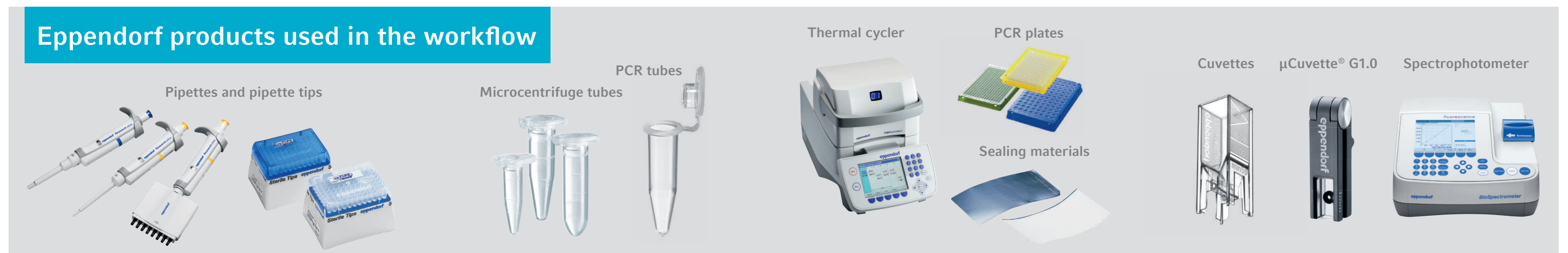


Getting the Most Out of Your PCR

A best practice and optimization guide



Your PCR workflow ...

1

DNA concentration
Measure your DNA (gDNA/ cDNA) for concentration and purity.
Ideal DNA purity range (A_{260}/A_{280}) = ~1.7 – 2.0

2

$M_1 V_1 = M_2 V_2$

Stock concentration Stock volume Final concentration Final volume

Calculate concentration
Calculate according to desired reaction volume.

3

Mastermix
Prepare a mastermix of all common components in 1 tube. Remember to always use suitable controls (positive, negative, no template, etc.).

4

Mix
Mix well to ensure homogeneous distribution of components to minimize well-to-well variation.
(See Eppendorf Application Note 130 for best mixing guide.)

5

Briefly spin down (short-spin)
Short spin can reduce contamination by pulling down the liquid from the rim of the tube.

6

Distribute mastermix
Hold pipette vertically during pipetting. Always dispense to the bottom of the tube.

7

- > Set cycler modes to match reaction volume (fast, standard, safe) for optimal heat transfer
- > **Denaturation:** temperature depends on enzyme; holding time depends on enzyme, DNA source and GC content
- > **Annealing:** temperature generally ~5-10°C below primer melting temperature. Long holding time not necessary.
- > **Extension:** optimal temperature for polymerase activity (e.g. Taq = 72°C. Incorporation rate of Taq: ~1000 bases/ min).

... and tips & tricks for some steps

1

- > Take note of the absorbance reading (not just concentration values). Aim for 0.1 - 1.0 A for reliable reading according to the Beer-Lambert Law.

2

- > Aliquot stock solutions to prevent multiple freeze-thawing events that can affect reproducibility of PCR.
- > Smaller reaction volume saves reagents and costs, but when working in small volumes it is important to protect against evaporation during PCR.
- > Use low retention tips or low binding tubes to minimize sample and reagent loss.

3

- > Prepare each mastermix in a single tube to prevent pipetting variation
- > Use bigger tube sizes when preparing mastermixes: (e.g. 1 x 5 mL tube is better than 2 x 2 mL tubes).

Use PCR clean consumables that are certified:

- > Human DNA-free
- > RNase-free
- > DNase-free
- > Free of PCR inhibitors

- > Use a dedicated set of pipettes for PCR.
- > Use the appropriate tips for the pipettes.
- > Calibrate the pipettes at least once a year to ensure accuracy and precision.
- > Prevent aerosol contamination by using either positive displacement pipette or filtered tips.

7

Always take note of ramp rates when:

- > Transferring protocols between different cyclers.
- > Transferring from optimization protocol to standard protocol.
- > Set cycler temperature modes to match reaction volume (e.g. safe, fast, standard in Eppendorf cyclers).

Troubleshooting your PCR

Problems	Possible solutions
1. Non-specific amplifications	<ol style="list-style-type: none"> Use Hot-start strategies: <ol style="list-style-type: none"> Manual hot-start Use devices with thermal sample protection (TSP) lid Use devices with "Impulse PCR" function Use hot-start reagents For new primers, run optimization with single-primer (e.g. forward primer only) controls to determine non-specificity Alternative strategies: <ol style="list-style-type: none"> Mg²⁺ titration (concentration optimization) Touchdown PCR
2. No / low amplifications	<ol style="list-style-type: none"> Optimize denaturation and/or annealing temperature with gradient function Use PCR enhancers (e.g. DMSO, BSA). These require empirical testing for each combination of template and primer