

# uMELT<sup>SM</sup> : 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, PhD <sup>2</sup>; Carl Wittwer, MD, PhD <sup>3</sup>

<sup>1</sup>School of Computing, <sup>2</sup>Department of Mathematics, <sup>3</sup>Department of Pathology, Univ. of Utah, Salt Lake City, UT

## Introduction

uMelt is a flexible web-based tool for predicting DNA melting curves and denaturation profiles of PCR (polymerase chain reaction) products. DNA melting curves are produced by high resolution melting : a process in which DNA samples taken from patients are heated rapidly in a mixture of fluorescing dyes. As the DNA “melts” or the two strands come apart, the fluorescence will decrease. By comparing melting curves of patients and populations, scientists can differentiate between healthy DNA and possible mutations that can lead to cystic fibrosis or muscular dystrophy.

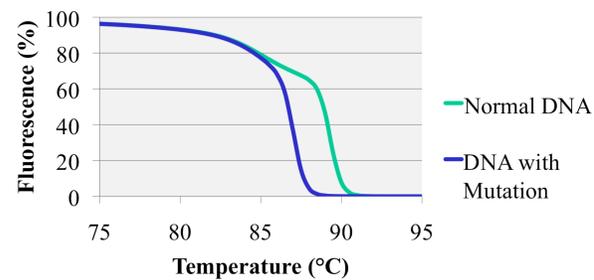


Figure 1: Experimental melting curves

## Materials and Methods

uMelt builds on existing models of DNA dissociation and hybridization using nearest neighbor thermodynamics and recursive calculations using statistical mechanics. Integrating techniques that reduce operation time in hopes of offering the user fast results and meaningful data, stability factors are used in the two-phase recursive calculation of vectors whose entries contain partition functions that relate relative probabilities of helicity vs. random coiling along segments of the molecule of increasing lengths. In uMelt, the resulting exact  $O(N^2)$  algorithm is implemented, where  $N$  is the length of the DNA sequence.

uMelt was designed and implemented in Actionscript utilizing the FLEX web application framework, providing an accessible, interactive, and accurate clinical diagnostic tool. Using a rich web application framework allows the user to have a ‘desktop’ experience within the browser.

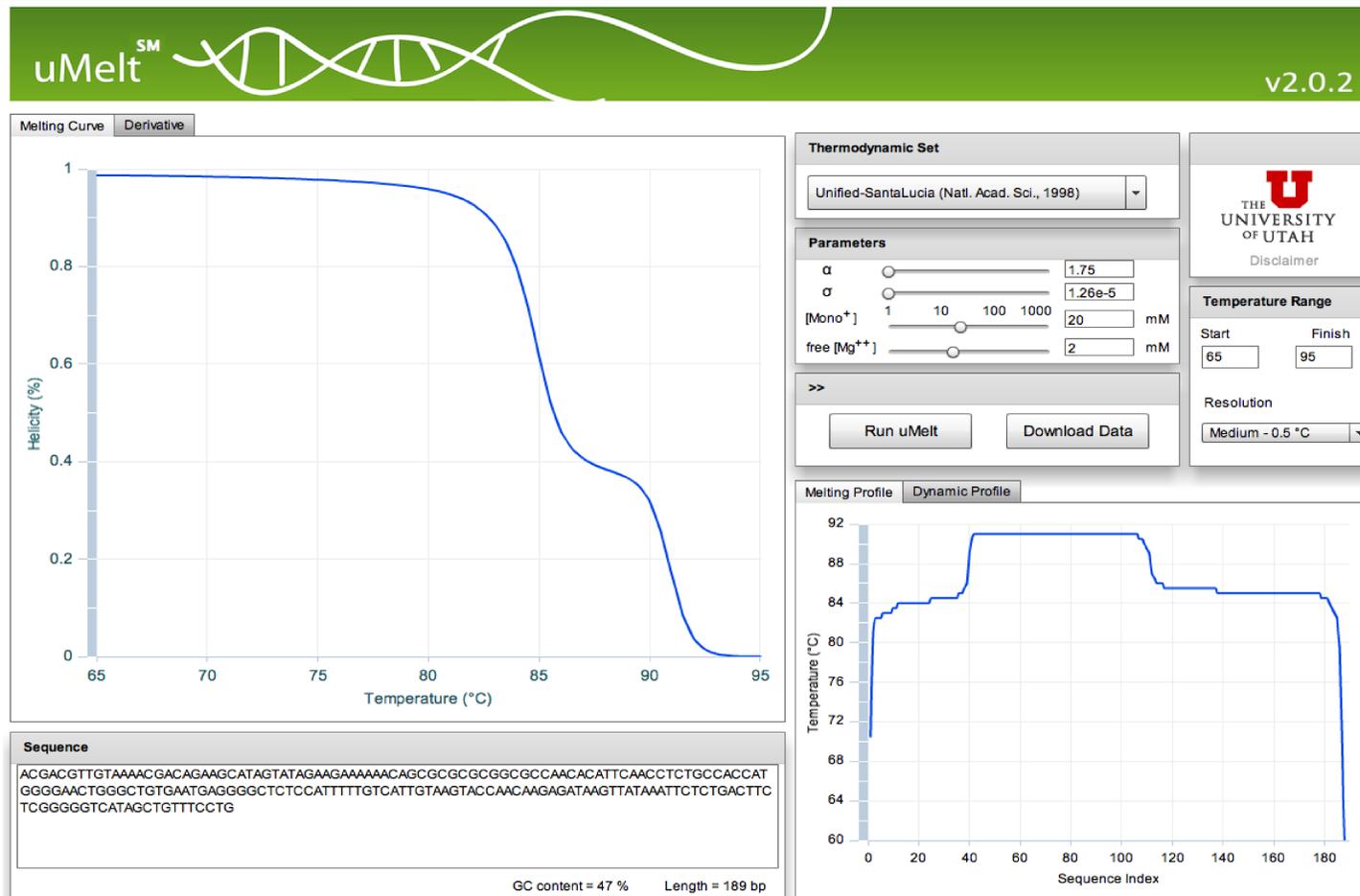
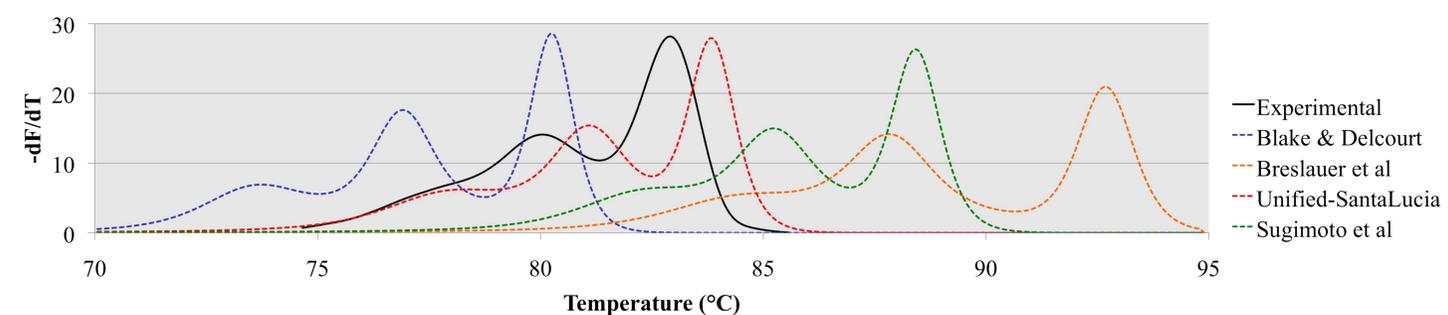


Figure 2: Screenshot of uMELT GUI

## Results

In Figure 4, a comparison is presented between predicted derivative melting curves (per thermodynamic set) and an experimental curve (MCAD gene, exon 3). The experimental shape agrees with all predictions in the number of melting domains (3), yet melting temperatures of domains varies with the user selected thermodynamic library.

Figure 4: Predicted and Experimental Derivative Melting curve comparison for MCAD



## Discussion

Prediction of polymer domain melting is more computationally intensive than simple 2-state oligomer melting. Lab conditions, experimental protocols, generalized thermodynamics, and secondary structures all have the ability to change shape, melting temperature, and slope of a melting domain.

uMelt predicts shape of melting curves accurately, yet more work is needed in nearest neighbor thermodynamics to gain better melting temperature predictions.

Even with current limitations, uMelt provides a convenient tool for design and optimization of high resolution melting experiments by predicting PCR product melting curves and reducing both laboratory costs and repeat experiments.

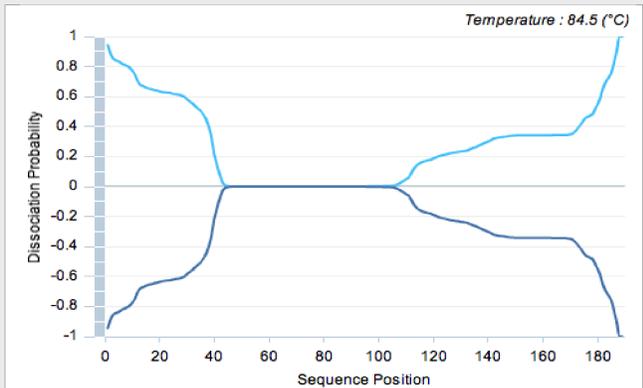


Figure 3: Dynamic Melting Profile

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## Contact Information

ZACH.DWIGHT@PATH.UTAH.EDU

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