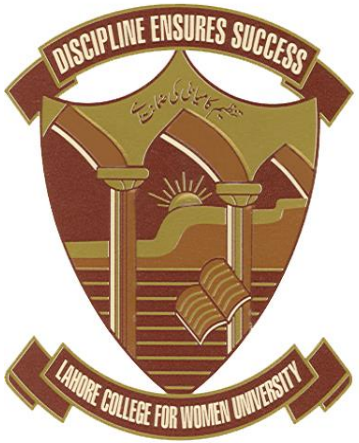

بسم الله الرحمن الرحيم

TYPES OF POLYMERASE CHAIN REACTION



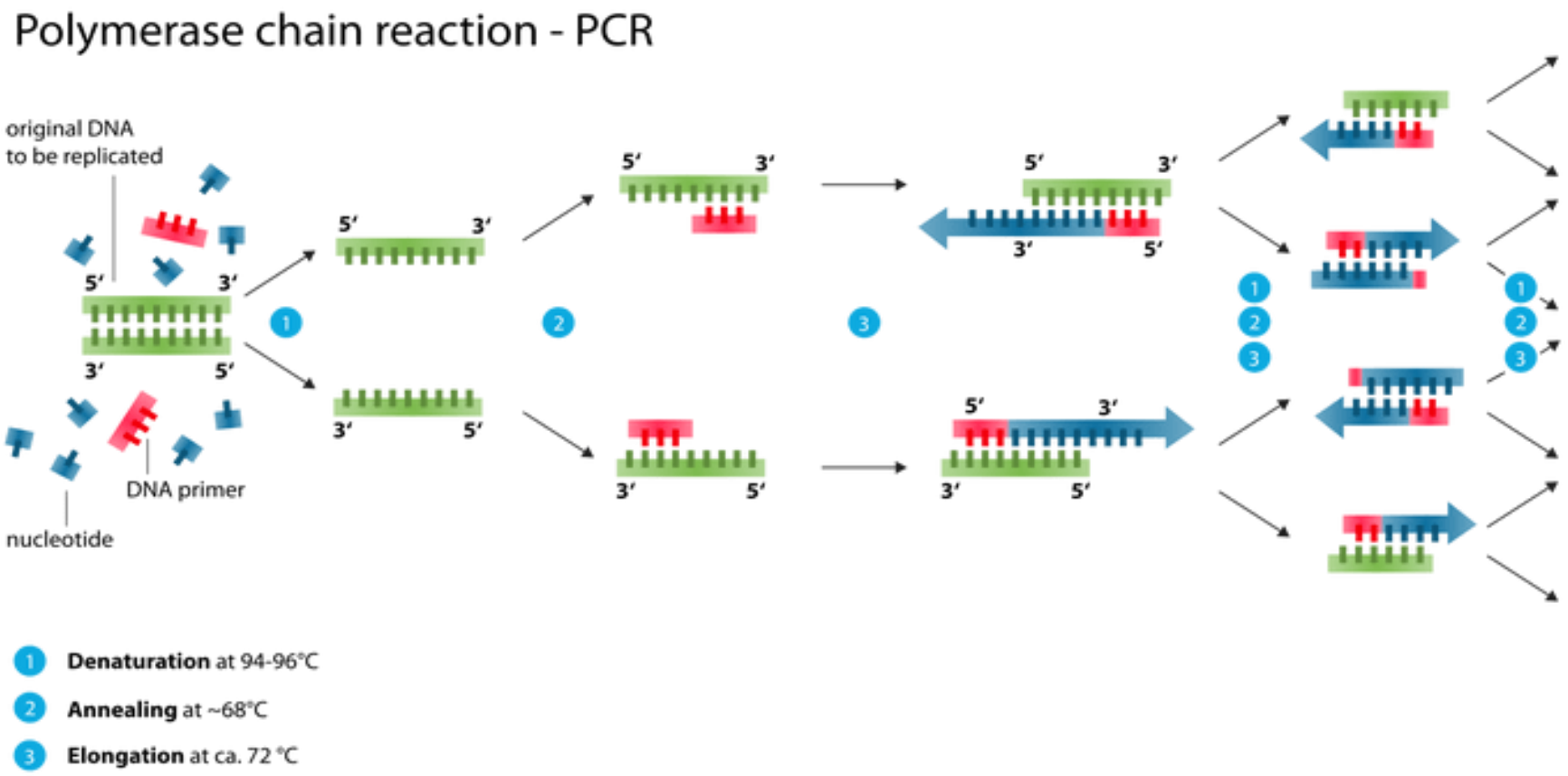
Department of Zoology,

Lahore College for Women University

What is PCR

- History
- Introduction
- Components
- Applications

How it works

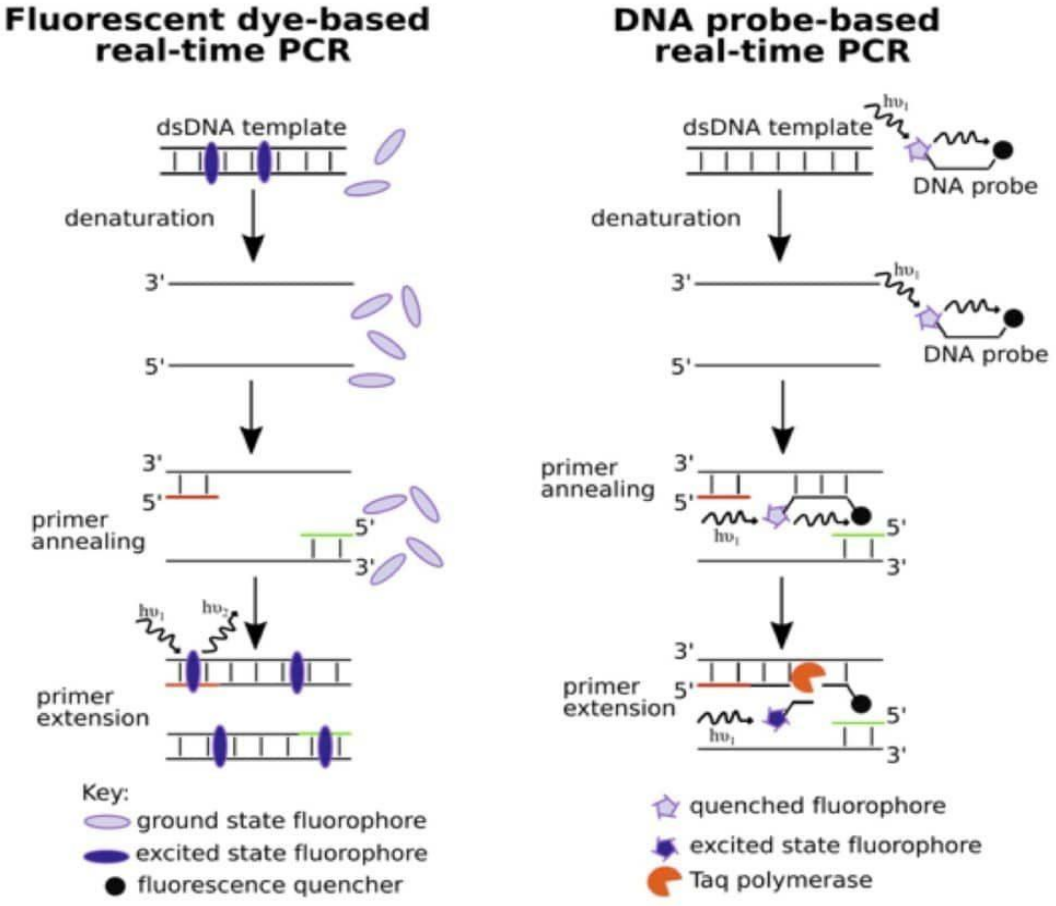

Polymerase chain reaction - PCR
original DNA
to be replicated
5′
3′
3′
5′
DNA primer
nucleotide
1
2
3
1 Denaturation at 94-96°C
2 Annealing at ~68°C
3 Elongation at ca. 72 °C

Different types of PCR

- Quantitative PCR
- Multiplex PCR
- Nested-semi nested PCR
- Standard PCR
- RT-PCR
- Hot start PCR
- Asymmetric PCR
- Touchdown PCR
- Colony PCR
- COLD PCR
- Suicide PCR

Quantitative PCR

- **Real-Time PCR**
- PCR with few improvements
- Types of qPCR
- Applications



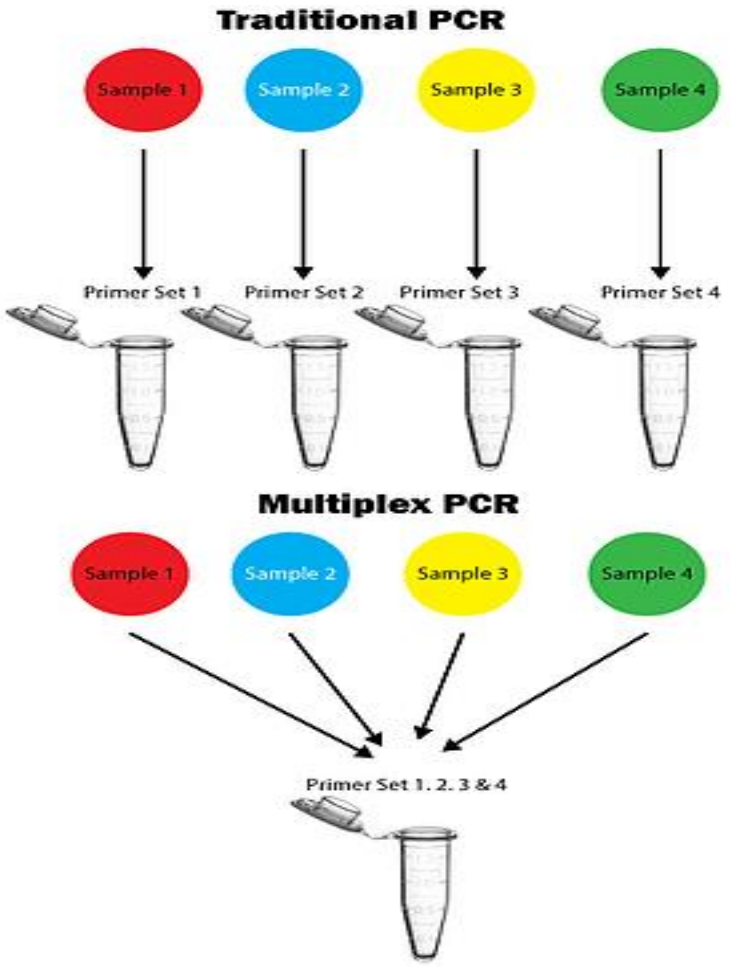
Fluorescent based and probe based qPCR

Multiplex PCR

- More than one target sequence can be amplified

Types of Multiplex PCR

- Single template PCR
- Multiple Template PCR



Traditional versus Multiplex PCR

Primer design parameters

- Primer length
- Melting temperature
- Specificity
- Avoidance of primer-dimer formation

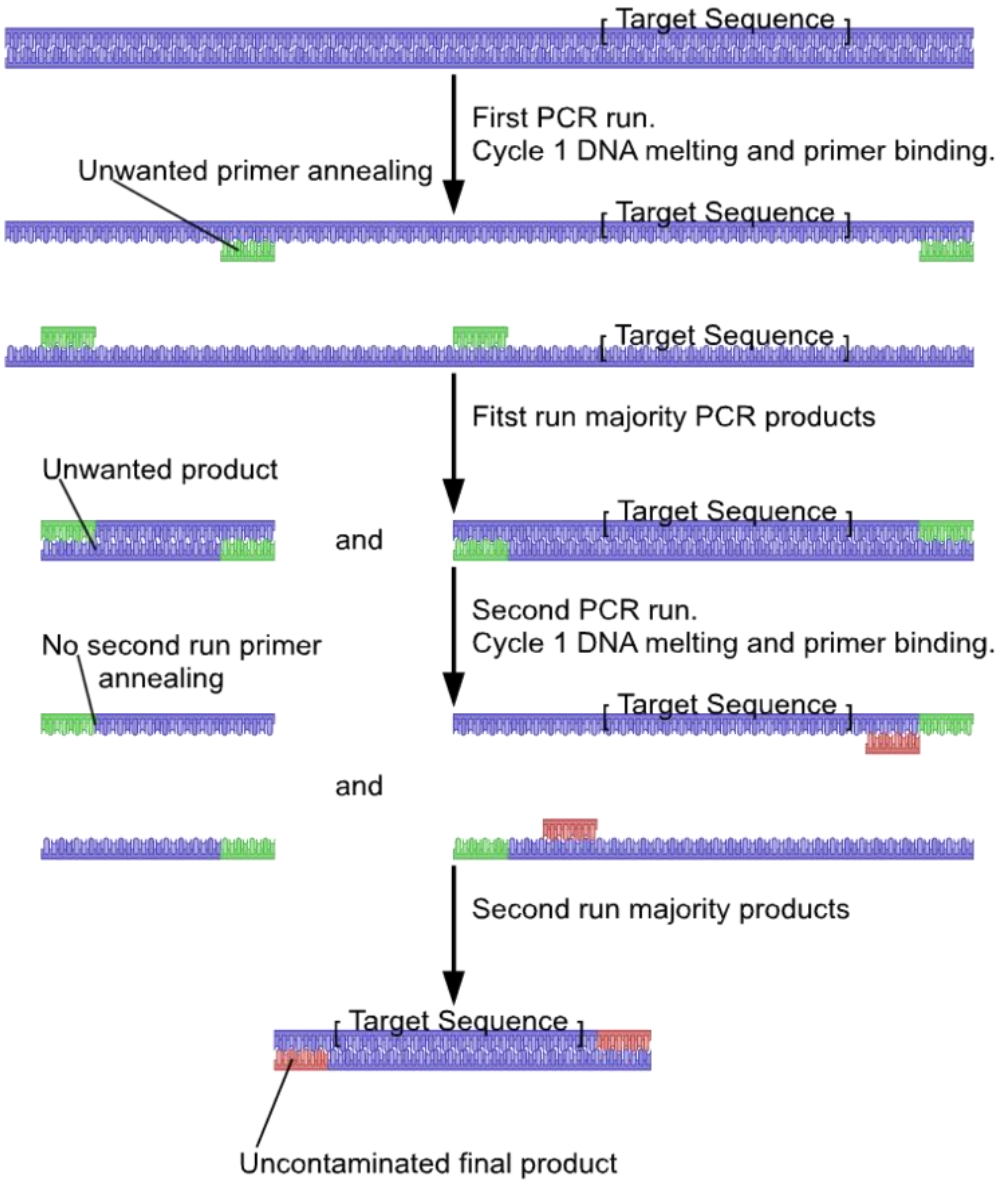
Advantages

- Internal controls
- Efficiency
- Indication of template quality
- Indication of template quantity

Application

Nested-semi nested PCR

- To reduce contamination in products
- Use of **two sets of primers**
- First set is an amplified sequence
- Second set is complementary to the first set


Target Sequence
First PCR run.
Cycle 1 DNA melting and primer binding.
Unwanted primer annealing
Target Sequence
Target Sequence
Fitst run majority PCR products
Unwanted product
and
Target Sequence
Second PCR run.
Cycle 1 DNA melting and primer binding.
No second run primer annealing
Target Sequence
and
Second run majority products
Target Sequence
Uncontaminated final product

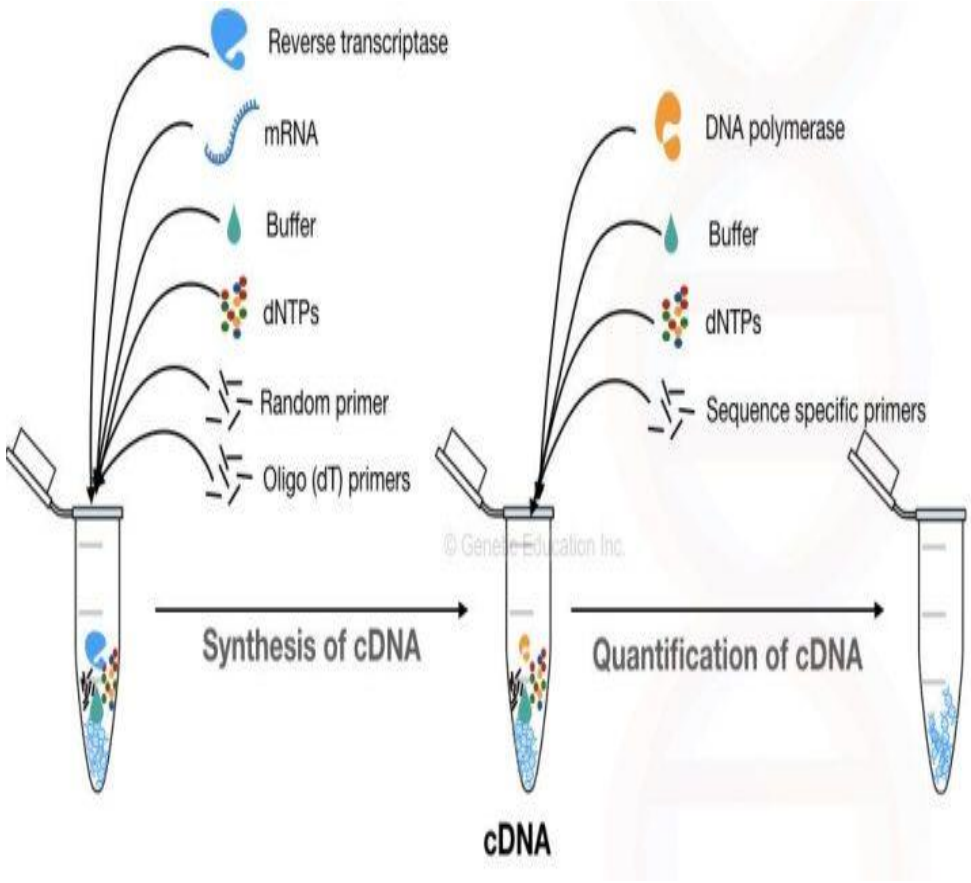
Nested PCR

Standard PCR

- Simple **efficient** and **sensitive** technique
- Use of **one pair of primers**
- Helps in early diagnosis of **Brucella**
- Used to determine **no of leukocytes DNA/heamo compounds**

RT-PCR

- Measures **RNA expression level**
- Production of **complementary DNA**
- Use of **Reverse transcriptase**
- Applications



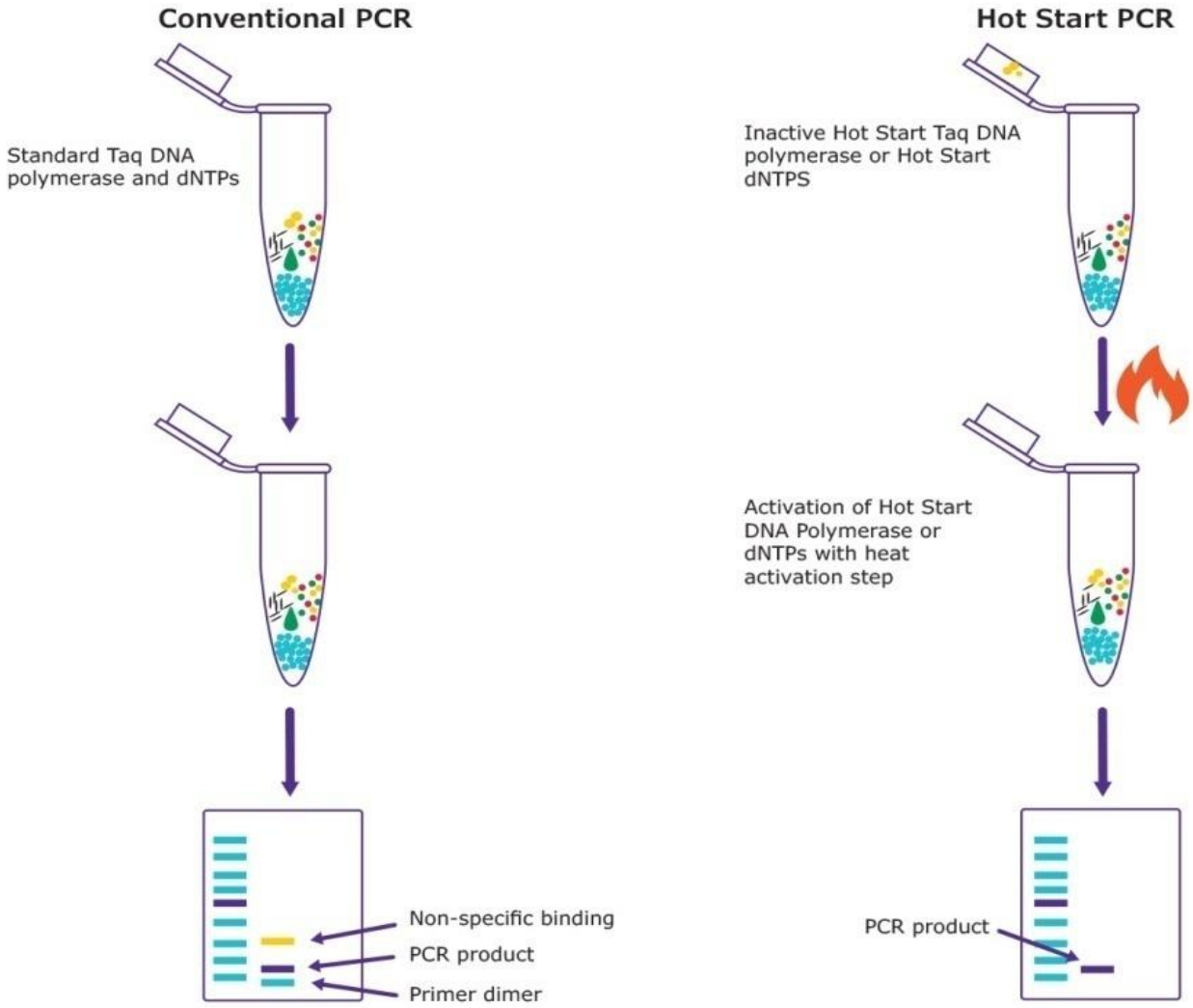
RT-PCR

Hot Start PCR

- Allows reaction setup at room temperature
- Without non-specific amplification and dimer formation

Method

- Physical separation
- DNA polymerase inactivation
- dNTP modifications



Comparison of conventional and Hot start PCR

Asymmetric PCR

- Amplifies one strand of target DNA
- **Thermocycling** with limiting amount or leaving out primer

Touchdown PCR

- **Annealing temperature** is decreased in later cycles
- In early cycles **3-5 degree above** the standard Tm
- Later cycles **3-5 degree below** Tm
- Initial higher T leads to **greater specificity for primer binding**
- Lower T permit more **efficient amplification** at the end of reaction

(A) Normal PCR program:

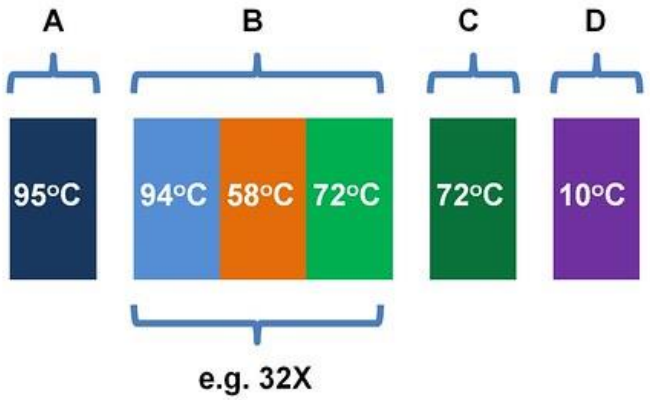
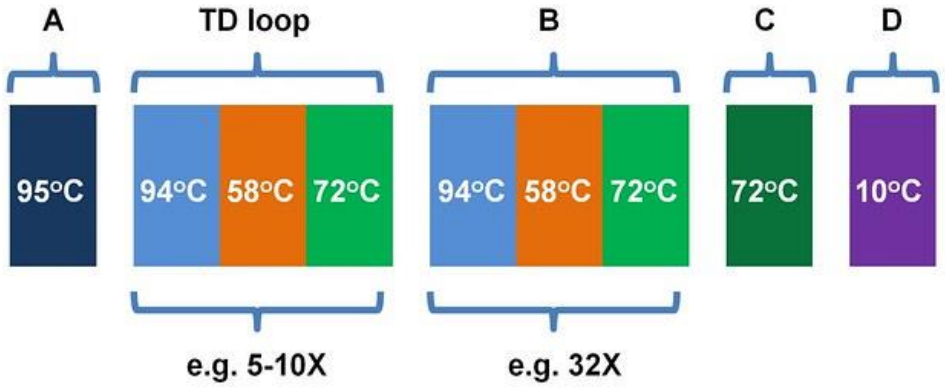


Figure legends:

A: Denaturation temperature.
B: PCR cycle.
C: Final extension.
D: Holding temperature.

TD loop: Touch down loop cycle.

(B) PCR program with "touch-down loop":



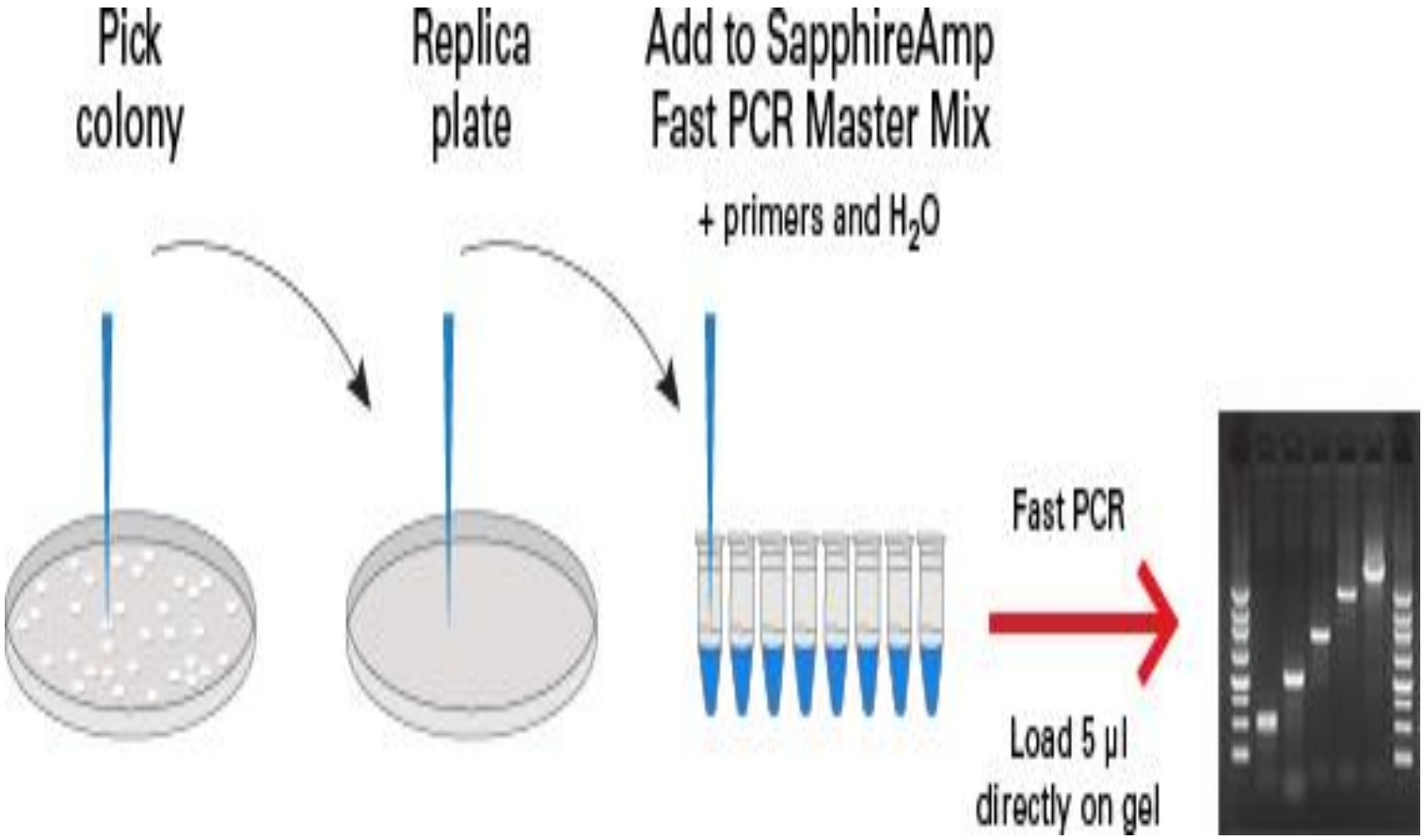
Normal versus Touchdown PCR

Colony PCR

- Bacterial colonies are screened directly
- Colonies are separated with sterile pipette tip
- Cells are transferred into a PCR mix

To release DNA from cells PCR is started either by:

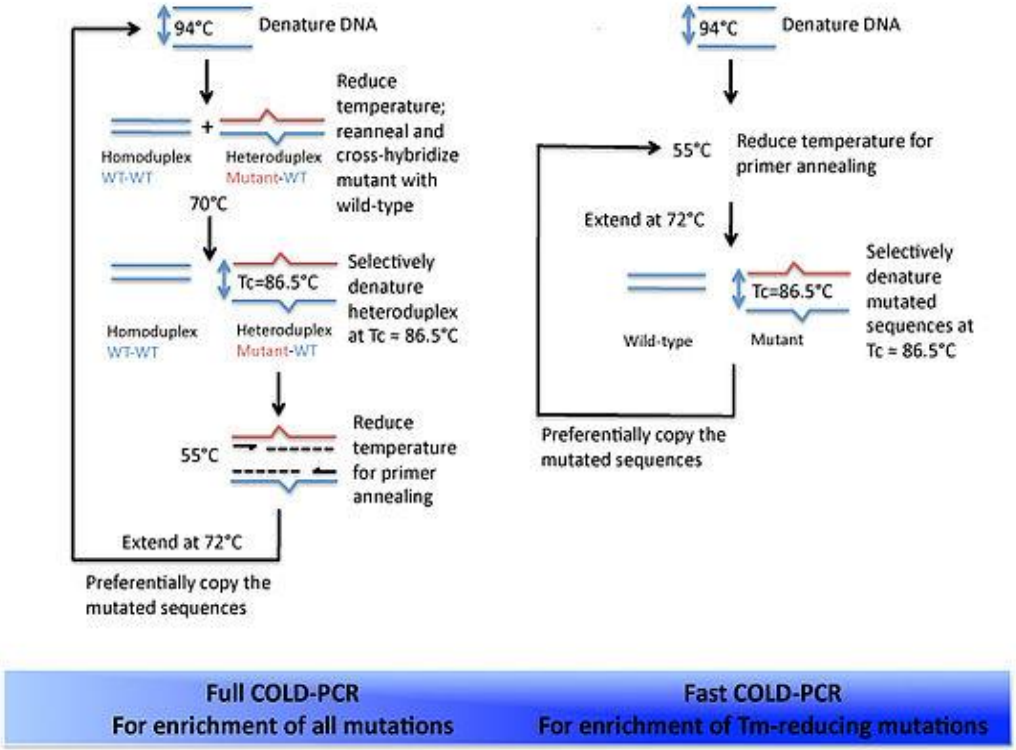
- Extended time at 95 degree
- Shortened denaturation step at 100 degree
- Special chimeric DNA


Pick colony
Replica plate
Add to SapphireAmp Fast PCR Master Mix
+ primers and H_2O
Fast PCR
Load 5 µl directly on gel

Colony PCR

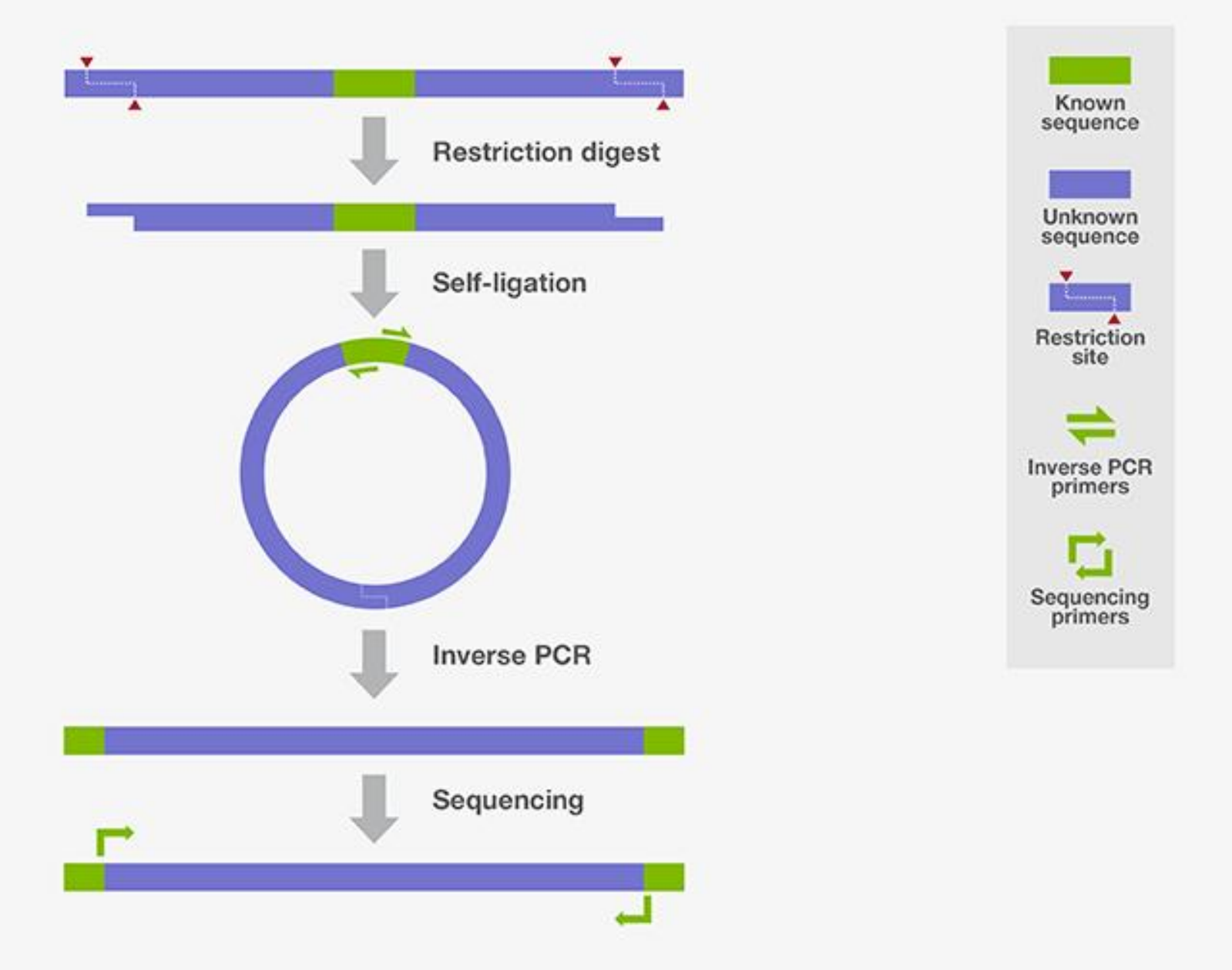
COLD-PCR

- It is a modified protocol that enriches variant alleles from a mixture of wildtype and mutation-containing DNA.



Full COLD and Fast COLD PCR

Inverse PCR


Restriction digest
Self-ligation
Inverse PCR
Sequencing
Known sequence
Unknown sequence
Restriction site
Inverse PCR primers
Sequencing primers


thanks!