INTEGRATED DNA TECHNOLOGIES

Calculation of T_m for Oligonucleotide Duplexes

Contents

1. Introduction

The melting temperature of an oligonucleotide duplex (T_m) refers to the temperature at which the oligonucleotide is 50% annealed to its exact complement. Due to the cooperativity seen in DNA hybridization and melting this means that, at the melting temperature, 50% of the molecules are single-stranded (SS) while 50% of the molecules are in the double-stranded (DS) form. This simple two-state model seems to hold true for most short oligonucleotide sequences where partially melted duplexes are present in negligible amounts. . Accurate estimation of the T_m of an oligonucleotide probe-target duplex is important for a wide variety of applications including PCR, hybridization, sequencing, and antisense/RNAi applications.

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2. Factors Influencing Melting Temperature

Melting temperature is the key parameter for designing oligonucleotides. Inaccurate prediction of T_m will increase the probability of failed assay design. The IDT website has a number of free software tools that can predict T_m values from oligonucleotide sequence and solvent composition. Melting temperature is commonly misunderstood to be a property solely of the oligonucleotide sequence and not to vary with experimental conditions. However, melting temperature depends on the base sequence as well as the oligonucleotide concentration and presence of cations in the buffer – specifically on both the monovalent (Na^+) and the divalent (Mg^{2+}) salt concentrations (Figure 1). For this reason, the melting temperature for specific experimental situations should be calculated using IDT SciTools available on IDT web site (www.idtdna.com/scitools/scitools.aspx). (Please use reference 11 to cite IDT SciTools in your published work.)

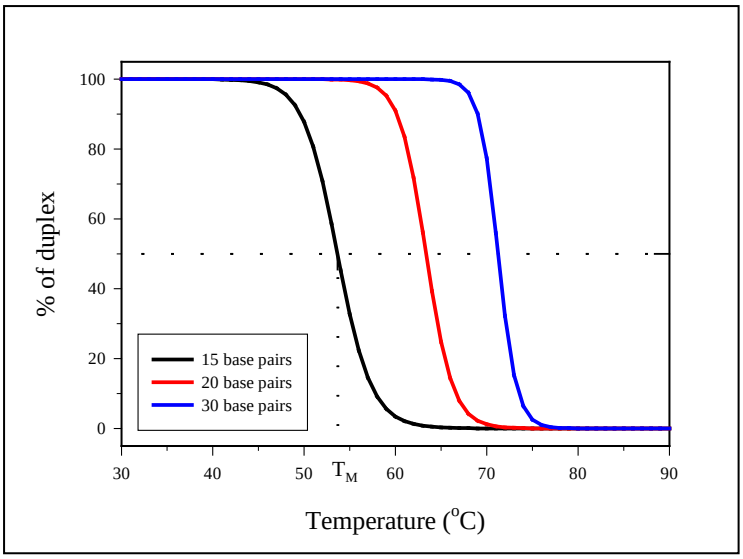

Figure 1. Effect of oligonucleotide length on melting profile. Melting profiles were plotted for oligonucleotides of 3 different lengths, each with ~50% GC content, and resuspended in a typical PCR buffer (1 mM Mg^{2+}, 50 mM KCl, 10 mM Tris, pH 8.3).

In the absence of destabilizing agents, such as urea or formamide, the T_m of an oligonucleotide will depend upon three major factors:

1. Oligonucleotide concentration (C_t): High DNA concentrations favor duplex formation which will increase the T_m.

2. Salt concentration: Higher ionic concentrations of the solvent will increase T_m due to the stabilizing effects that cations have on DNA duplex formation. More cations bind to duplex DNA than to the component single strands. Different cations may have different effects on T_m. IDT scientists have found that both Na^+ and Mg^{2+} have effects on oligonucleotide duplex stability.

3. Oligonucleotide sequence: Generally, sequences with a higher fraction of GC base pairs have a higher T_m than do AT-rich sequences. However, the T_m of an oligonucleotide is not simply the sum of AT and GC base content. Base stacking interactions must also be taken into account, so the actual specific sequence must be known to accurately predict T_m. The effects of neighboring bases as contributed through base stacking are called "nearest neighbor effects" and are mathematically accounted for using experimentally determined nearest neighbor (NN) thermodynamic parameters.

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3. Determining Melting Temperature

For oligonucleotides of the length ranges used today (6-60 base pairs), the best method to estimate the T_m of an oligonucleotide probe-target duplex takes into account all the above factors, including nearest-neighbor interactions [1-6], salt concentration, and oligonucleotide concentration. Assuming that the concentration of the oligonucleotide probe is much higher

than concentration of 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 a 1 M Na^+ solution. R is the ideal gas constant (1.987 $cal.K^{-1}.mole^{-1}$) and C_t is the molar concentration of oligonucleotide probe.

In recent years, predictive algorithms have been significantly improved; the latest nearest-neighbor method predicts T_m with a higher degree of accuracy than previously used methods [7]. Older formulas did not take interactions between neighboring base pairs into account and so did not provide T_m predictions that are accurate enough for design of some applications, such as real-time PCR. IDT scientists have published experimental studies on the effects of Na^+, K^+, and Mg^{2+} on oligonucleotide duplex stability and have proposed a model with increased accuracy [8,9]. Although the linear T_m correction has been used in the past to account for salt stabilizing effects, melting data of a large oligonucleotide set demonstrated that non-linear effects are substantial and must be considered [8,9] (Figure 2).

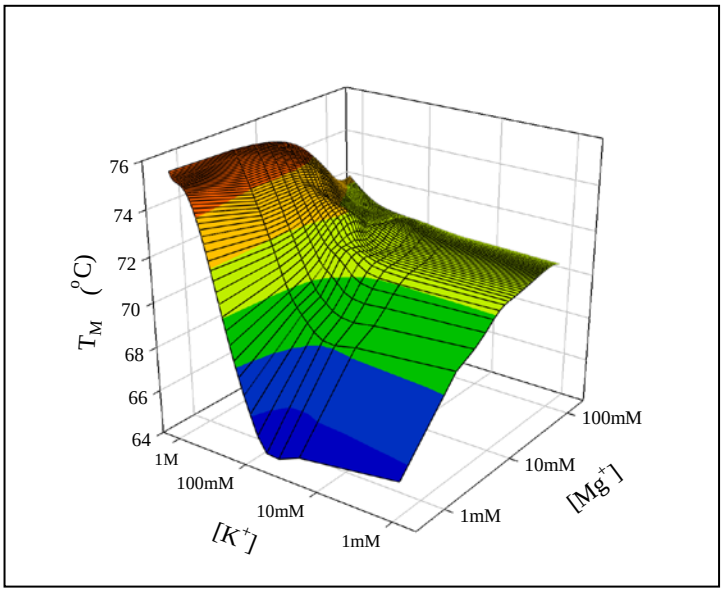

Figure 2. Ion concentration affects oligonucleotide stability. The stability of a 25 base pair long duplex (CTG GTC TGG ATC TGA GAA CTT CAG G) varies with K^+ and Mg^{2+} concentrations. Competitive binding of these cations to DNA is observed.

IDT scientists showed that T_m depends on both the monovalent (Na^+, K^+) and the divalent (Mg^{2+}) salt concentration of the solvent. The OligoAnalyzer tool on the IDT website employs the improved T_m salt correction function [9].

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

Table 1. Parameters for above equation 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}

Multivariate linear regression was used to fit these seven parameters in one step. The experimental data set consisted of 680 T_m values for 92 unique duplex DNAs measured in various Mg^{2+} concentrations. Table 1 presents the set of empirically derived parameters and their errors.
PCR buffers also contain deoxynucleoside triphosphates (dNTPs), which bind magnesium ions (Mg^{2+}) with much higher affinity than DNA. Since they decrease free Mg^{2+} activity, the T_m may be also decreased [9] (Figure 2). The best predictive algorithm considers this effect as well.

IDT SciTools [11] uses nearest-neighbor parameters obtained from the most currently available publications [2,3,5,6,10]. T_M calculations for oligonucleotides containing non-consecutive LNA nucleotides hybridized to a DNA template use LNA energetic parameters from McTigue [6]. Consecutive LNA nucleotides on a DNA template use parameters based on IDT research data not yet published. LNA modifications on an RNA template are approximated because nearest-neighbor parameters for these specific combinations are not yet available.

4. References

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