Citing uAnalyze℠

Example in text:

‘Normalization and analysis of melting curves was performed with the uAnalyze software at dna-utah.org.’

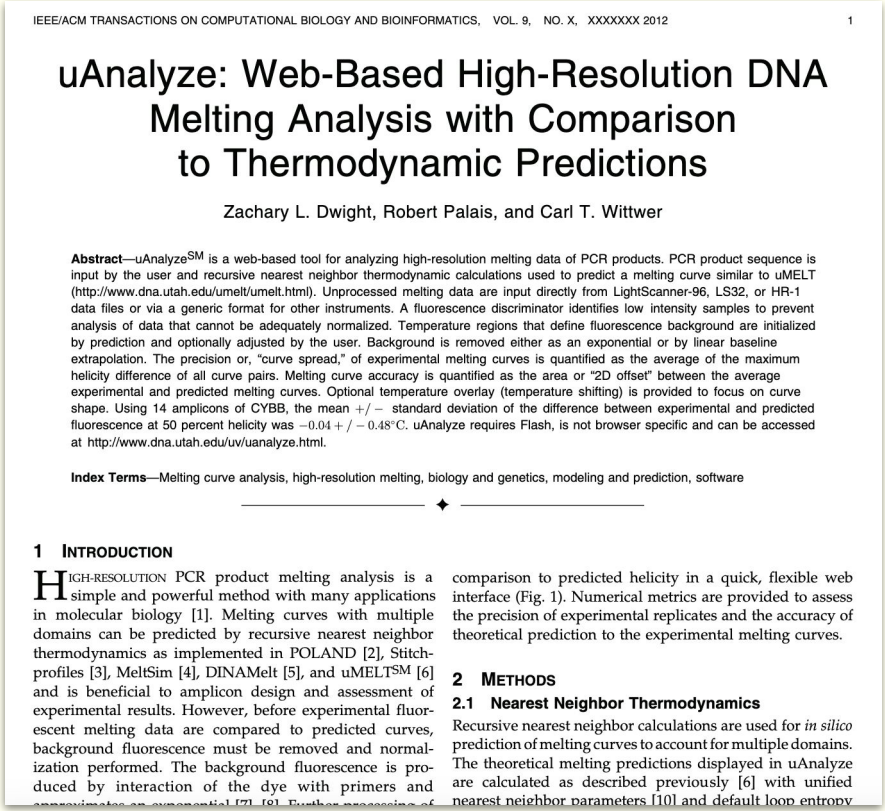
In References:

AS SOFTWARE…

Dwight Z. uAnalyze – High resolution melting normalization and analysis tool [Online]. DNA-UTAH.ORG. Available from: https://dna-utah.org/uv/uanalyze.html

OR CITE…

Dwight ZL, Palais R, Wittwer CT. uAnalyze: web-based high-resolution DNA melting analysis with comparison to thermodynamic predictions. *IEEE/ACM Trans Comput Biol Bioinform*. 2012;9(6):1805-1811. doi:10.1109/TCBB.2012.112



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uAnalyze: Web-Based High-Resolution DNA Melting Analysis with Comparison to Thermodynamic Predictions

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Abstract—uAnalyze℠ is a web-based tool for analyzing high-resolution melting data of PCR products. PCR product sequence is input by the user and recursive nearest neighbor thermodynamic calculations used to predict a melting curve similar to uMELT (http://www.dna.utah.edu/umelt/umelt.html). Unprocessed melting data are input directly from LightScanner-96, LS32, or HR-1 data files or via a generic format for other instruments. A fluorescence discriminator identifies low intensity samples to prevent analysis of data that cannot be adequately normalized. Temperature regions that define fluorescence background are initialized by prediction and optionally adjusted by the user. Background is removed either as an exponential or by linear baseline extrapolation. The precision or, “curve spread,” of experimental melting curves is quantified as the average of the maximum helicity difference of all curve pairs. Melting curve accuracy is quantified as the area or “2D offset” between the average experimental and predicted melting curves. Optional temperature overlay (temperature shifting) is provided to focus on curve shape. Using 14 amplicons of CYBB, the mean $+/-$ standard deviation of the difference between experimental and predicted fluorescence at 50 percent helicity was $-0.04 +/- 0.48°C$. uAnalyze requires Flash, is not browser specific and can be accessed at http://www.dna.utah.edu/uv/uanalyze.html.

Index Terms—Melting curve analysis, high-resolution melting, biology and genetics, modeling and prediction, software

1 Introduction

High-resolution PCR product melting analysis is a simple and powerful method with many applications in molecular biology [1]. Melting curves with multiple domains can be predicted by recursive nearest neighbor thermodynamics as implemented in POLAND [2], Stitch-profiles [3], MeltSim [4], DINAMelt [5], and uMELT℠ [6] and is beneficial to amplicon design and assessment of experimental results. However, before experimental fluorescent melting data are compared to predicted curves, background fluorescence must be removed and normalization performed. The background fluorescence is produced by interaction of the dye with primers and approximates an exponential [7], [8]. Further processing of comparison to predicted helicity in a quick, flexible web interface (Fig. 1). Numerical metrics are provided to assess the precision of experimental replicates and the accuracy of theoretical prediction to the experimental melting curves.

2 Methods

2.1 Nearest Neighbor Thermodynamics

Recursive nearest neighbor calculations are used for *in silico* prediction of melting curves to account for multiple domains. The theoretical melting predictions displayed in uAnalyze are calculated as described previously [6] with unified nearest neighbor parameters [10] and default loop entropy