DNA Hybridization Reaction

Part 3: Fraction of hybridized template as a function of temperature

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

In the dynamics of the hybridization reaction of two DNA single strands it is interesting to estimate the amount of hybridized template as a function of temperature.

As explained in the previous chapters:
Hybridization reaction of two strands (ssA and ssB) to form the hybrid (D)
ssA + ssB ↔ dsD

If C_A, C_B, C_D are the instantaneous concentrations of the individual elements of the reaction,
The instant free energy associated with this reaction is:

$$\Delta G = \Delta H - T\,\Delta S + R\,T \ln\left(\frac{C_D}{C_A\ C_B}\right)$$ General equation

Using this general equation, we will now see a method to estimate the amount of single strand that hybridizes in the duplex at a given temperature.

Here, for simplicity, I will define A and B as the initial concentrations of the two filaments (called C_{Ai}, C_{Bi} in the previous pages), we will have:

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

General equation also as a function of the initial concentrations (A and B)

Development of the hybridization equations

In this chapter, we want to see how the concentrations of the components (in particular of the template) vary at equilibrium (ΔG =0) as a function of temperature.
By fixing the temperature, and since entropy and enthalpy are unchangeable parameters for the same reaction, what will change will be only the concentrations of the reagents / products.

Let's start from the equation described above and (explained in the first chapter):

$$\Delta G = \Delta H - T\,\Delta S + R\,T \ln\left(\frac{C_D}{(A-C_D)(B-C_D)}\right) = 0$$

to simplify, we put $Cr = \frac{C_D}{(A-C_D)(B-C_D)}$

then $\Delta G = \Delta H - T\,\Delta S + RT \ln (C_r) = 0$

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

$$\ln\left(\frac{1}{Cr}\right) - \frac{\Delta H - T\,\Delta S}{R\,T} = 0$$

$$\frac{1}{Cr} - e^{\left(\frac{\Delta H - T\,\Delta S}{R\,T}\right)} = 0$$

$$\frac{(A-C_D)(B-C_D)}{C_D} - e^{\left(\frac{\Delta H - T\,\Delta S}{R\,T}\right)} = 0$$

If we take the ssB reagent as a reference (the least concentrated, such as the template in the PCR reaction), we can write the other components as a function of the fraction of ssB hybridized in the duplex.

If we define ‘f’ as the fraction of ssB hybridized in the duplex, we will have:

$f = C_D / B$

Instantaneous concentrations can be written as:

$C_D = f\ B$

$C_A = A - f\ B$

$C_B = B - f\ B$

$$\frac{1}{Cr} = \frac{(A - C_D)(B - C_D)}{C_D} = \frac{(A - f\ B)(B - f\ B)}{f\ B}$$

By developing and simplifying we will have:

$$\frac{1}{Cr} = \frac{A\ B - f\ A\ B - f\ B^2 + f^2\ B^2}{f\ B} = \frac{A\ - f\ A\ - f\ B + f^2\ B}{f}$$

But, as written above:

$$\frac{1}{Cr} - e^{\left(\frac{\Delta H - T\ \Delta S}{R\ T}\right)} = 0$$

$$\frac{A\ - f\ A\ - f\ B + f^2\ B}{f} - e^{\left(\frac{\Delta H - T\ \Delta S}{R\ T}\right)} = 0$$

that is $A\ - f\ A\ - f\ B + f^2 B\ - f e^{\left(\frac{\Delta H - T\ \Delta S}{R\ T}\right)} = 0$

We can rearrange this equation as a quadratic equation in standard form:

$$B\ f^2 + \left(-A - B - e^{\left(\frac{\Delta H - T\ \Delta S}{R\ T}\right)}\right) f\ + A = 0$$

As is known, a quadratic equation has two solutions:

$a\ x^2 + b\ x + c$ and the 2 resolutions are given by $x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$

Referring to the standard quadratic equation:

a = B

b = $-A - B - e^{\left(\frac{\Delta H - T\ \Delta S}{R\ T}\right)}$

c = A

The relative solutions are obtained:

$$f(B) = \frac{\left(A + B + e^{\left(\frac{\Delta H - T\ \Delta S}{R\ T}\right)}\right) \pm \sqrt{\left(A + B + e^{\left(\frac{\Delta H - T\ \Delta S}{R\ T}\right)}\right)^2 - 4\ B\ A}}{2B}$$

As is known, a quadratic equation has two values that satisfy it. In this case only one of the two values is possible (the one obtained with the minus sign). The other, the one obtained with the plus sign, turns out to be greater than 1, which is not possible (the fraction is always less than one).

$$f(B) = \frac{\left(A + B + e^{\left(\frac{\Delta H - T\,\Delta S}{R\,T}\right)}\right) - \sqrt{\left(A + B + e^{\left(\frac{\Delta H - T\,\Delta S}{R\,T}\right)}\right)^2 - 4\,B\,A}}{2B}$$

Equation of the hybridized template fraction as a function of temperature

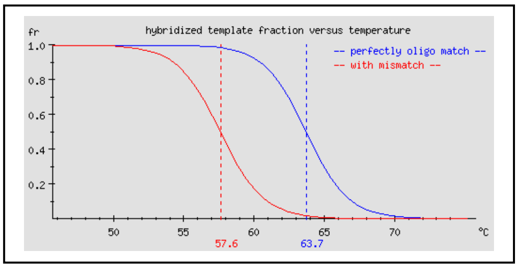
I remember that A and B are the initial concentrations of the two filaments (B is the lower one)

This is the equation that governs the hybrid fraction as a function of temperature. Tables and/or graphs can be obtained.

See examples on our platform (biozarivan.it or dna_promix.it) in the [Oligo Melting] app.

This is an example obtained with Oligo Melting

Fraction of hybridized template as a function of temperature (at equilibrium)




```
5'          ACCTTGACCGATTGAACCCA ->   3'
            ||||||||||| ||||||||
3' nn--     TGGAACTGGCTTACTTGGGT --nn 5'
```

Length oligo = 20   n.GC = 10 (50.00 %)   n.mismatch = 1

Template Fraction in duplex form (at equilibrium)

Temp. °C	41.64	43.64	45.64	47.64	49.64	51.64	53.64	55.64	57.64	59.64	61.64	63.64	65.64	67.64	69.64	71.64	73.64
Oligo only match % template in duplex	100.00	100.00	100.00	100.00	100.00	99.98	99.91	99.63	98.52	94.30	80.72	51.85	21.96	6.95	1.97	0.55	0.15
Duplex with mismatch % template in duplex	100.00	99.99	99.97	99.88	99.54	98.21	93.42	78.89	50.00	21.38	6.99	2.06	0.60	0.17	0.05	0.02	0.00

Note: I developed this equation many years ago, but it is difficult to find this equation or other equations cited for determining the fraction of hybridized template.

Useful Notes and Observations: In the next part I will share the experiences gained together with the group of Prof. Giorgio Valle at the Department of Biology of the University of Padua (.pdf)