DEPARTMENT OF BIOTECHNOLOGY

PCR (Polymerase chain reaction)

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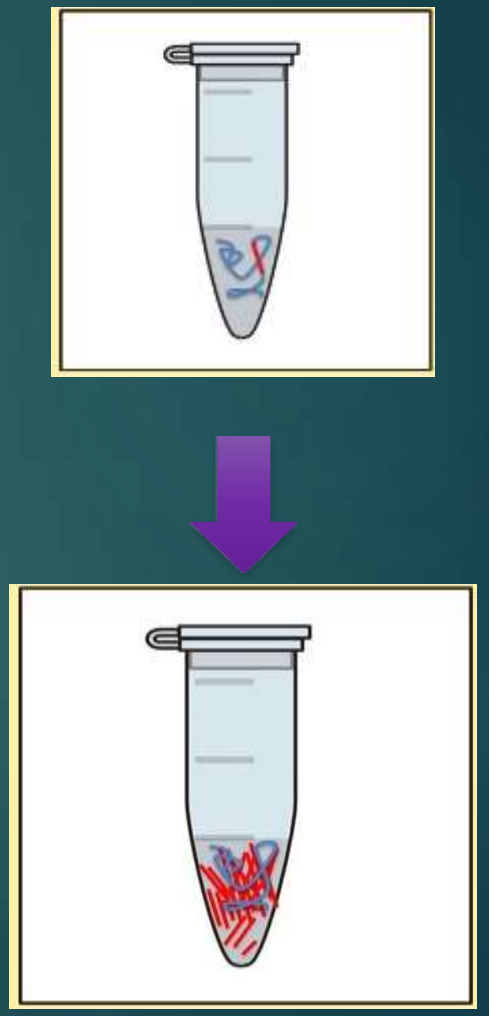
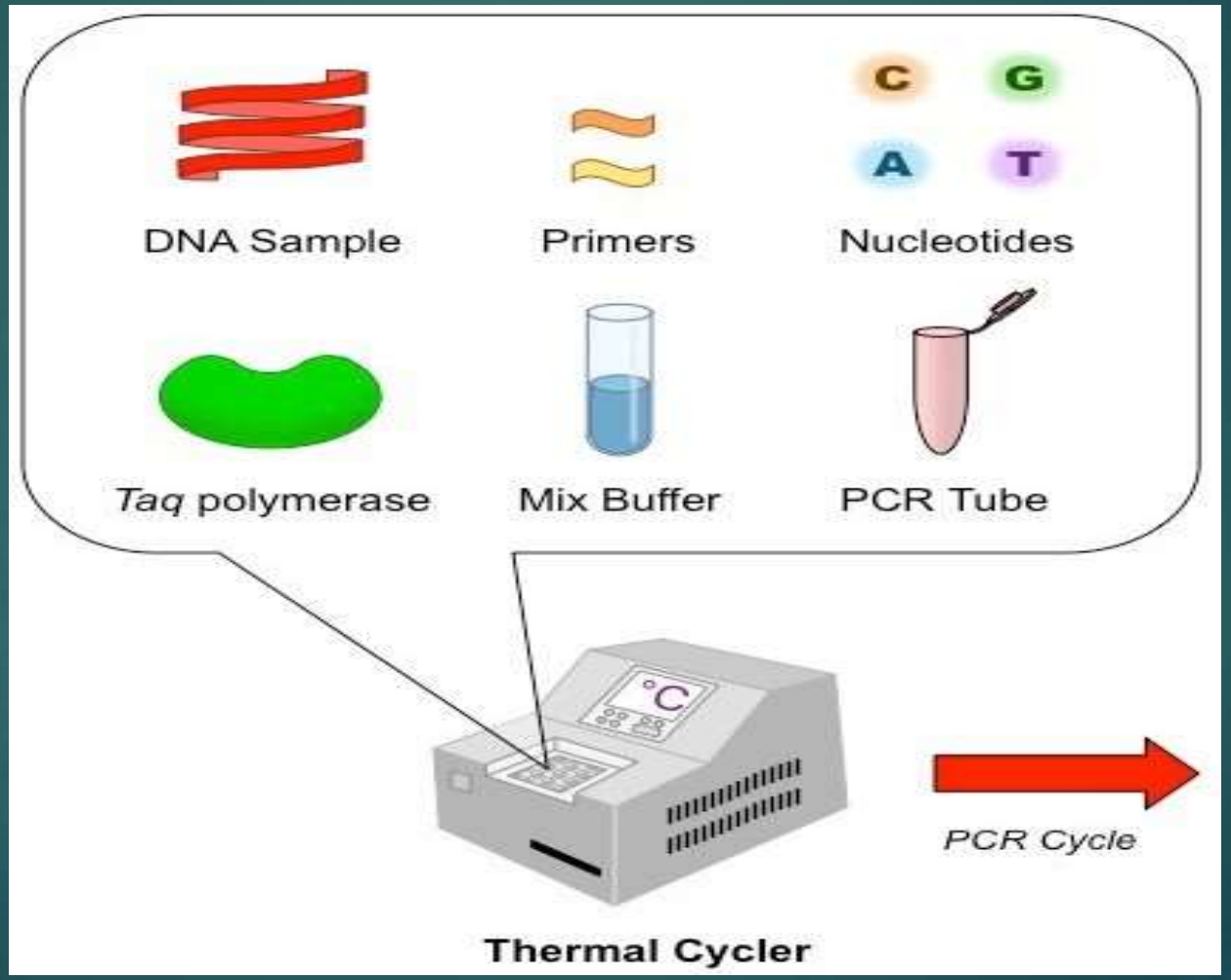
PCR

- PCR is a means to amplify a particular piece of DNA .
- PCR can make billions of copies of a target sequence of DNA in short time.
- It is a laboratory level of DNA replication.




DNA
XEROX
DNA
DNA
DNA

Component of PCR



PRIMER GUIDELINES

- Primer design
- Primer length
- GC %
- Melting temperature
- Annealing temperature.

PRIMER 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.

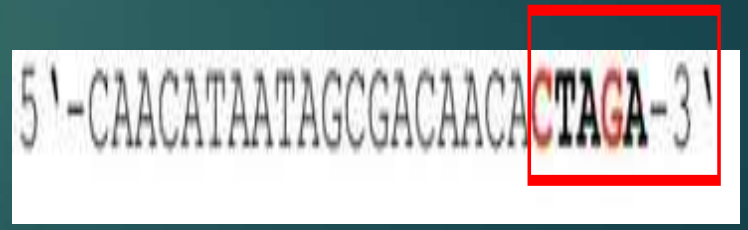
2. **Primer length:**
   - It is generally accepted that the optimal length of primers is **18-25 bp.**
   - Not too long nor too short

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. 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) x 4] + [(A + T) x 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.

STEPS

The double stranded DNA template DNA is denatured by heating, typically to 95℃ to separate the double stranded DNA

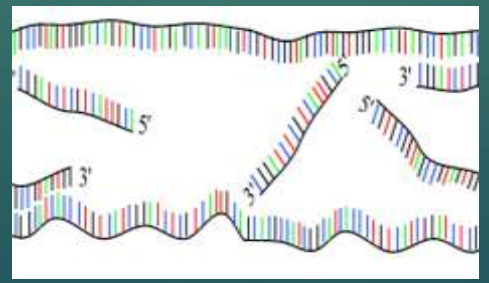
Denaturation (92-95℃)

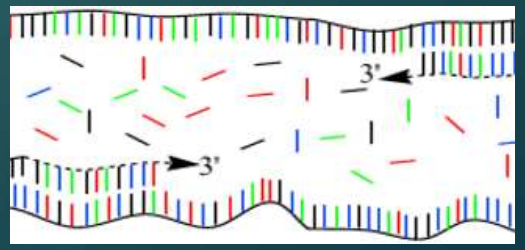
The reaction is rapidly cooled to an annealing temperature to allow the the oligonucleotide to hybridize the template.

Annealing (50-65℃)

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

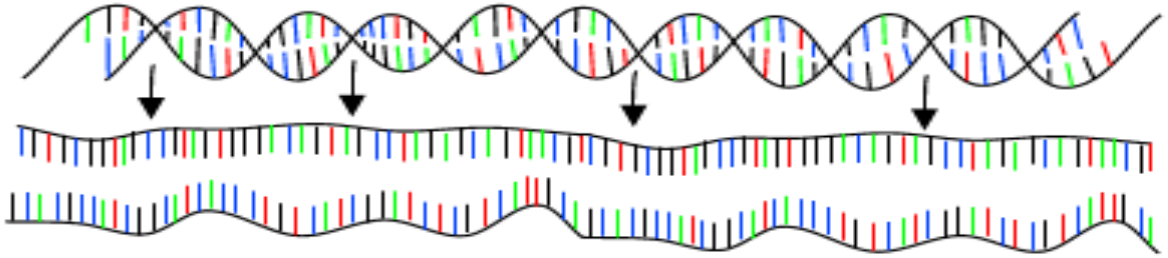
Extension: (72 ºC)





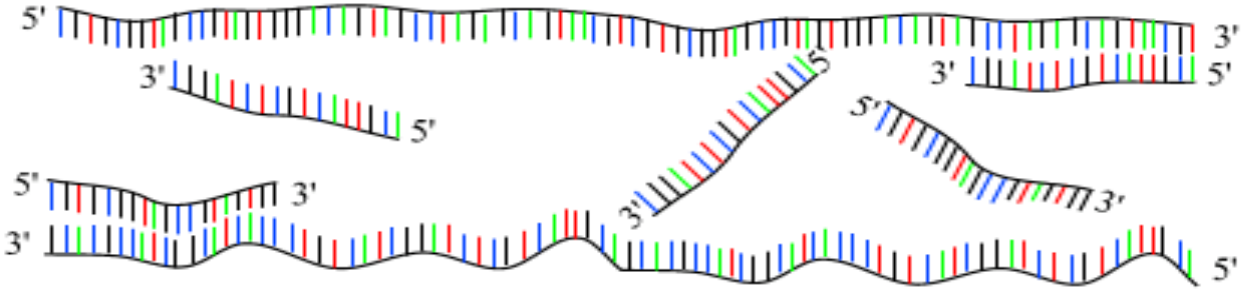
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

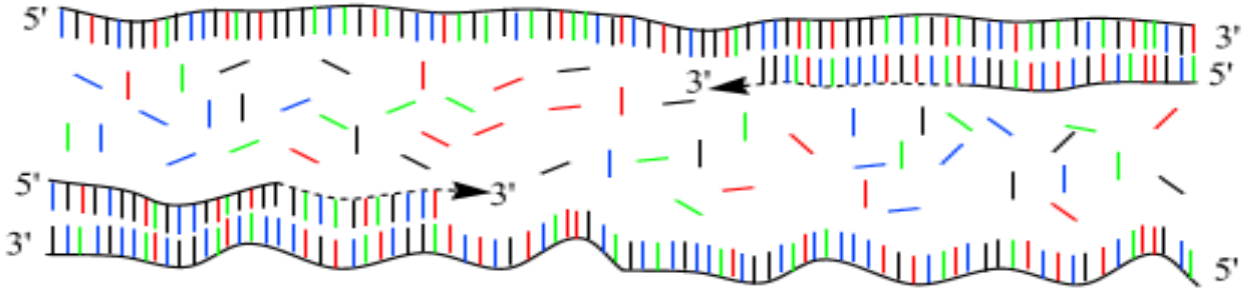
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

2 minutes 72 °C

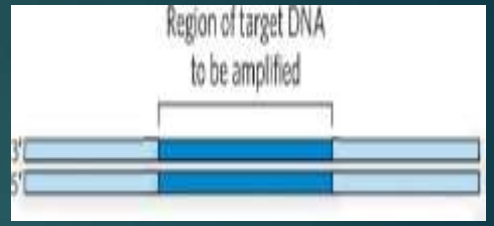
only dNTP's

(Andy Vierstraete 1999)

1. Denaturation:

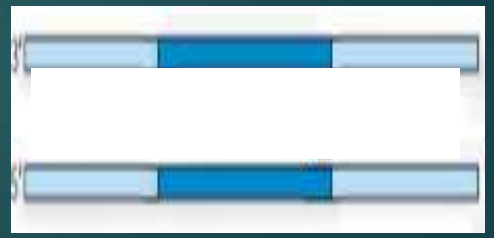
The double-stranded template DNA is denatured by heating, typically to **95°C**, to separate thedouble stranded DNA (why?).

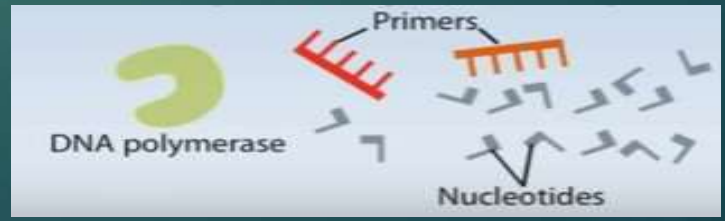
Break the H bonds between the strands.



5’ CATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAA GAGTGATTGAGGT 3’

3’ GTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATT CTCACTAACTCCA 5’

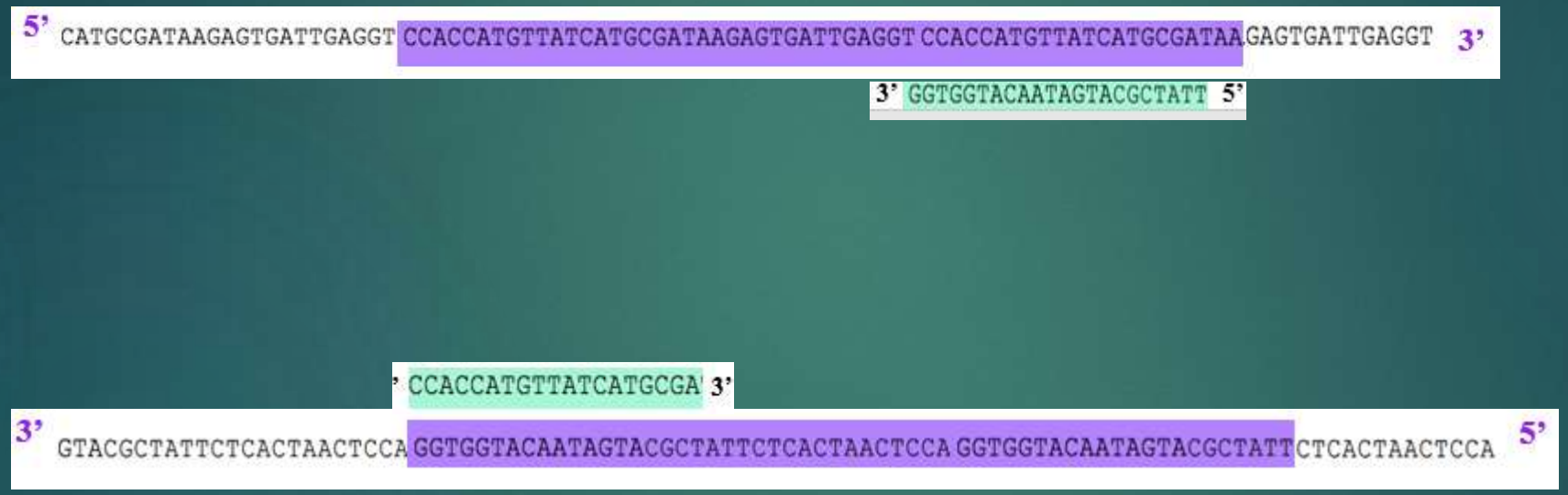




2. ANNEALING:

- 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).

Annealing:

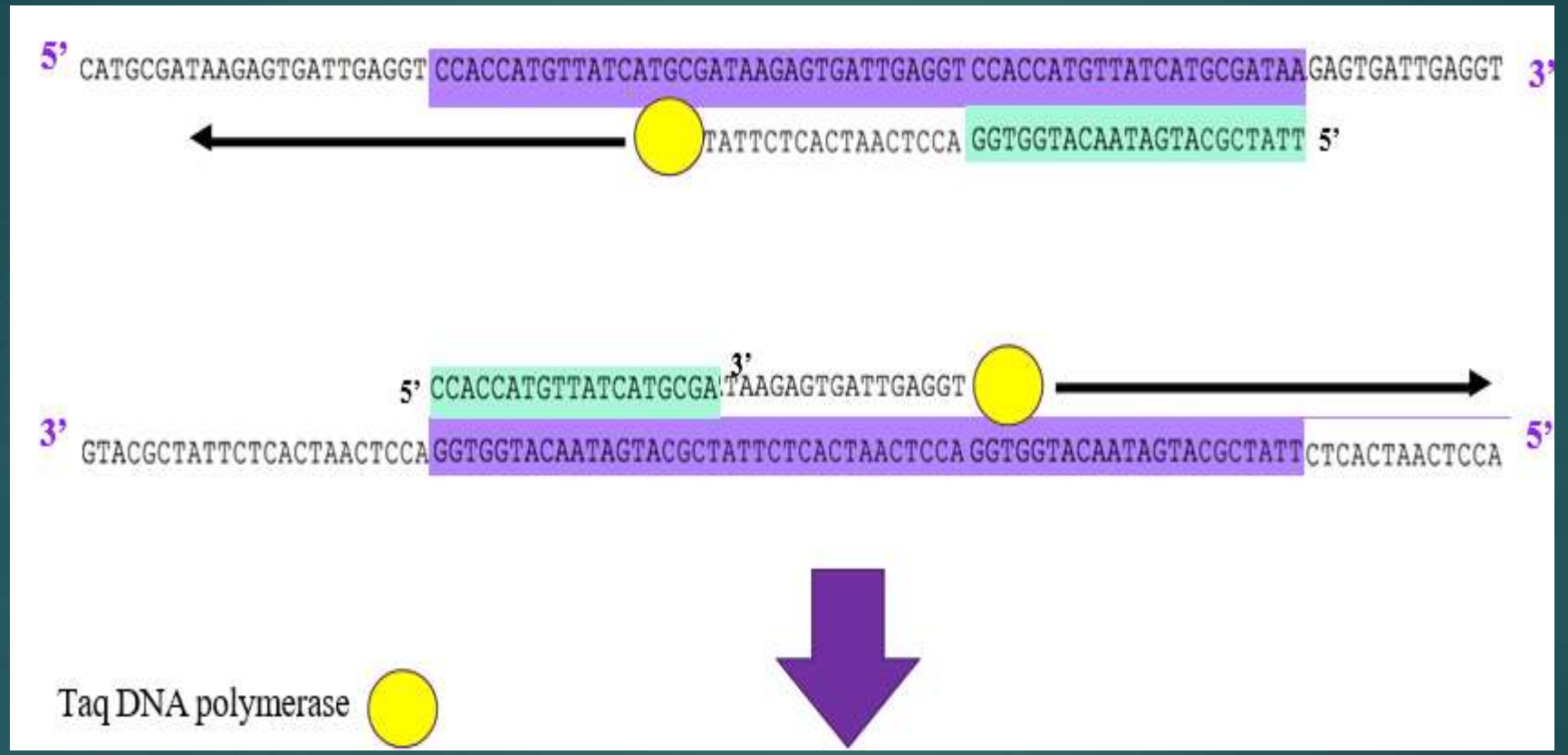


Forward primer: 5’ CCACCATGTTATCATGCGA 3’

Reverse primer: 3’ GGTGGTACAATAGTACGCTATT 5’

EXTENSION:

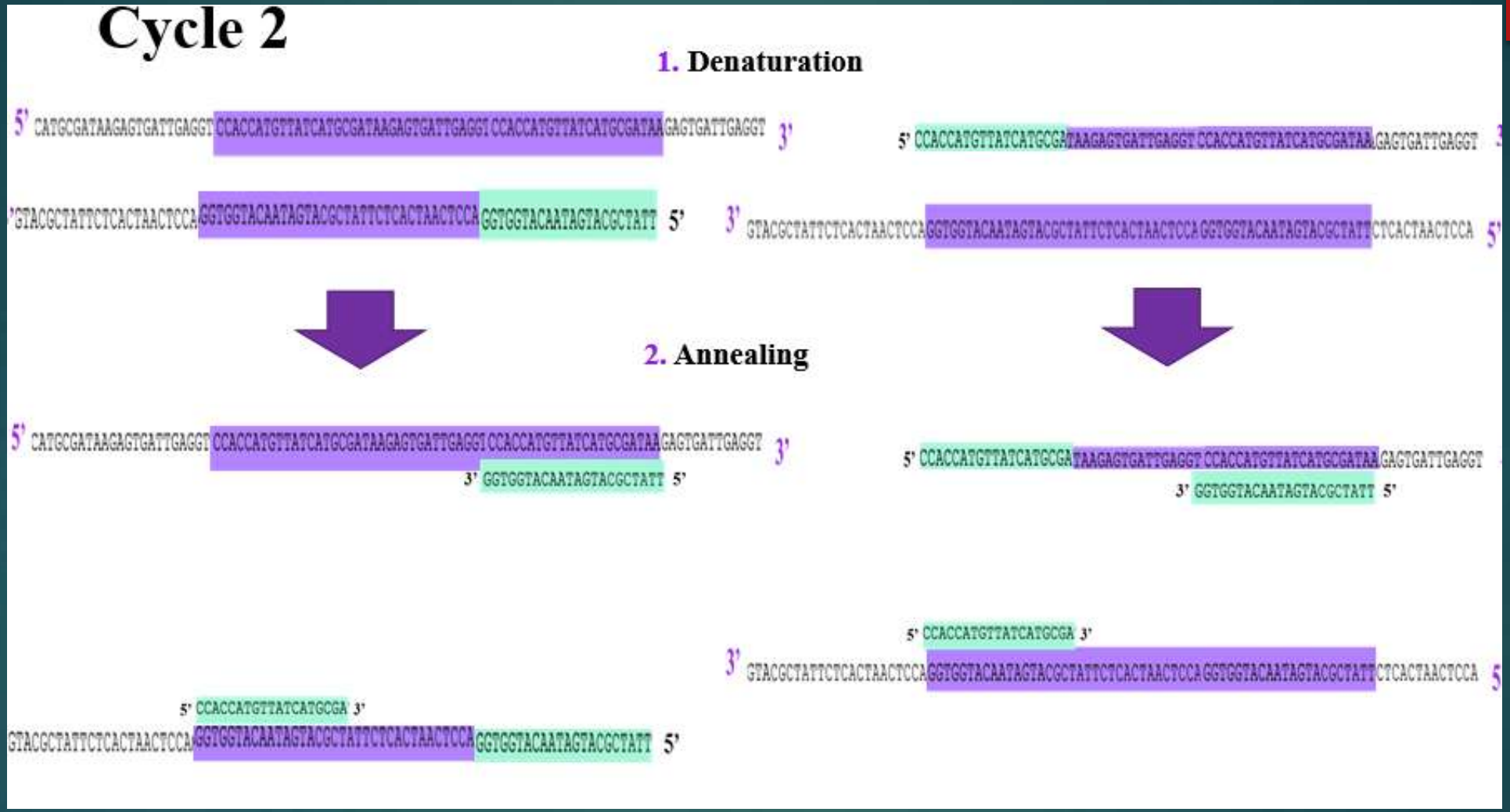
- The reaction is heated to a temperature depends on the DNA polymerase used.
- **Commonly** a temperature of 72°C is used with this enzyme.
- This means that 72°C is the optimum temperature of DNA polymerase.
- At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNAtemplate.


5’ CATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT 3’
TATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATT 5’
5’ CCACCATGTTATCATGCGA 3’ TAAGAGTGATTGAGGT
3’ GTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATT CTCACTAACTCCA 5’
Taq DNA polymerase

3. Extension :

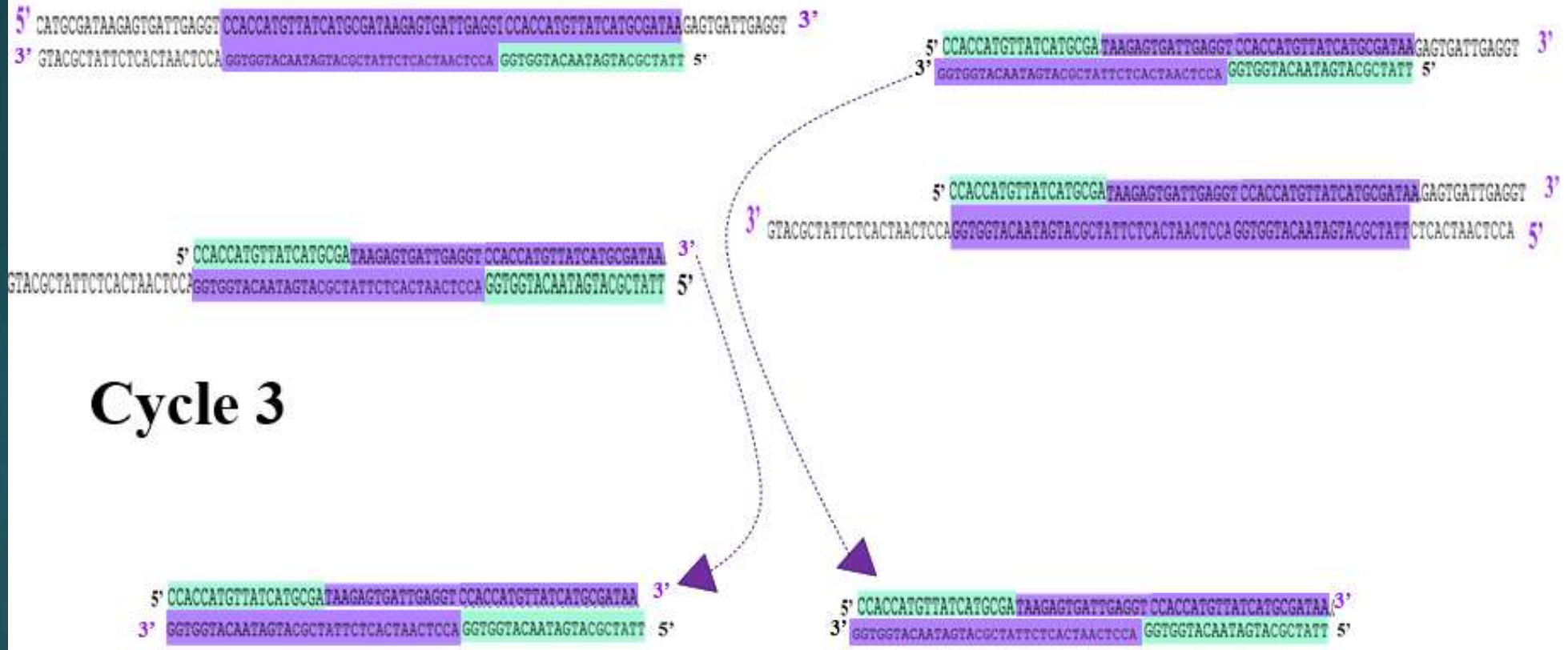
Cycle # 1:
1 DNA amplified to 2 DNA

Cycle 2

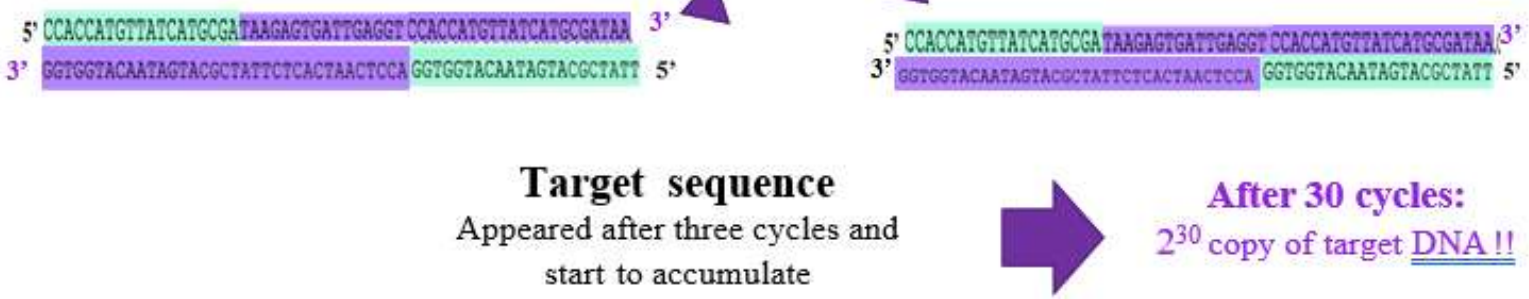

1. Denaturation
5' CATGCGATAAGAGTGATTGAGGTCCACCATGTTATCATGCGATAAGAGTGATTGAGGTCCACCATGTTATCATGCGATAAGAGTGATTGAGGT 3'
'GTACGCTATTCTCACTAACTCCAGGTGGTACAATAGTACGCTATTCTCACTAACTCCAGGTGGTACAATAGTACGCTATT 5'
3' GTACGCTATTCTCACTAACTCCAGGTGGTACAATAGTACGCTATTCTCACTAACTCCAGGTGGTACAATAGTACGCTATTCTCACTAACTCCA 5'
2. Annealing
5' CATGCGATAAGAGTGATTGAGGTCCACCATGTTATCATGCGATAAGAGTGATTGAGGTCCACCATGTTATCATGCGATAAGAGTGATTGAGGT 3'
3' GGTGGTACAATAGTACGCTATT 5'
5' CCACCATGTTATCATGCGATAAGAGTGATTGAGGTCCACCATGTTATCATGCGATAAGAGTGATTGAGGT
3' GGTGGTACAATAGTACGCTATT 5'
5' CCACCATGTTATCATGCGA 3'
GTACGCTATTCTCACTAACTCCAGGTGGTACAATAGTACGCTATTCTCACTAACTCCAGGTGGTACAATAGTACGCTATT 5'
5' CCACCATGTTATCATGCGA 3'
3' GTACGCTATTCTCACTAACTCCAGGTGGTACAATAGTACGCTATTCTCACTAACTCCAGGTGGTACAATAGTACGCTATTCTCACTAACTCCA 5

Cycle 2

3. Extension



Cycle 3

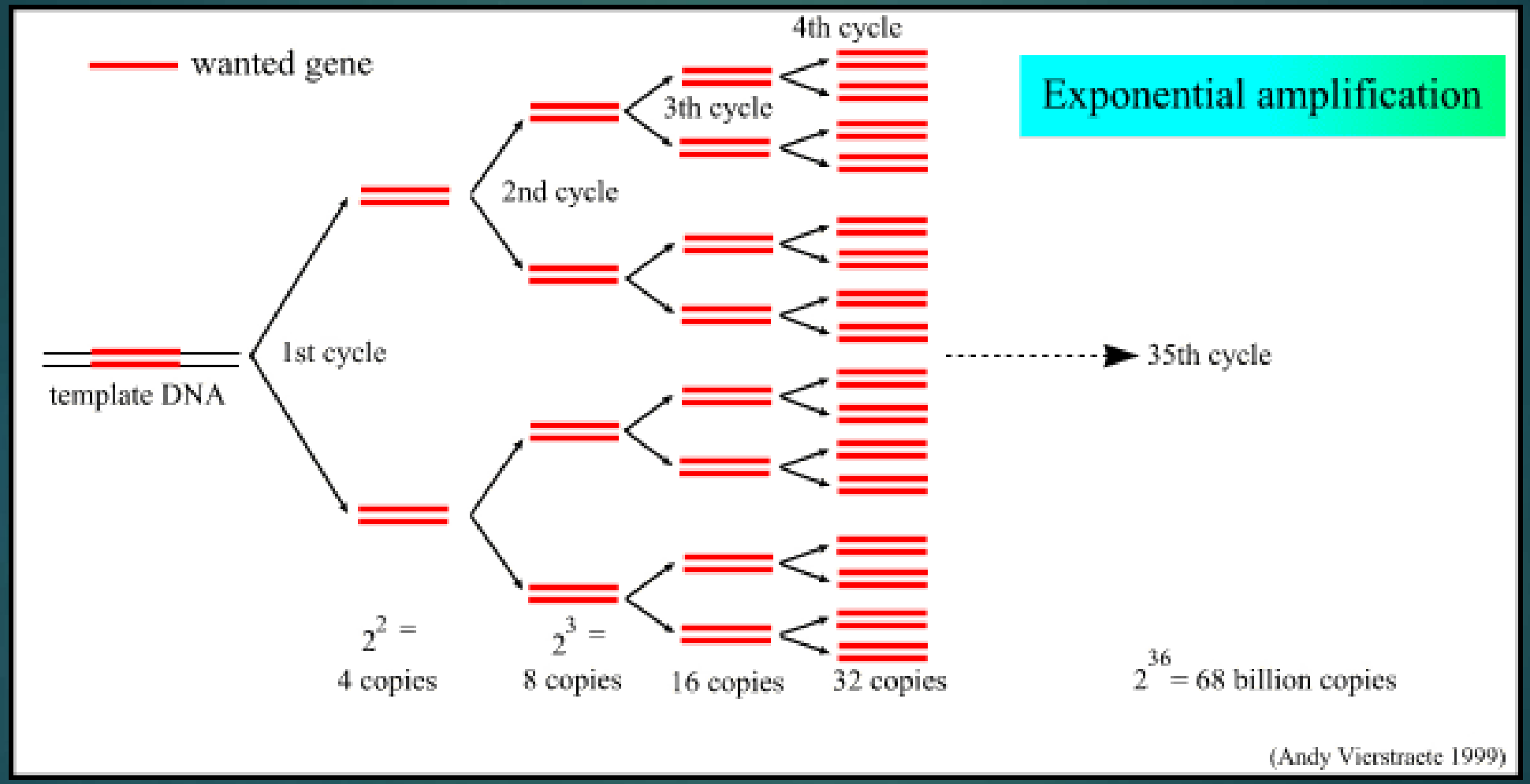


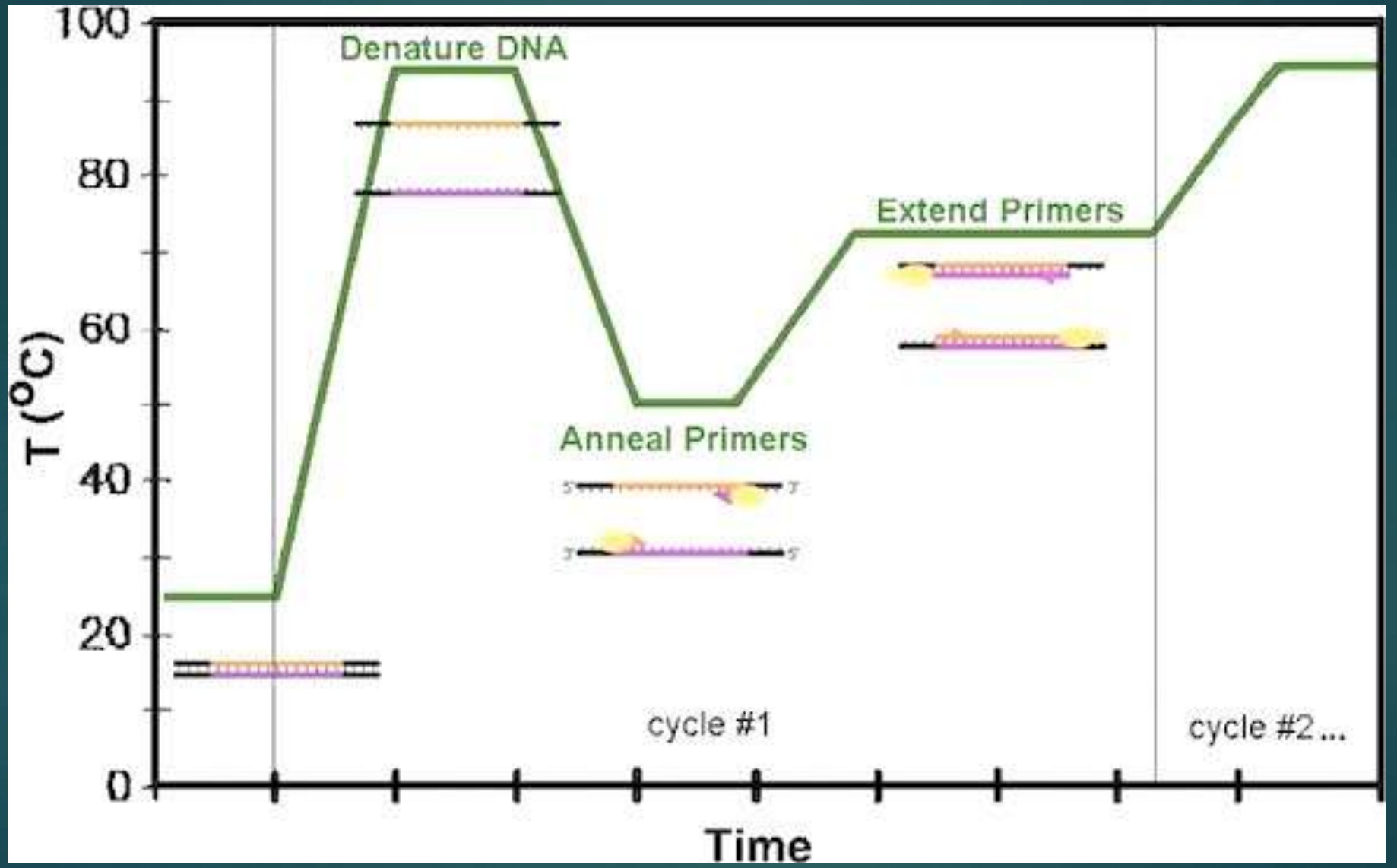
Target sequence

Appeared after three cycles and start to accumulate

After 30 cycles:

2^{30} copy of target DNA !!


wanted gene
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
Exponential amplification
35th cycle
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies
(Andy Vierstraete 1999)


100
80
60
40
20
0
T (°C)
Denature DNA
Extend Primers
Anneal Primers
cycle #1
cycle #2...
Time

Applications of PCR

Basic Research

- Mutation screening
- Drug discovery
- Classification of organisms
- Genotyping
- Molecular Archaeology
- Molecular Epidemiology
- Molecular Ecology
- Bioinformatics
- Genomic cloning
- Site-directed mutagenesis
- Gene expression studies

Applied Research

- Genetic matching
- Detection of pathogens
- Pre-natal diagnosis
- DNA fingerprinting
- Gene therapy