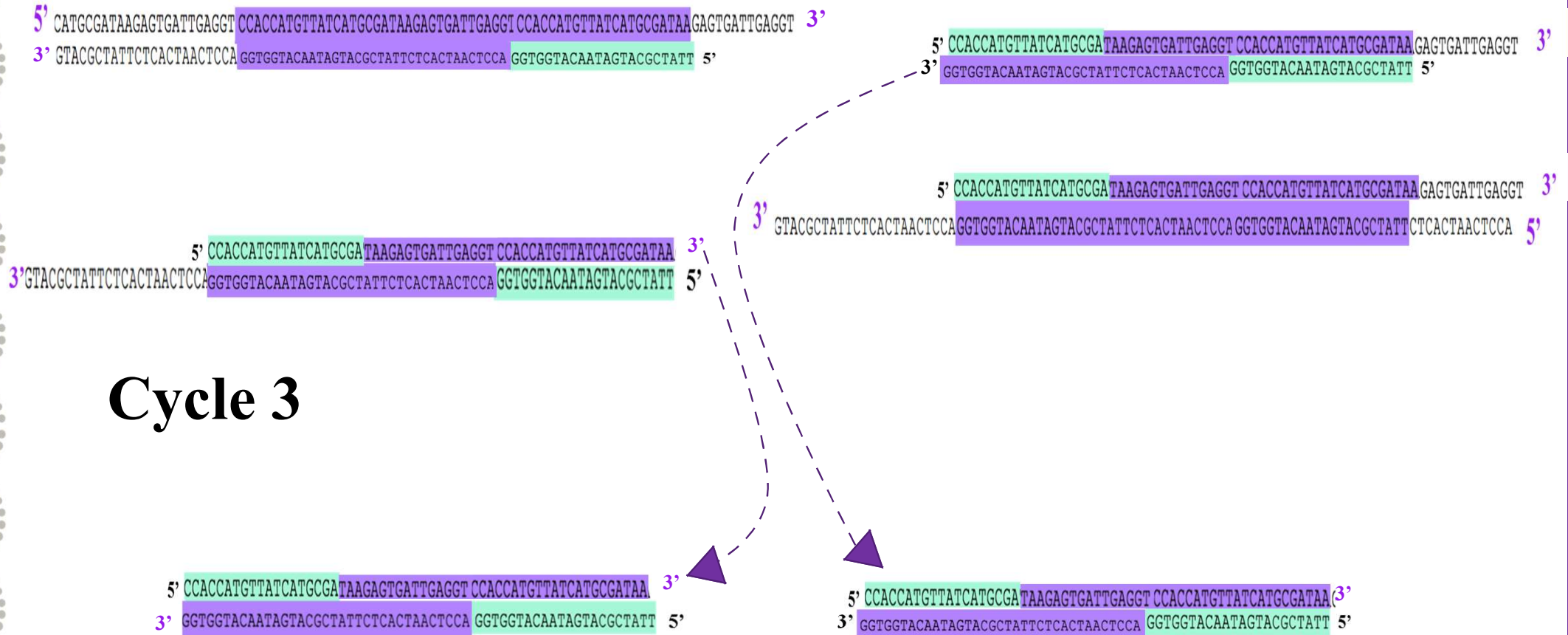


2. Annealing



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 !!