

POLYMERASE CHAIN REACTION (PCR)

Components, the Three-Step Cycle, Exponential Amplification, and Applications

1. Overview

The **Polymerase Chain Reaction (PCR)** is an in vitro enzymatic technique used to **exponentially amplify** a specific segment of DNA. Starting from only a minute amount of template DNA, PCR can generate millions to billions of copies of a target sequence within a few hours, making it one of the most widely used tools in molecular biology, medicine, and forensic science.

2. Essential Components

Component	Role	Description
Template DNA	Target	The genomic DNA containing the specific region to be amplified.
Taq Polymerase	Catalyst	A heat-stable DNA polymerase (from <i>Thermus aquaticus</i>) that synthesises new DNA strands at high temperatures.
Primers	Specification	Short, synthetic single-stranded DNA sequences (18-25 bp) that flank the target region and provide a 3'-OH group for polymerase.
dNTPs	Building blocks	Deoxynucleotide triphosphates (dATP, dTTP, dCTP, dGTP) used to construct the new DNA strand.
Mg ²⁺ / Buffer	Environment	MgCl ₂ acts as a cofactor for Taq polymerase; the buffer maintains optimal pH.

3. The PCR Cycle

A single PCR cycle consists of three temperature-dependent steps, repeated 25-35 times in an automated thermal cycler:

The PCR Cycle: Three Repeating Steps

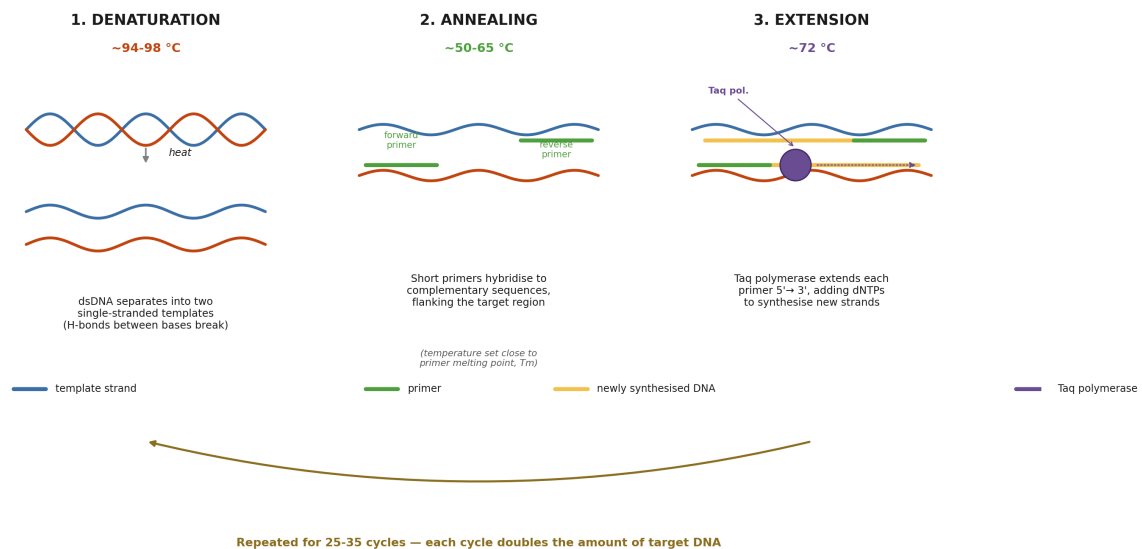


Fig. 1 — The three repeating steps of a PCR cycle: denaturation, annealing, and extension.

I. Denaturation (~94-98 °C)

- **Action:** High heat is applied to the reaction.
- **Result:** Hydrogen bonds between complementary base pairs break, separating the double-stranded DNA (dsDNA) into two single-stranded DNA (ssDNA) templates.

II. Annealing (~50-65 °C)

- **Action:** Temperature is lowered to allow primers to bind.
- **Result:** Primers hybridise to their complementary sequences at the 3' ends of the target region. The exact temperature depends on the primer's melting temperature (T_m).

III. Extension (~72 °C)

- **Action:** Temperature is raised to the optimal activity point of Taq polymerase.
- **Result:** Taq polymerase adds dNTPs to the 3' end of the primers, synthesising a complementary strand in the 5' → 3' direction.

4. Exponential Amplification

PCR amplification follows a **geometric progression**. The number of DNA copies doubles with every cycle:

Amplification formula

Number of copies = 2^n (where n is the number of cycles)

Cycle (n)	Approximate number of copies
1	2 copies
10	1,024 copies ($\sim 1.0 \times 10^3$)
20	$\sim 1.05 \times 10^6$
30	$\sim 1.07 \times 10^9$ copies

Exponential Amplification of Target DNA

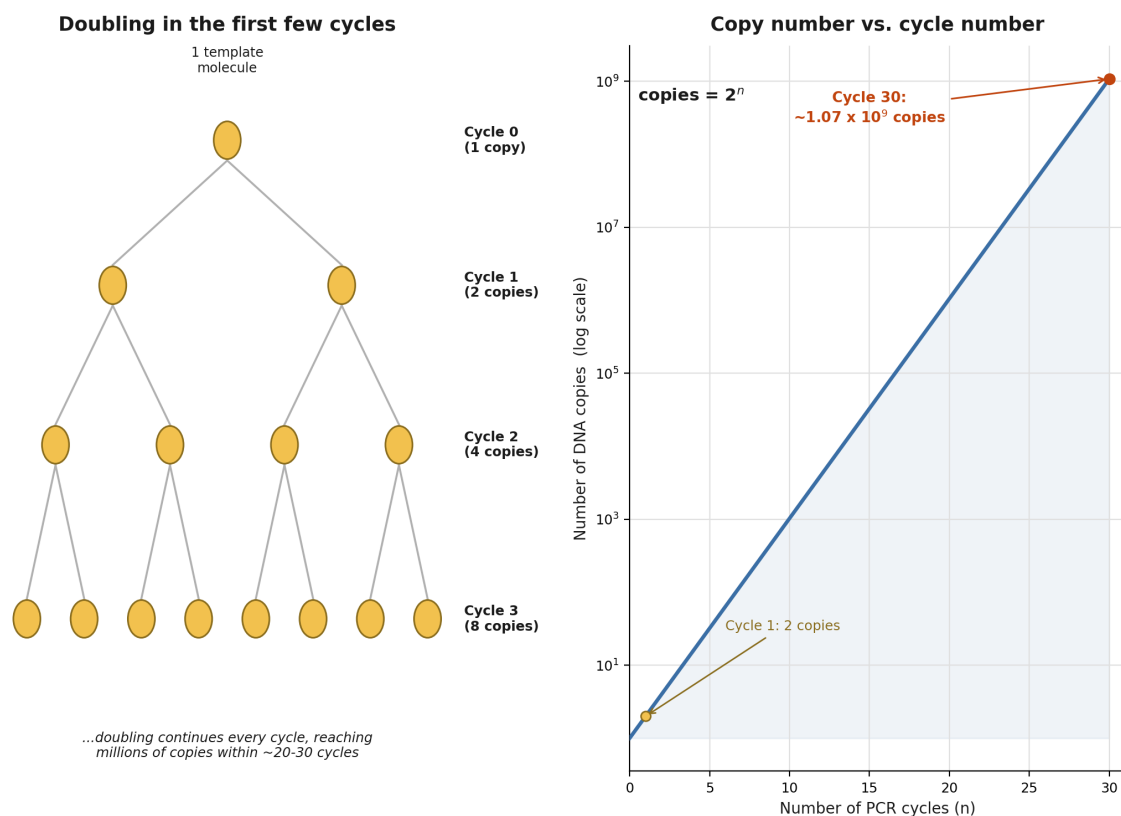


Fig. 2 — Exponential doubling of template DNA across successive PCR cycles, shown as a branching tree (early cycles) and as a growth curve on a log scale (up to cycle 30).

Note — the plateau effect

In practice, amplification is exponential only for the first ~25-30 cycles; beyond this, reagents (primers, dNTPs, polymerase) become limiting and the reaction enters a linear, then plateau, phase.

5. Applications

Medical Diagnostics

- Detection of pathogens (e.g. SARS-CoV-2, HIV) and genetic mutations (e.g. cystic fibrosis).

Forensic Science

- DNA profiling/fingerprinting using Short Tandem Repeats (STRs) from crime scene samples.

Research

- Gene cloning, sequencing, and site-directed mutagenesis.

Paternity Testing

- Comparing alleles between child and suspected parents.

Palaeogenetics

- Amplifying DNA from ancient fossils or mummified remains.

Compiled study note — molecular biology reference. Figures are original schematic diagrams prepared for this note and are not to scale.