

**T<sub>M</sub> TOOL<sup>SM</sup>**  
Melting Temperature  
Calculation



User Guide  
DNA.UTAH.EDU  
University of Utah



# Type of Calculation

- There are three options for the calculation that depend on the conditions of melting.

## CALCULATION TYPE

- ✓ **Primer (Before PCR)**
- Probe (After PCR)
- Oligo ( ≤ 50 bp)



# Type of Calculation

- Primer (Before PCR)

## SEQUENCE INFORMATION

Primer Sequence (5'-3')

aagtgcacttagctacgtagcatcg

@

0.5

μM

Length: 25 bp | 48% GC

- Since the primer concentration is so much greater than the template concentration before PCR, the primer T<sub>m</sub> depends only on the primer concentration.



# Type of Calculation

- Probe (After PCR)

## SEQUENCE INFORMATION

Probe Sequence (5'-3')	aagtgcaacttagctacgtagcatcg	@	0.5	μM
[Primers]	0.5	μM		
	0.1	μM		
[Excess Primer]	0.4	μM		
Fraction Converted	0.6			



# Type of Calculation

- Oligo (<= 50 bp)

## SEQUENCE INFORMATION

Oligo Sequence (5'-3')

gaaaaggagtgcaacttagctacgta

@

0.5

μM

Complement Sequence (3'-5')

CTTTTCCTCACGTGAATCGA

@

0.5

μM

Length: 31 bp | 48% GC



# Parameters

- There are also parameters which apply to all calculation types

## PARAMETERS

[Mono <sup>+</sup> ] (Na <sup>+</sup> + K <sup>+</sup> + Tris <sup>+</sup> )	<input type="text" value="20.0"/>	mM
[Mg <sup>++</sup> ]	<input type="text" value="3.0"/>	mM
[Total dNTPs]	<input type="text" value="0.8"/>	mM
Free [Mg <sup>++</sup> ]	<input type="text" value="2.2"/>	mM
Thermodynamic Library	<input type="text" value="1998 Unified SL"/> ▼	
Salt Correction	<input type="text" value="2004 SL Hicks"/> ▼	



# Calculation

- Once all inputs are defined, the calculation can be performed by pushing the button underneath the parameters section

## PARAMETERS

[Mono <sup>+</sup> ] (Na <sup>+</sup> + K <sup>+</sup> + Tris <sup>+</sup> )	<input type="text" value="20.0"/>	mM
[Mg <sup>++</sup> ]	<input type="text" value="3.0"/>	mM
[Total dNTPs]	<input type="text" value="0.8"/>	mM
Free [Mg <sup>++</sup> ]	<input type="text" value="2.2"/>	mM
Thermodynamic Library	<input type="text" value="1998 Unified SL"/>	▼
Salt Correction	<input type="text" value="2004 SL Hicks"/>	▼



# Calculation

- The final T<sub>m</sub> calculation is then displayed below.

## PARAMETERS

[Mono <sup>+</sup> ] (Na <sup>+</sup> + K <sup>+</sup> + Tris <sup>+</sup> )	<input type="text" value="20.0"/>	mM
[Mg <sup>++</sup> ]	<input type="text" value="3.0"/>	mM
[Total dNTPs]	<input type="text" value="0.8"/>	mM
Free [Mg <sup>++</sup> ]	<input type="text" value="2.2"/>	mM
Thermodynamic Library	<input type="text" value="1998 Unified SL"/>	▼
Salt Correction	<input type="text" value="2004 SL Hicks"/>	▼

Calculate T<sub>m</sub>

T<sub>m</sub>(°C):  
66.44



Navigation and info bar



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Melting Temperature Calculation

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Select calculation type from the drop down



CALCULATION TYPE

Oligo (<= 50 bp) ▾

Input sequence and concentration values



SEQUENCE INFORMATION

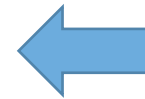
Oligo Sequence (5'-3')	<input type="text" value="gaaaaggagtgcaacttagctacgta"/>	@	<input type="text" value="0.5"/>	μM
Complement Sequence (3'-5')	<input type="text" value="CTTTTCCTCACGTGAATCGA"/>	@	<input type="text" value="0.5"/>	μM

Length: 31 bp | 48% GC

PARAMETERS

[Mono <sup>+</sup> ] (Na <sup>+</sup> + K <sup>+</sup> + Tris <sup>+</sup> )	<input type="text" value="20.0"/>	mM
[Mg <sup>++</sup> ]	<input type="text" value="3.0"/>	mM
[Total dNTPs]	<input type="text" value="0.8"/>	mM
Free [Mg <sup>++</sup> ]	<input type="text" value="2.2"/>	mM
Thermodynamic Library	<input type="text" value="1998 Unified SL"/>	
Salt Correction	<input type="text" value="2004 SL Hicks"/>	

Change laboratory conditions here



Calculate T<sub>m</sub>

T<sub>m</sub>(°C):  
70.89

Final T<sub>m</sub> displayed here





# Feedback and Contact

- Feedback is always appreciated as well as bug reporting and feature requests.
- Contact us at [zach.dwight \[at\] path.utah.edu](mailto:zach.dwight@path.utah.edu)



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# Credits

- Tm Tool was built with the PURE CSS Library (<http://purecss.io/>)
  - See Yahoo BSD License for more information
- DNA strand illustrations courtesy of

Kelly Dern  
<http://www.kellydern.com/>



# References

The following publications were used in the development of this application.

- [Owzcarzy et al. \(2008\)](#)
- [SantaLucia, Jr. \(1998\)](#)
- [SantaLucia, Jr. & Hicks \(2004\)](#)
- [Sugimoto et al. \(1996\)](#)
- [Weber \(2014\)](#)



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