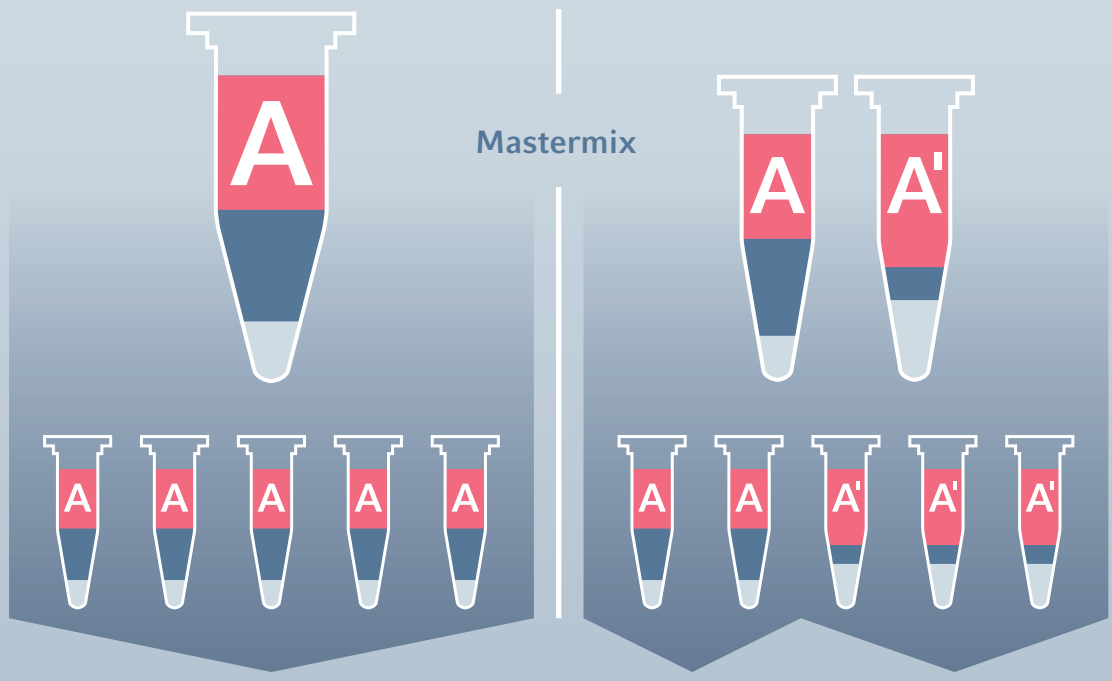
Stay Informed

Reproducible DNA Amplification in PCR

Nowadays, PCR can be robust and easy—but you can still run into difficulties. Make sure to get the most out of your PCR with some simple tricks.



1 Measure your DNA concentration

Determine the concentration and purity of your DNA. Ideal DNA purity range (A_{260}/A_{280}) = ~1.7–2.0

Take note of the absorbance reading (not just concentration values). It should be between 0.1–1.0 A for reliable reading in accordance with the Beer-Lambert Law.

2 Preparation of Mastermix

Prepare the mastermix in only one tube to prevent pipetting variations that can occur from preparing multiple mastermixes. Use a tube large enough (e.g. 5 mL) to sufficiently hold the entire volume of the mastermix.

Aliqout immediately afterwards to avoid multiple-thawing that can have a negative impact on the reproducibility of your PCR.

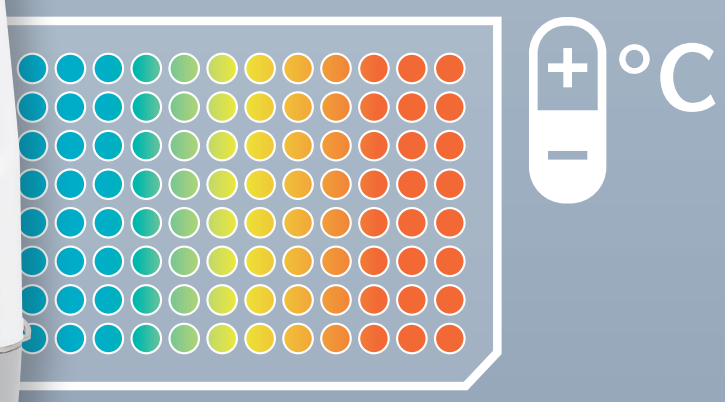
3 In case of non-specific amplifications

Use Hot-start protocols. Make sure your cycler is properly calibrated and reaches the designated temperatures quickly during the run.

For new primers, run optimization with a single primer (i.e. forward or reverse primer only) controls to determine non-specificity

Titrate Mg^{2+} to optimize the concentrations for your PCR reaction.

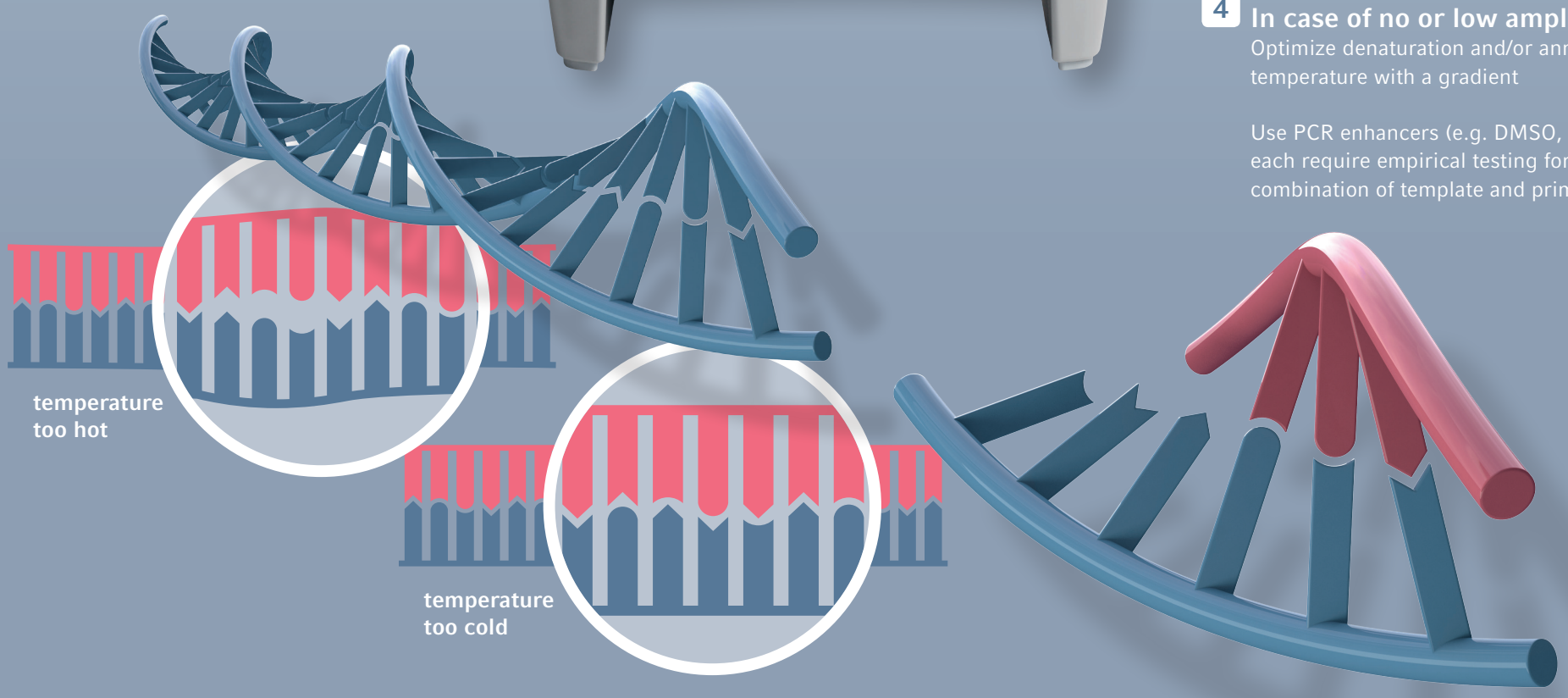
12
Mg
MAGNESIUM
24.305



4 In case of no or low amplification

Optimize denaturation and/or annealing temperature with a gradient

Use PCR enhancers (e.g. DMSO, BSA, etc.) each require empirical testing for the specific combination of template and primer



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