

T_m Calculations

Wallace Method (for oligos < 18mers): $T_m = 2 \times (A + T) + 4 \times (G + C)$

%GC Method: $T_m = 81.5 + 16.6 (\log_{10}[\text{Na}^+] + 0.41[\%GC] - [625/N])$

N = length of oligo

Nearest neighbor (1) $T_m = (\Delta H - 3.4 \text{ kcal}) / ((A + \Delta S) + (R \ln(C/4))) - 273.15 + 16.6 \log_{10}[\text{salt}]$

ΔH is the sum of nearest neighbor enthalpy changes

A is the initiation constant of -10.8 cal/K⁰ mole for non-self complementary sequences, -12.4 cal/K⁰ mole for complementary sequences

ΔS is the sum of nearest neighbor entropy changes

R is the gas constant 1.987 cal/K⁰ mole

C is the concentration of oligonucleotide (generally fixed at 250 pM) (2)

References:

1. Breslauer, K.J. *et al.* (1986) *Proc. Natl. Acad. Sci. USA.* **83**: 3746-3750.
2. Rychlik, W. *et al.* (1990) *Nucleic Acids Res.* **18**: 6409-6412.