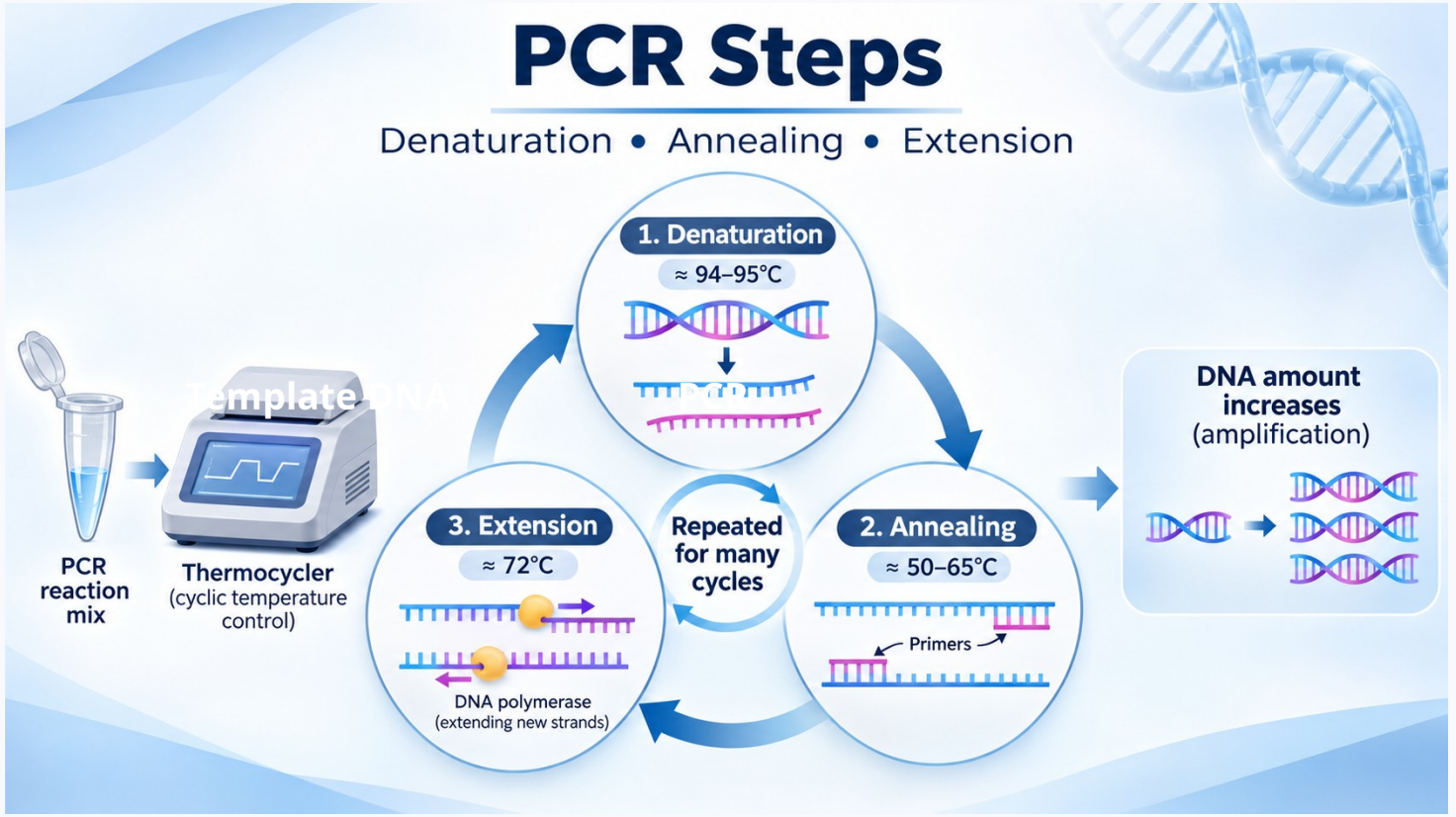
PCR steps

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PCR setup at the bench

Precision and contamination control matter because PCR is highly sensitive.



Micropipette
Eppendorf Research® plus pipettes



Microcentrifuge tubes
Eppendorf microcentrifuge tubes



PCR tube strips
Bio-Rad PCR tube strips

Use clean tips • separate pre- and post-PCR areas when possible • keep reagents organized

The instrument repeatedly changes the reaction temperature according to a programmed protocol.

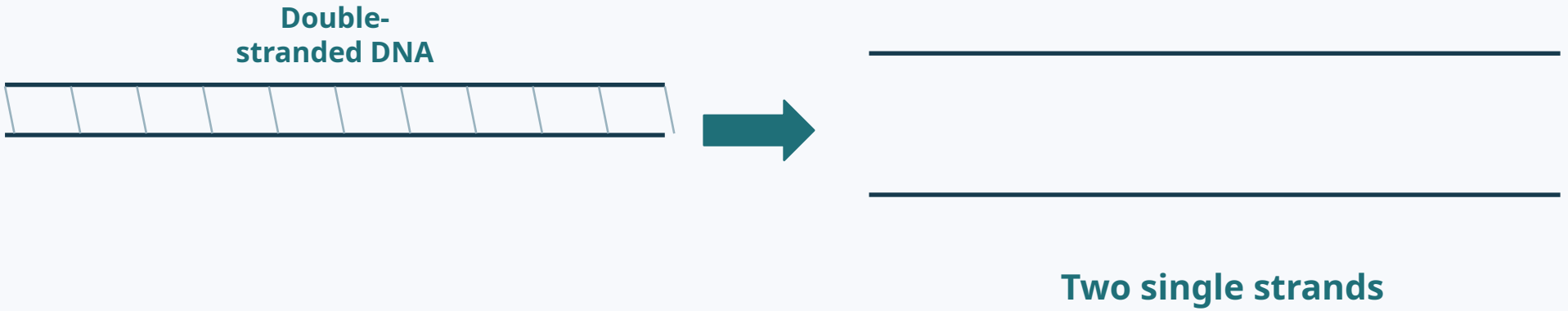


Bio-Rad T100 thermal cycler

Initial denaturation	**94–98°C**
Denaturation	**94–98°C**
Annealing	**primer-dependent**
Extension	**~68–72°C**
Final extension	**~68–72°C**

Initial denaturation

Typical: 94–98°C for ~1–5 min, depending on polymerase and template.



High temperature disrupts hydrogen bonding and base stacking interactions that stabilize the duplex.

The covalent sugar–phosphate backbone remains intact.

Step 1 — Denaturation

DENATURATION

94–98°C

dsDNA → single strands

Purpose

Separate the DNA strands so primers can access complementary sequences.

Before



Step 2 — Annealing

ANNEALING

~50–65°C

primers bind the template

The exact temperature depends on primer sequence and melting temperature (Tm).

Too high

Primers may bind poorly → weak or no amplification.

Too low

Primers may bind non-specifically → extra products.

Annealing temperature is a major determinant of PCR specificity.

Step 3 — Extension

EXTENSION

~68–72°C

polymerase extends primers

DNA polymerase adds complementary dNTPs to the 3′ end of each bound primer.

New DNA synthesis **5′ → 3′**

For standard Taq polymerase, ~72°C is a common extension temperature.

One complete PCR cycle

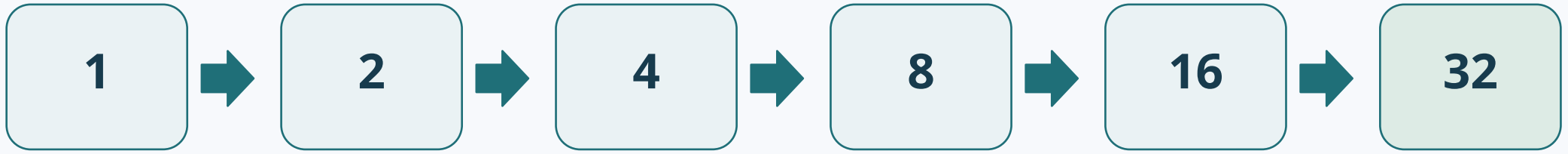


Separate → Bind → Copy

This three-step sequence is repeated many times.

Repeated cycling drives amplification

Typical conventional PCR uses about 25–40 cycles, depending on the assay.



Early cycles

Exponential phase

Plateau later

Real reactions eventually plateau because amplification efficiency decreases and reagents become limiting.

Final extension and hold

Final extension

Often ~68–72°C for several minutes. Allows incompletely extended products to finish.

Hold

The instrument commonly holds the reactions at 4–10°C until samples are removed.

PCR is complete — but the product is not yet interpreted.

Next: analyze the amplicon by agarose gel electrophoresis.

Positive control

Known DNA that should amplify with the selected primer pair.

POSITIVE CONTROL

Expected PCR product

- Confirms reagents are functional
- Confirms the primer pair can amplify
- Helps distinguish technical failure from a negative sample

If the positive control fails, do not interpret a negative sample as “target absent.”

Negative control — No Template Control (NTC)

Contains all PCR reagents except template DNA.

NTC

Expected: NO PCR product

- Detects contamination of reagents or workflow
- Template is replaced by nuclease-free water
- A band in the NTC is a warning sign

A positive NTC usually means the run should be treated as contaminated until the source is resolved.

What does a successful conventional PCR look like?

After PCR, products are commonly checked on an agarose gel.



A band of the expected size supports successful amplification; sequence identity may require additional confirmation.

Ideal pattern: expected-size band in the positive control and samples; no band in NTC.

Problem: no PCR product

01
Poor template quality

02
Primer mismatch or design problem

03
Annealing temperature too high

04
Missing or inactive reagent

05
PCR inhibitors

06
Too little target DNA

First question: did the positive control work?

Problem: multiple bands

Usually indicates non-specific amplification.

Low annealing temperature

Primers may bind unintended sequences.

Poor primer specificity

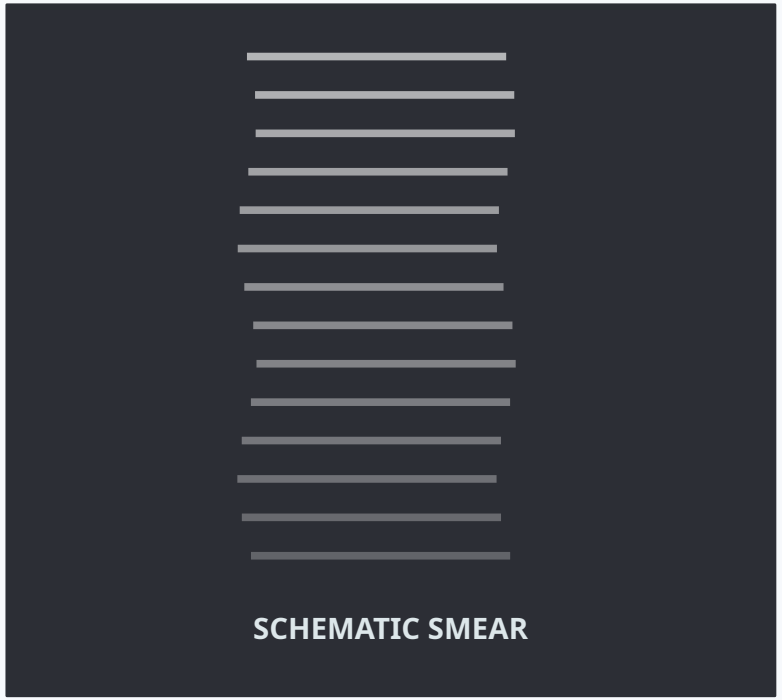
Primer sequences may match more than one region.

Reaction conditions

Excess Mg^{2+} or excessive cycling can increase non-specific products.

Optimization goal one dominant amplicon at the expected size

Problem: smear or weak product



- Degraded DNA or poor template quality
- Too much template
- Non-specific amplification
- Suboptimal electrophoresis conditions

Interpret the whole workflow: PCR and gel conditions can both contribute.

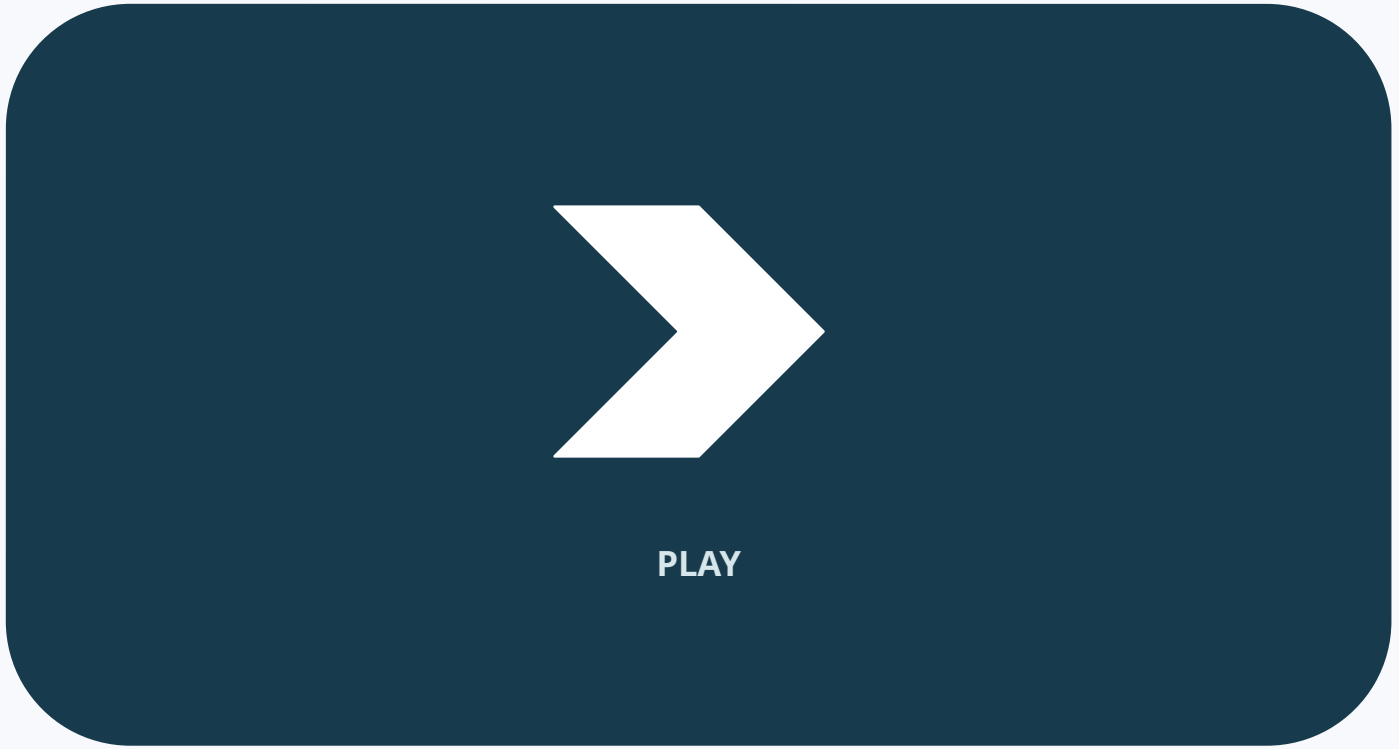
A systematic troubleshooting sequence

1. Did the positive control work?
2. Is the NTC clean?
3. Is the band at the expected size?
4. Is the product specific?
5. Is template DNA suitable?
6. Are primer and cycling conditions appropriate?

Troubleshooting should follow evidence, not random changes to the protocol.

Video checkpoint — Overview of PCR

Recommended short animation: New England Biolabs (NEB).



NEB — Overview of PCR

Use the video after students know the three cycle steps.

Ask them to identify:

- denaturation
- annealing
- extension
- role of primers
- role of polymerase

Take-home messages

PCR

selectively amplifies a chosen DNA region

Primers

define the target and provide 3′-OH ends

Polymerase

synthesizes new DNA 5′→3′

Cycle

denaturation → annealing → extension

Controls

are required for valid interpretation

Next step

analyze PCR products by gel electrophoresis