Getting the Most Out of Your PCR

A best practice and optimization guide

Eppendorf products used in the workflow

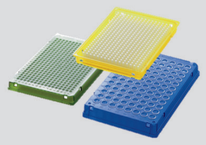
Pipettes and pipette tips

Microcentrifuge tubes

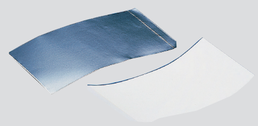
PCR tubes

Thermal cycler

PCR plates



Sealing materials



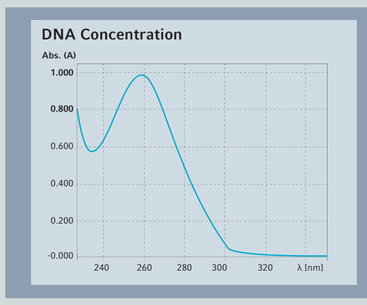
Cuvettes

µCuvette® G1.0

Spectrophotometer

Your PCR workflow ...

1



DNA concentration

Measure your DNA (gDNA/ cDNA) for concentration and purity.
Ideal DNA purity range $(A_{260}/A_{280}) = \sim 1.7 - 2.0$

2

$M_1V_1 = M_2V_2$

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

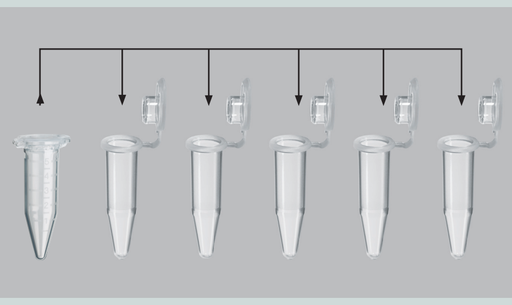
Mix well to ensure homogeneous distribution of components to minimize well-to-well variation.
(*See Eppendorf Application Note 130 for best mixing guide.*)

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
> DNase-free
> RNase-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	1. Use Hot-start strategies: a) Manual hot-start b) Use devices with thermal sample protection (TSP) lid c) Use devices with "Impulse PCR" function d) Use hot-start reagents 2. For new primers, run optimization with single-primer (e.g. forward primer only) controls to determine non-specificity 3. Alternative strategies: a) Mg2+ titration (concentration optimization) b) Touchdown PCR
2. No / low amplifications	1. Optimize denaturation and/or annealing temperature with gradient function 2. Use PCR enhancers (e.g. DMSO, BSA). These require empirical testing for each combination of template and primer

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