

uMeltSM User Guide

v2.0

Author

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Location

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Wittwer DNA Lab

<http://www.dna.utah.edu/>

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1) Input Sequence

2) Select Thermodynamic Set

3) Adjust Parameters

4) Run uMelt

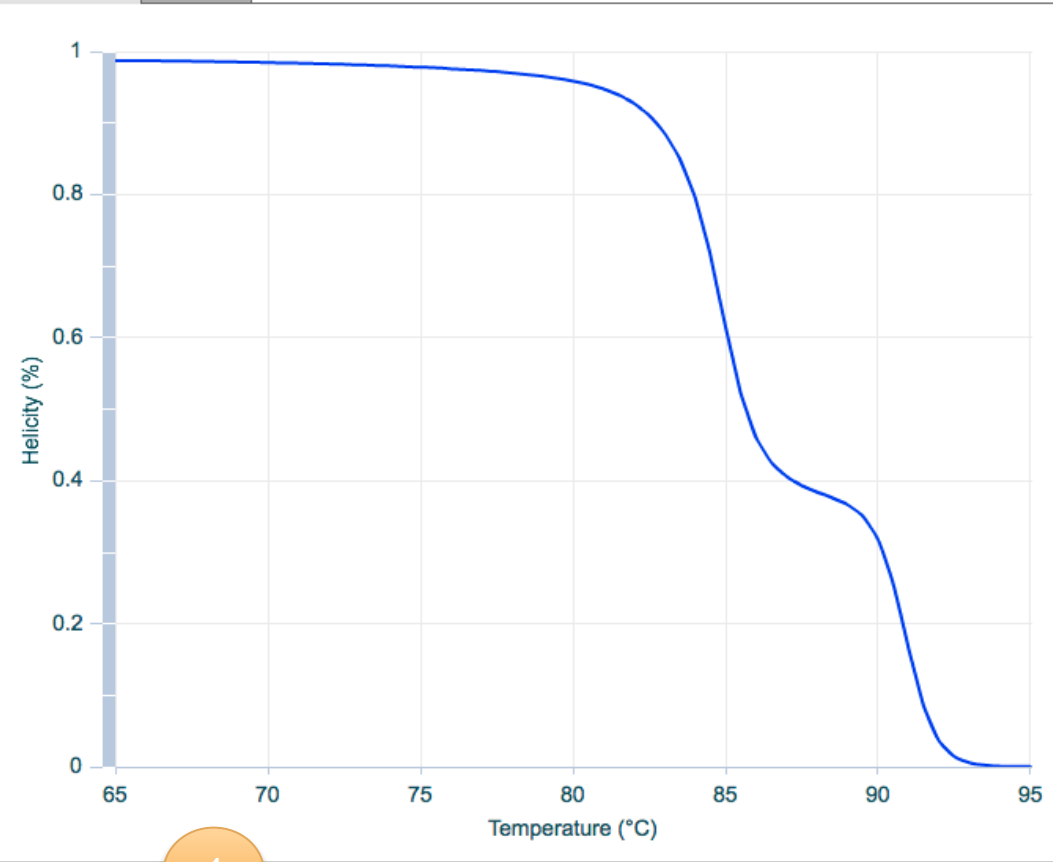
5) Download Results

uMeltSM



v2.0.2

Melting Curve Derivative



Sequence
ACGACGTTGTAAAACGACAGAAGCATAGTATAGAAGAAAAACAGCGCGCGGGCGCCAACACATTCAACCTCTGCCAC
CATGGGGAACGGGCTGTGAATGAGGGGCTCTCCATTTTTGTCATTGTAAGTACCAACAAGAGATAAGTTATAAATTCTCT
GACTTCTCGGGGGTCATAGCTGTTTCCTG

GC content = 47 % Length = 189 bp

Thermodynamic Set 2
Unified-SantaLucia (Natl. Acad. Sci., 1998)

Parameters 3

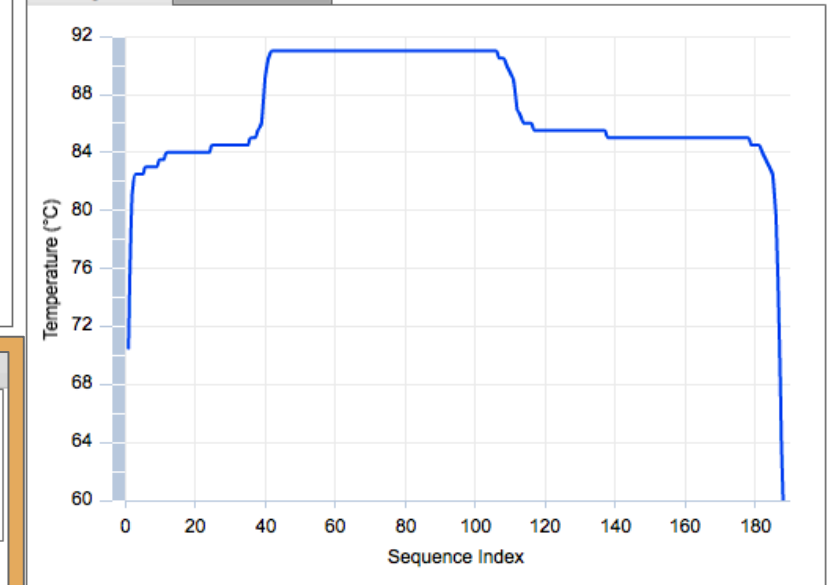
α 1.75
 σ 1.26e-5
[Mono⁺] 1 10 100 1000 20 mM
free [Mg⁺⁺] 2 mM
DMSO % 0 %

Temperature Range

Start 65 Finish 95
Resolution Medium - 0.5 °C

4 Run uMelt 5 Download Data

Melting Profile Dynamic Profile



Input Sequence

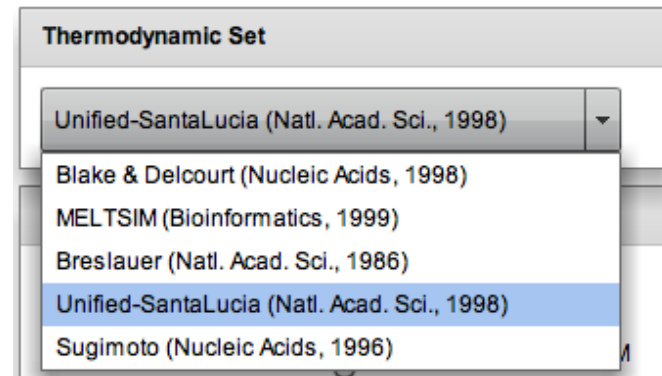
- Copy/Paste amplicon sequence into text box below:

Sequence
ACGACGTTGTAAAACGACAGAAGCATAGTATAGAAGAAAGGCAAACACAACACATTCAACCTCTGCCACCATGGGGAAGTGGGCTGTGAATGAGGGGCTCTCCATTTTTGTCATTGTAAGTACCAACAAGAGATAAGTTATAAATTCTCTGACTTCTCGGGGGTCATAGCTGTTTCCTG

- Note: uMeltSM only accepts the nucleotides A, T, G, C – any other characters will be deleted. Both capital and small letters are accepted.
- White or blank space is ignored

Thermodynamic Parameter Sets

- uMeltSM includes a variety of nearest neighbor parameter sets from the literature (see Technical Guide for references).
- By default, SantaLucia's unified parameter set is selected.



Parameters

- To define your experimental conditions and better match predicted to observed melting curves, parameter sliders are provided for the following:

- α is the loop closure exponent. Loops are more likely at high α compared to low (default).
- σ defines the cooperativity of melting. Higher σ (closer to 1) results in more gradual melting transitions.

*Blossey & Carlon (2003) further detail use of α and σ (see Technical Guide)

- ¹ [Mono⁺] : [monovalent cations] (mM)
- ² Free [Mg⁺⁺] : [Mg - dNTPs] (mM)
- ³ DMSO % : dimethylsulfoxide (%)

Parameters	
α	<input type="text" value="1.75"/>
σ	<input type="text" value="1.26e-5"/>
[Mono ⁺]	<input type="text" value="20"/> mM
free [Mg ⁺⁺]	<input type="text" value="2"/> mM
DMSO %	<input type="text" value="0 %"/>

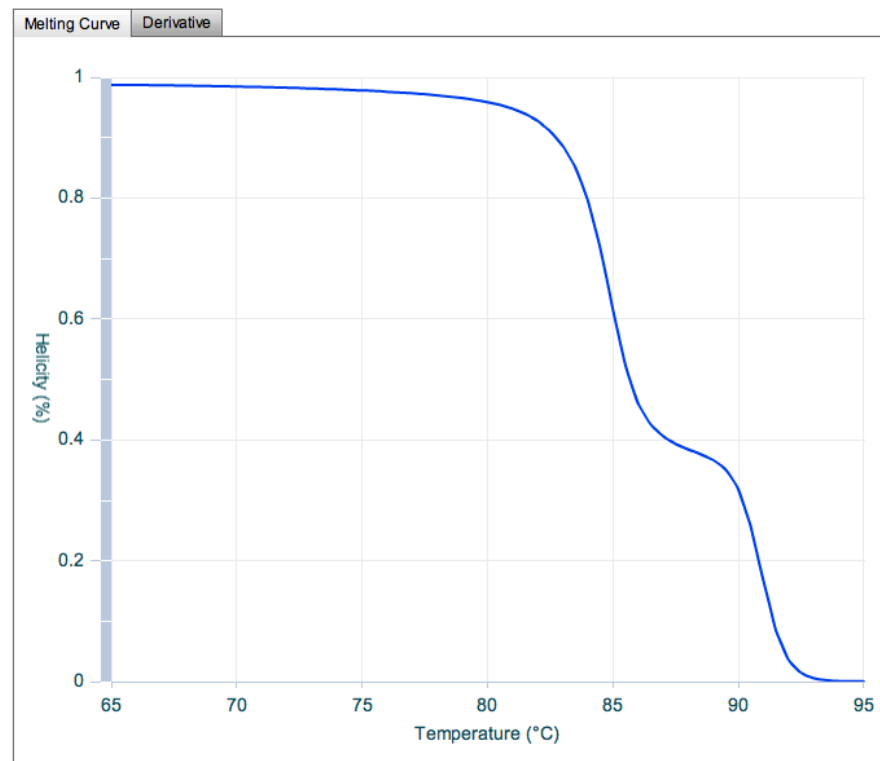
Temperature Range

- Specify a temperature range to help reduce calculation time. Graphs will adjust to set range:

Temperature Range

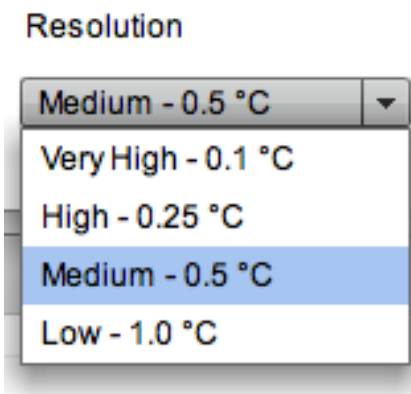
Start: 65 Finish: 95

Resolution: Medium - 0.5 °C



Resolution

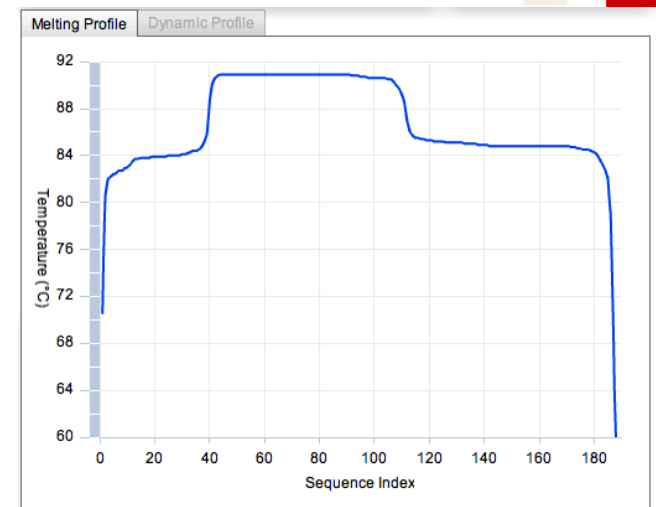
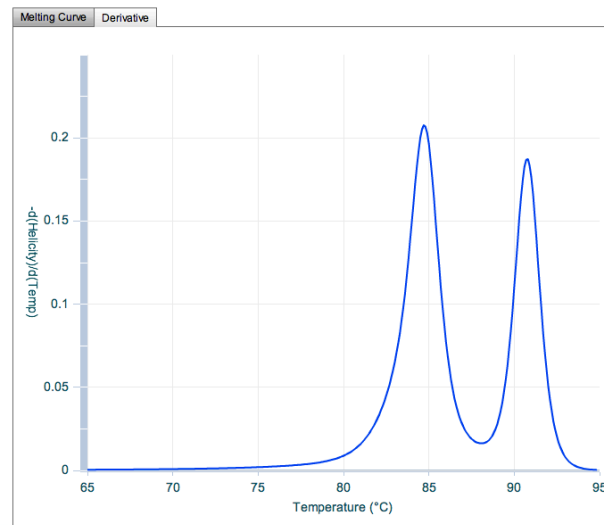
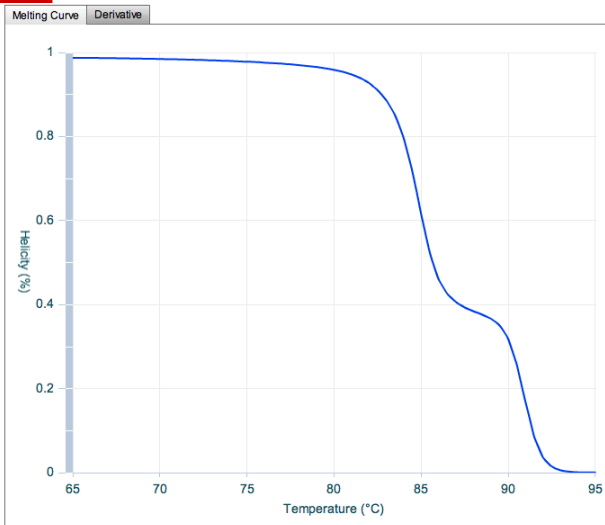
- Four temperature resolutions can be selected :
 - Very high = 10 points/°C
 - High = 4 points/°C
 - Medium = 2 points/°C
 - Low = 1 point/°C



Run uMeltSM & Download Data

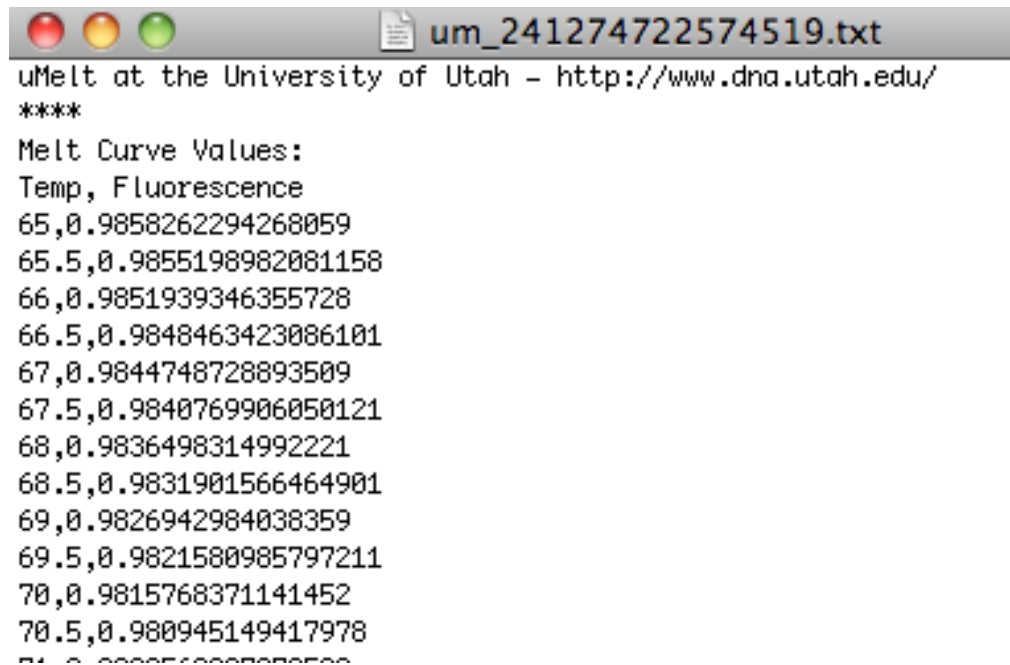
- Once a melting curve is calculated, the data may be downloaded.
- Three sets of values are available in a text file
 - Melting Curve : Temperature & Fluorescence
 - Derivative : Temperature & $-d(\text{Helicity})/d(\text{Temperature})$
 - Melting Profile : Sequence Index & T_m

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Data File

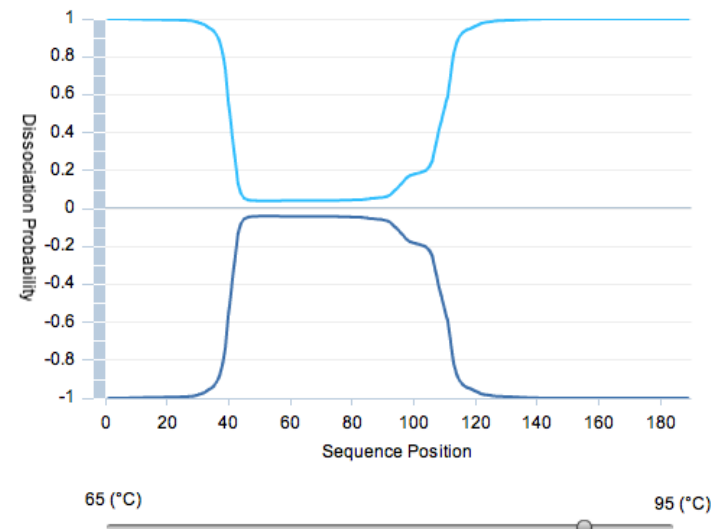
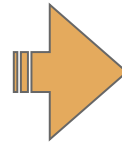
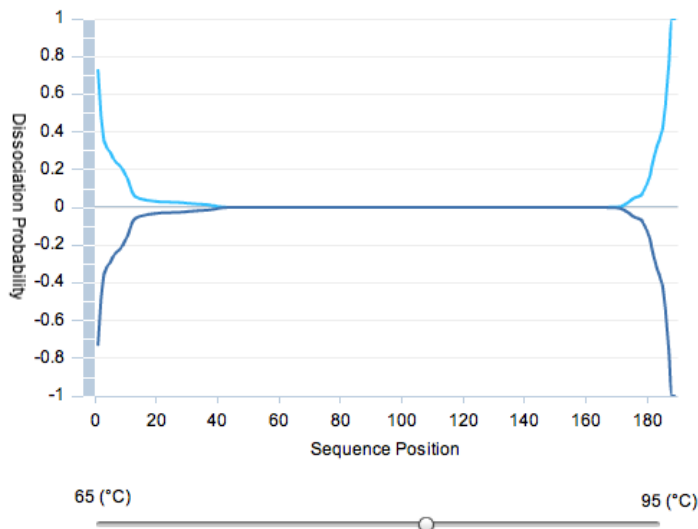
- Data is downloaded as a *.txt.



```
um_241274722574519.txt
uMelt at the University of Utah - http://www.dna.utah.edu/
****
Melt Curve Values:
Temp, Fluorescence
65,0.9858262294268059
65.5,0.9855198982081158
66,0.9851939346355728
66.5,0.9848463423086101
67,0.9844748728893509
67.5,0.9840769906050121
68,0.9836498314992221
68.5,0.9831901566464901
69,0.9826942984038359
69.5,0.9821580985797211
70,0.9815768371141452
70.5,0.980945149417978
```

Dynamic Melting Profile

- In addition to melting curve, derivative plot, and static melting profile – a dynamic representation of the melting profile is provided to visualize strand dissociation
- User may control visualization with a temperature slider



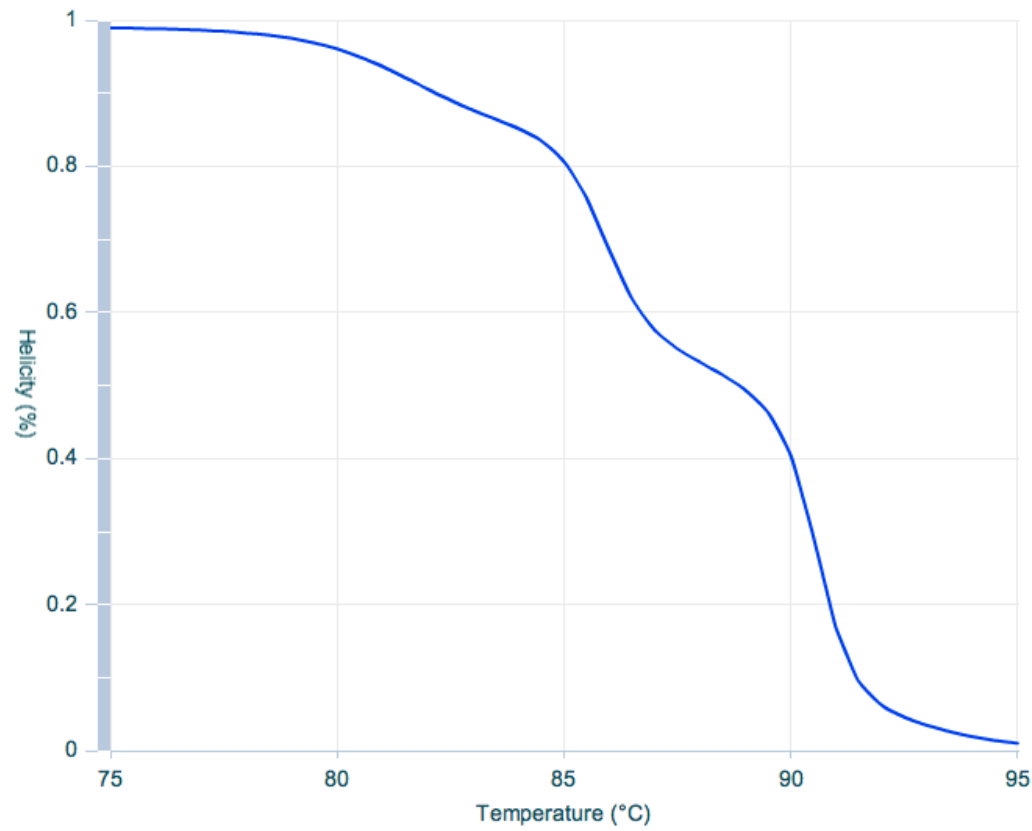
uMeltSM Suggested Practices

- 1) Copy/Paste Sequence
 - Between 50-1000 base pairs works well, but uMeltSM has been stress tested to 3600 bps.
- 2) Select thermodynamic set
 - Depends on your experimental conditions
- 3) Adjust parameters
 - Default parameters reflect our PCR conditions
 - Temperature range between 65 & 95 often works well
- 4) Run uMeltSM
 - Calculation time depends on the sequence length as well as the resolution.
 - If the program times out or freezes, try refreshing the application from the browser toolbar.
- 5) Download Data File
 - The data file is comma delimited for easy import.

Provide Feedback

- We welcome your feedback, questions, bugs, improvements, ideas to zach.dwight@path.utah.edu
 - Users provide great insight and we are always looking to improve.
 - Even a quick email describing how you are using our software is very beneficial.
 - More importantly, enjoy the software !

Thanks and Enjoy!



uMeltSM

<http://www.dna.utah.edu>

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