

# Citing uMelt<sup>SM</sup>

## Example in text:

'Melting curve predictions were generated by the uMelt software (v. Quartz) at [dna-utah.org](http://dna-utah.org).'

## In References:

### AS SOFTWARE...

Dwight Z. uMelt – Melting curve prediction software for single, batch, and multiplex PCR products [Online]. DNA-UTAH.ORG. Available from: <https://dna-utah.org/umelt/>

### OR CITE...

Zachary Dwight, Robert Palais, Carl T. Wittwer, uMELT: prediction of high-resolution melting curves and dynamic melting profiles of PCR products in a rich web application, *Bioinformatics*, Volume 27, Issue 7, April 2011, Pages 1019–1020, <https://doi.org/10.1093/bioinformatics/btr065>

## BIOINFORMATICS APPLICATIONS NOTE

Vol. 27 no. 7 2011, pages 1019–1020  
doi:10.1093/bioinformatics/btr065

### Sequence analysis

Advance Access publication February 7, 2011

## uMELT: prediction of high-resolution melting curves and dynamic melting profiles of PCR products in a rich web application

Zachary Dwight<sup>1,\*</sup>, Robert Palais<sup>2</sup> and Carl T. Wittwer<sup>3</sup>

<sup>1</sup>School of Computing, University of Utah, 50 South Central Campus Drive, Room 3190, <sup>2</sup>Department of Mathematics, University of Utah, 155 South 1400 East, Room 233 and <sup>3</sup>Department of Pathology, University of Utah, 15 North Medical Drive East, Salt Lake City, UT 84112, USA

Associate Editor: John Quackenbush

### ABSTRACT

**Summary:** uMelt<sup>SM</sup> is a flexible web-based tool for predicting DNA melting curves and denaturation profiles of PCR products. The user defines an amplicon sequence and chooses a set of thermodynamic and experimental parameters that include nearest neighbor stacking energies, loop entropy effects, cation (monovalent and Mg<sup>++</sup>) concentrations and a temperature range. Using an accelerated partition function algorithm along with chosen parameter values, uMelt interactively calculates and visualizes the mean helicity and the dissociation probability at each sequence position at temperatures within the temperature range. Predicted curves display the mean helicity as a function of temperature or as derivative plots. Predicted profiles display stability as a function of sequence position either as 50% helicity temperatures or as the helicity probability at specific temperatures. The loss of helicity associated with increasing temperature may be viewed dynamically to visualize domain formation within the molecule. Results from fluorescent high-resolution melting experiments match the number of predicted melting domains and their relative temperatures. However, the absolute melting temperatures vary with the selected thermodynamic parameters and current libraries do not account for the rapid melting rates and helix stabilizing dyes used in fluorescent melting experiments. uMelt provides a convenient platform for simulation and design of high-resolution melting assays.

**Availability and implementation:** The application was developed in Actionscript and can be found online at <http://www.dna.utah.edu/>

Gotoh, 1983; Markham and Zuker, 2005; Poland, 1974; Steger, 1994; Tostesen, 2003; Zimm, 1960) to predict fluorescent melting analysis of PCR products in a rich web application.

### 2 METHODS

Temperature-dependent stability factors for each base tetrad are used to calculate probabilities of helicity for each position at each temperature. Averaging over the entire sequence gives the predicted helicity at each temperature. Stability factors of the 10 possible nearest neighbor tetrads are calculated using Equation (1).

$$\text{Stability factor} = \frac{e^{-(\Delta H - T\Delta S)}}{RT} \quad (1)$$

Enthalpy ( $\Delta H$ ) and entropy ( $\Delta S$ ) parameters are taken from one of several thermodynamic libraries,  $T$  is the absolute temperature and  $R$  is Boltzmann's constant. The entropy parameters are modified for monovalent cation (Blake and Delcourt, 1998) and Mg<sup>++</sup> (Nakano *et al.*, 1999; von Ahsen *et al.*, 2001) concentrations.

Tetrad stability factors are used in the two-phase recursive calculation of vectors whose entries contain partition functions that relate relative probabilities of helicity versus random coiling along segments of the molecule of increasing lengths. The algorithm, described in Tostesen *et al.* (2003), accelerates both the exact  $O(N^3)$  and approximate  $O(N^2)$  method described previously (Yeremian *et al.*, 1990) by one order ( $O$ ) in the oligo