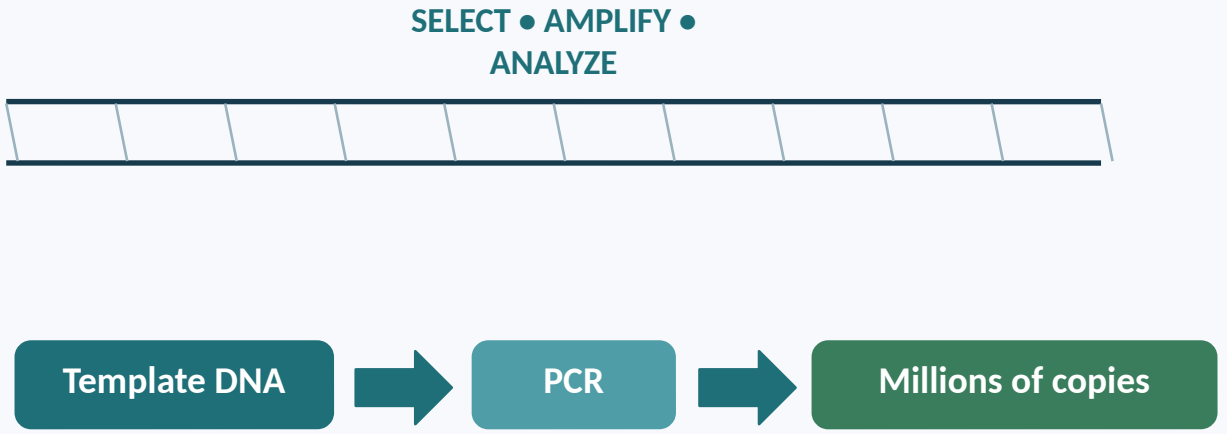
POLYMERASE CHAIN REACTION

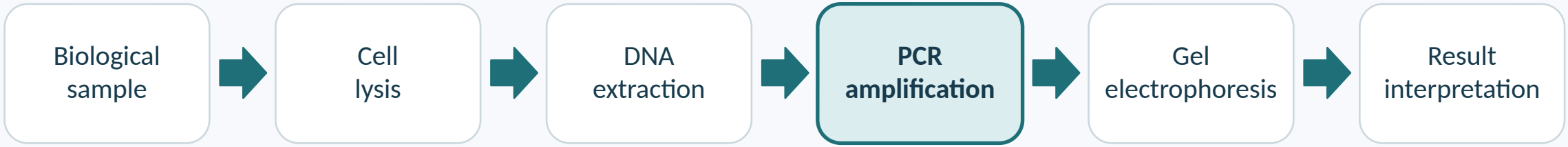
Principles, Components

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Where does PCR fit?

A PCR experiment is one step in a larger molecular biology workflow.



Question

We already extracted genomic DNA. Why do we still need PCR?

Because we usually need to detect or analyze one specific DNA region, not the entire genome.

What is PCR?

PCR is an in vitro technique used to selectively amplify a specific DNA sequence.

in vitro reaction performed outside a living cell, in a controlled laboratory syster

amplify increase the number of copies of a selected DNA fragment



PCR reaction tubes

What does amplification mean?



Ideal model

$$N = N_0 \times 2^n$$

30 cycles ≈ 1.07×10^9-fold under ideal doubling

Each new PCR product can become a template in the next cycle.

Real PCR eventually deviates from ideal doubling as reagents become limiting and the reaction approaches a plateau.

Target DNA

The target is the specific DNA region selected for amplification.



Examples

- 16S rRNA gene
- acdS gene
- nifH gene
- resistance gene
- pathogen-specific DNA

Template DNA vs target DNA

TEMPLATE DNA

DNA added to the PCR tube

May contain the whole genome

Provides the original sequence information

TARGET DNA

Specific region to be amplified

Defined by the positions of the primers

Usually a small part of the template

Analogy: genomic DNA = a whole book ● target DNA = one selected paragraph

PCR requires two primers



- Short single-stranded DNA oligonucleotides
- Bind to complementary sequences
- Define the boundaries of the amplicon
- Provide a free 3′-OH for DNA synthesis

Primer orientation and the 5′→3′ rule

DNA polymerase synthesizes new DNA only in the 5′ → 3′ direction.



The primers face inward so synthesis proceeds through the target region.

DNA polymerase: the copying enzyme

Uses a template

Reads the existing DNA sequence as a guide.

Extends a primer

Adds nucleotides to the primer’s 3′ end.

Synthesizes 5′→3′

The new DNA strand always grows in one direction.

DNA polymerase does not start a new strand from nothing.

A primer must already be bound to the template.

Why use a thermostable polymerase?

PCR repeatedly heats the reaction to about 94–98°C during denaturation.

Taq DNA polymerase

A thermostable enzyme originally isolated from the thermophilic bacterium Thermus aquaticus.

Thermostability allows the same enzyme to remain active through repeated heating cycles.



Bio-Rad T100 thermal cycler

dNTPs: building blocks of new DNA



DNA polymerase selects the nucleotide complementary to the template base.

Base pairing: A–T • G–C

Mg^{2+}: an essential cofactor

Mg^{2+} is required for DNA polymerase catalysis and strongly influences PCR performance.

Too little Mg^{2+}

Low enzyme activity → weak or absent amplification.

Appropriate Mg^{2+}

Good amplification with suitable specificity.

Excess Mg^{2+} can promote non-specific primer binding and unwanted products.

PCR buffer: the chemical environment

pH

Maintains a suitable acid–base environment for enzyme activity.

Ionic strength

Provides appropriate salt conditions for primer-template interactions.

Enzyme stability

Supports polymerase activity throughout cycling.

Commercial PCR master mixes often combine buffer, Mg^{2+}, dNTPs and polymerase in one reagent.

What is inside a PCR tube?

Template DNA	contains the target sequence
Forward primer	defines one boundary
Reverse primer	defines the opposite boundary
DNA polymerase	synthesizes new DNA
dNTPs	provide the building blocks
Mg^{2+} + buffer	support enzyme activity
Nuclease-free water	adjusts the final volume



Eppendorf microcentrifuge tubes

Think function, not just names: what is copied ● where copying starts ● who copies ● what it is built from