*Tm Tool v1.0b*

*DNA.UTAH.EDU*

# *Software Methods and Equations*

***V1.3***

UNIVERSITY OF UTAH
HEALTH SCIENCES

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# METHODS

## 0.1 $T_M$

$T_m$ is the melting temperature of a DNA duplex in a thermodynamically closed system. This allows the use of thermodynamic parameters to define it. We start with two different definitions of Gibbs free energy.

$$\Delta G = \Delta H - T\Delta S$$

$$\Delta G = -RTlnK$$

## 0.2 SUBSTITUTION OF ΔG

$$-RTlnK = \Delta H - T\Delta S$$

$$\therefore T = \frac{\Delta H}{\Delta S - RlnK}$$

## 0.3 ΔH AND ΔS

The ΔH and ΔS terms are sums of nearest neighbor thermodynamic parameters. The most common set is Santalucia-Hicks (2004), but many others exist.

## 0.4 K

K is the equilibrium constant. For DNA there are two strands that are in the single strand (A, B) or double strand (AB) state.

$$A + B \rightleftharpoons AB$$

$$K = \frac{[AB]}{[A][B]}$$

Substitute K

$$T = \frac{\Delta H}{\Delta S - Rln\left(\frac{[AB]}{[A][B]}\right)} = \frac{\Delta H}{\Delta S + Rln\left(\frac{[A][B]}{[AB]}\right)}$$

## 0.5 CONCENTRATION FOR DIFFERENT CALCULATION TYPES

#1 $T_m$ is defined as 50% conversion to the double strand state, where the '50' subscript indicates 50% of the maximum possible concentration.

*What is* $\overline{\left(\frac{[A_{50}][B_{50}]}{[AB_{50}]}\right)}$ *?*

If [A] ≥ [B], then the maximum concentration of the duplex that can be formed is [B]/2.

$$\left(\frac{[A_{50}][B_{50}]}{[AB_{50}]}\right) = \frac{\left([A]-\frac{[B]}{2}\right)\left(\frac{C[B]}{2}\right)}{\left(\frac{C_B}{2}\right)} = [A]-\frac{[B]}{2}$$

When [A]=[B]:

$$\left(\frac{[A_{50}][B_{50}]}{[AB_{50}]}\right) = \frac{[A]}{2}$$

This is equivalent to ( [A] + [B ]) / 2, sometimes written as ( Ct / 4 ) in other publications.

#2 For Primer $T_m$ at the beginning of PCR (no probe):

$$[A]-\frac{[B]}{2} = [primer]-\frac{[template]}{2}$$

$$[primer] \gg [template]$$

$$= [primer]$$

#3 For Probe $T_m$ at the end of PCR, we need to first estimate the concentration of the available product strand complementary to the probe. This is difficult, because it varies according to the experiment performed. As a default we use 60% of the excess primer remaining after duplex product formation. That is:

$$Available\ product = 0.6([excess\ primer]-[limiting\ primer])$$

If $0.6([excess\ primer]-[limiting\ primer]) > [probe]$:

$$[A]-\frac{[B]}{2} = 0.6([excess\ primer]-[limiting\ primer])-\frac{[probe]}{2}$$

If $[probe] > ([excess\ primer]-[limiting\ primer])$:

$$[A]-\frac{[B]}{2} = [probe]-\frac{0.6([excess\ primer]-[limiting\ primer])}{2}$$

## 0.6 SALT CORRECTION

#1 ΔS is adjusted with respect to salt in the buffer solution when predicting $T_m$. Monovalent cations [$Mono^+$] and magnesium cations [$Mg^{++}$] will affect ΔS and thus modify $T_m$. The common salt correction published in Santalucia 2004 is as follows (N is the number of bases of the sequence):

$$(0.368)(N-1)\ln\left([mono^+] + 3.795\sqrt{[free\ Mg^{++}]}\right)$$

#2 Owczarzy et. al. (2008) have published their own salt correction that is dependent on $\frac{\sqrt{Mg^{++}}}{Mono^+}$ with 3 possible cases: A, B, & C.

**A)** $0.22 < \frac{\sqrt{Mg^{++}}}{Mono^+} < 6.0$

The following equation is used:

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

Coefficients are used for *b*, *c*, *e*, *f*:

| | |
|---|---|
| *b* | $-9.11 \times 10^{-6}$ |
| *c* | $6.26 \times 10^{-5}$ |
| *e* | $-4.82 \times 10^{-4}$ |
| *f* | $5.25 \times 10^{-4}$ |

Where the parameters *a*, *d*, *g* are defined as:

$$a = 3.92 \times 10^{-5}(0.843 - 0.352\sqrt{[Mon^+]} \times \ln[Mon^+])$$

$$d = 1.42 \times 10^{-5}[1.279 - 4.03 \times 10^{-3}\ln[Mon^+] - 8.03 \times 10^{-3}(\ln[Mon^+])^2]$$

$$g = 8.31 \times 10^{-5}[0.486 - 0.258\ln[Mon^+] + 5.25 \times 10^{-3}(\ln[Mon^+])^3]$$

**B)** $\frac{\sqrt{Mg^{++}}}{Mono^{+}}$ > 6.0 or [*Mono*$^{+}$] = 0.0

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

With parameters as follows:

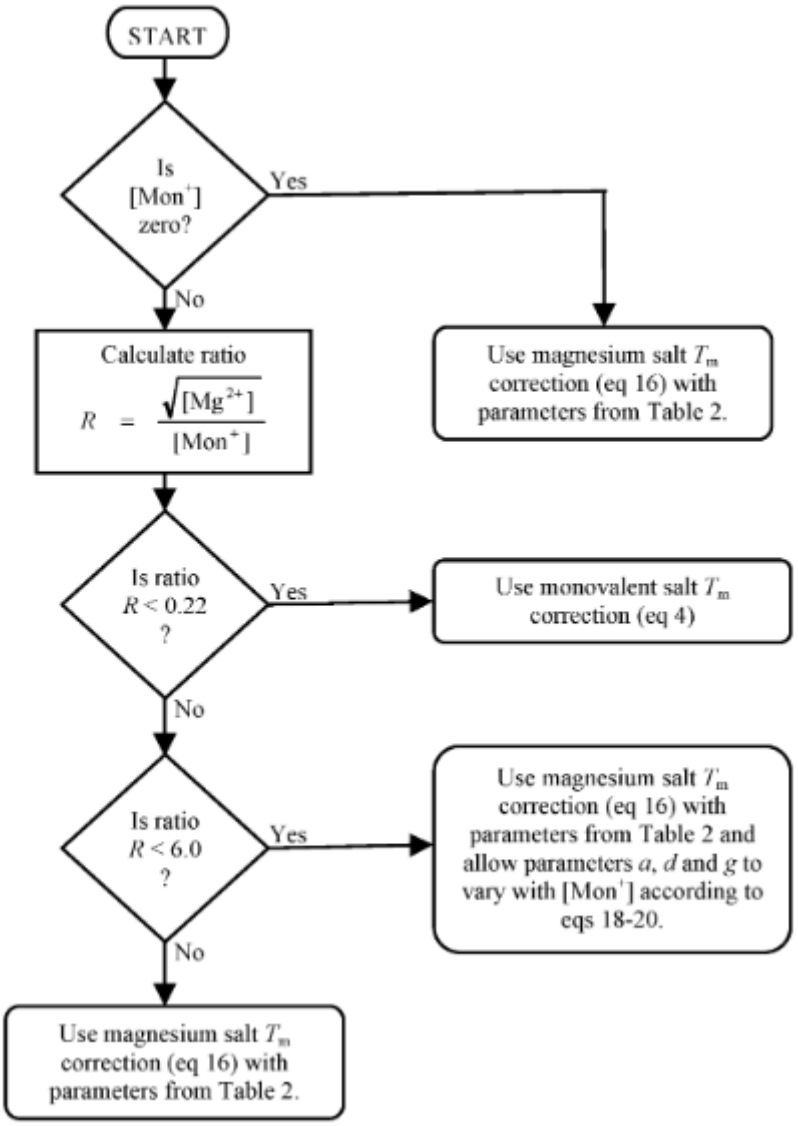
| | |
|---|---|
| $a$ | $3.92 \times 10^{-5}$ |
| $b$ | $-9.11 \times 10^{-6}$ |
| $c$ | $6.26 \times 10^{-5}$ |
| $d$ | $1.42 \times 10^{-5}$ |
| $e$ | $-4.82 \times 10^{-4}$ |
| $f$ | $5.25 \times 10^{-4}$ |
| $g$ | $8.31 \times 10^{-5}$ |

**C)** $\frac{\sqrt{Mg^{++}}}{Mono^{+}}$ < 0.22

The following is equation used:

$$\frac{1}{T_m(\mathrm{Mon}^{+})} = \frac{1}{T_m(1\ M\ \mathrm{Na}^{+})} + (4.29 f_{GC} - 3.95) \times 10^{-5}\ \ln[\mathrm{Mon}^{+}] + 9.40 \times 10^{-6}(\ln[\mathrm{Mon}^{+}])^2$$

The published work (cited in references) also includes a useful flowchart to easily illustrated the 3 cases and which equation is most applicable to the reader:



# 1 FULL $T_M$ EQUATION

Units:
ΔH in kcal/mole
ΔS in cal / (K * mole)
R (gas constant) as 1.9872 cal/K•mol
Salt Correction as cal/mol
-273.15 as K to ˚C conversion.
[A] and [B] in mol with [A] greater or equal to [B]
$T_m$ in ˚C

$$T_m = \frac{\Delta H \bullet 1000}{\Delta S + (salt\ correction) + 1.9872 \ln\left([A] - \frac{[B]}{2}\right)} - 273.15$$

# 2 REFERENCES

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