

# 1 Name

MELTING - nearest-neighbor computation of nucleic acid hybridation

## 2 Synopsis

**melting** [*options* ]

## 3 Description

MELTING computes, for a nucleic acid duplex, the enthalpy and the entropy of the helix-coil transition, and then its melting temperature. Three types of hybridisation are possible: DNA/DNA, DNA/RNA, and RNA/RNA. The program uses the method of nearest-neighbors. The set of thermodynamic parameters can be easily changed, for instance following an experimental breakthrough. Melting is a free program in both sense of the term. It comes with no cost and it is open-source. In addition it is coded in ISO C and can be compiled on any operating system. Some perl scripts are provided to show how melting can be used as a block to construct more ambitious programs.

If you use MELTING, please quote

Le Novère. MELTING, a free tool to compute the melting temperature of nucleic acid duplex. *Bioinformatics*, 17: 1226-1227.

## 4 Options

The options are treated sequentially. If there is a conflict between the value of two options, the latter normally erases the former.

**-A***file.nn*

Informes the program to use *file.nn* as an alternative set of nearest-neighbor parameters, rather than the default for the specified hybridisation type (option **-H**). The standard distribution of melting provides some files ready-to-use: *all97a.nn* (Allawi et al 1997), *bre86a.nn* (Breslauer et al 1986), *san96a.nn* (SantaLucia et al 1996), *sug96a.nn* (Sugimoto et al 1996), *san04a.nn* (SantaLucia et al 2004) (DNA/DNA), *fre86a.nn* (Freier et al 1986), *xia98a.nn* (Xia et al 1998) (RNA/RNA) and *sug95a.nn* (Sugimoto et al 1995) (DNA/RNA). The program will look for the file in a directory specified during the installation. However, if an environment variable NN\_PATH is defined, melting will search in this one first. Be careful, the option **-A** changes the default parameter set defined by the option **-H**.

**-C***complementary\_sequence*

Enters the complementary sequence, from 3' to 5'. This option is mandatory if there are mismatches between the two strands. If it is not used, the program will compute it as the complement of the sequence entered with the option **-S**

### **-Ddnadnade.nn**

Informs the program to use the file *dnadnade.nn* to compute the contribution of dangling ends to the thermodynamic of helix-coil transition. The dangling ends are not taken into account by the approximative mode.

### **-Ffactor**

This is the a correction factor used to modulate the effect of the nucleic acid concentration in the computation of the melting temperature. See section ALGORITHM for details.

### **-Gx.xxe-xx**

Magnesium concentration (No maximum concentration for the moment). The effect of ions on thermodynamic stability of nucleic acid duplexes is complex, and the correcting functions are at best rough approximations. The published  $T_m$  correction formula for divalent  $Mg^{2+}$  ions of Owczarzy (2008) can take in account the competitive binding of monovalent and divalent ions on DNA. However this formula is only for DNA duplexes.

### **-h**

Displays a short help and quit with EXIT\_SUCCESS.

### **-Hhybridisation\_type**

Specifies the hybridisation type. This will set the nearest-neighbor set to use if no alternative set is provided by the option **-A** (remember the options are read sequentially). Moreover this parameter determines the equation to use if the sequence length exceeds the limit of application of the nearest-neighbor approach (arbitrarily set up by the author). Possible values are *dnadna*, *dnarna* and *rnadna* (synonymous), and *rnarna*. For reasons of compatibility the values of the previous versions of melting *A,B,C,F,R,S,T,U,W* are still available although **strongly** deprecated. Use the option **-A** to require an alternative set of thermodynamic parameters. **Important:** If the duplex is a DNA/RNA heteroduplex, the sequence of the DNA strand has to be entered with the option **-S**

### **-Iinput\_file**

Provides the name of an input file containing the parameters of the run. The input has to contain one parameter per line, formatted as in the command line. The order is not important, as well as blank lines. example:

```
-Hdnadna
-Asug96a.nn
-SAGCTCGACTC
-CTCGAGGTGAG
-N0.2
-P0.0001
-v
-Ksan96a
```

### **-ifile.nn**

Inform the program to use file.nn as an alternative set of inosine pair parameters, rather than the default for the specified hybridisation type. The standard distribution of melting provides some files ready-to-use: san05a.nn (Santalucia et al 2005) for deoxyinosine in DNA duplexes, bre07a.nn (Brent M Znosko et al 2007) for inosine in RNA duplexes. Note that not all the inosine mismatched wobble's pairs have been investigated. Therefore it could be impossible to compute the  $T_m$  of a duplex with inosine pairs. Moreover, those inosine pairs are not taken into account by the approximative mode.

### **-Ksalt\_correction**

Permits to choose another correction for the concentration in sodium. Currently, one can choose between *wet91a*, *san96a*, *san98a*. See section ALGORITHM

### **-kx.xxe-xx**

Potassium concentration (No maximum concentration for the moment). The effect of ions on thermodynamic stability of nucleic acid duplexes is complex, and the correcting functions are at best rough approximations. The published  $T_m$  correction formula for sodium ions of Owczarzy (2004) is therefore also applicable to buffers containing Tris or KCl. Monovalent  $K^+$ ,  $Na^+$ ,  $Tris^+$  ions stabilize DNA duplexes with similar potency, and their effects on duplex stability are additive. However this formula is only for DNA duplexes.

### **-L**

Prints the legal informations and quit with EXIT\_SUCCESS.

### **-Mdnadnamm.nn**

Inform the program to use the file *dnadnamm.nn* to compute the contribution of mismatches to the thermodynamic of helix-coil transition. Note that not all the mismatched Crick's pairs have been investigated. Therefore it could be impossible to compute the  $T_m$  of a mismatched duplex. Moreover, those mismatches are not taken into account by the approximative mode.

### **-Nx.xxe-xx**

Sodium concentration (between 0 and 10 M). The effect of ions on thermodynamic stability of nucleic acid duplexes is complex, and the correcting functions are at best rough approximations. Moreover, they are generally reliable only for  $[Na^+]$  belonging to  $[0.1, 1 \text{ M}]$ . If there are no other ions in solution, we can use only the sodium correction. In the other case, we use the Owczarzy's algorithm.

### **-Ooutput\_file**

The output is directed to this file instead of the standard output. The name of the file can be omitted. An automatic name is then generated, of the form *meltingYYYYMMDD\_HHhMMm.out* (of course, on POSIX compliant systems, you can emulate this with the redirection of stdout to a file constructed with the program date).

### **-Px.xxe-xx**

Concentration of the nucleic acid strand in excess (between 0 and 0.1 M).

### **-p**

Return the directory supposed to contain the sets of calorimetric parameters and quit with EXIT\_SUCCESS. If the environment variable NN\_PATH is set, it is returned. Otherwise, the value defined by default during the compilation is returned.

### **-q**

Turn off the interactive correction of wrongly entered parameter. Useful for run through a server, or a batch script. Default is OFF (i.e. interactive on). The switch works in both sens. Therefore if **-q** has been set in an input file, another **-q** on the command line will switch the quiet mode OFF (same thing if two **-q** are set on the same command line).

### **-Ssequence**

Sequence of one strand of the nucleic acid duplex, entered 5' to 3'. **Important:** If it is a DNA/RNA heteroduplex, the sequence of the DNA strand has to be entered. Uridine and thymidine are considered as identical. The bases can be upper or lowercase.

### **-Txxx**

Size threshold before approximative computation. The nearest-neighbour approach will be used only if the length of the sequence is inferior to this threshold.

### **-tx.xxe-xx**

Tris buffer concentration (No maximum concentration for the moment). The effect of ions on thermodynamic stability of nucleic acid duplexes is complex, and the correcting functions are at best rough approximations. The published T<sub>m</sub> correction formula for sodium ions of Owczarzy (2004) is therefore also applicable to buffers containing Tris or KCl. Monovalent K<sup>+</sup>, Na<sup>+</sup>, Tris<sup>+</sup> ions stabilize DNA duplexes with similar potency, and their effects on duplex stability are additive. However this formula is only for DNA duplexes. Be careful, the Tris<sup>+</sup> ion concentration is about half of the total tris buffer concentration.

### **-v**

Control the verbose mode, issuing a lot more information about the current run (try it once to see if you can get something interesting). Default is OFF. The switch works in both sens. Therefore if **-v** has been set in an input file, another **-v** on the command line will switch the verbose mode OFF (same thing if two **-v** are set on the same command line).

### **-V**

Displays the version number and quit with EXIT\_SUCCESS

Force the program to compute an approximative tm, based on G+C content. This option has to be used with caution. Note that such a calcul is increasingly incorrect when the length of the duplex decreases. Moreover, it does not take into account nucleic acid concentration, which is a strong mistake.

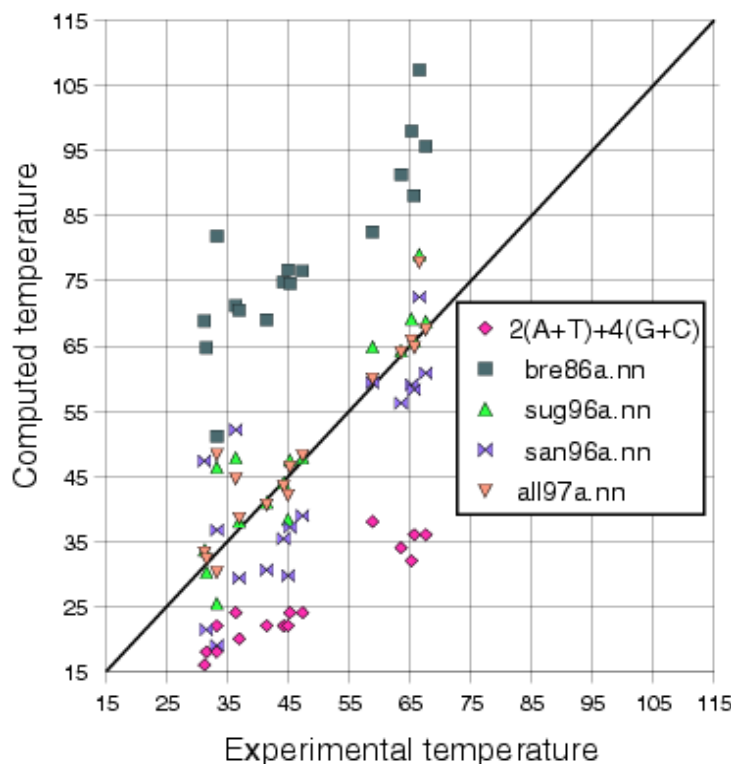
## 5 Algorithm

### 5.1 Thermodynamics of helix-coil transition of nucleic acid

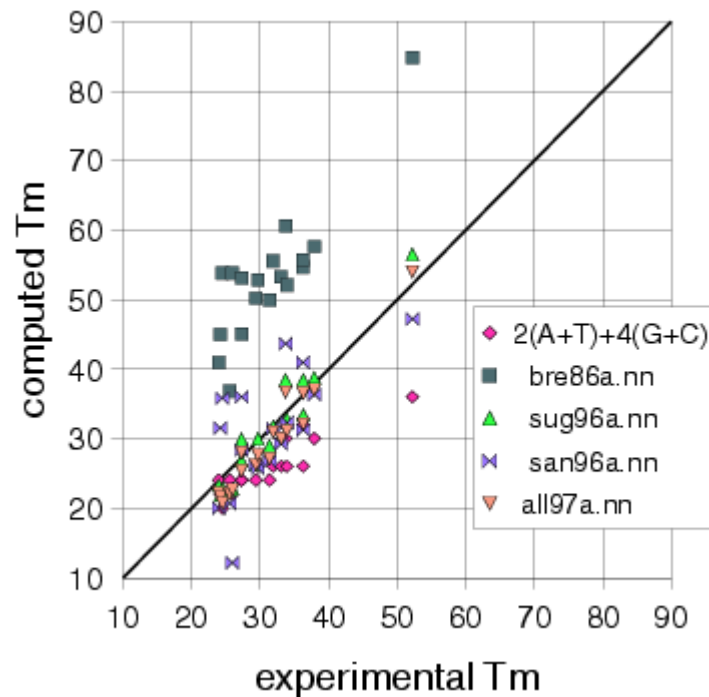
The nearest-neighbor approach is based on the fact that the helix-coil transition works as a zipper. After an initial attachment, the hybridisation propagates laterally. Therefore, the process depends on the adjacent nucleotides on each strand (the Crick's pairs). Two duplexes with the same base pairs could have different stabilities, and on the contrary, two duplexes with different sequences but identical sets of Crick's pairs will have the same thermodynamics properties (see Sugimoto et al. 1994). This program first computes the hybridisation enthalpy and entropy from the elementary parameters of each Crick's pair.

$$\begin{aligned}\Delta H &= \delta h_{\text{initiation}} + \sum \delta h_{\text{Crick's pair}} \\ \Delta S &= \delta s_{\text{initiation}} + \sum \delta s_{\text{Crick's pair}}\end{aligned}$$

See Wetmur J.G. (1991) and SantaLucia (1998) for deep reviews on the nucleic acid hybridisation and on the different set of nearest-neighbor parameters.



**Figure 1:** Comparison of experimental and computed Tm for various sets of nearest-neighbor parameters. [Na+]=1M, [nucleic acid]=4.10-4M



**Figure 2:** Comparison of experimental and computed  $T_m$  for various sets of nearest-neighbor parameters.  $[Na^+]=0.11M$ ,  $[nucleic\ acid]=8.10^{-6}M$

## 5.2 Effect of mismatches and dangling ends

The mismatching (inosine mismatches included) pairs are also taken into account. However the thermodynamic parameters are still not available for every possible cases (notably when both positions are mismatched). In such a case, the program, unable to compute any relevant result, will quit with a warning. The two first and positions cannot be mismatched. in such a case, the result is unpredictable, and all cases are possible. for instance (see Allawi and SanLucia 1997), the duplex

```

A           T
  GTGAGCTCAT
  TACTCGAGTG
T           A

```

is more stable than

```

AGTGAGCTCATT
TTACTCGAGTGA

```

The dangling ends, that is the unmatched terminal nucleotides, can be taken into account.

## 5.3 Example

$$\begin{aligned}
\Delta H \left( \begin{array}{c} \text{AGCGATGAA} \\ \cdot \text{CGCTGCTTT} \end{array} \right) &= \Delta H \left( \begin{array}{c} \text{AG} \\ \cdot \text{C} \end{array} \right) + \Delta H \left( \begin{array}{c} \text{A} \\ \text{TT} \end{array} \right) \\
&\quad + \Delta H \left( \begin{array}{c} \text{G} \\ \text{C} \end{array} \right)_{\text{init}} + \Delta H \left( \begin{array}{c} \text{A} \\ \text{T} \end{array} \right)_{\text{init}} \\
&\quad + \Delta H \left( \begin{array}{c} \text{GC} \\ \text{CG} \end{array} \right) + \Delta H \left( \begin{array}{c} \text{CG} \\ \text{GC} \end{array} \right) + 2x \Delta H \left( \begin{array}{c} \text{GA} \\ \text{CT} \end{array} \right) + \Delta H \left( \begin{array}{c} \text{AA} \\ \text{TT} \end{array} \right) \\
&\quad + \Delta H \left( \begin{array}{c} \text{AT} \\ \text{TG} \end{array} \right) + \Delta H \left( \begin{array}{c} \text{TG} \\ \text{GC} \end{array} \right)
\end{aligned}$$

(The same computation is performed for  $\Delta G$ )

## 5.4 The melting temperature

Then the melting temperature is computed by the following formula:

$$T_m = \frac{\Delta H}{\Delta S + R \ln(C_T/F)} + \mathcal{F}([Na^+]) - 273.15$$

$T_m$  in K (for  $[Na^+] = 1$  M)

*correction* for the salt concentration (if there are only  $Na^+$  cations in the solution) and to get the temperature in degree Celsius. (In fact some corrections are directly included in the  $\Delta S$ . See that of SanLucia 1998)

## 5.5 Correction for the concentration of nucleic acid

Many thanks to Ivano Zara (zarivan@cribi.unipd.it), who gave me most of the following explanation.

In a reaction  $A + B \rightleftharpoons D$ , where  $A$  is the strand in excess,  $B$  the other strand, and  $D$  the duplex, and where the oligonucleotides *are not self-complementary*,

$$\Delta G = \Delta G_0 - RT \ln K = \Delta H_0 - T \Delta S_0 - RT \ln K$$

and

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

If  $\xi$  is the fraction of molecules  $B$  that forms the duplex,

$$[D] = \xi [B]_0$$

$$[B] = [B]_0 - [D] = [B]_0 - \xi [B]_0 = [B]_0(1 - \xi)$$

$$[A] = [A]_0 - [D] = [A]_0 - \xi [B]_0$$

Therefore,

$$K = \frac{([A]_0 - \xi[B]_0)[B]_0(1 - \xi)}{\xi[B]_0} = \frac{([A]_0 - \xi[B]_0)(1 - \xi)}{\xi}$$

and, at melting temperature, when  $\xi = 1/2$ ,

$$K = [A]_0 - \frac{1}{2}[B]_0$$

If both strands are present in equivalent amount,  $[A]_0 = [B]_0$  and  $C_T = [A]_0 + [B]_0$ , then

$K = \frac{C_T}{4}$  ( $F = 4$ ). If  $[A]_0 \gg [B]_0$ , then  $C_T \approx [A]_0$  and  $K \approx [A]_0 \approx C_T$  ( $F = 1$ ). If the oligonucleotides are self-complementary,  $C_T = [A]_0$  and ( $F = 1$ ).

Note however that MELTING makes the assumption of no self-assembly, *i.e.* the computation does not take any entropic term to correct for self-complementarity.

## 5.6 Correction for the concentration of salt

If there are only sodium ions in the solution, we can use the following corrections. the correction can be chosen between *wet91a*, presented in Wetmur 1991 *i.e.*

$$16.6 \log \frac{[Na^+]}{1 + 0.7[Na^+]} + 3.85$$

*san96a* presented in SantaLucia et al. 1996 *i.e.*

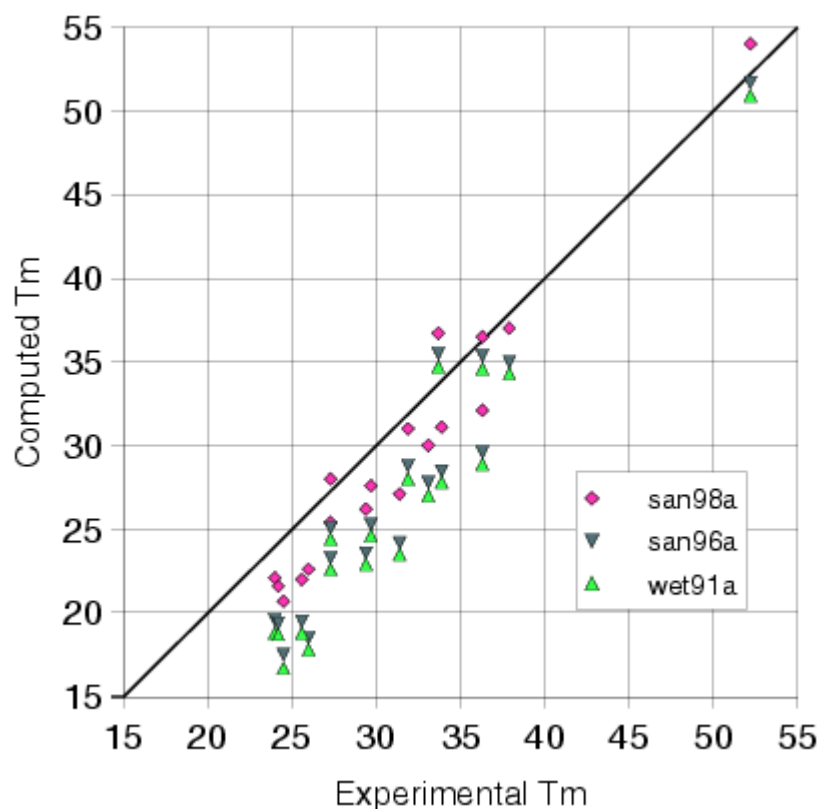
$$12.5 \log[Na^+]$$

and *san98a* presented in SantaLucia 1998 *i.e.* a correction of the entropic term without modification of enthalpy

$$\Delta S = \Delta S_{[Na^+] = 1 \text{ M}} + 0.368(N - 1) \ln[Na^+]$$

Where  $N$  is the length of the duplex (SantaLucia 1998 actually used 'N' the number of non-terminal phosphates, that is effectively equal to our  $N-1$ ).





**Figure 3:** Comparison of experimental and computed Tm for various correction of salt concentration.

### **5.7 Correction for the concentration of ions when other monovalent ions such as Tris<sup>+</sup> and K<sup>+</sup> or divalent Mg<sup>2+</sup> ions are added**

If there are only Na<sup>+</sup> ions, we can use the correction for the concentration of salt (see above). In the opposite case, we will use the magnesium and monovalent ions correction from Owczarzy *et al* (2008). (only for DNA duplexes)

$$[\text{Mon}^+] = [\text{Na}^+] + [\text{K}^+] + [\text{Tris}^+]$$

Where [Tris<sup>+</sup>] is equal to half of total tris buffer concentration. (*in the option -t, it is the Tris buffer concentration which is entered*).

When the divalent ions are the only ions present, the melting temperature is :

$$\frac{1}{T_m(\text{Mg}^{2+})} = \frac{1}{T_m(1\text{M Na}^+)} + a - b \times \ln([\text{Mg}^{2+}]) + F_{gc} \times (c + d \times \ln([\text{Mg}^{2+}])) + \frac{1}{2 \times (\text{Nbp} - 1)} \times (-e + f \times \ln([\text{Mg}^{2+}]) + g \times \ln([\text{Mg}^{2+}]^2))$$

where :  $a = 3.92 \times 10^{-5}$

$$b = 9.11 \times 10^{-6}$$

$$c = 6.26 \times 10^{-5}$$

$$d = 1.42 \times 10^{-5}$$

$$e = 4.82 \times 10^{-4}$$

$$f = 5.25 \times 10^{-4}$$

$$g = 8.31 \times 10^{-5}.$$

Fgc is the fraction of GC base pairs in the sequence and Nbp is the length of the sequence (Number of base pairs).

When there are both monovalent and divalent ions, there are several cases because we can have a competitive DNA binding between monovalent and divalent cations :

If the following ratio :

$$\frac{[Mg^{2+}]^{0.5}}{[Mon^+]}$$

is inferior to 0.22, monovalent ion influence is dominant, divalent cations can be disregarded and the melting temperature is :

$$\frac{1}{Tm(Mg^{2+})} = \frac{1}{Tm(1M Na^+)} + (4.29 \times Fgc - 3.95) \times 10^{-5} \times \ln([Mon^+]) + 9.40 \times 10^{-6} \times \ln([Mon^+]^2)$$

If the ratio is included in [0.22, 6[, we must take in account both  $Mg^{2+}$  and monovalent cations concentrations. The melting temperature is calculated with the first equation but with monovalent ions concentration dependent parameters a, d and g :

$$a = 3.92 \times 10^{-5} \times (0.843 - 0.352 \times [Mon^+]^{0.5} \times \ln([Mon^+]))$$

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

$$g = 8.31 \times 10^{-5} \times (0.486 - 0.258 \times \ln([mon^+]) + 5.25 \times 10^{-4} \times \ln([mon^+]) \times \ln([mon^+]) \times \ln([mon^+]))$$

. and b, c, e, f are constant.

Finally, if the ratio is superior to 6, divalent ion influence is dominant, monovalent cations can be disregarded and the melting temperature is calculated with the first equation and the constant parameters a, b, c, d, e, f, g.

## 5.8 Long sequences

It is important to realise that the nearest-neighbor approach has been established on small oligonucleotides. Therefore the use of MELTING in the non-approximative mode is really accurate only for relatively short sequences (Although if the sequences are too short, let's say  $< 6$  bp, the

influence of extremities becomes too important and the reliability decreases a lot). For long sequences an approximative mode has been designed. This mode is launched if the sequence length is higher than the value given by the option -T (the default threshold is 60 bp).

The melting temperature is computed by the following formulas:

ADN/ADN:

$$Tm = 81.5 + 16.6 \log \frac{[Na^+]}{1 + 0.7[Na^+]} + 0.41\%GC - \frac{500}{size}$$

ADN/ARN:

$$Tm = 67 + 16.6 \log \frac{[Na^+]}{1 + 0.7[Na^+]} + 0.8\%GC - \frac{500}{size}$$

ARN/ARN:

$$Tm = 78 + 16.6 \log \frac{[Na^+]}{1 + 0.7[Na^+]} + 0.7\%GC - \frac{500}{size}$$

The usage of this mode is nevertheless **strongly disencouraged**.

## 5.9 Miscellaneous comments

MELTING is currently accurate only when the hybridisation is performed at pH 7.1. The computation is valid only for the hybridisations performed in aqueous medium. Therefore the use of denaturing agents such as formamide completely invalidates the results.

# 6 References

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Xia T., SantaLucia J., Burkard M.E., Kierzek R., Schroeder S.J., Jiao X., Cox C., Turner D.H. (1998). Thermodynamics parameters for an expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick base pairs. *Biochemistry* 37: 14719-14735

For review see:

SantaLucia J. (1998) A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. *Proc Natl Acad Sci USA* 95: 1460-1465

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## 7 Files

*\*.nn*

Files containing the nearest-neighbor parameters, enthalpy and entropy, for each Crick's pair. They have to be placed in a directory defined during the compilation or targeted by the environment variable NN\_PATH.

*tkmelting.pl*

A Graphical User Interface written in perl/tk is available for users who prefer the 'button and menu' approach.

*\*.pl*

Scripts are available to use MELTING iteratively. For instance, the script multi.pl permits to predict the T<sub>m</sub> of several duplexes in one shot. The script profil.pl allow an interactive computation along a sequence, by sliding a window of specified width.

## 8 See Also

New versions and related material can be found at

<http://www.ebi.ac.uk/~lenov/meltinghome.html>

You can use MELTING through a web server at <http://bioweb.pasteur.fr/seqanal/interfaces/melting.html>

## 9 Known Bugs

The infiles have to be ended by a blank line because otherwise the last line is not decoded.

If an infile is called, containing the address of another input file, it does not care of this latter. If it is

its own address, the program quit (is it a bug or a feature?).

In interactive mode, a sequence can be entered on several lines with a backslash

```
AGCGACGAGCTAGCCTA\
```

```
AGGACCTATACGAC
```

If by mistake it is entered as

```
AGCGACGAGCTAGCCTA\AGGACCTATACGAC
```

The backslash will be considered as an illegal character. Here again, I do not think it is actually a bug (even if it is unlikely, there is a small probability that the backslash could actually be a mistyped base).

## 10 Copyright

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## 11 Acknowledgements

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## 12 Author

Nicolas Le Novère,  
EMBL-EBI,  
Wellcome-Trust Genome Campus  
Hinxton Cambridge, CB10 1SD, UK  
lenov@ebi.ac.uk

## 13 History

The version 4 has been entirely rewritten from zero. Therefore an history of previous versions would be of little interest.

See the file ChangeLog for the changes of the versions 4 and more recent.

# About this document ...

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The command line arguments were:

```
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```

The translation was initiated by Nicolas Le Novère on 2003-02-11

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*Nicolas Le Novère 2004-05-10*