Optimizing Tm and Annealing

Important instructions on annealing temperature

When using Phusion, Phire, DyNAzyme or DyNAmo polymerases or kits, we recommend calculating primer Tm with the modified Breslauer's method[1]. To determine the annealing temperature for the actual PCR run, please see the table below. A few notes about primer design are also included.

Phusion High-Fidelity DNA Polymerase

Primer concentration (nM)	Use the actual primer concentration in the calculation. Recommendation for Phusion DNA Polymerase is 500 nM, but it can be varied between 200 nM–1000 nM.
Salt (mM)	Always use the default 50 mM salt concentration in the calculation.
Notes	• For primers max 20 nt use the lower Tm given by the calculator for annealing. • For primers >20 nt use an annealing temperature 3°C higher than the lower Tm given by the calculator. Example: Tm's given by the calculator are 66.5°C and 65.0°C => Use an annealing temperature of 68.0°C in the actual run. • With Phusion Hot Start DNA Polymerase, use primers with Tm 60°C or higher. • With Phusion Hot Start II DNA Polymerase and all non-hot start Phusion DNA Polymerases primers with lower Tm can also be used. • With Phusion Flash DNA Polymerase and Phusion Blood DNA Polymerase, do not perform annealing below 50°C. • If the amplification fails with the recommended annealing temperature, use a temperature gradient to optimize the annealing. The annealing gradient should range from the original annealing temperature to the extension temperature (two-step PCR). • If high DMSO concentration is used, the annealing temperature determined by the guidelines above must be lowered, as DMSO decreases the melting point of the primers. It has been reported that 10% DMSO decreases the melting temperature by 5.5–6.0°C.[2]

Phire Hot Start DNA Polymerase

DyNAzyme I, DyNAzyme II and DyNAzyme EXT DNA Polymerases

DyNAmo SYBR Green qPCR kits

DyNAmo Probe qPCR Kits

References

1. **Breslauer KJ, Frank R, Blöcker H, Marky LA (1986) *Proc Nat Acad Sci* 83:3746–3750.**
2. **Chester N, Marshak DR (1993) *Analytical Biochemistry* 209: 284–290.**

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