Factors Influencing the Tm of Nucleic Acid Hybrids

Factor	Influence on Tm
Ionic strength	Tm increases 16.6°C for each one-fold increase in monovalent cations, between 0.01 and 0.40 M NaCl.
Base composition	AT base pairs are less stable than GC base pairs in aqueous solutions containing NaCl. The difference is negligible in tetramethyl ammonium chloride.
Destabilizing agents	Each 1% formamide reduces the Tm by ~0.6°C. 6M urea reduces the Tm by about 30°C.
Mismatched base pairs	Tm is reduced by 10°C for each 1% of mismatch.
Duplex length	Negligible effect with probes > 500 bp.