INTEGRATED DNA TECHNOLOGIES

Calculation of T_m for Oligonucleotide Duplexes
Quick Look

This is a modified, quick look version of the full Technical Report "Calculation of Tm for Oligonucleotide Duplexes." Please see the full version for a more comprehensive explanation.

The melting temperature (T_m) of an oligonucleotide duplex refers to the temperature at which the oligonucleotide is 50% annealed to its complement. This means that 50% of the molecules are single-stranded (SS) while 50% of the molecules are in the double-stranded (DS) form. In the absence of destabilizing agents such as urea or formamide, the T_m of an oligonucleotide will depend on three major factors:

- Oligonucleotide concentration (C_1) – high DNA concentrations favor duplex formation and increase T_m.
- Salt concentration – higher ionic concentrations of the solvent leads to increases in T_m due to the stabilizing effects that cations have on DNA duplex formation.
- Oligonucleotide sequence – generally, sequences with a higher fraction of G-C base pairs have a higher T_m than do AT-rich sequences.

IDT's online Oligo Analyzer estimates T_m from the nearest-neighbor two-state model, which is applicable to short DNA duplexes. Assuming that the concentration of the oligonucleotide probe is much higher than the concentration of the DNA target, the following thermodynamic relationship can be used to predict T_m:

$$T_m\,(\text{Kelvin}) = \frac{\Delta H^o}{\Delta S^o + R\ln C_t}$$

Where ΔH^o is the change in standard enthalpy and ΔS^o is the change in entropy associated with duplex formation in 1M Na^+ solution. R is the ideal gas constant (1.987 $cal.K^{-1}.mole^{-1}$) and C_t is the molar concentration of the oligonucleotide probe.

Although the linear T_m correction for salt concentration has been typically used in the past, scientists at IDT performed a large scale study and determined that this linear function is inaccurate. IDT showed that T_m depends on both the monovalent (Na^+, K^+) and the divalent (Mg^{2+}) salt concentration of the solvent. Oligo Analyzer employs the improved T_m salt correction function [1].

$$\frac{1}{T_m(Mg^{2+})} = \frac{1}{T_m(1M\ Na^+)} + a + b\ln[Mg^{2+}] + f_{GC}\times(c + d\ln[Mg^{2+}])$$
$$+ \frac{1}{2(N_{bp}-1)}\times\left[e + f\ln[Mg^{2+}] + g(\ln[Mg^{2+}])^2\right]$$

Table 1. Parameters for Equation Above in Reciprocal Kelvins.

parameter	value (K^{-1})	standard error (K^{-1})
a	3.92×10^{-5}	0.2×10^{-5}
b	-9.11×10^{-6}	0.5×10^{-6}
c	6.26×10^{-5}	0.4×10^{-5}
d	1.42×10^{-5}	0.08×10^{-5}
e	-4.82×10^{-4}	0.7×10^{-4}
f	5.25×10^{-4}	0.2×10^{-4}
g	8.31×10^{-5}	0.2×10^{-5}

References

1. Owczarzy R, Moreira BG, et al. (2008) Predicting stability of DNA duplexes in solutions containing magnesium and monovalent cations. Biochemistry, 47(19): 5336-5353.