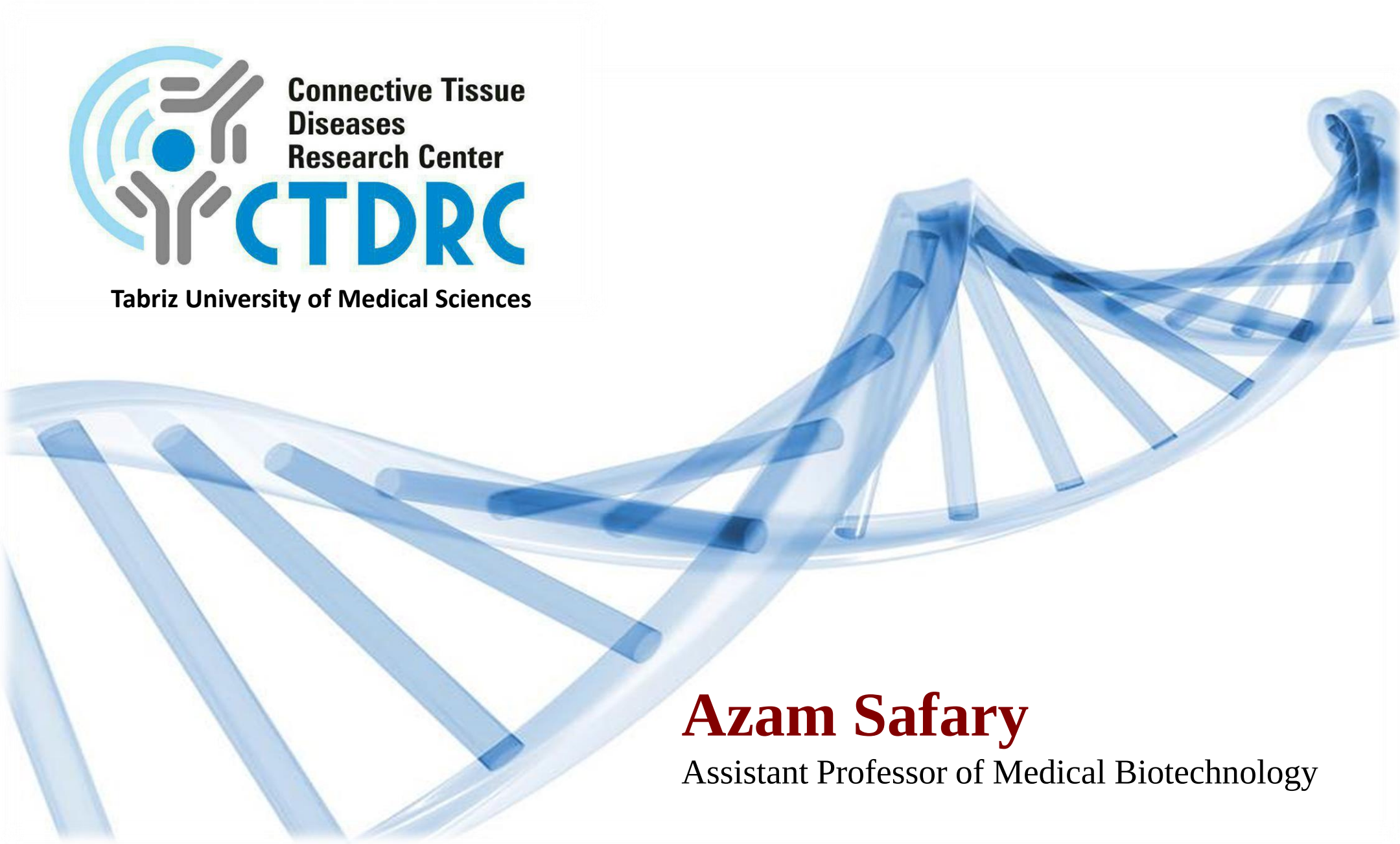
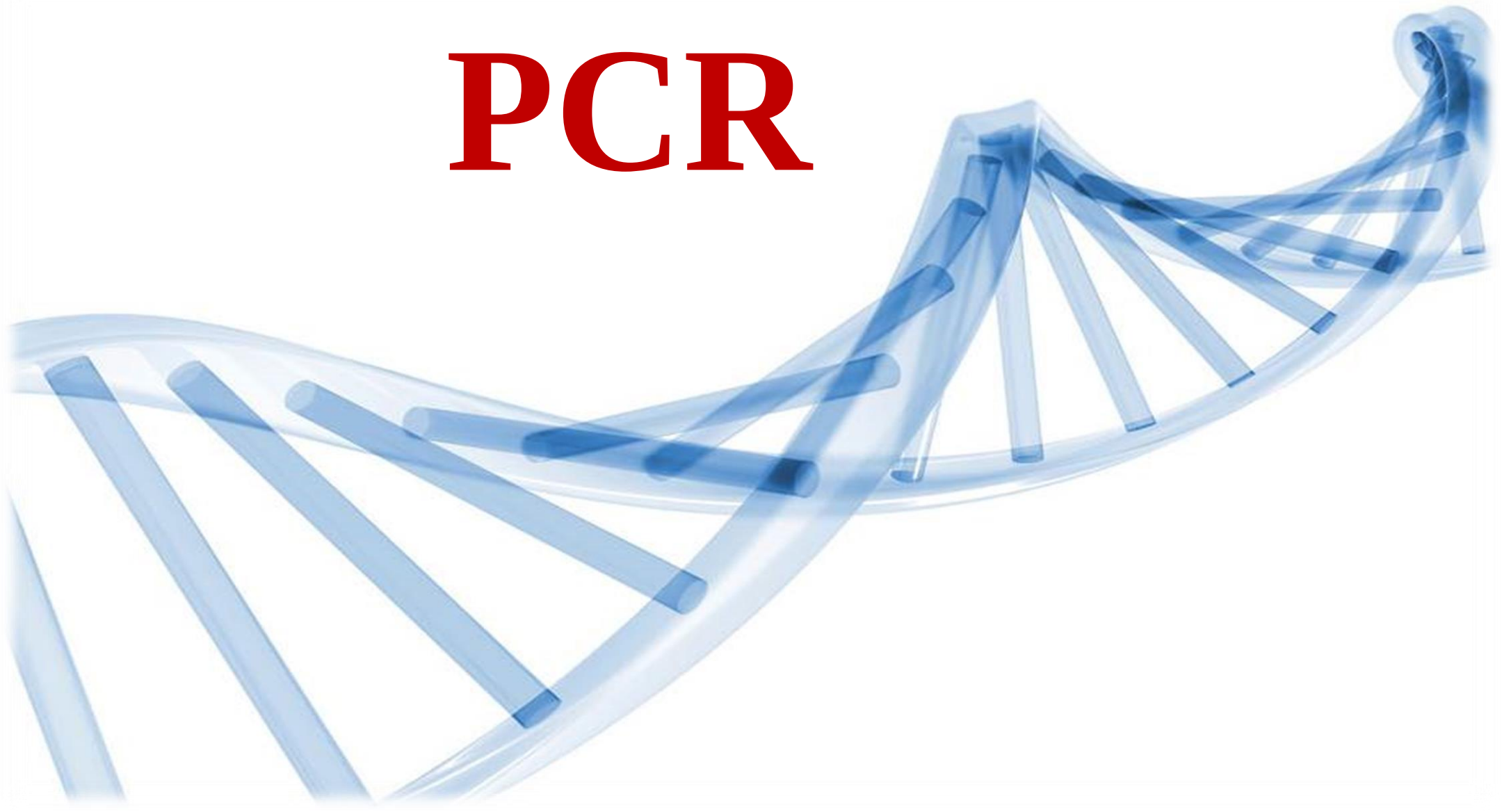


Azam Safary

Assistant Professor of Medical Biotechnology

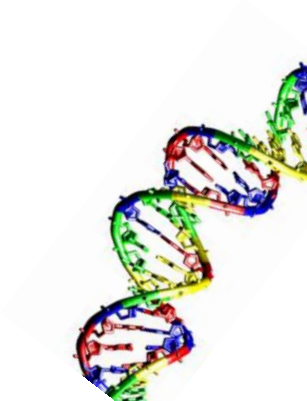
Polymerase Chain Reaction

PCR

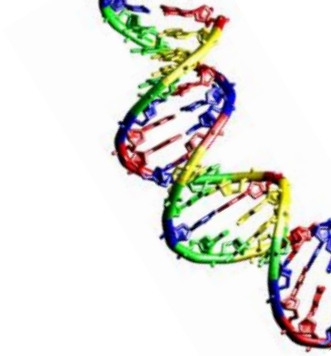


Content

1. What is the PCR?
2. Principle of PCR
3. PCR Program
4. PCR Reaction Components
5. Application of PCR
6. PCR Types



What is the PCR?



- The **P**olymerase **C**hain **R**eaction (PCR) is an exponentially progressing synthesis of the defined target DNA sequences *in vitro*.

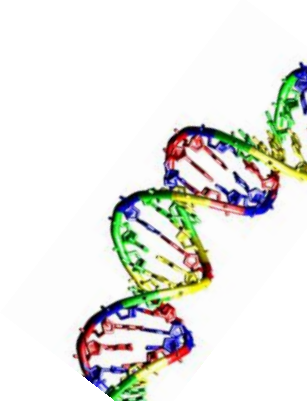
- It was invented in 1983 by Dr. Kary Mullis, for which he received the Nobel Prize in Chemistry in 1993.

What is the PCR?

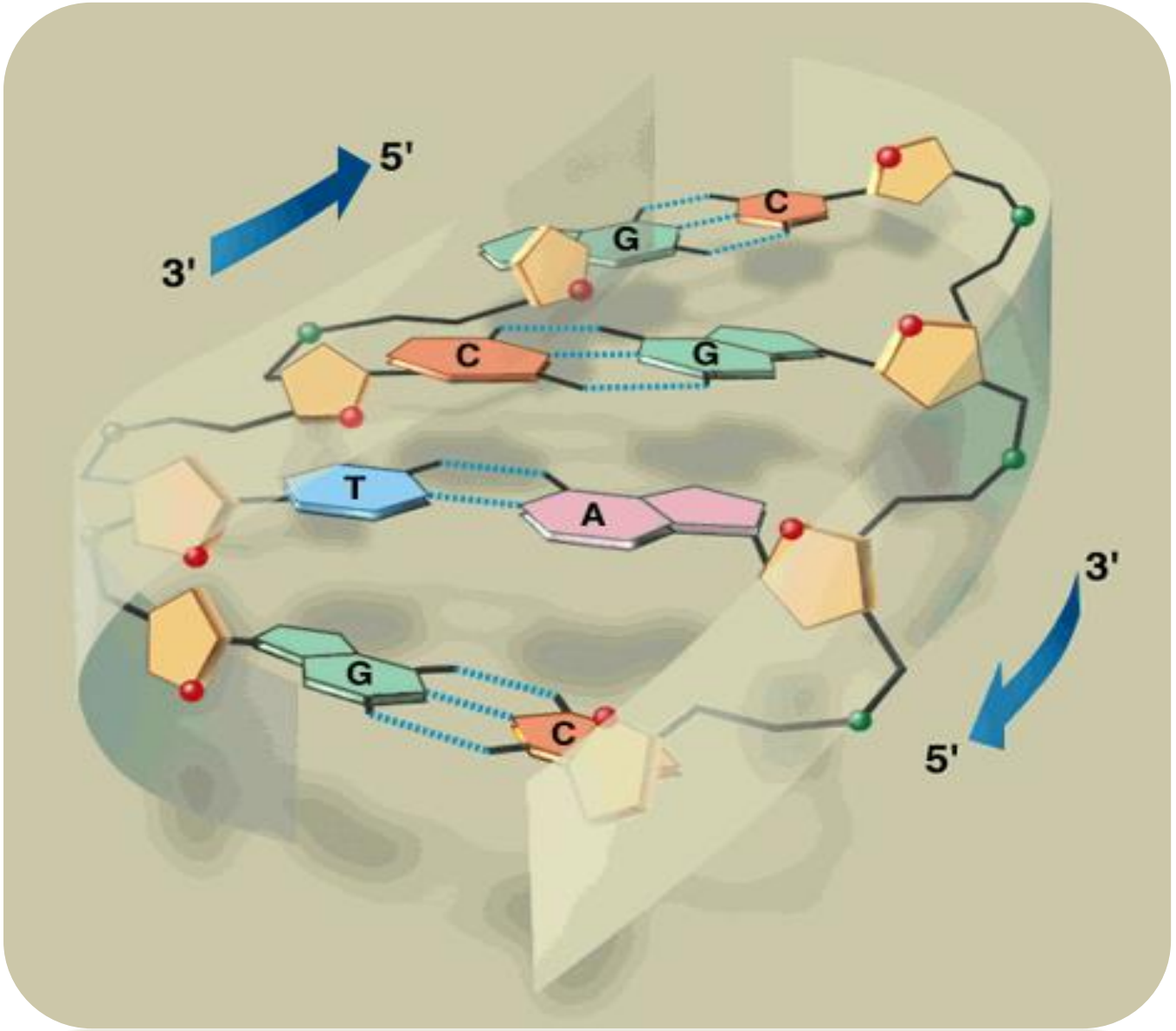
Why “Polymerase” and “chain”?

- It is called “polymerase” because the only enzyme used in this reaction is DNA polymerase.

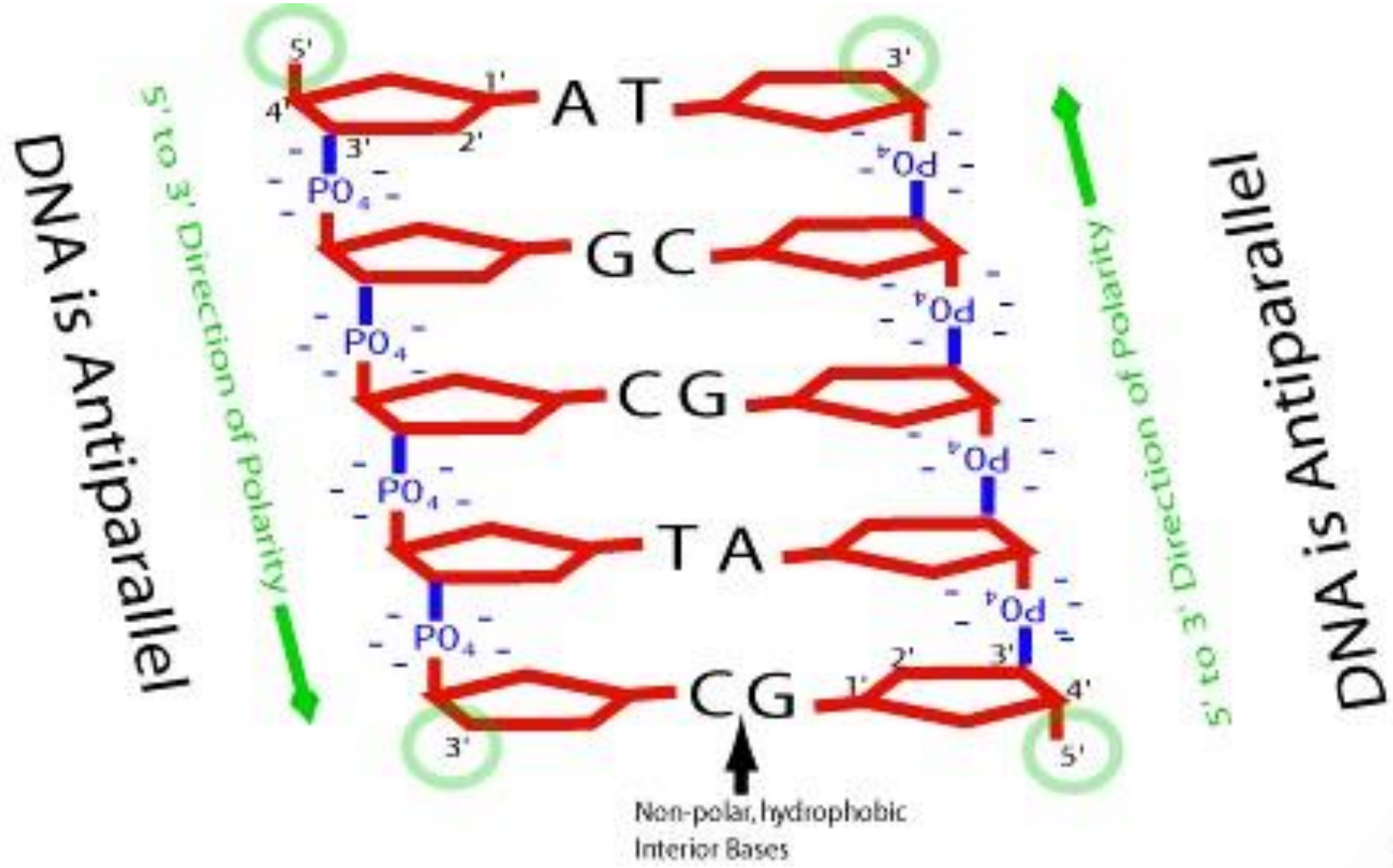
- It is called “chain” because the products of the first reaction become substrates of the following one, and so on.



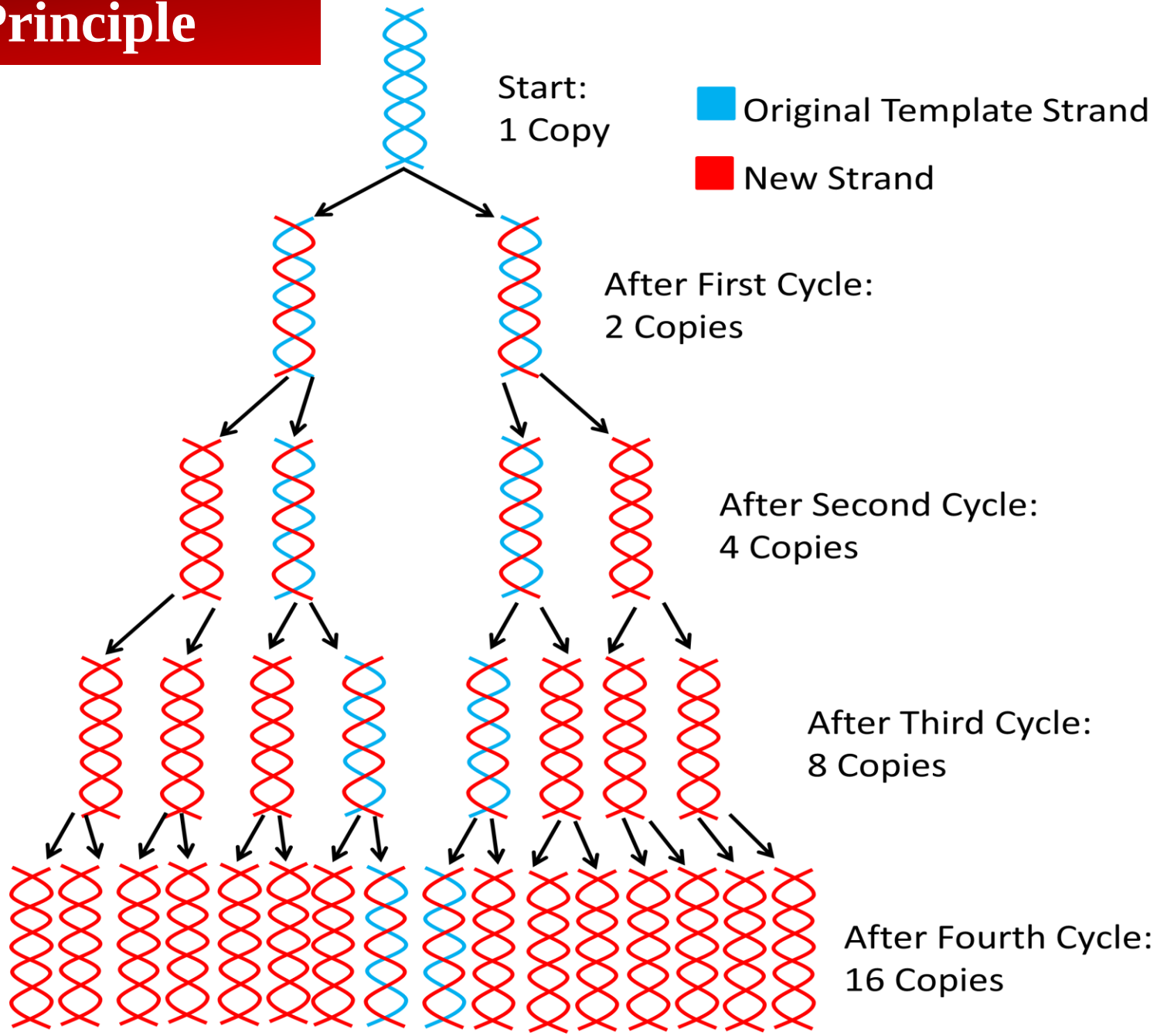
DNA structure



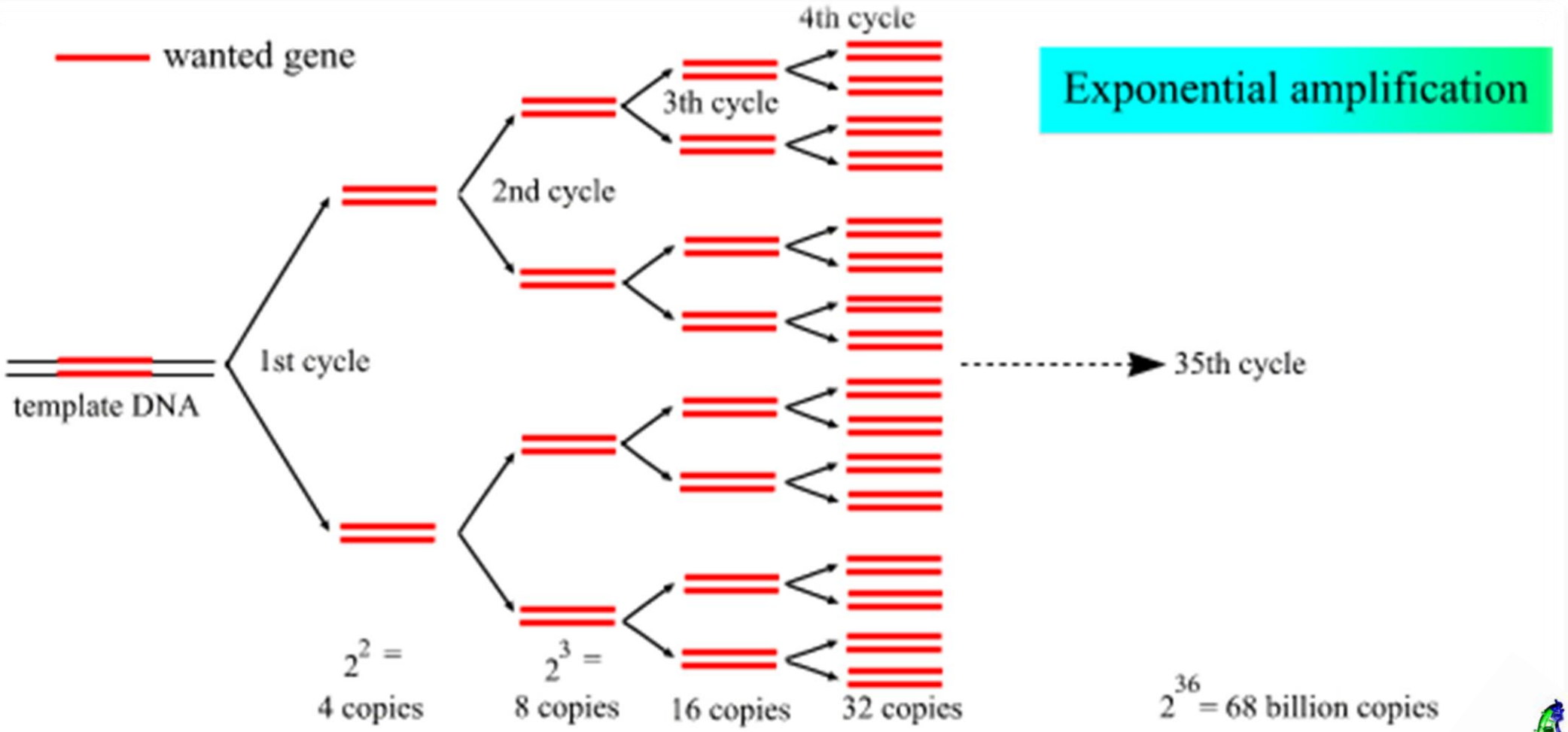
DNA structure



PCR Principle

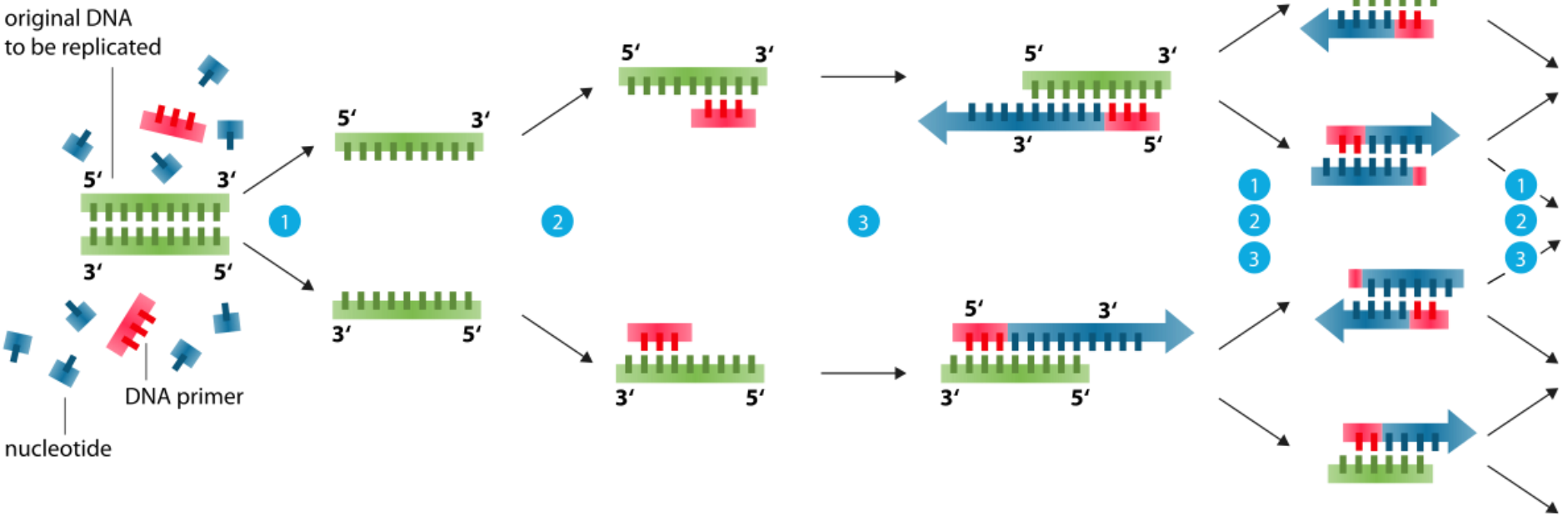


PCR Principle



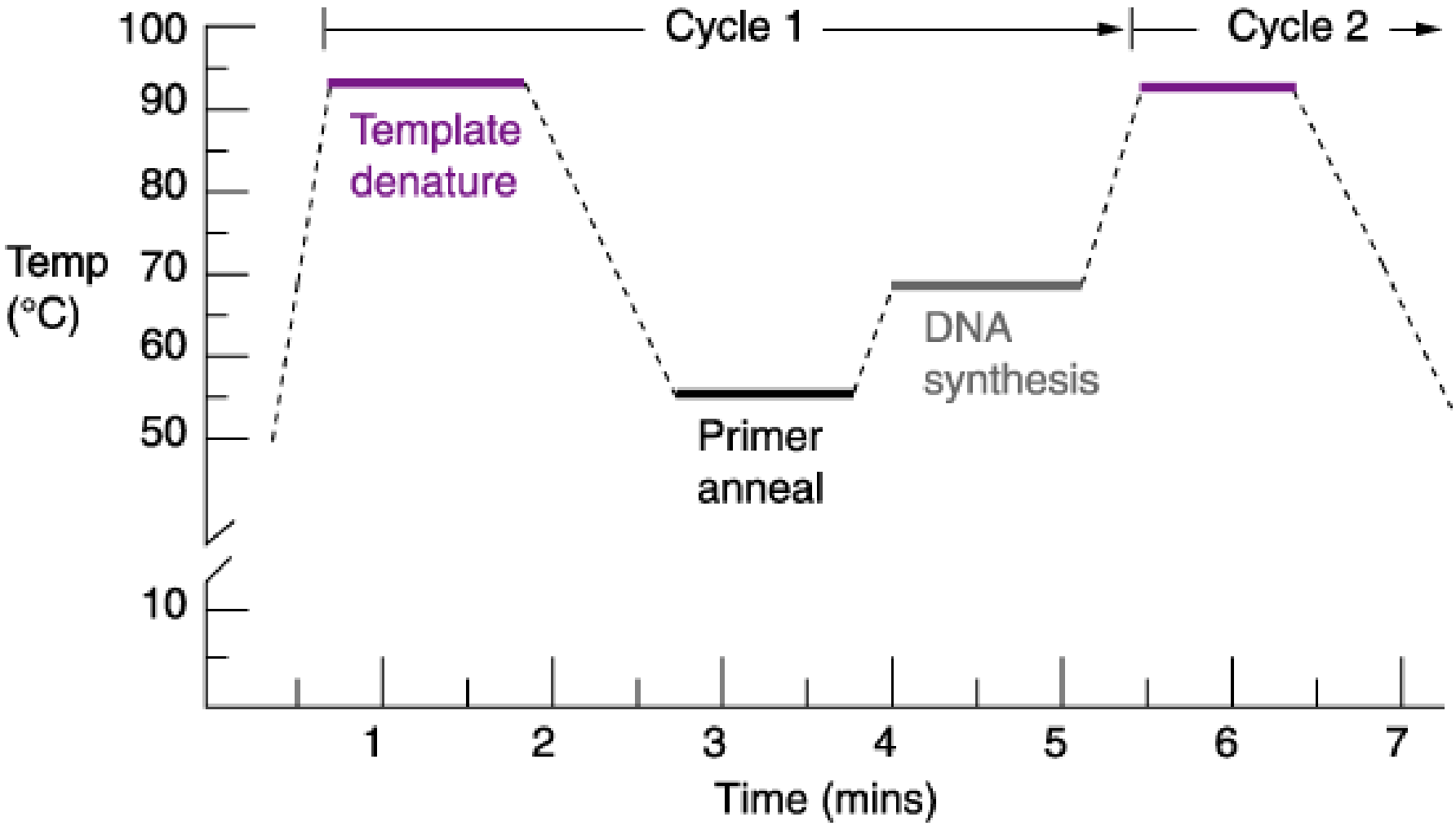
PCR Principle

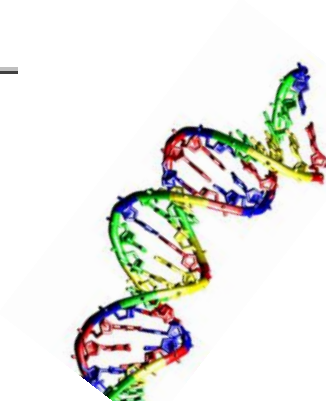
Polymerase chain reaction - PCR



1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

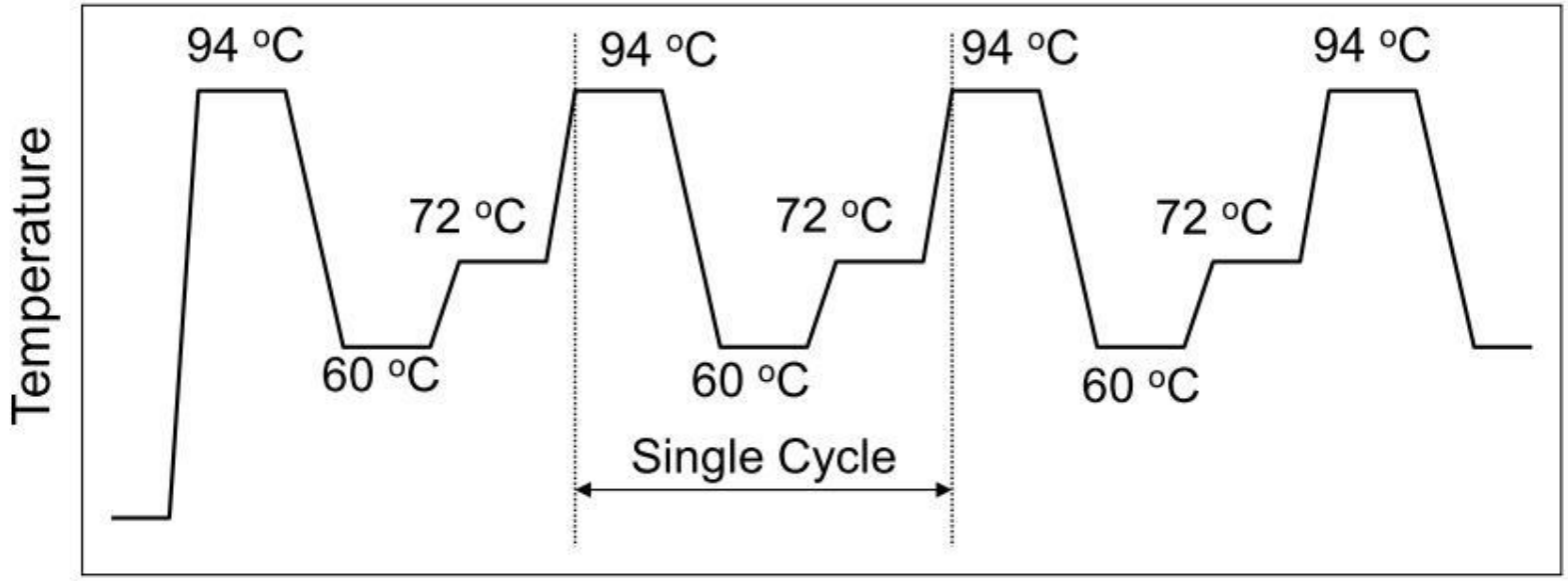
PCR Program


Cycle 1
Cycle 2
100
90
80
70
60
50
10
Temp (°C)
Template denature
Primer anneal
DNA synthesis
1
2
3
4
5
6
7
Time (mins)



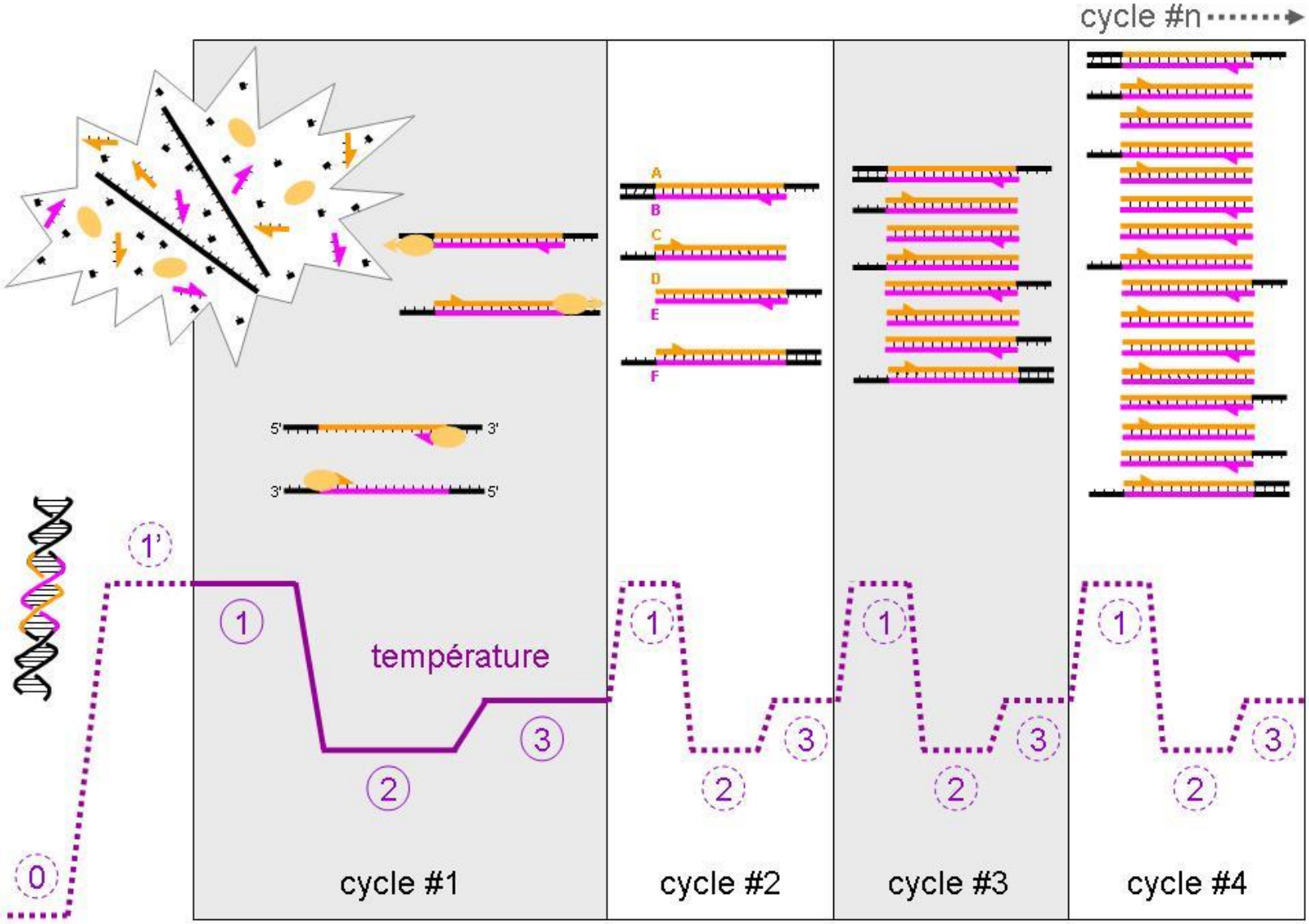
PCR Program

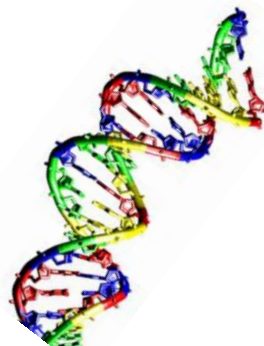
Thermal cycling temperature profile for PCR



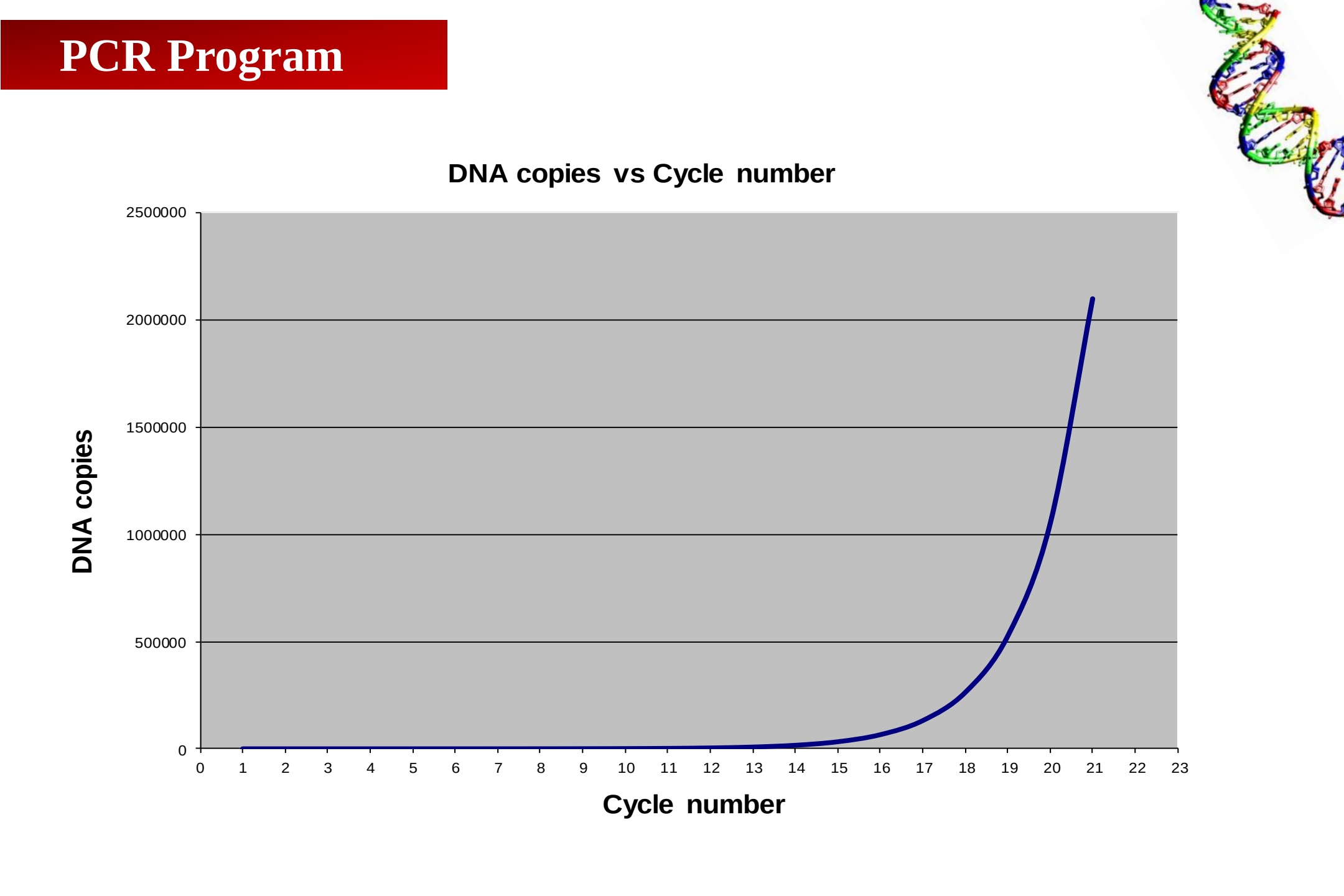
Typically 25-35 cycles performed during PCR

PCR Program


cycle #n
A
B
C
D
E
F
5'
3'
3'
5'
1'
1
2
3
0
température
cycle #1
cycle #2
cycle #3
cycle #4



PCR Program


DNA copies vs Cycle number
DNA copies
2500000
2000000
1500000
1000000
500000
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Cycle number

PCR- Before Thermocycler

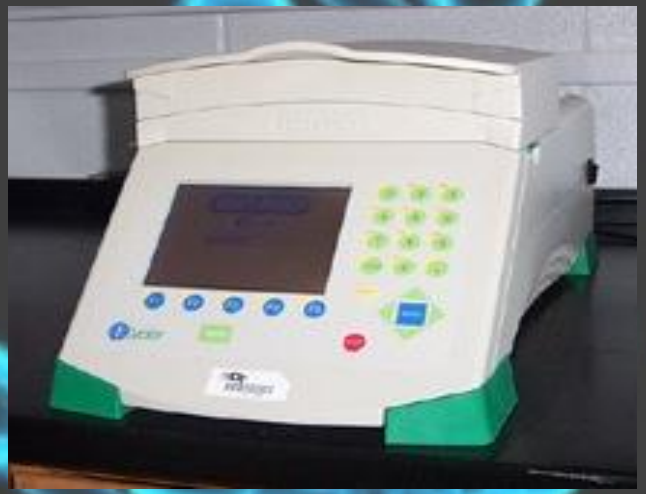
95° C
5 min

55° C
3 min

72° C
5 min

35 times

8 BORING hours per PCR!

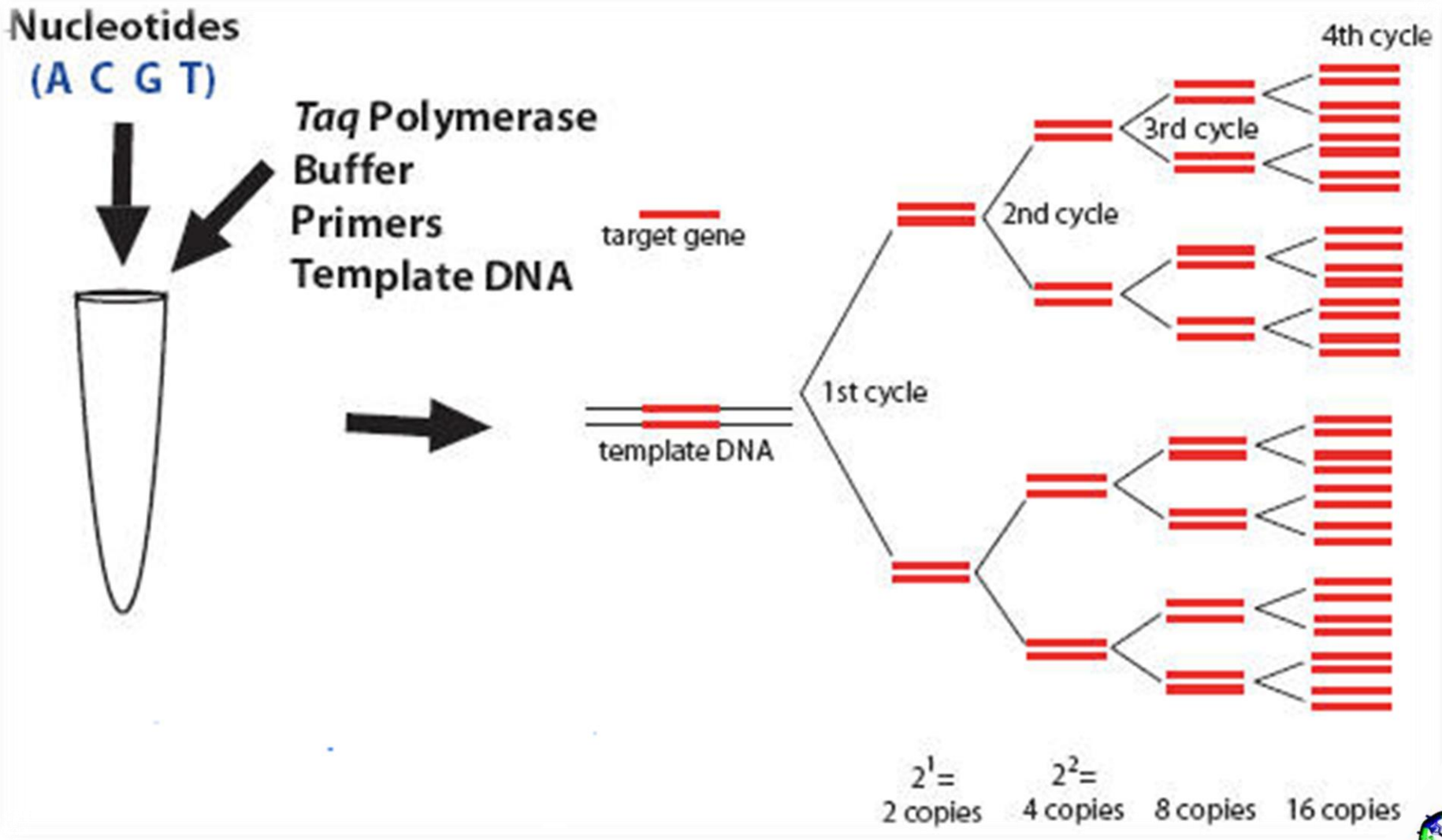


- Heated lids
- Adjustable ramping times
- Single/multiple blocks
- Gradient thermocycler blocks

- ↑volume
- ↑cost
- Evaporation
- heat transfer

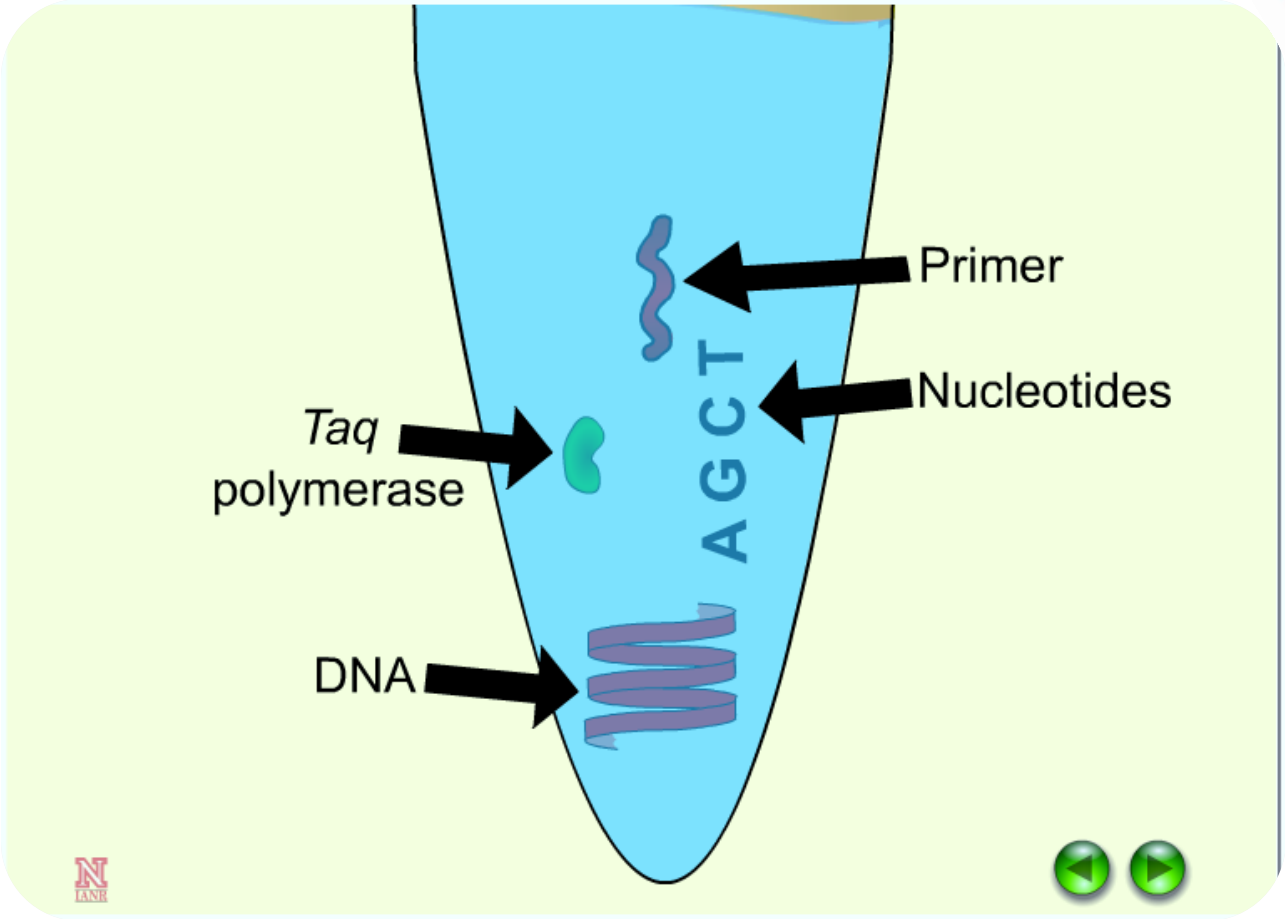
- Thin walled tube
- ↓volume
- ↓cost
- ↓evaporation & heat transfer

PCR Reaction Components

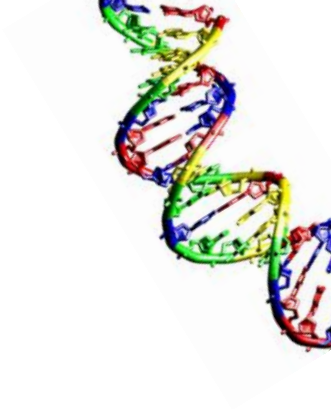


PCR Reaction Components

- Water
- Buffer
- DNA template
- Primers
- Nucleotides
- Mg^{++} ions
- DNA Polymerase



PCR Reaction Components



1) Target DNA: contains the sequence to be amplified.

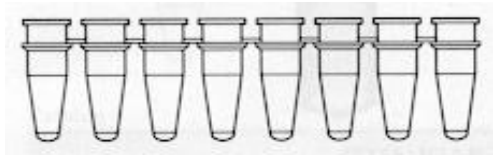
2) Pair of Primers: oligonucleotides that define the sequence to be amplified.

3) dNTPs: deoxy.nucleotide.triphosphates: DNA building blocks.

4) Thermostable DNA Polymerase: enzyme that catalyses the reaction.

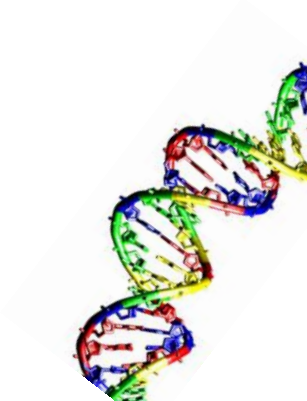
5) Mg^{++} ions: cofactor of the enzyme.

6) Buffer solution: maintains pH and ionic strength of the reaction solution suitable for the activity of the enzyme.



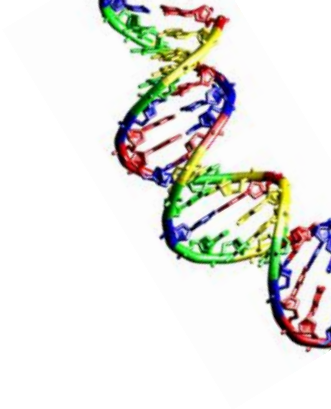
Applications of PCR

- Classification of organisms
- Genotyping
- Mutagenesis
- Mutation detection
- Sequencing
- Detection of pathogens
- Pre-natal diagnosis
- Drug discovery
- Genetic matching
- Genetic engineering



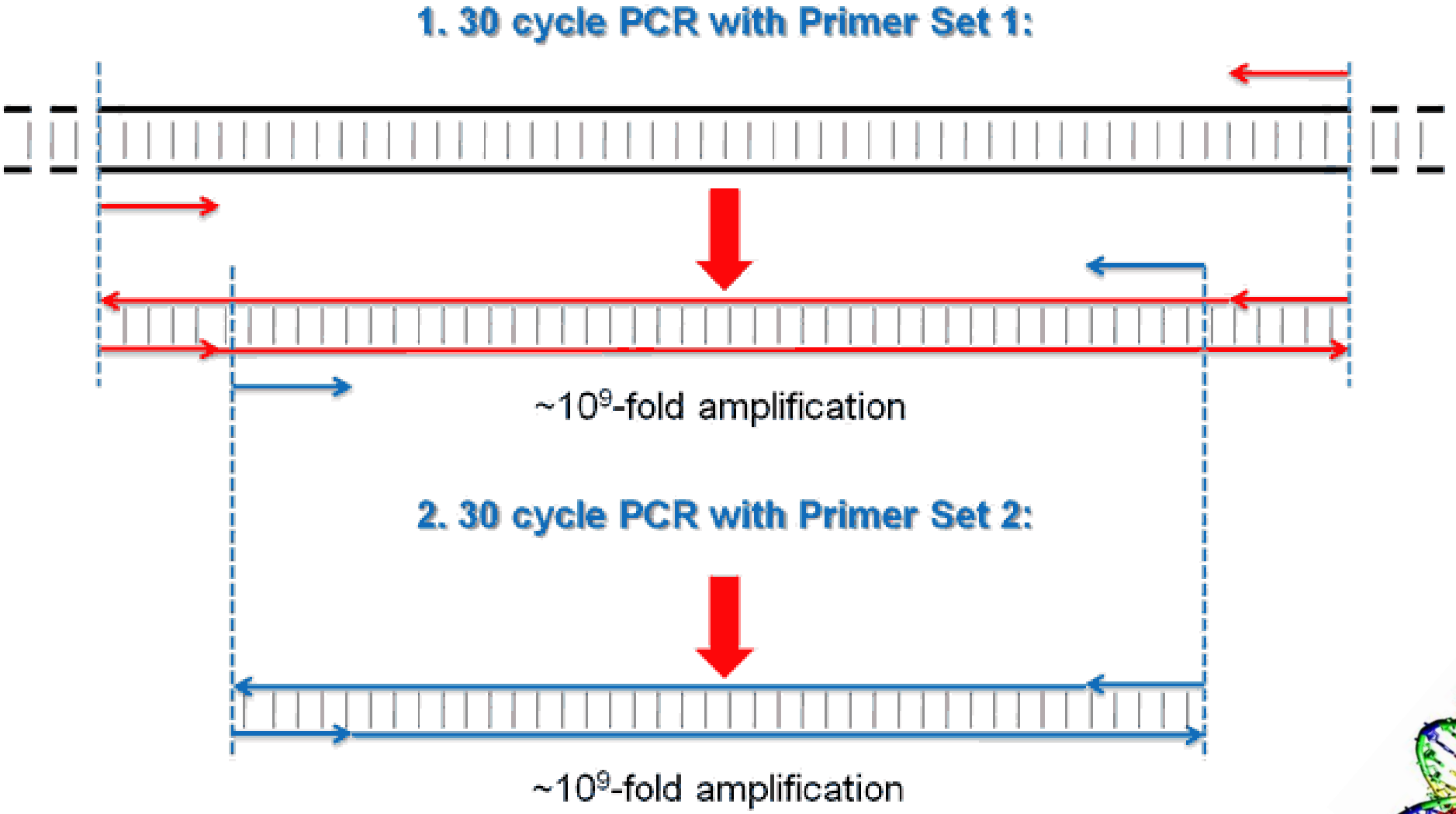
PCR Types

Nested PCR



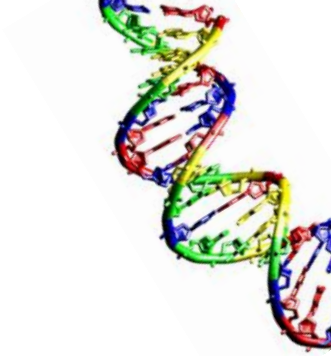
- Increases the specificity of DNA amplification, by **reducing background** due to non-specific amplification of DNA.
- Two sets of primers are being used in two successive PCR reactions. In the first reaction, one pair of primers is used to generate DNA products, which besides the intended target, may still consist of non-specifically amplified DNA fragments.
- The products are then used in a second PCR reaction with a set of primers whose binding sites are completely or partially different from and located 3' of each of the primers used in the first reaction.
- Nested PCR is often more successful in specifically amplifying long DNA fragments than conventional PCR, but it requires more detailed knowledge of the target sequences.

Nested Primer PCR:



PCR Types

RT-PCR

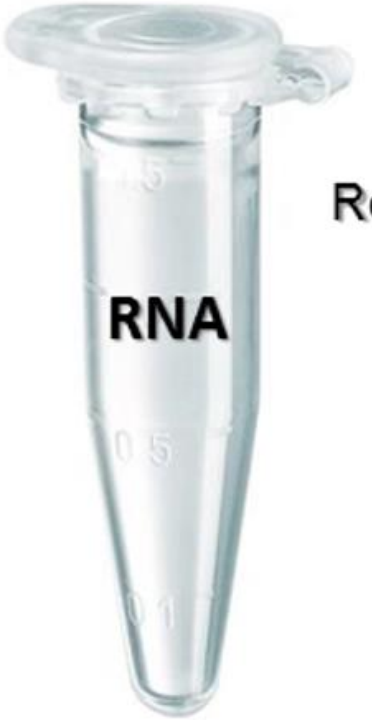


- RT-PCR (**R**everse **T**ranscription **P**CR) is a method used to amplify, isolate or identify a known sequence from a cellular or tissue **RNA**.

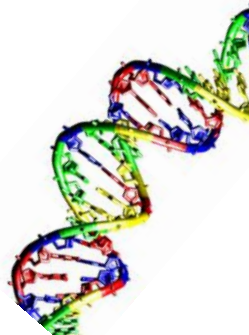
- The PCR is preceded by a reaction using reverse transcriptase to convert RNA to cDNA. RT-PCR is widely used in expression profiling to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start and termination sites and, if the genomic DNA sequence of a gene is known, to map the location of exons and introns in the gene.

RT-PCR

Extract RNA

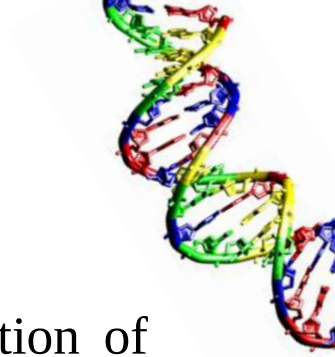


Reverse Transcription



PCR Types

Multiplex-PCR

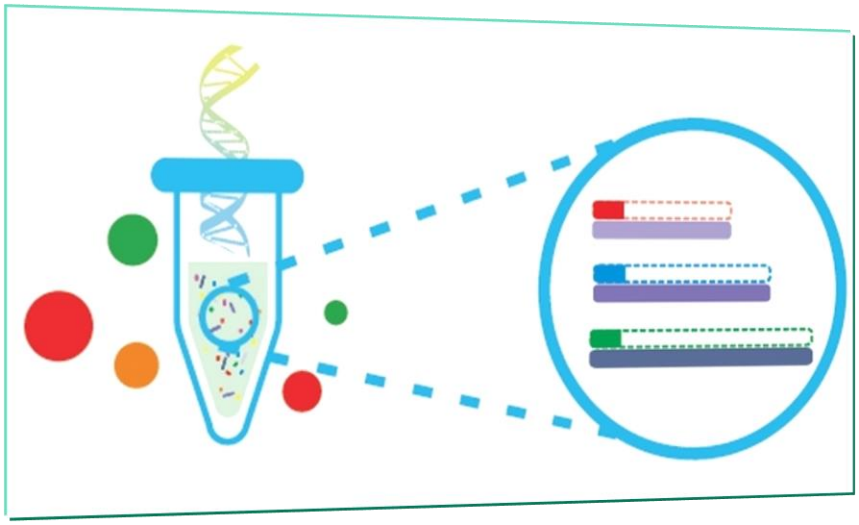


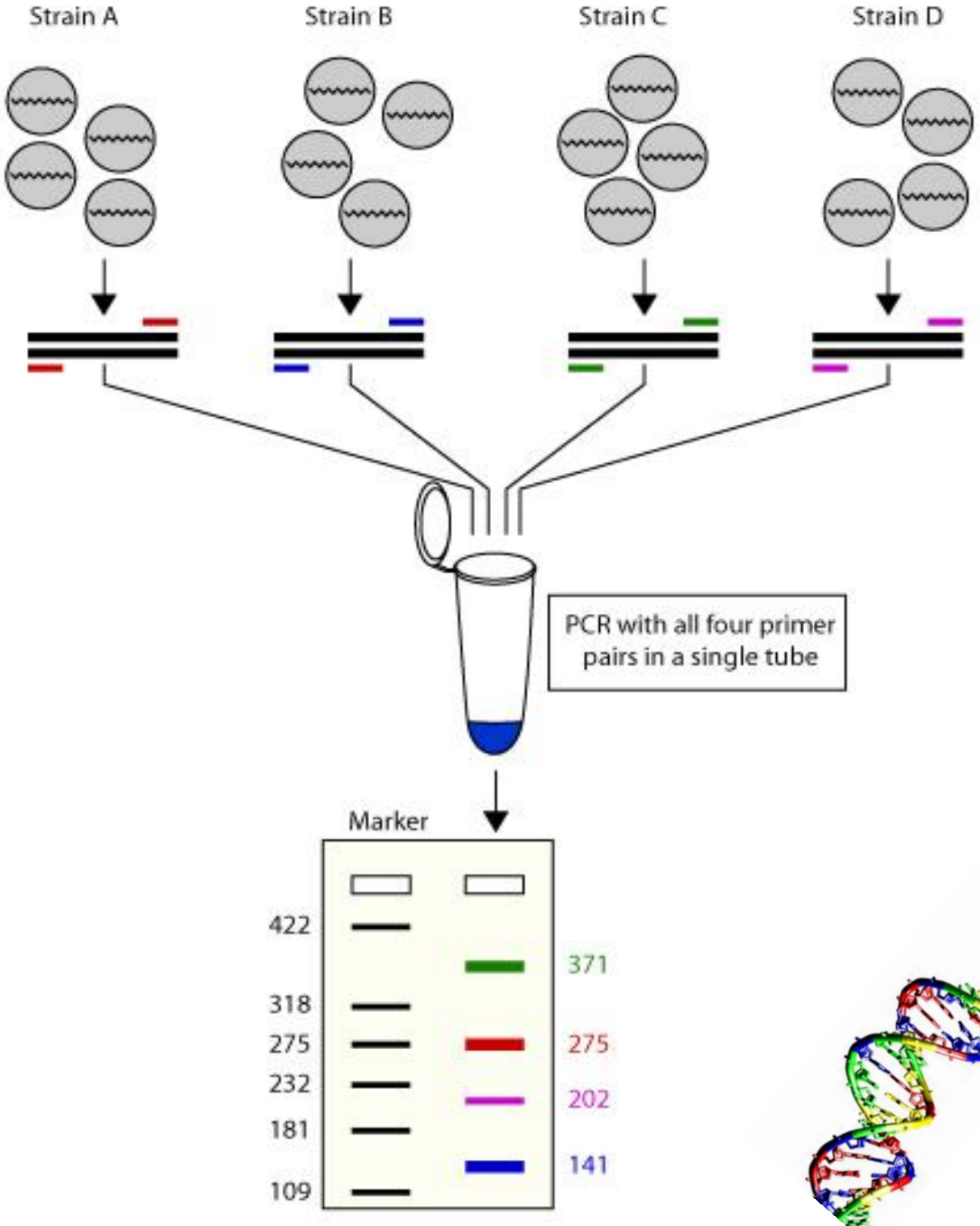
- Multiplex PCR is a widespread molecular biology technique for amplification of **multiple targets in a single PCR** experiment.

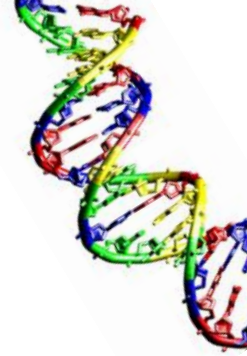
- In a multiplexing assay, more than one target sequence can be amplified by using multiple primer pairs in a reaction mixture.

- As an extension to the practical use of PCR, this technique has the potential to produce considerable savings in time and effort within the laboratory without compromising on the utility of the experiment.

Multiplex-PCR







Multiplex RT-PCR

RNA ⇨ cDNA synthesis ⇨ PCR with many primer sets

Nested RT-PCR

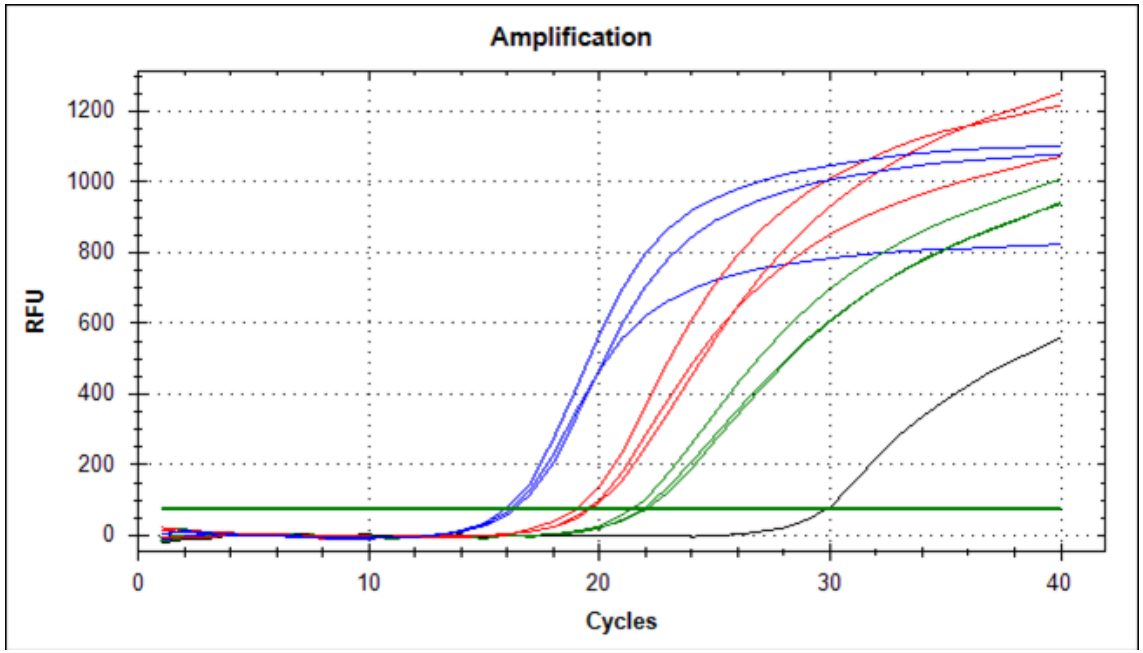
RNA ⇨ cDNA synthesis ⇨ PCR with Outer primers ⇨ PCR with Inner primers

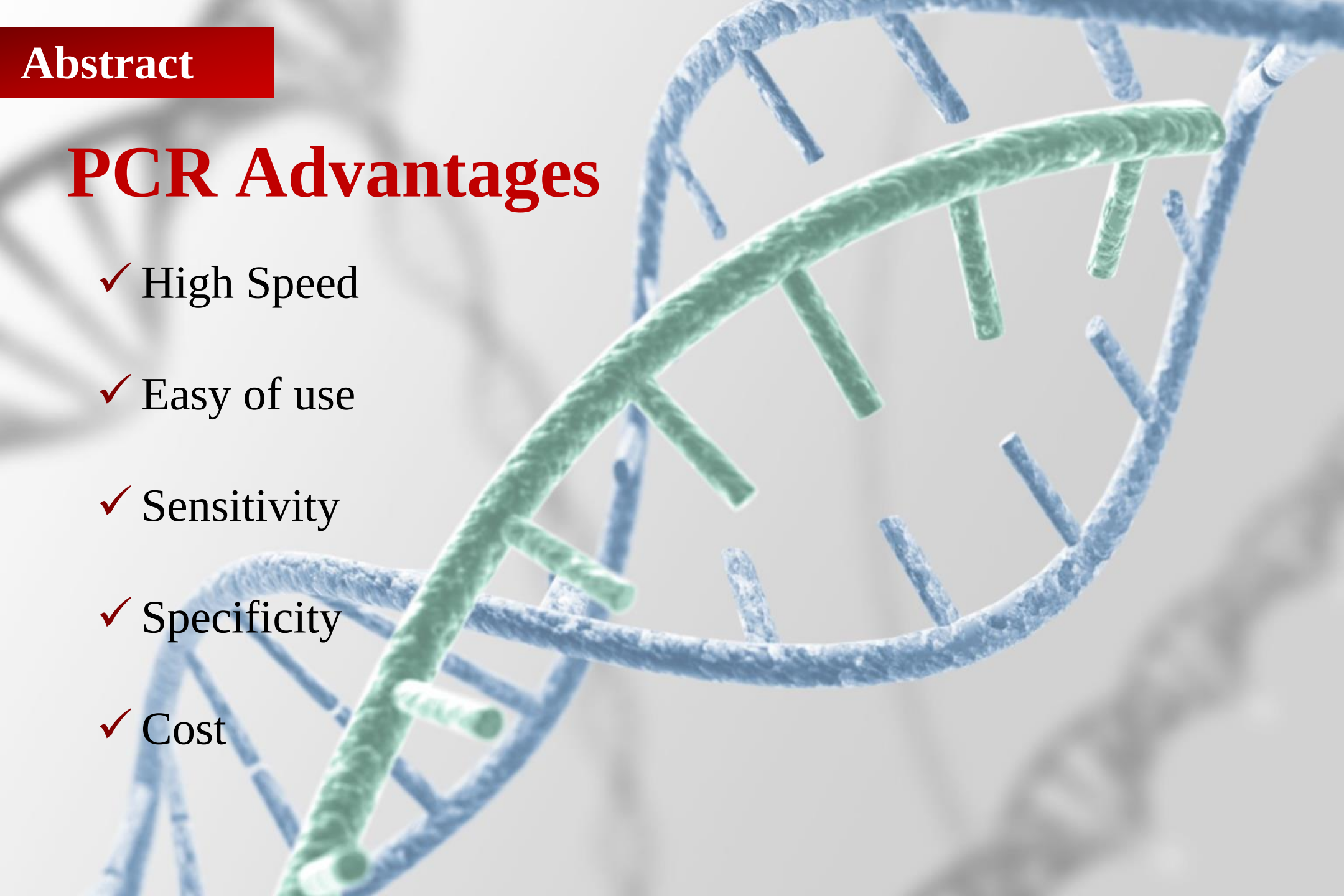
PCR Types

Real Time PCR

- Real-Time PCR a specialized technique that allows a PCR reaction to be visualized “in real time” as the reaction progresses.

- As we will see, Real-Time PCR allows us to measure amounts of DNA sequences in a sample!





Abstract

PCR Advantages

- ✓ High Speed
- ✓ Easy of use
- ✓ Sensitivity
- ✓ Specificity
- ✓ Cost

References

- PCR (مبانی و کاربردهای آزمایشگاهی)/ تالیف ام. جی. مک فرسون، اس. جی. مولر، ترجمه محسن کریمی، سیروس زینلی. تهران: اندیشه ظهور، ۱۳۸۳.
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- مقدمه ای بر کلون سازی ژن ها و آنالیز DNA/ تالیف براون، ترنس اوستن. ترجمه مجتبی طباطبایی یزدی، غلامرضا زرینی، ضرغام سپهری زاده. تهران: خانه زیست شناسی، ۱۳۹۰.