DNA Hybridization Reaction

Part 2 Melting Temperature (Tm)

dr. Ivano Zara (ivano.zara.bio@gmail.com) web: dna_promix.it or biozarivan.it.

In the hybridization reaction seen previously: ssA + ssB ↔ dsD

And starting from the general equation seen previously, depending on the initial concentrations of reagents

$$\Delta G = \Delta H - T\,\Delta S + R\,T \ln\left(\frac{C_D}{(C_{Ai} - C_D)(\,C_{Bi} - C_D\,)}\right)$$

In a hybridization reaction the Melting temperature (Tm) parameter is used to estimate the ability of two strands to associate.
This parameter (at equilibrium, i.e. when ΔG=0) corresponds to the temperature at which half of the strand (with the lowest concentration) is associated in the duplex form. This value is used, for example, to set the annealing temperature in the PCR reaction

Let's see all the steps to get to the general equation of Tm

For ease, Placing $Cr = \frac{C_D}{(C_{Ai} - C_D)(\,C_{Bi} - C_D\,)}$

$$\Delta G = \Delta H - T\,\Delta S + R\,T \ln(Cr)$$

And breaking down and regrouping
$\Delta H = T\,\Delta S - RT \ln(\,C_r\,)$
$\Delta H = T\,(\,\Delta S - R \ln(\,C_r\,))$

$$T = \frac{\Delta H}{\Delta S - \mathrm{R} \ln(Cr)}$$

or by the property of logarithms

$$T = \frac{\Delta H}{\Delta S + \mathrm{R} \ln\left(\frac{1}{Cr}\right)}$$

As written above, for the melting temperature the following condition must exist:
the concentration of the duplex (dsD) must be equal to half the initial concentration of the less concentrated oligo (ssB) (e.g. the template in the PCR reaction).

Under these conditions $C_D = ½\ C_{Bi}$ Then:

$$Cr = \frac{½\ C_{Bi}}{(C_{Ai} - ½\ C_{Bi})(\,C_{Bi} - ½\ C_{Bi})} = \frac{½\ C_{Bi}}{(C_{Ai} - ½\ C_{Bi})(\,½\ C_{Bi})} = \frac{1}{(C_{Ai} - ½\ C_{Bi})}$$

Or $\frac{1}{Cr} = C_{Ai} - ½\ C_{Bi}$

Thus

$$Tm = \frac{\Delta H}{\Delta S + \mathrm{R}\ln(C_{Ai} - \frac{1}{2}C_{Bi})}$$

general equation to determine the Tm (in °Kelvin)

To convert to degrees Celsius Tm(°C)= Tm-273

ΔH in J/mol°K (or cal/mol°K), ΔS in J/mol (or cal/mol), concentrations in molar units, 'R' is the gas constant 8.314 J/Mol°K (or 1.987 cal/Mol°K);

PARTUCULAR CASES
Tm in PCR reaction

There are cases that simplify this equation.
For example: in the PCR reaction, the template DNA is at very low concentrations (even fM) and primer is 7-10 times higher, therefore, in the Cr parameter, C_{Bi} (which corresponds to the template concentration) can be omitted,
If $C_{Bi} <<< C_{Ai}$

$$\frac{1}{Cr} = C_{Ai} - \frac{1}{2}\ C_{Bi}\ =\ C_{Ai} = primer\ concentration$$

then $$Tm = \frac{\Delta H}{\Delta S + \mathrm{R}\ln(C_{primer})}$$

Another special case: if the initial concentration of the two filaments ssA and ssB were equal

$C_{Ai} = C_{Bi} = C$, then

$$\frac{1}{Cr} = C_{Ai} - \frac{1}{2}\ C_{Bi}\ = \frac{C}{2}$$ then $$Tm = \frac{\Delta H}{\Delta S + \mathrm{R}\ln\left(\frac{C}{2}\right)}$$

IMPORTANT NOTES
The Tm determined with the above equation is valid if the reagents are immersed in a standard solution (Na+ 1M)

In different conditions it is necessary to modify the thermodynamic parameters and therefore the calculated Tm.
Some methods, based on empirical experiences, modify the melting temperature considering only the concentration of the ions, others also consider the length of the oligo, others instead modify the entropy of the oligo.

See in the Attachment the methods commonly used.
In our programs we generally use the methods described in SantaLucia et al.(1998) and von Ahsen et al. (2001):

Another interesting question is the fraction of DNA strands that hybridize as a function of temperature.
This issue will be explained in the next document (.pdf)