



Using uMelt algorithm to predict fluorescent melting curves for the ABI7500.

Created: Jun 29, 2017; Last modified: Jun 29, 2017, Version: 1.0

uMelt is an online internet application used to predict high-resolution fluorescent DNA melting temperature curves for PCR products of known sequence. The uMELT Melting Curve Predictions Software was developed and is maintained by The Wittwer Lab for DNA analysis and hosted in the University of Utah website at <https://www.dna.utah.edu/umelt/umelt.html>. Melting curve analysis assesses the dissociation-characteristics of double-stranded DNA molecules subjected to heating. As the temperature increases, the double strand begins to dissociate leading to an increase in absorbance intensity. The temperature at which 50% of DNA is denatured is known as the melting point. The graph of the negative first derivative of the melting-curve may make it easier to pin-point the temperature of dissociation, by virtue of the peak thus formed. Salts concentration, presence of DNA intercalators (such as ethidium bromide), monovalent ions, free magnesium, DMSO, FBS and other parameters such as pH affect melting curves. The uMelt algorithm allows for optimisation of some of these parameters to better reflect the contents of different commercial mastermixes. This protocol describes the best uMelt parameters for melting curve analysis for applications using the Applied Biosystems 7500 thermocycler and the following mastermixes: 1) VeriQuest SYBR Green One-step qRT-PCR Mix by USB Affymetrix (Cat. No.: 75700) and 2) Script One step RT-qPCR Greenmaster Mix by Jena Bioscience (Cat. No.: PCR-514S).

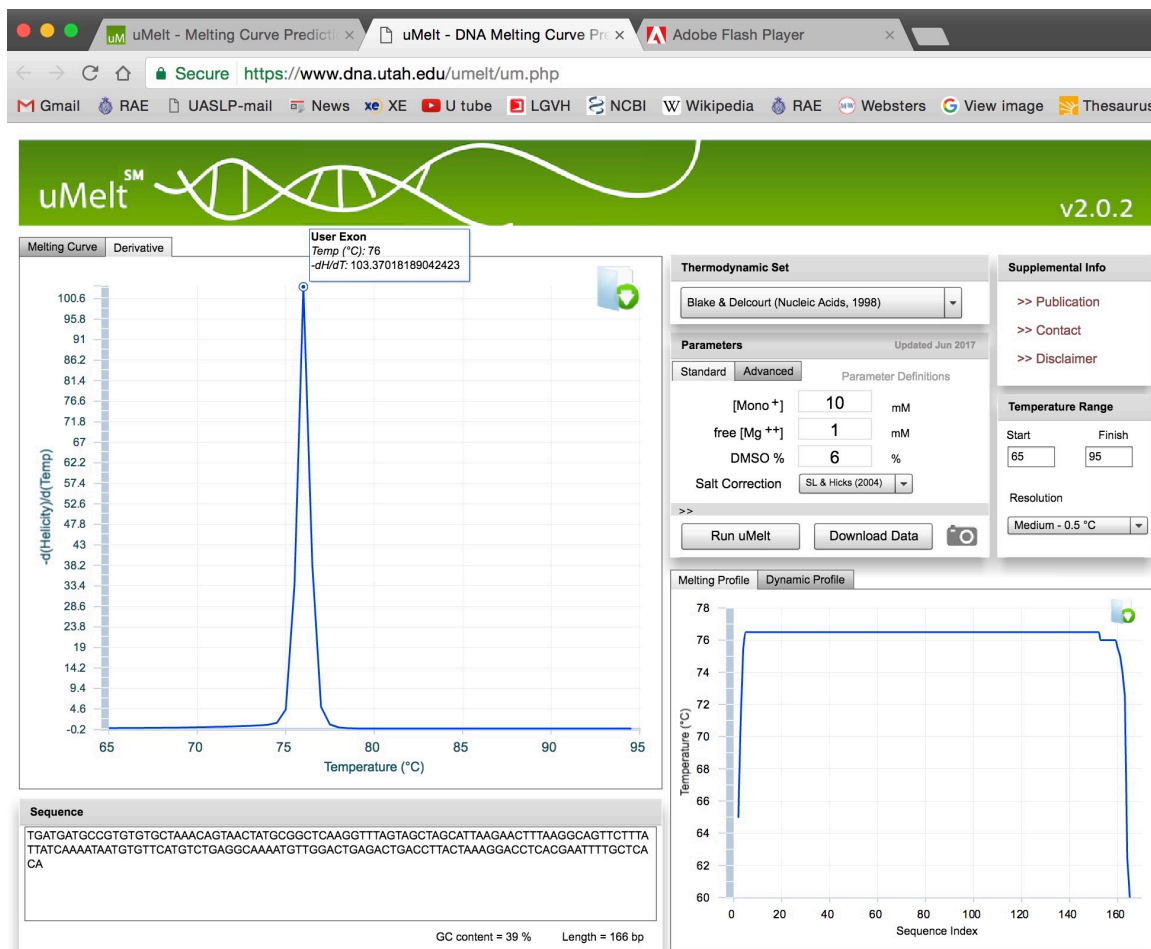
Procedure

1. In a flash supported browser go to <https://www.dna.utah.edu/umelt/umelt.html>.
2. Launch the uMelt by clicking on the link indicated with the arrow on the image below:



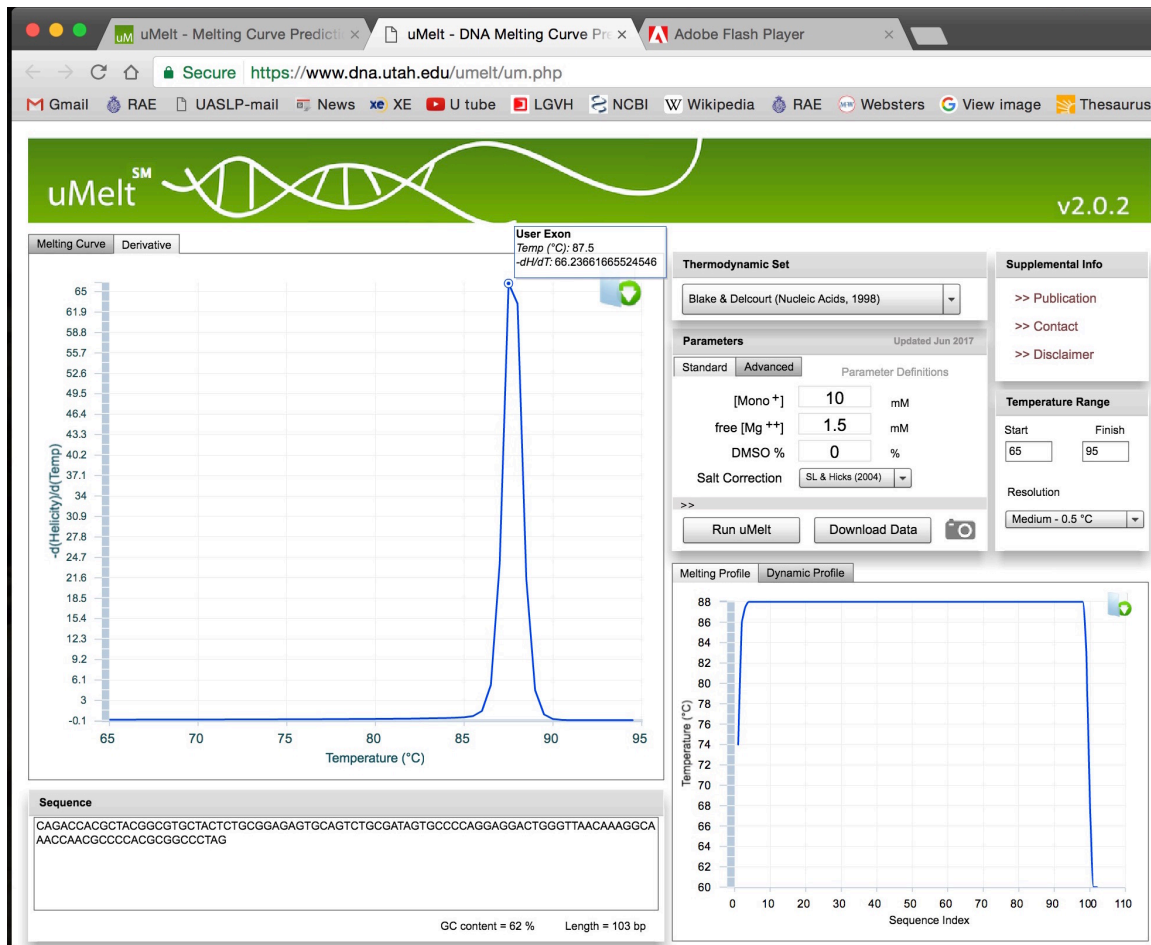
For fragments amplified using VeriQuest SYBR Green One-step qRT-PCR (SYBRgreen)

1. Use the **Blake & Delcourt Nucleic Acids 1998** thermodynamic set.
2. Use a **10 mM** monovalent cation concentration.
3. Use a **1 mM** free magnesium concentration.
4. Use **6%** DMSO content.
5. Select **SL & Hicks (2004)** salt correction
6. Introduce target sequence in window in the lower part of screen and select **RUN uMELT**.
7. In upper left portion of screen select **Derivative**.
8. Check estimated T_m by hovering cursor above melting curve peak.



For fragments amplified using Script One step RT-qPCR Greenmaster (EVAgreen)

1. Use the **Blake & Delcourt Nucleic Acids 1998** thermodynamic set.
2. Use a **10 mM** monovalent cation concentration.
3. Use a **1.5 mM** free magnesium concentration.
4. Use **0%** DMSO content.
5. Select **SL & Hicks (2004)** salt correction
6. Introduce target sequence in window in the lower part of screen and select **RUN uMELT**.
7. In upper left portion of screen select **Derivative**.
8. Check estimated T_m by hovering cursor above melting curve peak.





Notes

1. uMelt estimated melting temperatures may vary somewhat from the ABI7500 melting curve analysis. We have determined that these variations deviate between 0.1 to 0.3 °C for the Jena Bioscience Script One step RT-qPCR Greenmaster (EVAgreen) assay and between 0.2 to 1.4 °C for the USB Affymetrix VeriQuest SYBR Green One-step qRT-PCR (SYBRgreen) assay.
2. uMelt parameters should be optimized for other commercial brands and components such as for qPCR only and two-step RT-qPCR reactions setups.

References

1. Dwight Z, Palais R, Wittwer CT. uMELT: prediction of high-resolution melting curves and dynamic melting profiles of PCR products in a rich web application. *Bioinformatics*. 2011 Apr 1;27(7):1019-20. doi: 10.1093/bioinformatics/btr065. Epub 2011 Feb 7. PubMed PMID: 21300699.
2. <https://www.dna.utah.edu/umelt/umelt.html>

Revision history

- 1.0 Original document.

