Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Physical Parameters & Oligo Analysis

For research use only. Not for use in diagnostic procedures for clinical purposes.

The Custom Oligonucleotide Synthesis (COS) Report accompanying each custom oligonucleotide contains extensive thermodynamic and physical data for each oligo. This technical sheet explains each of the values and the method used for arriving at a particular physical data. The figures may differ for the same oligo sequence by using different software applications, the reason being the selection of details and constants for arriving at the calculated value. An example is the salt concentration for calculating Tm; Gene Link software uses 50 mM monovalent (Na^+ and K^+) salt concentration as a default, which reasonably approximates the total monovalent salt concentration commonly used in PCR reactions.

Gene Link has an oligo analysis calculator at this link. The algorithm at this link is revised periodically and is more current as compared to this report.
Copy and paste the following sequence to familiarize the various calculations and analysis parameters.
Example: [BHQ-1-5][Acrd]TGGCTGGCC[Sp18]CTGGGATG[mC][Ps][mU][Ps][mG][Ps][SpC3-3]

1. Size

The size is the length of the oligo. For unmodified oligos, this is equal to the number of bases in the oligonucleotide sequence. For modified bases, including 3'- and 5'-modifications. The software counts bases and modified bases (examples; inosine, dU, amino C6 dT, Fam-dT, etc.) and does not count non-base modifications (examples; quenchers, fluorophores, amino linkers, spacers, etc.).

2. MW

MW is the molecular weight of the oligonucleotide. The molecular weight of a substance is the mass of one atom or molecule of that substance, in atomic mass units or Daltons (1 Dalton = exactly 1/12th the mass of an atom of Carbon-12). One mole (6.02 x 10^{23} particles) of a substance (the molar mass) has a mass in grams equal to its molecular weight.

The molecular weight of an oligonucleotide is equal to the sum of the molecular weights of the individual ribo/deoxyribonucleotides, plus any modifications. For nucleotides, these values are as follows:

Deoxyribonucleotide	Molecular Wt (Da)	Ribonucleotide	Molecular Wt (Da)
dA	313.20	rA	329.20
dC	289.19	rC	305.19
dG	329.21	rG	345.21
dT	304.20	rU	306.17
dI	314.20	rI	330.20

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Using these values, the MW of a 20mer DNA oligo with the following sequence:

5'-GAAAGCGTCTAGCCATGGCG-3'

is (5 x MW_A) + (7 x MW_G) + (5 x MW_C) + (3 x MW_T) = (5 x 313.20) + (7 x 329.21) + (5 x 289.19) + (3 x 304.20) = 1566.00 + 2304.47 + 1445.95 + 912.60 = **6229.02 Da**. The 5'-terminal base has hydroxyl (OH) and does not have a phosphate and subtracting the mw of 62 for phosphate will yield a mw of **6167.02**

3. %GC

The %GC is the percentage of G+C in the oligo. The calculation is the total number of G and C divided by the total number of bases in the oligo.

4. Oligonucleotide Melting Temperature (Tm)

The melting temperature (Tm) of an oligonucleotide duplex is defined as the temperature at which exactly 50% of the double-helix is denatured into single-stranded form. The value of Tm provides a good estimation of the stability of a particular duplex, with a higher Tm implying greater stability. Using Tm to reliably estimate the stability of an oligonucleotide primer-template duplex is critically important for optimizing the reaction conditions (especially the annealing temperature) used in a variety of molecular biology applications, such as PCR, DNA sequencing, DNA/RNA target detection using labeled probes, and RNAi/anti-sense experiments. For oligonucleotides $\leq$ 60 bases long, we calculate Tm using the salt-corrected nearest neighbor method (see Appendix for the method used prior to July 21, 2009). The utility of the nearest-neighbor method for calculating the Tm of oligonucleotides has been firmly established by several research groups (Breslauer *et al.*, 1986; Sugimoto *et al.*,
1995, 1996; Xia *et al.*, 1998; Santa-Lucia *et al.*, 1998). In combination with the recently published salt correction equations, which adjust nearest-neighbor Tm values based on the concentration of monovalent and divalent ions present (Owczarzy *et al.*, 2004, 2008), the resulting salt-corrected nearest-neighbor method is currently the best available for estimating Tm for oligonucleotides within this size range.

For oligonucleotides, the nearest neighbor formula for Tm is the following:

$$Tm(^{o}C) = \frac{\Delta H^0}{\Delta S^0 + R \ln C} - 273.15$$

where

R: ideal gas constant = 0.0019865 kcal/mol K

C: the concentration of oligonucleotide primer or probe strand (mol/L)

ΔH^0 (kcal/mole): ΔH^0 is the change in enthalpy, that is, the change in the amount of heat energy contained in a chemical or physical system due to a change (such as a chemical reaction or melting) in the system. Here ΔH^0 is calculated by summing together the enthalpy values of each of the dinucleotide nearest neighbor base pairs, and including the values for solvent-terminal base end effects and symmetry effects.

ΔS^0 (kcal/mole): ΔS^0 is the change in entropy, that is, the change in the amount of disorder a chemical or physical system exhibits due to a change in the system. Here ΔS^0 is calculated by summing together the

entropy values of each of the dinucleotide nearest neighbor base pairs and including values for solvent-terminal base end effects and symmetry effects.

For DNA oligos, we use the dinucleotide nearest-neighbor thermodynamic values reported by Allawi and SantaLucia (1997). For RNA oligos, we use those reported by Xia *et al.* (1998).

Salt Correction (°C): The nearest neighbor Tm value needs to be corrected for the actual concentration of salt present in the reaction mixture. This is because the nearest neighbor parameters for ΔH^0 and ΔS^0 were obtained from DNA melting experiments performed in 1M Na^+ buffer. This salt correction accounts for the effect on Tm of different concentrations of free monovalent (Owczarzy *et al.*, 2004) and Mg^{2+} (Owczarzy *et al.*, 2008) cations in solution. The nature of the effect is governed by the ratio R,

$$R = \frac{\sqrt{[Mg^{2+}]}}{[Na^+]}$$

where $[Mg^{2+}]$ and $[Na^+]$ are the concentrations of Mg^{2+} and Na^+, respectively.

If R < 0.22, monovalent cations exert a dominant effect on Tm. For this case, the following monovalent cation salt correction equation is used:

$$\frac{1}{Tm(\mathrm{Na^+})} = \frac{1}{Tm(1\mathrm{M\ Na^+})} + [(4.29 f_{GC} - 3.95)\, ln[\mathrm{Na^+}] + 0.940\, ln^2[\mathrm{Na^+}]] x 10^{-5}$$

where f_{GC} is the fraction of GC base pairs in the oligonucleotide.

If 0.22 < R < 6.0, both monovalent and divalent cations affect the Tm, and so the divalent cation salt correction equation is used (Owczarzy, R. et al., *Biochemistry*, **47**, 5336):

$$\frac{1}{Tm(\mathrm{Mg^{2+}})} = \frac{1}{Tm(1\mathrm{M\ Na^+})} + [(a - 0.911\, ln[\mathrm{Mg^{2+}}]) + (f_{GC} \times (6.26 + d\, ln[\mathrm{Mg^{2+}}]))$$
$$+ \frac{1}{2(Nbp - 1)}(-48.2 + 52.5\, ln[\mathrm{Mg^{2+}}] + g\, ln^2[\mathrm{Mg^{2+}}])] \times 10^{-5}$$

where N_{bp} is the number of base pairs and the three parameters *a*, *d*, and *g* show the following dependence on Na^+ concentration:

$$a = 3.92(0.843 - 0.352\sqrt{[\mathrm{Na^+}]} \times \ln[\mathrm{Na^+}])$$
$$d = 1.42(1.279 - 0.00403\, ln[\mathrm{Na^+}] - 0.00803\, ln^2[\mathrm{Na^+}])$$
$$g = 8.31(0.486 - 0.258\, ln[\mathrm{Na^+}] + 0.00525\, ln^3[\mathrm{Na^+}])$$

If R ≥ 6.0, divalent cations exert a dominant effect on Tm, and so the divalent cation salt correction equation shown above is used, but with the three parameters *a*, *d*, and *g* having the constant values 3.92, 1.42, and 8.31, respectively.

For our COS Report, we use the following default values for calculating the reported oligonucleotide Tm:

C = 0.25 μM

$[Na^+]$ = 50. mM

$[Mg^{2+}]$ = 0 μM

These values were chosen because they are fairly common primer and sodium ion concentrations used in routine PCR reactions. Using these values, the calculated Tm of a 20mer DNA oligo with the following sequence:

5'-GAAAGCGTCTAGCCATGGCG-3'

is

Salt-corrected nearest-neighbor method: Tm=**60.59°C**.

By comparison, the corresponding calculated Tm values using the salt-adjusted method and a default $[Na^+]$

= 0.1M (previously used by Gene Link prior to July 21, 2009—see Appendix for the equation) and the classical 4+2 Rule ((4°C x (#G+C)) + (2°C x (#A+T))) rule are

Salt-adjusted method: Tm = **59.50 °C**

4+2 Rule: Tm = (4°C x (12)) + (2°C x (8)) = **64°C**

5. Extinction Coefficient (EC_{260} or ε_{260})

The extinction coefficient (ε_λ) is defined as the fraction of light of a given wavelength (λ) lost to both scattering and absorption per unit distance traveled in a specific medium (for example, a dilute aqueous solution of DNA). Extinction coefficient is an intrinsic property of the medium. The relationship between the extinction coefficient and absorbance (A_λ) of a medium for a given wavelength of light is given by the Beer-Lambert Law:

$$A_\lambda = \varepsilon_\lambda \times b \times c$$

where b = path length (the distance the light travels through the medium), c = concentration of the medium, and $A = -\log_{10}(I/I_0)$, where I_0 = initial intensity of light just before entering the medium, and I = final intensity of light just after passing through the medium.

The absorbance of a particular sample is typically determined using a spectrometer sensitive to the desired wavelength range (such as UV-Visible) and a sample cuvette having a path length of 1 cm. For a particular wavelength λ, the absorbance of a set of concentration standards of the medium is measured, and an absorbance vs. concentration calibration curve plotted, with the slope of the curve being equal to the extinction coefficient ε_λ. Once its extinction coefficient is known, the particular concentration of any sample of that medium can be calculated from the measured absorbance and the path length using the Beer-Lambert Law.

When there is more than one absorbing species in a solution, the total absorbance is the sum of the individual absorbances of each species:

$$A_T = (\varepsilon_1 c_1 + \varepsilon_2 c_2 + \varepsilon_3 c_3 + \ldots) \times b$$

`For an oligonucleotide, the absorbing species are not simply its individual bases, but actually its nearest-neighbor base pairs, since the individual bases are close enough together to significantly interact with each other. In other words, for an oligonucleotide, both its base composition and its base order effect the value of its extinction coefficient. Consequently, the extinction coefficient for an oligonucleotide of length n turns out to be the sum of the extinction coefficients of all the nearest-neighbor base pairs minus a factor that corrects for multiple countings of internal bases that occur while summing the nearest-neighbor pairs.

$$\varepsilon\text{oligo} = \sum_{1}^{n-1} \varepsilon\text{nearestneighbor} - \sum_{2}^{n-1} \varepsilon\text{individual base}$$

For calculating the extinction coefficient of DNA/RNA oligos, we use previously published ε_{260} dinucleotide nearest-neighbor values (Warshaw and Tinoco,1966), and individual ε_{260} nucleotide values (Cantor and Warshaw, 1970; Cantor, *et al.*, 1970), using $mM^{-1}cm^{-1}$ for units. For DNA, the values of $\varepsilon_{nearestneighbor}$ ($mM^{-1}cm^{-1}$) are

Nearest-neighbor pair	$\varepsilon_{nearestneighbor}$ ($mM^{-1}cm^{-1}$)	Nearest-neighbor pair	$\varepsilon_{nearestneighbor}$ ($mM^{-1}cm^{-1}$)
AA	27.4	AG	25.0
CA	21.2	CG	18.0
GA	25.2	GG	21.6
TA	23.4	TG	19.0
AC	21.2	AT	22.8
CC	14.6	CT	15.2
GC	17.6	GT	20.0
TC	16.2	TT	16.8

And the values of $\varepsilon_{individualbase}$ ($mM^{-1}cm^{-1}$) are

A = 15.4; C = 7.4; G = 11.5; T = 8.7

For RNA, the values of $\varepsilon_{nearestneighbor}$ ($mM^{-1}cm^{-1}$) are

Nearest-neighbor pair	$\varepsilon_{nearestneighbor}$ ($mM^{-1}cm^{-1}$)	Nearest-neighbor pair	$\varepsilon_{nearestneighbor}$ ($mM^{-1}cm^{-1}$)
AA	27.4	AG	25.0
CA	21.0	CG	17.8
GA	25.2	GG	21.6
UA	24.6	UG	20.0

AC	21.0	AU	24.0
CC	14.2	CU	16.2
GC	17.4	GU	21.2
UC	17.2	UU	19.6

And the values of $\varepsilon_{individualbase}$ ($mM^{-1}cm^{-1}$) are

A = 15.4; C = 7.2; G = 11.5; U = 9.9

Using these values, the ε_{260} of a 20mer DNA oligo with the following sequence:

5'-GAAAGCGTCTAGCCATGGCG-3'

is ($\varepsilon_{GA} + \varepsilon_{AA} + \varepsilon_{AA} + \varepsilon_{AG} + \varepsilon_{GC} + \varepsilon_{CG} + \varepsilon_{GT} + \varepsilon_{TC} + \varepsilon_{CT} + \varepsilon_{TA} + \varepsilon_{AG} + \varepsilon_{GC} + \varepsilon_{CC} + \varepsilon_{CA} + \varepsilon_{AT} + \varepsilon_{TG} + \varepsilon_{GG} + \varepsilon_{GC} + \varepsilon_{CG}$) – ($\varepsilon_A + \varepsilon_A + \varepsilon_A + \varepsilon_G + \varepsilon_C + \varepsilon_G + \varepsilon_T + \varepsilon_C + \varepsilon_T + \varepsilon_A + \varepsilon_G + \varepsilon_C + \varepsilon_C + \varepsilon_A + \varepsilon_T + \varepsilon_G + \varepsilon_G + \varepsilon_C$) = (25.2 +27.4 + 27.4 +25.0 + 17.6 + 18.0 + 20.0 + 16.2 + 15.2 + 23.4 + 25.0 + 17.6 + 14.6 + 21.2 + 22.8 + 19.0 + 21.6 + 17.6 + 18.0) – (15.4 + 15.4 + 15.4 + 11.5 + 7.4 + 11.5 + 8.7 + 7.4 + 8.7 + 15.4 + 11.5 + 7.4 + 7.4 + 15.4 + 8.7 + 11.5 + 11.5 + 7.4) = (392.8) - (197.6) = **195.2 $mM^{-1}cm^{-1}$ (1 dp)**

6. µg/A_{260}

The µg/A_{260} is the concentration of the oligo in micrograms per OD at 260 nm. The formula is

$$\mu g/A_{260} = \frac{MW}{\varepsilon_{260}}$$

7. nmol /A_{260}

The nmol /A_{260} is the concentration of the oligo in number of nanomoles (10^{-9} mol) per OD at 260 nmol. The formula is

$$nmol/A_{260} = \frac{1000}{\varepsilon_{260}}$$

Appendix: Comparison of Current Salt-Corrected Nearest-Neighbor Method with Previous Methods for Calculating Tm.

Prior to July 21, 2009, the value of the oligonucleotide Tm appearing in the COS Report was calculated using the salt-adjusted formula (Rychlik *et al.*, 1990):

$$T_m = 81.5 + 16.6\,log_{10}([\mathrm{Na}^+] + [K^+]) + 0.41(\%GC) - (600/N)$$

where N = size of oligo, $[Na^+] + [K^+]$ = 0.1 M (default).

The following table show the comparable calculated Tm values (^oC) of four oligonucleotides of different length for three methods: (a) salt-corrected nearest-neighbor (new method), (b) salt-adjusted method (old method), (c) 4+2 Rule (Tm = ((4^oC x (#G+C)) + (2^oC x (#A+T))). For a) and b), the total concentration of monovalent cation is set to the previous default value of 0.1 M, in order to establish a more direct comparison between these methods.

Sequence (5'-->3')	Size (mer)	Tm Salt Corrected NN	Tm (old)	Tm (4+2)
GAAAGCGTCTAGCCATGGCG	20	60.60	59.50	64
GAGGGTTTCCCTGCCACAGTCGAGCTCGAC	30	70.90	69.10	98
ATCTTTCACAAATTTTGTAATCCAGAGGTTGATTGTCGAC	40	61.00	63.1	108
TTGTTGTTTGTTGTTTACTGGCCGTCGTTTTACAGCTATGCTGTAAAACG	50	66.9	68.4	140

The 4+2 Rule was originally developed to estimate the Tm of probe-target duplexes formed during membrane hybridization experiments. Thus, it is generally not recommended for estimating Tm of duplexes formed in PCR reactions, which is a solution-based method. Even for membrane experiments, the above results indicate that the 4+2 Rule is not a reliable way to estimate Tm for probe-target duplexes longer than about 20 mer.

The salt-corrected nearest-neighbor method consistently yields a Tm value that is a few degrees higher than that generated by the simpler salt-adjusted method.

Appendix 2: Comparison of Current Extinction Coefficient (EC260 or ε 260) Method with Previous Calculation Method.

Prior to July 31, 2009, the value of the oligonucleotide extinction coefficient (EC_{260} or ε_{260}) appearing in the COS Report was calculated using the classical formula:

$$\varepsilon 260 = \sum_{1}^{n} \varepsilon \text{individual base}$$

where the values of $\varepsilon_{individualbase}$ ($mM^{-1}cm^{-1}$) at 260 nm are those shown in section 5 above for DNA and RNA.

The following table show the comparable calculated ε_{260} values ($mM^{-1}cm^{-1}$) of four oligonucleotides of different length for two methods: (a) nearest-neighbor paired base (new method), (b) classical individual base method (old method).

Sequence (5'-->3')	Size (mer)	ε_{260} (new)	ε_{260} (old)
GAAAGCGTCTAGCCATGGCG	20	195.2	220.6

GAGGGTTTCCCTGCCACAGTCGAGCTCGAC	30	277.4	306.7
ATCTTTCACAAATTTTGTAATCCAGAGGTTGATTGTCGAC	40	387.8	432.2
TTGTTGTTTGTTGTTTACTGGCCGTCGTTTTACAGCTATGCTGTAAAACGA	50	476.3	527.2

The nearest-neighbor paired base method consistently yields an extinction coefficient value that is lower than that produced using the classical method. Unlike the classical method, the nearest-neighbor method takes into account the ability of the individual nucleotides to rotate with respect to one another, which results in greater light transmission through the absorbing medium.

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