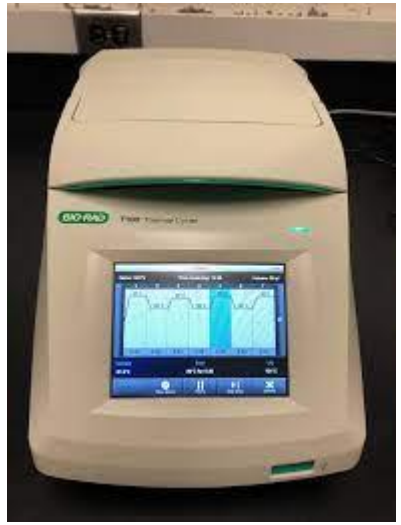
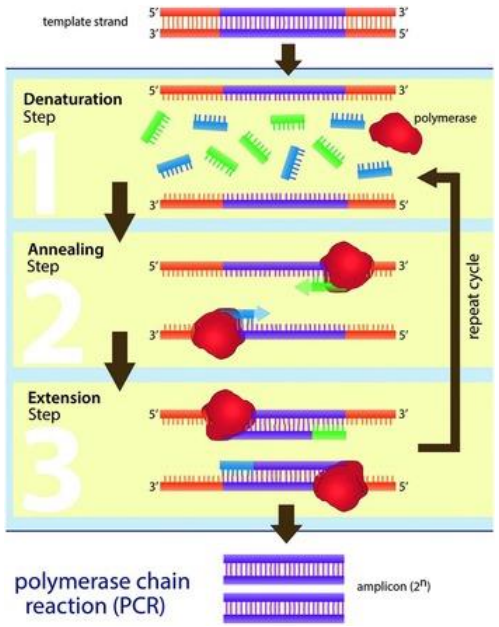




FDE 330
FOOD BIOTECHNOLOGY

Polymerase Chain Reaction
Gel Electrophoresis

Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR)

- **PCR** is based on the enzymatic amplification of a DNA fragment from oligonucleotide primers that flank the target region in duplex DNA.

- This technique in a way mimics the *in vivo* DNA replication in that the number of DNA molecules generated by the PCR doubles after each cycle.

- This method repeats a set of three steps: **denaturing the DNA**, **annealing the primers to their complementary sequences**, and **extending the annealed primers with a DNA polymerase**.

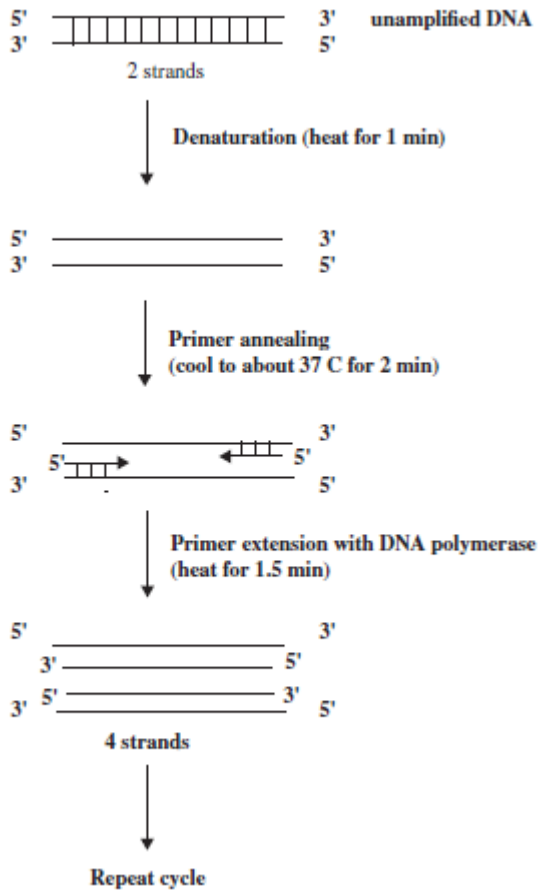
- Typically, PCR work proceeds in three steps-denaturation, annealing, and extension, referred to as a «**cycle**».

Polymerase chain reaction (PCR)

1. In denaturation, a double-stranded DNA sample (the template) is heated at nearly 100 ◦C, whereupon the two strands separate and remain in solution until the temperature is cooled (annealed) in the presence of two primers.

2. In primer annealing the separated strands are cooled in the presence of two short primers (≈20 bases each).

3. Next DNA polymerase mediates the 5′-to-3′ extension of the primer–template complex (i.e., the pairs of synthetic oligonucleotides that anneal to sites flanking the region to be amplified). The primers then initiate the synthesis of two new strands, complementary to the two primers in the presence of the DNA polymerase.



Polymerase chain reaction (PCR)

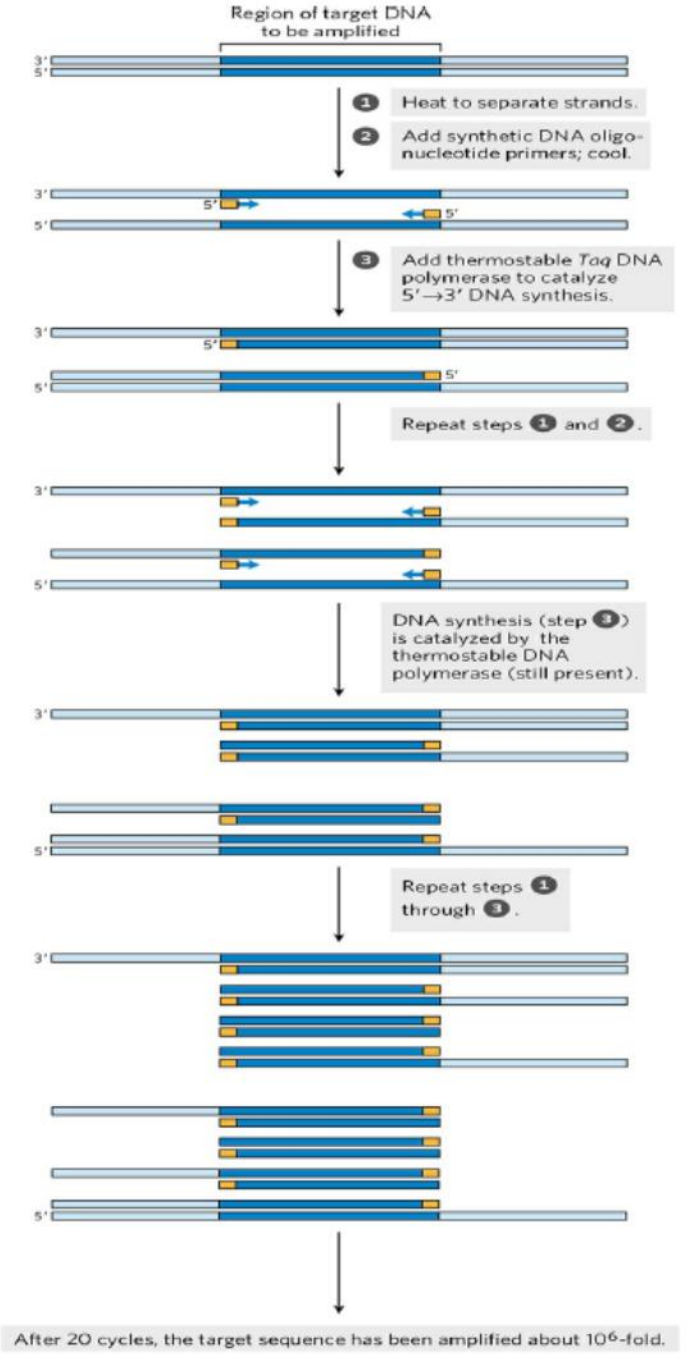
- If we know the sequence of at least the end portions of a DNA segment we are interested in, we can hugely amplify the number of copies of that DNA segment with the **polymerase chain reaction (PCR)**.

- **The polymerase chain reaction (PCR)**, now a central part of biotechnology, was developed by Kary Mullis in 1983.

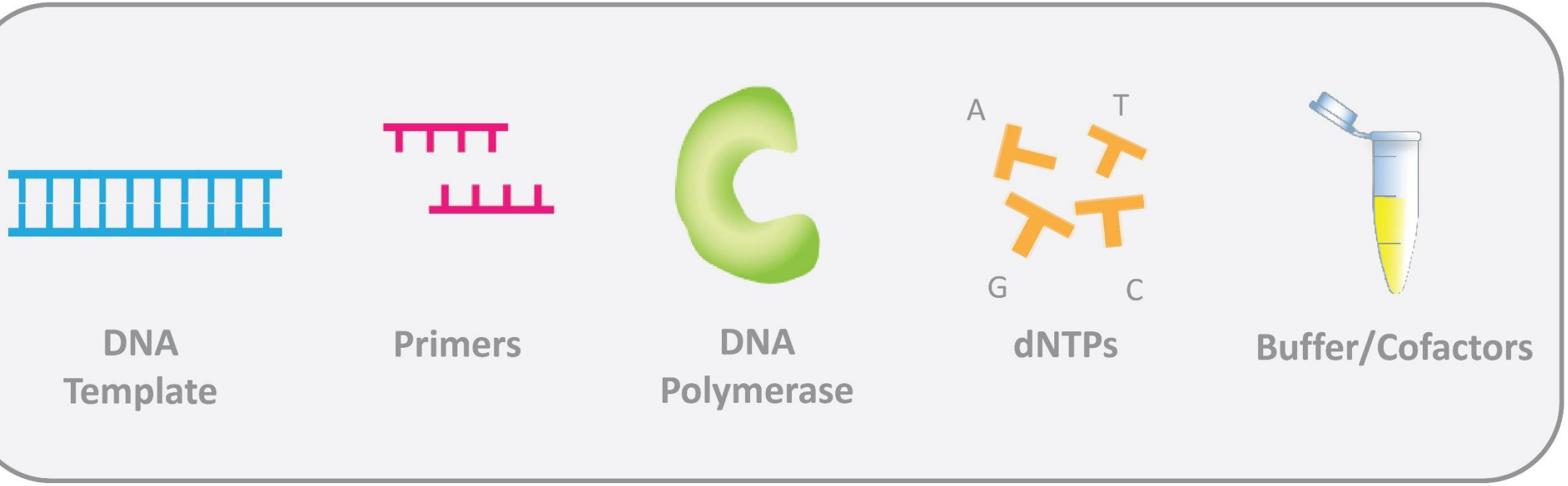
- The amplified DNA can then be used for a multitude of purposes.

Polymerase chain reaction (PCR)

- The PCR procedure relies on enzymes called **DNA polymerases**.
- These enzymes synthesize DNA strands from **deoxyribonucleotides (dNTPs)**, using **a DNA template**.
- DNA polymerases do not synthesize DNA de novo, but instead must add nucleotides to preexisting strands, referred to as **primers**.
- In PCR, two synthetic oligonucleotides are prepared for use as replication primers that can be extended by a DNA polymerase.
- These oligonucleotide primers are complementary to sequences on opposite strands of the target DNA, positioned so that their 5′ ends define the ends of the segment to be amplified, and they become part of the amplified sequence.
- The 3′ ends of the annealed primers are oriented toward each other and positioned to prime DNA synthesis across the targeted DNA segment.



PCR Components



The key ingredients of a PCR reaction are **Taq polymerase**, **primers**, **template DNA**, and **nucleotides** (DNA building blocks). The ingredients are assembled in a tube, along with **cofactors** needed by the enzyme, and are put through repeated cycles of heating and cooling that allow DNA to be synthesized.

PCR Components

Basic PCR requires four components:

1. a DNA sample containing segment to be amplified (**DNA template**)
2. the pair of synthetic oligonucleotide primers (**PCR primers/Oligonucleotide primers**)
3. a DNA polymerase (**Taq polymerase**)
4. a pool of deoxynucleoside triphoshates (**dNTPs**)

* What are Forward and Reverse Primers?

- Forward and reverse primers are the two types of primers used in the PCR to amplify a specific part of a DNA strand.
- Both are oligonucleotides used for the initiation of PCR.
- Also, their length varies between 18 to 25 base pairs.
- Additionally, they run in the 5′ to 3′ direction from left to the right.
- Besides, they are complementary DNA, which anneals to the single-stranded DNA during the annealing step.
- Moreover, their annealing occurs at higher temperatures and their melting temperatures (Tm) should be within 55°C and 65°C.
- Importantly, the maximum difference between the melting temperatures of both primers should be 5°C.
- Also, their GC content should be between 40 and 60%, with the 3′ of a primer ending in C or G to promote binding.
- They should not contain regions forming secondary structures.
- Furthermore, they should avoid self-dimers/hairpins and primer-dimer formation.
- The **main difference** between forward and reverse primers is that **forward primers anneal to the antisense strand of the double-stranded DNA, which runs from 3′ to 5′ direction, whereas reverse primers anneal to the sense strand of the double-stranded DNA, which runs from 5′ to 3′ direction**.
- Furthermore, 5′ primers refer to forward primers, while 3′ primers refer to reverse primers.
- Forward primers refer to the PCR primers, which are complementary to the antisense strand of double-stranded DNA, while reverse primers refer to the PCR primers, which are complementary to the sense strand of the double-stranded DNA. Thus, this is the main difference between forward and reverse primers.
- Also Known as 5′ primers refer to forward primers, while 3′ primers refer to reverse primers.

The three main steps involved in a PCR

Denaturation

- Melting DNA duplex into two single strands by heating to 94-95°C.

Annealing

- The binding of the forward and reverse primers to the complementary sequences on the template. The temperature of this step depends on the melting temperature of the primer combination.

Extension

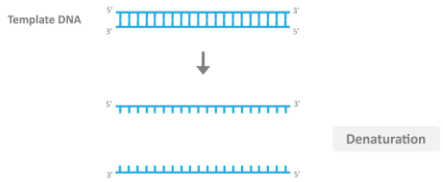
- DNA polymerase enzyme extends each of the primers at their 3'end by adding complementary bases to the growing strand. The optimum temperature of *Taq* polymerase, 72 °C is used as the temperature in the extension step. The time of the extension depends on the number of base pairs in the template strand.

➢ Generally, the three steps are repeated for 30-40 times during the PCR to obtain an exponential growth of the DNA fragment of interest.

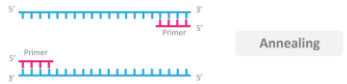
PCR Process-The steps of PCR

- Basic PCR can be split into three general stages:

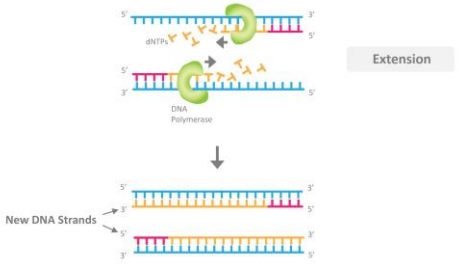
1. **Denaturation**

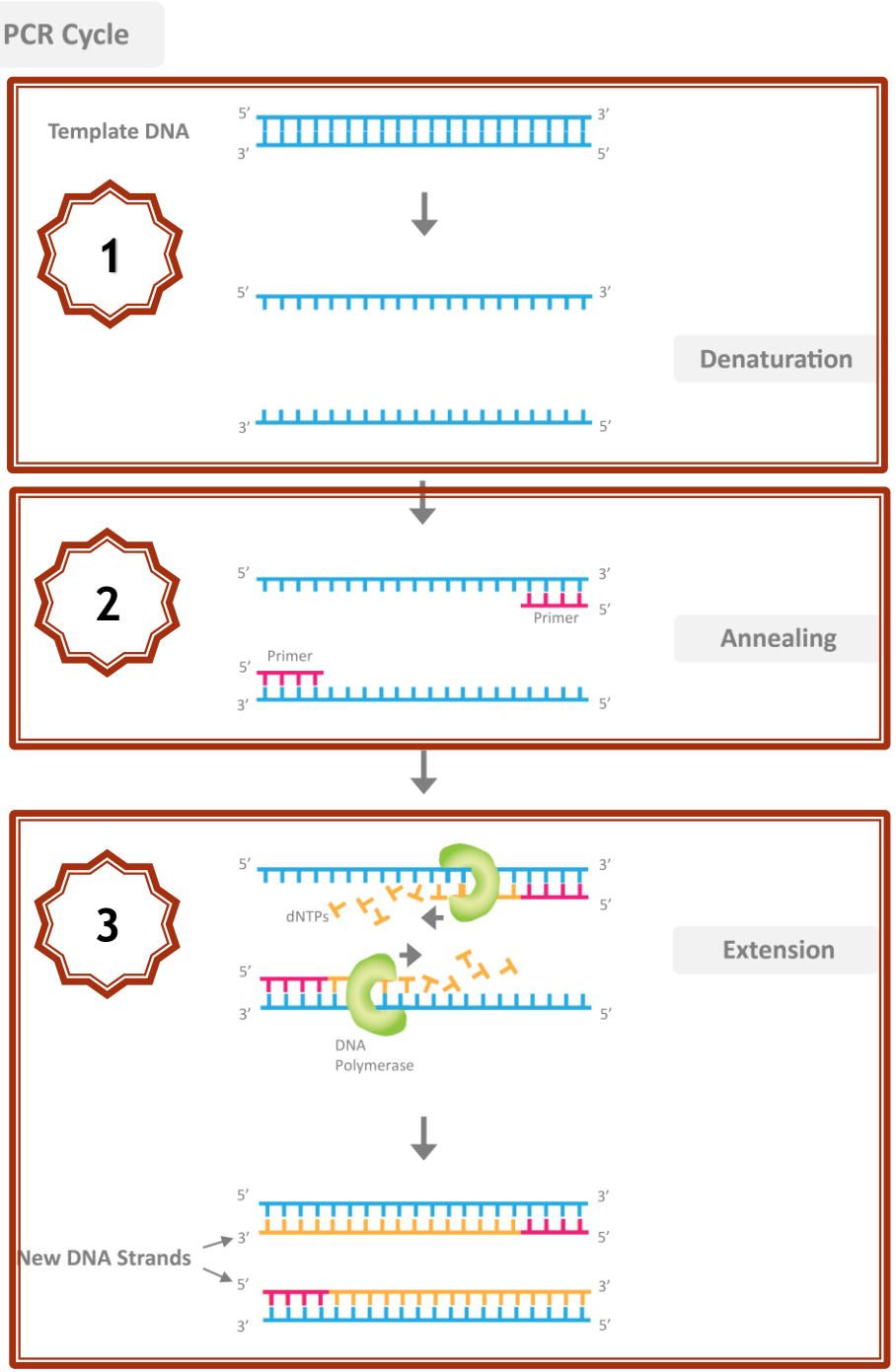


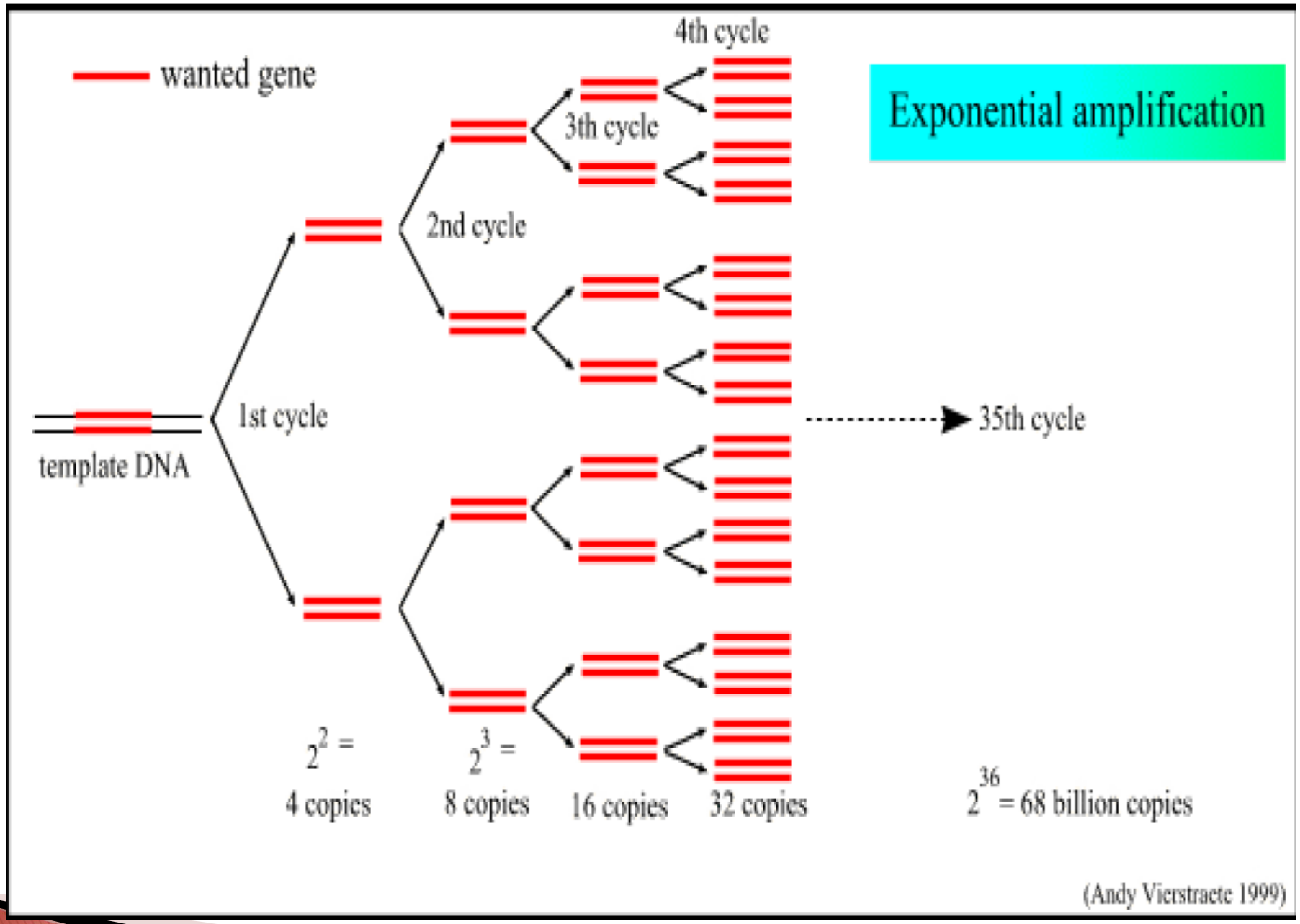
2. **Annealing**



3. **Extension**






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

Applications of PCR

- Cloning of gene or gene fragments
- Genetic diagnosis-Detection mutations
- Monitoring the continuity of mutant genes
- DNA Sequence Analysis
- Calculation of polymorphism between species
- Evolution studies (in fossil samples)
- Determination of seed purity
- Maternity-paternity determination
- Identification in forensic medicine
- Determination of hereditary diseases before birth
- Determination of GMO
- Identification of microorganisms
- Detection of pathogens

Applications of PCR

- Using PCR, a DNA sequence can be amplified millions or billions of times, producing enough DNA copies to be analyzed using other techniques. For instance, the DNA may be visualized by gel electrophoresis, sent for sequencing, or digested with restriction enzymes and cloned into a plasmid.

- PCR is used in many research labs, and it also has practical applications in forensics, genetic testing, and diagnostics. For instance, PCR is used to amplify genes associated with genetic disorders from the DNA of patients (or from fetal DNA, in the case of prenatal testing). PCR can also be used to test for a bacterium or DNA virus in a patient's body: if the pathogen is present, it may be possible to amplify regions of its DNA from a blood or tissue sample.

- **In food analysis, PCR techniques are used for;**
  - **detection of genetically modified organisms (GMO) in different matrices,**
  - **identification of different animal species in meat and dairy products,**
  - **the detection of food infection with food-borne pathogens and toxicogenic fungi.**

What is the Difference Between PCR and DNA Replication

- The **main difference** between PCR and DNA replication is that **PCR is an *in vitro* process which synthesizes DNA, while DNA replication is the *in vivo* process of DNA synthesis.**

- PCR and DNA replication are two processes responsible for DNA synthesis. The enzyme responsible for DNA synthesis in PCR is a thermophilic DNA polymerase such as *Taq* polymerase while the enzyme responsible for DNA replication is DNA polymerase. Moreover, PCR uses DNA primers while DNA replication uses RNA primers synthesized by RNA primase.