uMelt^{SM} Technical Guide v2.0

Author
Zach Dwight

Location
University of Utah
Wittwer DNA Lab
http://www.dna.utah.edu/

TOGETHER WE REACH

Table of Contents

TOGETHER WE REACH

Technical Overview

- Base pair stability factors are calculated based on thermodynamic values (entropy, enthalpy) for each nearest neighbor pair in the sequence

- Recursive relations are then used to account for all the possible configurations of helix to random coil states and their probabilities

- The sum of these probabilities is the helicity (fluorescence) of the sequence at the specified temperature

- Helicity is then plotted against the associated temperature.

Loop Entropy Parameter (LEP)

- A loop entropy parameter (LEP) is calculated to adjust base pair stability factors.

- The LEP consists of two variables

  - σ (Cooperativity Parameter)
  - α (Loop Closure Exponent)
  - For the equation below, N is the sequence length and x is the base pair index

$$LEP(x) = \sigma \times (N+1)^{-\alpha}$$

Base Pair Stability Factors

- Using a thermodynamic nearest neighbor set, base pair stability factors are calculated <u>for each nearest neighbor interaction at each temperature step</u>:

  – Blake & Delcourt's (1998) Tij and dTdLog equations are used to calculate the stability factor (s) with a modified version of the equation :

  - $s = \exp{-(\Delta H - T \times \Delta S)} / R \times T$

  – Modifications include

  - [Monovalent cation] (See SantaLucia, 1998)
  - Free [Mg^{++}] (See *von Ahsen, 2001*)
  - Thermodynamic Set

  – Each thermodynamic parameter set is incorporated into Blake & Delcourt's stability equations.

TOGETHER WE REACH

Recursive Relations

- Statistical mechanics are implemented according Poland (1974), Yeramian (1990), and Tostesen (2003).
    - A set of arrays are created which correspond to different helix to coil configurations. A series of iterations occur, in which individual elements of these arrays are added to one another in order to find the sum, which is the probability that the base pair is in the helix state.
        - By adding up the probabilities from one end of the sequence to the other, the degree of helicity is calculated for that temperature.
        - A great visual representation (Figure 1) and explanation can be found in the Tostesen (2003) publication.
        - uMelt^{SM} does not use Fixman and Friere's approximation.

Application and Graphics

- uMelt is constructed with the Adobe™ FLEX framework using Flash Builder 4.

- Why Adobe™ Flex?
  - Customizable interface components
  - XML based framework
  - ActionScript is quick yet powerful
  - Sharp colors and visually appealing features
  - Easy to design and organize interface
  - Compiles into a Flash file for easy launch
  - Chart library is robust
  - Adobe™ was co-founded by a University of Utah graduate (Dr. John Warnock)
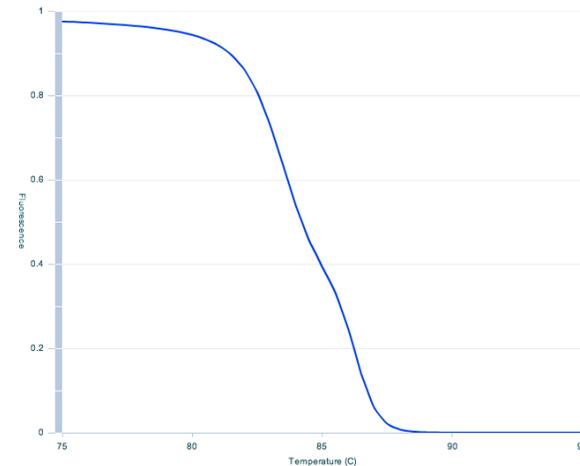
Acknowledgements

Special thanks to the following for sharing their knowledge of melting curves:

Dr. Gerhard Stegar

Institut fur Physikalische Biologie at the Heinrich-Heine-Universitat

Dr. Eivind Tostesen

University of Oslo

Dr. Nicholas Markham

Rensselaer Polytechnic Institute

Current citation for uMelt℠ :

Zachary Dwight, Robert Palais, and Carl T. Wittwer. *uMELT: prediction of high-resolution melting curves and dynamic melting profiles of PCR products in a rich web application*. **Bioinformatics (2011) 27(7): 1019-1020**

TOGETHER WE REACH

Other Related Software

- uMelt℠ is focused on predicting high resolution DNA melting curves. A variety of applications are available that offer additional unique and valuable features:

    - Poland
        - http://www.biophys.uni-duesseldorf.de/local/POLAND/poland.html

    - Meltsim
        - http://www.bioinformatics.org/meltsim/wiki/

    - Dinamelt
        - http://dinamelt.bioinfo.rpi.edu/

    - Stitchprofiles
        - http://stitchprofiles.uio.no/index.php

References

Blake, R.D., Bizzaro, J.W., Blake, J.D., Day, G.R., Delcourt, S.G., Knowles, J., Marx, K.A. and SantaLucia, J., Jr. (1999). Statistical Mechanical Simulation of Polymeric DNA Melting with MELTSIM. *Bioinformatics 15(5):370-375.* [PMID: 10366657]

Blake, R.D. and Delcourt, S.G. (1998). Thermal stability of DNA. Nucleic Acids Research 26 (14):3323-3332. [PMID: 9649614]

Blossey, R. and Carlon, E. (2003). Reparametrizing the loop entropy weights: Effect on DNA melting curves. Phys Rev E 68:061911. [PMID: 14754238]

Breslauer, K.J., Blocker, H., Frank, R., and Marky, L.A. (1986). Predicting DNA duplex stability from the base sequence. Proc. Natl. Acad. Sci. USA. 83(11),3746-50. [PMID: 3459152]

Crothers, D.M. (1968). Calculation of Melting Curves for DNA. Biopolymers. 6, 1391-1404. [PMID: 5685101]

Huguet, J.M., et al. Single-molecule derivation of salt dependent base-pair free energies in DNA. Proc Natl Acad Sci U S A, 2010. 107(35):15431-6. [PMID: 20716688]

Markham, N. R. & Zuker, M. (2005) DINAMelt web server for nucleic acid melting prediction. Nucleic Acids Res., 33, W577-W581. [PMID: 15980540]

Musielski, H. Mann, W. Lauer, R. and Michel S. (1981) Influence of dimethylsulfioxide on transcription by bacteriophage T3 induced RNA polymerase. Z Allg Mikobiolo., 22, 447-56. [PMID: 442703]

References Continued

Poland, D. (1974). Recursion Relation Generation of Probability Profiles for Specific-Sequence Macromolecules with Long-Range Correlations. Biopolymers. 13, 1859-1871. [PMID: 4415504]

SantaLucia, J., Jr. (1998). A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. Proc. Natl. Acad. Sci. USA. 95, 1460-1465.
[PMID: 9465037]

Steger, G. (1994). Thermal denaturation of double-stranded nucleic acids: prediction of temperatures critical for gradient gel electrophoresis and polymerase chain reaction. Nucleic Acids Res. 22, 2760-2768. [PMID: 8052531]

Sugimoto N, Nakano S, Katoh M, Matsumura A, Nakamuta H, Ohmichi T, Yoneyama M, Sasaki M. (1995) Thermodynamic parameters to predict stability of RNA/DNA hybrid duplexes. Biochemistry. Sep 5;34(35):11211–11216. [PMID: 7545436]

Tøstesen, E., Liu, F., Hovig, E. and Jenssen, T. (2003): Speed-Up of DNA Melting Algorithm with Complete Nearest Neighbor Properties. Nucl. Acids Res., 33, w573- w576. [PMID: 14579309]

von Ahsen, N., C.T. Wittwer, and E. Schutz. (2001) Oligonucleotide melting temperatures under PCR conditions: nearest-neighbor corrections for Mg(2+), deoxynucleotide triphosphate, and dimethyl sulfoxide concentrations with comparison to alternative empirical formulas. Clin Chem. 47(11):1956-61. [PMID: 11673362]

Yeramian, E.; Schaeffer, F.; Caudron, B.; Claverie, P.; Buc, H. (1990). Biopolymers. 30, 481– 497.

Zimm, B.H. (1960). Theory of 'Melting' of the Helical Form in Double Chains of the DNA Type. Journal of Chemical Physics. Volume 33 (5).

THE
UNIVERSITY
OF UTAH™

Disclaimer

The following disclaimers apply to any use of uMelt[SM] and related materials provided on dna.utah.edu. Access to uMelt and related materials are provided as a courtesy, free of charge, and are intended for informational and educational purposes only. Any use of uMelt and such related materials shall be at the sole and exclusive risk of the user(s).

The University of Utah and its affiliates make no representations or warranties of any kind, express or implied, about the completeness, accuracy, reliability, suitability or availability of uMelt or such related materials, or any related products and services. Any reliance you place on uMelt or such related materials is therefore strictly at your own risk.

In no event will the University of Utah or its affiliates be liable for any loss or damage arising in connection with your use of uMelt or such related information including, without limitation, any direct, indirect or consequential loss or damages.

Neither uMelt, nor results generated through the use of uMelt, may be incorporated into any publication or formal or official report or document without appropriate reference (See Acknowledgements in the Technical Guide) and use of the service mark ([SM]). Users of uMelt may not use the name or marks of the University of Utah, without the prior, written consent of the University of Utah.

TOGETHER WE REACH