ADVANCED LABORATORY TECHNIQUES

TYPES OF PCR

Sawa University — جامعـــــة ســـــــاوة الاهلية

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third Stage . — المرحلـــــــــــة الثالثة

محاضرة رقم9

Lecture No. 9

الجانب النظري

Theoretical

تدريسي المادة : م.م طيبة شاكر

(INTRODUCTION)

Polymerase chain reaction (PCR) is a molecular biology technique used to amplify specific DNA sequences. Developed by kary mullis in 1983, it allows the exponential amplification of DNA from a minimal starting sample. PCR revolutionized genetics, diagnostics, and forensic science due to its **speed, specificity, and sensitivity.**

The PCR process involves a series of temperature-dependent steps and utilizes a DNA polymerase enzyme to catalyze the synthesis of new DNA strands.

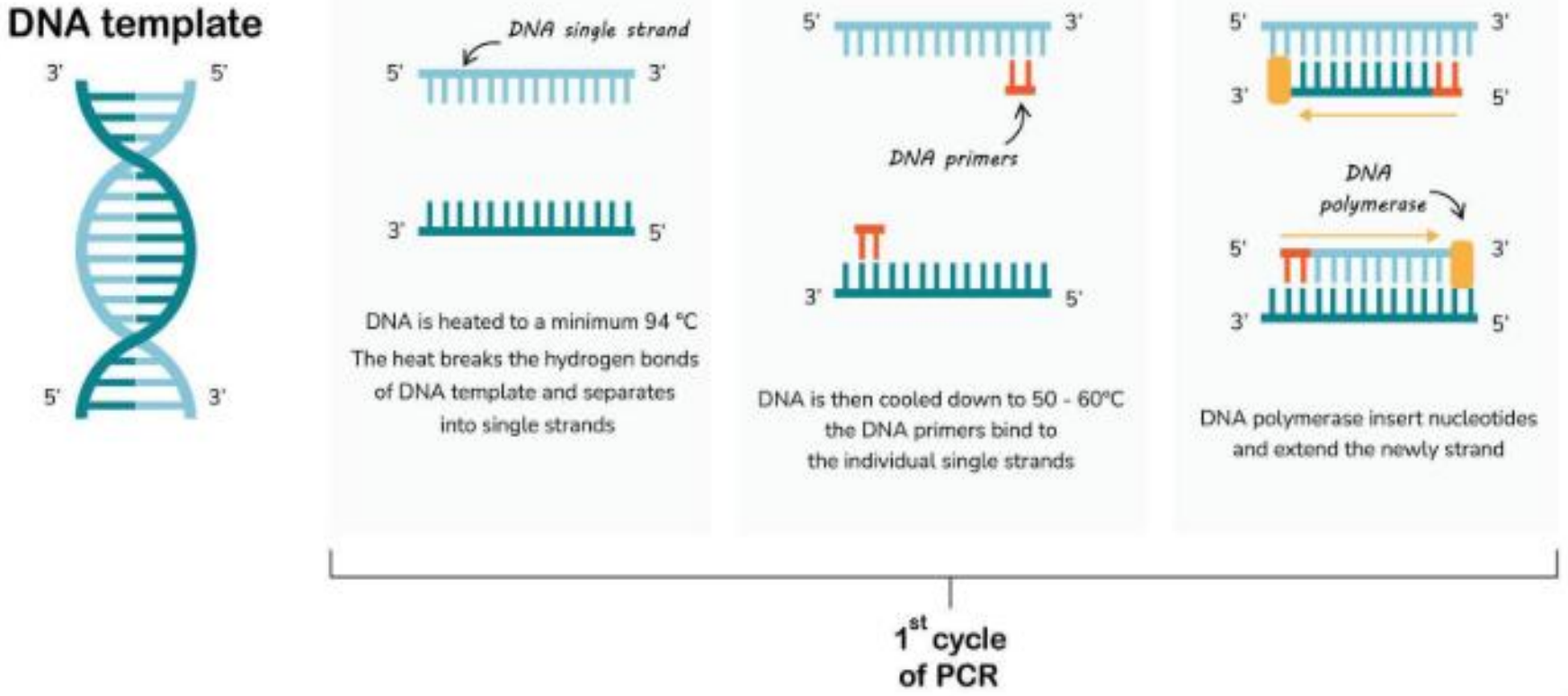
Basic principle of PCR

PCR involves three main steps repeated in cycles:

1. **Denaturation (94–96°C):** double-stranded DNA separates into single strands.
2. **Annealing (50–65°c):** primers bind (anneal) to target sequences.
3. **Extension (72°c):** taq DNA polymerase synthesizes new DNA strands.

These three steps—denaturation, annealing, and extension—are typically repeated for a specific number of cycles, usually around 20 to 40 cycles. Each cycle doubles the amount of DNA, resulting in an exponential increase in the target DNA segment.

Polymerase Chain Reaction (PCR)



Components of PCR (Enzymes used in PCR)

DNA

DNA Polymerase

DNA with Primers

dNTPs

PCR Buffers

PCR Machine

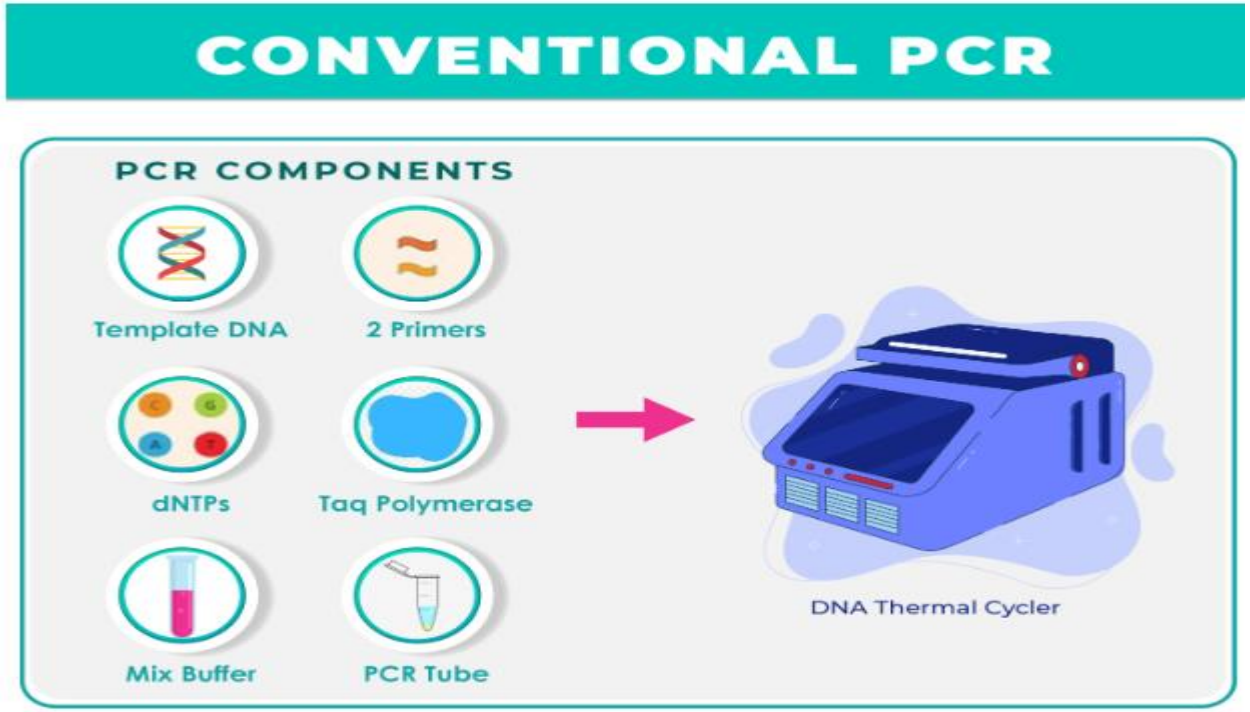
qPCR Machine

Droplet digital PCR System

MAJOR TYPES OF PCR:

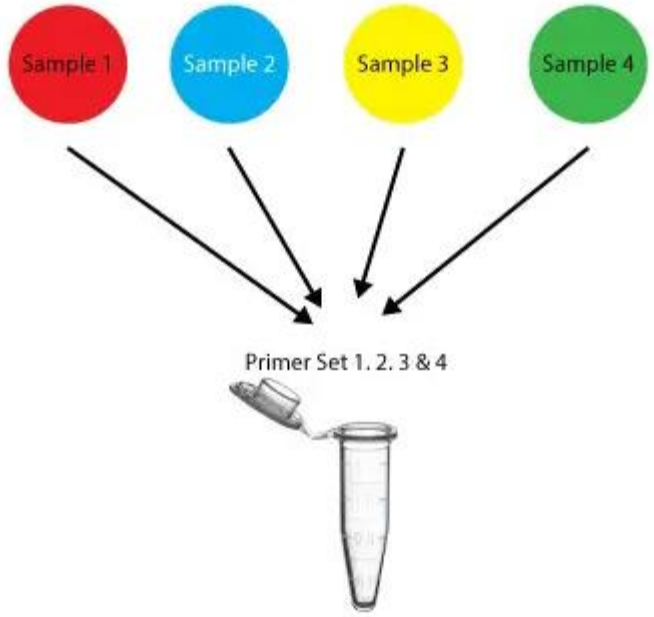
Conventional PCR :

this defined as a normal PCR process. Here the primers bind specifically to each other with 2 DNA strands. Primers also limit the sequence to be replicated and a particular DNA sequence is amplified with billions of copies.



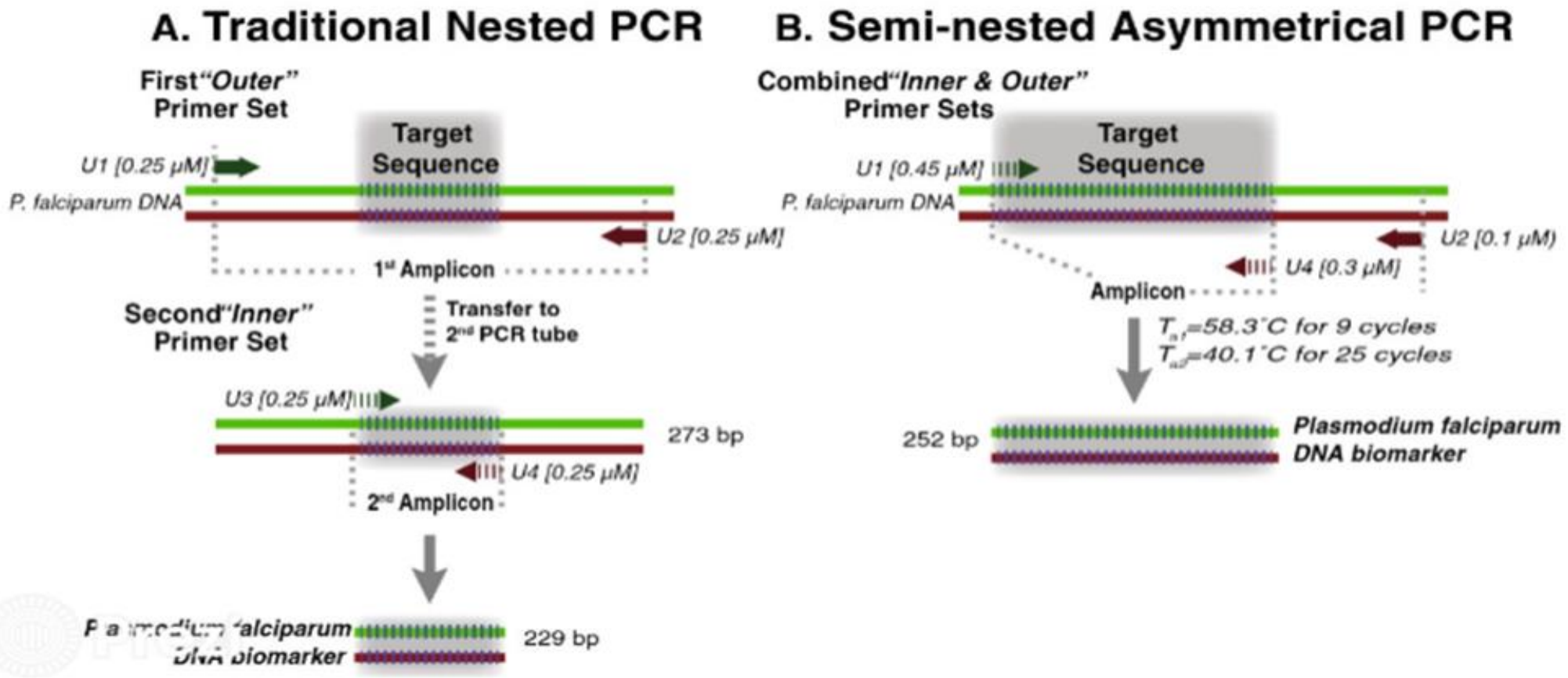
Multiplex PCR:

multiplex PCR technique detects different pathogens in a single sample, used to identify exonic/intronic sequence5 in specific genes. The designing of primers are different because they are meant to adhere to specific DNA sequence. Here in multiplex PCR the base pair lengths should be different to form distinct bands because varying sizes of different DNA genes are targeted in a single reaction to avoid higher expenses, time consumption and recognizes many pathogens at once .



Nested-semi nested PCR:

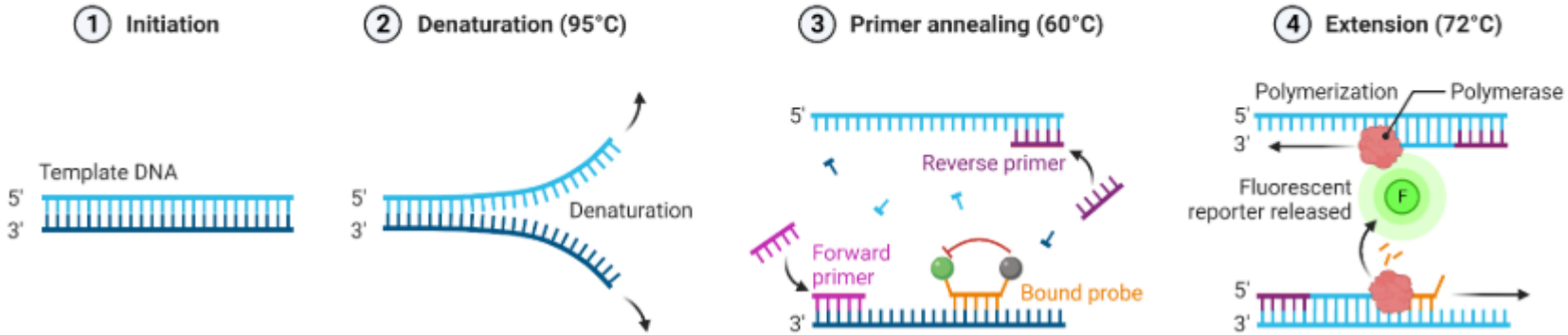
two sets of primers are used here for a single locus point. The first set is an amplified sequence and the second set is complementary to the first sequence which will be shorter than The first amplified product. Nested PCR is used because it intends to reduce the contaminations in products due to the amplification of unexpected primer binding sites.

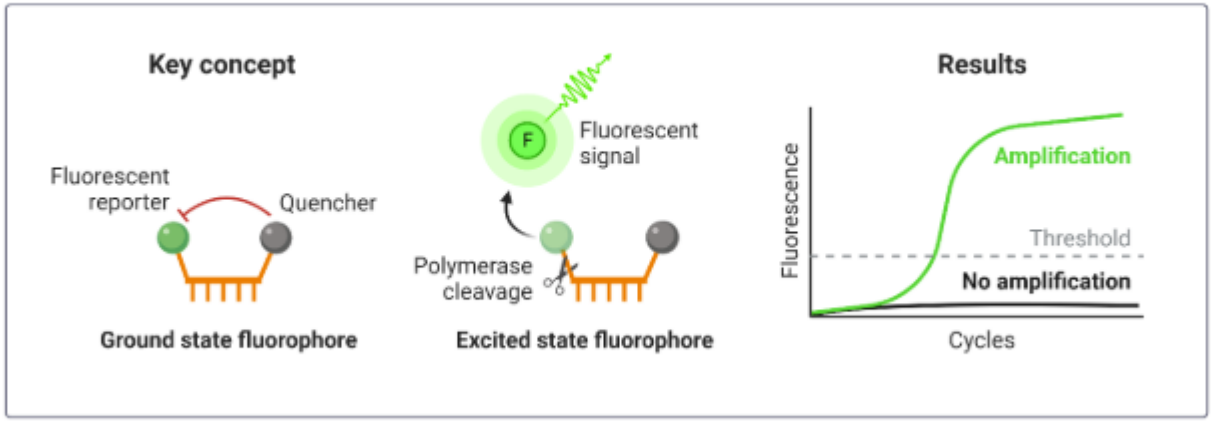

A. Traditional Nested PCR
First "Outer" Primer Set
Target Sequence
U1 [0.25 µM]
P. falciparum DNA
U2 [0.25 µM]
1st Amplicon
Second "Inner" Primer Set
Transfer to 2nd PCR tube
U3 [0.25 µM]
273 bp
U4 [0.25 µM]
2nd Amplicon
Plasmodium falciparum DNA biomarker
229 bp
B. Semi-nested Asymmetrical PCR
Combined "Inner & Outer" Primer Sets
Target Sequence
U1 [0.45 µM]
P. falciparum DNA
U2 [0.1 µM]
U4 [0.3 µM]
Amplicon
T_{a1}=58.3˚C for 9 cycles
T_{a2}=40.1˚C for 25 cycles
252 bp
Plasmodium falciparum DNA biomarker

Real-time PCR:

it is also called as quantitative pcr(q PCR)5, highly reproducaable ,rapid, sensitive and specific technique for automating data. Brucella species has been developed for targeting 16S23S gene by means of this technique.

Fluorescent Probe-Based Real Time PCR (qPCR)





Specialized variants

- **Allele-specific PCR:** detects single nucleotide polymorphisms (snps).
- **Long pcr:** amplifies very long dna fragments (up to 40 kb).
- **In situ pcr:** amplifies dna directly within fixed cells or tissues.
- **Quantitative rt-pcr (qrt-pcr):** combines reverse transcription and real-time quantification for rna analysis.

Applications

- **Medical diagnostics:** detecting infectious agents (HIV, HBV, COVID-19).
- **Genetic research:** mutation detection, cloning, gene expression.
- **Forensic science:** DNA fingerprinting.
- **Environmental studies:** microbial community analysis.
- **Food and agriculture:** GMO detection.

Type	Target	Key Feature	Main Use
Conventional PCR	DNA	Qualitative	Basic amplification
RT-PCR	RNA	Converts RNA to cDNA	Gene expression
qPCR	DNA/cDNA	Quantitative	Diagnostics
Multiplex PCR	Multiple DNA targets	Multi-target detection	Pathogen panels
Nested PCR	Low-copy DNA	High specificity	Infectious disease
Digital PCR	DNA/cDNA	Absolute quantification	Cancer, rare mutations

QUIZ:

What is the main difference between conventional PCR and real-time PCR (qPCR)?

ANY QUESTION?

Thank You