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MELTING, computing the melting temperature of nucleic acid duplex

Nicolas Le Novère

Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

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ABSTRACT

Summary: MELTING computes the enthalpy and entropy of an oligonucleotide duplex helix–coil transition, and then its melting temperature. The program uses the method of nearest-neighbours. The set of thermodynamic parameters can be easily customized. The program provides several correction methods for the concentration of salt. MELTING is a free program, available at no cost and open-source. Perl scripts are provided to show how MELTING can be used to construct more ambitious programs.

Availability: MELTING is available for several platforms (http://www.pasteur.fr/recherche/unites/neubiomol/meltinghome.html) and is accessible via a www server (http://bioweb.pasteur.fr/seqanal/interfaces/melting.html).

Contact: nl223@cus.cam.ac.uk

INTRODUCTION

The quality of numerous experiments in the field of molecular biology depends on the accurate characterization of the helix–coil transition of nucleic acid duplexes. The choice of optimal washing temperatures for *in situ* hybridizations or annealing temperatures for PCR requires the knowledge of the melting temperature (T_m). More recently, the fast growing of micro-arrays usage further emphasized the tremendous interest for such a calculation. MELTING was first written in 1997 to fulfil this need as a simple, but plastic, free and portable tool. Since then, it has been improved and extended.

HELIX–COIL TRANSITION THERMODYNAMICS

The nearest-neighbour approach assumes that the helix–coil transition works as a zipper. After an initial attachment, the hybridization propagates laterally. Therefore, the process depends on the adjacent nucleotides on each strand (the Crick's pairs); (Wetmur, 1991). Two duplexes with the same base pairs could have different stabilities, and conversely, two duplexes with different sequences but identical sets of Crick's pairs have the same thermodynamics properties (Sugimoto *et al.*, 1994). The enthalpy and entropy of the duplex can therefore be linearly deduced from the elementary parameters of each Crick's pair. Various sets of parameters are proposed with the program, those of Breslauer *et al.* (1986); SantaLucia *et al.* (1996); Sugimoto *et al.* (1996), and Allawi and SantaLucia (1997) (the default) for the homoduplexes DNA/DNA, of Freier *et al.* (1986) and Xia *et al.* (1998) (the default) for the homoduplexes RNA/RNA, and of Sugimoto *et al.* (1995) for the heteroduplexes DNA/RNA. See SantaLucia (1998) for a review of the different set of nearest-neighbour parameters.

The mismatched pairs can be taken into account, the parameters being provided for the DNA/DNA homoduplexes (Allawi and SantaLucia, 1997, 1998a,b,c; Peyret *et al.*, 1999). Note that all the parameters for the double mismatched Crick's pairs are not provided, preventing the use on long non-Watson–Crick stretches. Users can still add their own parameters to the appropriate configuration file. The dangling ends, that is the unmatched terminal nucleotides, can also be taken into account, the parameters being provided for the DNA/DNA homoduplexes (Bommarito *et al.*, 2000).

Here is an example of decomposition for the enthalpy:

$$\begin{aligned}\Delta H\begin{pmatrix}\texttt{AGCGATGA-}\\ \texttt{-CGCTGCTT}\end{pmatrix} = {} & \Delta H\begin{pmatrix}\texttt{AG}\\ \texttt{-C}\end{pmatrix} + \Delta H\begin{pmatrix}\texttt{A-}\\ \texttt{TT}\end{pmatrix} \\ & + \Delta H\begin{pmatrix}\texttt{G}\\ \texttt{C}\end{pmatrix}_{\text{init}} + \Delta H\begin{pmatrix}\texttt{A}\\ \texttt{T}\end{pmatrix}_{\text{init}} \\ & + \Delta H\begin{pmatrix}\texttt{GC}\\ \texttt{CG}\end{pmatrix} + \Delta H\begin{pmatrix}\texttt{CG}\\ \texttt{GC}\end{pmatrix} + 2\times\Delta H\begin{pmatrix}\texttt{GA}\\ \texttt{CT}\end{pmatrix} \\ & + \Delta H\begin{pmatrix}\texttt{AT}\\ \texttt{TG}\end{pmatrix} + \Delta H\begin{pmatrix}\texttt{TG}\\ \texttt{GC}\end{pmatrix}.\end{aligned}$$

MELTING TEMPERATURE

The melting temperature is computed as:

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

The first member represents the T_m in kelvin for 1 M of sodium ion and the second is a correction for the salt concentration and to get the temperature in degree celsius. C_T is the total concentration in strand. F is 1 in case of self-complementary oligonucleotides. Otherwise, F is 4 if the concentration of the two strands are similar and 2 if one strand is in excess (for instance in the PCR experiment). Note however, that MELTING does not use any entropic

term to correct for self-complementarity. Currently, the only available correction is for sodium (i.e. monovalent cations). Users can choose between several corrections, $16.6 \log \frac{[Na^+]}{1+0.7[Na^+]} + 3.85$ (Wetmur, 1991), $12.5 \log[Na^+]$ (SantaLucia *et al.*, 1996), and a correction of the entropic term without modification of enthalpy (default) $\Delta S = \Delta S_{[Na^+]=1\ M} + 0.368(N - 1) \ln[Na^+]$ where N is the length of the duplex (SantaLucia, 1998).

IMPLEMENTATION

The program is written in ISO C. Binaries for Digital Unix, GNU/Linux and Windows are provided together with the source code. The program is licensed under the General Public Licence (Free Software Foundation, 1991). Although the program can be easily used though the command-line, the distribution also provides a graphical interface written in Perl/Tk. In addition a www interface has been developed with PISE (Letondal, 2001).

The distribution contains a detailed documentation and several scripts showing how to extend the possibilities of the basal program, for instance to scan a sequence.

COMPARISON WITH SIMILAR TOOLS

Several programs have been developed concurrently to MELTING. DAN is a free tool provided together with the sequence analysis suite EMBOSS (http://www.uk.embnet.org/Software/EMBOSS/). The parameters used—those of Breslauer *et al.* (1986)—are outdated and can truly cope only with DNA/DNA duplexes. The program does not accurately take into account mismatches and dangling ends. In addition it offers very limited plasticity to the users (although being free, one can still modify the code itself). The WISCONSIN PACKAGE provides MELTTEMP, a commercial equivalent of DAN. Recently Peyret developed HYTHER, a very complete and accurate program (http://jsl1.chem.wayne.edu/Hyther/hythermenu.htm). Although its parameters are currently up-to-date, HYTHER does not allow the users to choose between different competing sets of nearest neighbours or salt correction. HYTHER is not open-source and exists only under Microsoft Windows. Finally, the POLAND server (Steger, 1994) offers an approach based on a different algorithm (http://www.biophys.uni-duesseldorf.de/POLAND/). However the program is mainly aimed at the long sequences and cannot cope with specific mismatches or dangling ends.

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