

# Package ‘rmelting’

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**Title** R Interface to MELTING 5

**Version** 1.22.0

**Description** R interface to the MELTING 5 program (<https://www.ebi.ac.uk/biomodels/tools/melting/>) to compute melting temperatures of nucleic acid duplexes along with other thermodynamic parameters.

**Depends** R (>= 3.6)

**Imports** Rdpack, rJava (>= 0.9-8)

**Suggests** readxl, knitr, rmarkdown, reshape2, pander, testthat

**SystemRequirements** Java

**biocViews** BiomedicalInformatics, Cheminformatics,

**License** GPL-2 | GPL-3

**Encoding** UTF-8

**LazyData** true

**RoxygenNote** 7.2.1

**RdMacros** Rdpack

**URL** <https://github.com/aravind-j/rmelting>,  
<https://aravind-j.github.io/rmelting/>

**BugReports** <https://github.com/aravind-j/rmelting/issues>

**VignetteBuilder** knitr

**git\_url** <https://git.bioconductor.org/packages/rmelting>

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```

"owcmix08", "tanmix07"),
method.Naeq = c("ahs01", "mit96", "pey00"),
correction.DMSO = c("ahs01", "cul76", "esc80", "mus81"),
correction.formamide = c("bla96", "lincorr"))

```

## Arguments

|                    |  |
|--------------------|--|
| sequence           | Sequence (5' to 3') of one strand of the nucleic acid duplex as a character string ( <b>Note:</b> Uridine and thymidine are not considered as identical).  |
| comp.sequence      | Complementary sequence (3' to 5') of the nucleic acid duplex as a character string.  |
| nucleic.acid.conc  | Concentration of the nucleic acid strand (M or mol L <sup>-1</sup> ) in excess as a numeric value.   |
| hybridisation.type | The hybridisation type. Either "dnadna", "rnarna", "dnarna", "rnadna", "mrnarna" or "rnamrna" (see <b>Hybridisation type options</b> ).  |
| Na.conc            | Concentration of Na ions (M) as a positive numeric value (see <b>Ion and agent concentrations</b> ).   |
| Mg.conc            | Concentration of Mg ions (M) as a positive numeric value (see <b>Ion and agent concentrations</b> ).   |
| Tris.conc          | Concentration of Tris ions (M) as a positive numeric value (see <b>Ion and agent concentrations</b> ).   |
| K.conc             | Concentration of K ions (M) as a positive numeric value (see <b>Ion and agent concentrations</b> ).  |
| dNTP.conc          | Concentration of dNTP (M) as a positive numeric value (see <b>Ion and agent concentrations</b> ).  |
| DMSO.conc          | Concentration of DMSO (%) as a positive numeric value (see <b>Ion and agent concentrations</b> ).  |
| formamide.conc     | Concentration of formamide (M or % depending on correction method) as a positive numeric value (see <b>Ion and agent concentrations</b> ).   |
| size.threshold     | Sequence length threshold to decide approximative or nearest-neighbour approach for computation. Default is 60.  |
| force.self         | logical. Enforces that sequence is self complementary and complementary sequence is not required (see <b>Self complementary sequences</b> ). Default is FALSE.   |
| correction.factor  | Correction factor to be used to modulate the effect of the nucleic acid concentration (nucleic.acid.conc) in the computation of melting temperature (see <b>Correction factor for nucleic acid concentration</b> ).  |
| method.approx      | Specify the approximative formula to be used for melting temperature calculation for sequences of length greater than size.threshold. Either "ahs01", "che93", "che93corr", "schdot", "owe69", "san98", "wetdna91", "wetrna91" or "wetdnarna91" (see <b>Approximative formulas</b> ).                  |
| method.nn          | Specify the nearest neighbor model to be used for melting temperature calculation for perfectly matching sequences of length lesser than size.threshold. Either "all97", "bre86", "san04", "san96", "sug96", "tan04", "fre86", "xia98", "sug95" or "tur06" (see <b>Perfectly matching sequences</b> ). |

- method.GU Specify the nearest neighbor model to compute the contribution of GU base pairs to the thermodynamic of helix-coil transition. Either "tur99" or "ser12" (see **GU wobble base pairs effect**).
- method.singleMM Specify the nearest neighbor model to compute the contribution of single mismatch to the thermodynamic of helix-coil transition. Either "allsanpey", "tur06", "zno07" "zno08" or "wat11" (see **Single mismatch effect**).
- method.tandemMM Specify the nearest neighbor model to compute the contribution of tandem mismatches to the thermodynamic of helix-coil transition. Either "allsanpey" or "tur99" (see **Tandem mismatches effect**).
- method.single.dangle Specify the nearest neighbor model to compute the contribution of single dangling end to the thermodynamic of helix-coil transition. Either "bom00", "sugdna02", "sugrna02" or "ser08" (see **Single dangling end effect**).
- method.double.dangle Specify the nearest neighbor model to compute the contribution of double dangling end to the thermodynamic of helix-coil transition. Either "sugdna02", "sugrna02", "ser05" or "ser06" (see **Double dangling end effect**).
- method.long.dangle Specify the nearest neighbor model to compute the contribution of long dangling end to the thermodynamic of helix-coil transition. Either "sugdna02" or "sugrna02" (see **Long dangling end effect**).
- method.internal.loop Specify the nearest neighbor model to compute the contribution of internal loop to the thermodynamic of helix-coil transition. Either "san04", "tur06" or "zno07" (see **Internal loop effect**).
- method.single.bulge.loop Specify the nearest neighbor model to compute the contribution of single bulge loop to the thermodynamic of helix-coil transition. Either "san04", "tan04", "ser07" or "tur06" (see **Single bulge loop effect**).
- method.long.bulge.loop Specify the nearest neighbor model to compute the contribution of long bulge loop to the thermodynamic of helix-coil transition. Either "san04" or "tur06" (see **Long bulge loop effect**).
- method.CNG Specify the nearest neighbor model to compute the contribution of CNG repeats to the thermodynamic of helix-coil transition. Available method is "bro05" (see **CNG repeats effect**).
- method.inosine Specify the specific nearest neighbor model to compute the contribution of inosine bases (I) to the thermodynamic of helix-coil transition. Either "san05" or "zno07" (see **Inosine bases effect**).
- method.hydroxyadenine Specify the nearest neighbor model to compute the contribution of hydroxyadenine bases (A\*) to the thermodynamic of helix-coil transition. Available method is "sug01" (see **Hydroxyadenine bases effect**).
- method.azobenzenes Specify the nearest neighbor model to compute the contribution of azobenzenes (X\_T for trans azobenzenes and X\_C for cis azobenzenes) to the thermodynamic of helix-coil transition. Available method is "asa05" (see **Azobenzenes effect**).

|                                    |  |
|------------------------------------|--|
| method.locked                      | Specify the nearest neighbor model to compute the contribution of single locked nucleic acids (AL, GL, TL and CL) to the thermodynamic of helix-coil transition. Either "owc11" or "mct04" (see <b>Single locked nucleic acids effect</b> ).   |
| method.consecutive.locked          | Specify the nearest neighbor model to compute the contribution of consecutive locked nucleic acids (AL, GL, TL and CL) to the thermodynamic of helix-coil transition. Available method is "owc11" (see <b>Consecutive locked nucleic acids effect</b> ).   |
| method.consecutive.locked.singleMM | Specify the nearest neighbor model to compute the contribution of consecutive locked nucleic acids (AL, GL, TL and CL) with a single mismatch to the thermodynamic of helix-coil transition. Available method is "owc11" (see <b>Consecutive locked nucleic acids with single mismatch effect</b> ).   |
| correction.ion                     | Specify the correction method for ions. Either one of the following: <ul style="list-style-type: none"> <li>• Na corrections "ahs01", "kam71", "owc1904", "owc2004", "owc2104", "owc2204", "san96", "san04", "schlif", "tanna06", "wetdna91", "tanna07", "wetrna91" or "wetdnarna91" (see <b>Sodium corrections</b>)</li> <li>• Mg corrections "owcmg08", "tanmg06" or "tanmg07" (see <b>Magnesium corrections</b>)</li> <li>• Mixed Na Mg corrections "owcmix08", "tanmix07" or "tanmix07" (see <b>Mixed Sodium and Magnesium corrections</b>)</li> </ul> |
| method.Naeq                        | Specify the ion correction which gives a sodium equivalent concentration if other cations are present. Either "ahs01", "mit96" or "pey00" (see <b>Sodium equivalent concentration methods</b> ).   |
| correction.DMSO                    | Specify the correction method for DMSO. Specify the correction method for DMSO. Either "ahs01", "mus81", "cu176" or "esc80" (see <b>DMSO corrections</b> ).  |
| correction.formamide               | Specify the correction method for formamide. Specify the correction method for formamide Either "bla96" or "lincorr" (see <b>Formamide corrections</b> ).  |

## Value

A list with the following components:

|             |   |
|-------------|---|
| Environment | A list with details about the melting temperature computation environment.  |
| Options     | A list with details about the options (default or user specified) used for melting temperature computation.                             |
| Results     | A list with the results of the melting temperature computation including the enthalpy and entropy in case of nearest neighbour methods. |
| Message     | Error and/or Warning messages, if any.  |

## Mandatory arguments

The following are the arguments which are mandatory for computation.

sequence 5' to 3' sequence of one strand of the nucleic acid duplex as a character string. Recognises A, C, G, T, U, I, X\_C, X\_T, A\*, AL, TL, GL and CL. U and T are not considered identical (see **Recognized nucleotides**).

`comp.sequence` Mandatory if there are mismatches, inosine(s) or hydroxyadenine(s) between the two strands. If not specified, it is computed as the complement of sequence. Self-complementarity in sequence is detected even though there may be (are) dangling end(s) and `comp.sequence` is computed (see **Self complementary sequences**).

`nucleic.acid.conc` See **Correction factor for nucleic acid concentration**.

`Na.conc`, `Mg.conc`, `Tris.conc`, `K.conc` At least one cation (Na, Mg, Tris, K) concentration is mandatory, the other agents (dNTP, DMSO, formamide) are optional (see **Ion and agent concentrations**).

`hybridisation.type` See **Hybridisation type options**.

### Recognized nucleotides

| Code | Type                |
|------|---------------------|
| A    | Adenine             |
| C    | Cytosine            |
| G    | Guanine             |
| T    | Thymine             |
| U    | Uracil              |
| I    | Inosine             |
| X_C  | Trans azobenzenes   |
| X_T  | Cis azobenzenes     |
| A*   | Hydroxyadenine      |
| AL   | Locked nucleic acid |
| TL   | "                   |
| GL   | "                   |
| CL   | "                   |

U and T are not considered identical.

### Hybridisation type options

The details of the possible options for hybridisation type specified in the argument `hybridisation.type` are as follows:

| Option               | Sequence       | Complementary sequence |
|----------------------|----------------|------------------------|
| <code>dnadna</code>  | DNA            | DNA                    |
| <code>rnarna</code>  | RNA            | RNA                    |
| <code>dnarna</code>  | DNA            | RNA                    |
| <code>rnadna</code>  | RNA            | DNA                    |
| <code>mrnarna</code> | 2-o-methyl RNA | RNA                    |
| <code>rnamrna</code> | RNA            | 2-o-methyl RNA         |

This parameter determines the nature of the sequences in the arguments `sequence` and `comp.sequence`.

### Ion and agent concentrations

Ion concentrations are specified by the arguments `Na.conc`, `Mg.conc`, `Tris.conc` and `K.conc`, while agent concentrations are specified by the arguments `dNTP.conc`, `DMSO.conc` and `formamide.conc`.

These values are used for different correction functions which approximately adjusts for effects of these ions (Na, Mg, Tris, K) and/or agents (dNTP, DMSO, formamide) on the thermodynamic stability of nucleic acid duplexes. Their concentration limits depends on the correction method used. All the concentrations must be in M, except for the DMSO (%) and formamide (% or M depending on the correction method). Note that  $[\text{Tris}^+]$  is about half of the total tris buffer concentration.

**Self complementary sequences**

Self complementarity for perfect matching sequences or sequences with dangling ends is detected automatically. However it can be enforced by the argument `force.self = TRUE`.

**Correction factor for nucleic acid concentration**

For self complementary sequences (Auto detected or specified by `force.self`) it is 1. Otherwise it is 4 if the both strands are present in equivalent amount and 1 if one strand is in excess.

**Approximative estimation formulas**

| Formula      | Type    | Limits/Remarks                                  | Reference  |
|--------------|---------|---|--|
| ahs01        | DNA     | No mismatch                                     | von Ahsen et al., 2001   |
| che93        | DNA     | No mismatch; Na=0, Mg=0.0015, Tris=0.01, K=0.05 | Marmur and Doty, 1962  |
| che93corr    | DNA     | No mismatch; Na=0, Mg=0.0015, Tris=0.01, K=0.05 | Marmur and Doty, 1962  |
| schdot       | DNA     | No mismatch                                     | Wetmur, 1991; Marmur and Doty, 1962; Chester and Marshak, 1993; Schildkraut and Lifson, 1965; Wahl et al., 1987; Britten et al., 1974; Hall et al., 1980 |
| owe69        | DNA     | No mismatch                                     | Owen et al., 1969; Frank-Kamenetskii, 1971; Blake, 1996; Blake and Delcourt, 1998  |
| san98        | DNA     | No mismatch                                     | SantaLucia, 1998; von Ahsen et al., 2001   |
| wetdna91*    | DNA     |   | Wetmur, 1991   |
| wetrna91*    | RNA     |   | Wetmur, 1991   |
| wetdnarna91* | DNA/RNA |   | Wetmur, 1991   |

\* Default formula for computation.

Note that calculation is increasingly incorrect when the length of the duplex decreases. Further, it does not take into account nucleic acid concentration.

**Nearest neighbor models****Perfectly matching sequences:**

| Model   | Type               | Limits/Remarks  | Reference                   |
|---------|--------------------|---|-----------------------------|
| all197* | DNA                |   | Allawi and SantaLucia, 1997 |
| tur06*  | 2'-O-MeRNA/<br>RNA | A sodium correction (san04) is automatically applied to convert the entropy (Na = 0.1M) into the entropy (Na = 1M). | Kierzek et al., 2006        |
| bre86   | DNA                |   | Breslauer et al., 1986      |
| san04   | DNA                |   | SantaLucia and Hicks, 2004  |

|        |             |                         |
|--------|-------------|-------------------------|
| san96  | DNA         | SantaLucia et al., 1996 |
| sug96  | DNA         | Sugimoto et al., 1996   |
| tan04  | DNA         | Tanaka et al., 2004     |
| fre86  | RNA         | Freier et al., 1986     |
| xia98* | RNA         | Xia et al., 1998        |
| sug95* | DNA/<br>RNA | SantaLucia et al., 1996 |

\* Default model for computation.

#### GU wobble base pairs effect:

| Model  | Type | Limits/Remarks | Reference            |
|--------|------|----------------|----------------------|
| tur99  | RNA  |                | Mathews et al., 1999 |
| ser12* | RNA  |                | Chen et al., 2012    |

\* Default model for computation.

GU base pairs are not taken into account by the approximative mode.

#### Single mismatch effect:

| Model      | Type    | Limits/Remarks                      | Reference   |
|------------|---------|-------------------------------------|---|
| allsanpey* | DNA     |                                     | Allawi and SantaLucia, 1997;<br>Allawi and SantaLucia, 1998;<br>Allawi and SantaLucia, 1998;<br>Allawi and SantaLucia, 1998;<br>Peyret et al., 1999 |
| wat11*     | DNA/RNA |                                     | Watkins et al., 2011  |
| tur06      | RNA     |                                     | Lu et al., 2006   |
| zno07*     | RNA     |                                     | Davis and Znosko, 2007  |
| zno08      | RNA     | At least one adjacent GU base pair. | Davis and Znosko, 2008  |

\* Default model for computation.

Single mismatches are not taken into account by the approximative mode.

#### Tandem mismatches effect:

| Model      | Type | Limits/Remarks                           | Reference   |
|------------|------|--|---|
| allsanpey* | DNA  | Only GT mismatches and TA/TG mismatches. | Allawi and SantaLucia, 1997;<br>Allawi and SantaLucia, 1998;<br>Allawi and SantaLucia, 1998;<br>Allawi and SantaLucia, 1998;<br>Peyret et al., 1999 |
| tur99*     | RNA  | No adjacent GU or UG base pairs.         | Mathews et al., 1999; Lu et al., 2006   |

\* Default model for computation.

Tandem mismatches are not taken into account by the approximative mode. Note that not all the mismatched Crick's pairs have been investigated.



**Single dangling end effect:**

| Model    | Type | Limits.Remarks   | Reference                                 |
|----------|------|--|---|
| bom00*   | DNA  |  | Bommarito et al., 2000                    |
| sugdna02 | DNA  | Only terminal poly A self complementary sequences.                               | Ohmichi et al., 2002                      |
| sugrna02 | RNA  | Only terminal poly A self complementary sequences.                               | Ohmichi et al., 2002                      |
| ser08*   | RNA  | Only 3' UA, GU and UG terminal base pairs only 5' UG and GU terminal base pairs. | O'Toole et al., 2006; Miller et al., 2008 |

\* Default model for computation.

Single dangling ends are not taken into account by the approximative mode.

**Double dangling end effect:**

| Model     | Type | Limits/Remarks   | Reference            |
|-----------|------|--|----------------------|
| sugdna02* | DNA  | Only terminal poly A self complementary sequences.                         | Ohmichi et al., 2002 |
| sugrna02  | RNA  | Only terminal poly A self complementary sequences.                         | Ohmichi et al., 2002 |
| ser05     | RNA  | Depends on the available thermodynamic parameters for single dangling end. | O'Toole et al., 2005 |
| ser06*    | RNA  |  | O'Toole et al., 2006 |

\* Default model for computation.

Double dangling ends are not taken into account by the approximative mode.

**Long dangling end effect:**

| Model     | Type | Limits/Remarks                                     | Reference            |
|-----------|------|--|----------------------|
| sugdna02* | DNA  | Only terminal poly A self complementary sequences. | Ohmichi et al., 2002 |
| sugrna02* | RNA  | Only terminal poly A self complementary sequences. | Ohmichi et al., 2002 |

\* Default model for computation.

Long dangling ends are not taken into account by the approximative mode.

**Internal loop effect:**

| Model  | Type | Limits.Remarks   | Reference                  |
|--------|------|--|----------------------------|
| san04* | DNA  | Missing asymmetry penalty. Not tested with experimental results. | SantaLucia and Hicks, 2004 |
| tur06  | RNA  | Not tested with experimental results.                            | Lu et al., 2006            |
| zno07* | RNA  | Only for 1x2 loop.   | Badhwar et al., 2007       |

\* Default model for computation.

Internal loops are not taken into account by the approximative mode.

**Single bulge loop effect:**

| Model  | Type | Limits/Remarks                                  | Reference                  |
|--------|------|---|----------------------------|
| tan04* | DNA  |   | Tan and Chen, 2007         |
| san04  | DNA  | Missing closing AT penalty.                     | SantaLucia and Hicks, 2004 |
| ser07  | RNA  | Less reliable results. Some missing parameters. | Blose et al., 2007         |
| tur06* | RNA  |   | Lu et al., 2006            |

\* Default model for computation.

Single bulge loops are not taken into account by the approximative mode.

**Long bulge loop effect:**

| Model  | Type | Limits/Remarks                        | Reference                             |
|--------|------|---------------------------------------|---------------------------------------|
| san04* | DNA  | Missing closing AT penalty.           | SantaLucia and Hicks, 2004            |
| tur06* | RNA  | Not tested with experimental results. | Mathews et al., 1999; Lu et al., 2006 |

\* Default model for computation.

Long bulge loops are not taken into account by the approximative mode.

**CNG repeats effect:**

| Model  | Type | Limits/Remarks                                    | Reference          |
|--------|------|---|--------------------|
| bro05* | RNA  | Self complementary sequences. 2 to 7 CNG repeats. | Broda et al., 2005 |

\* Default model for computation.

CNG repeats are not taken into account by the approximative mode. The contribution of CNG repeats to the thermodynamic of helix-coil transition can be computed only for 2 to 7 CNG repeats. N represents a single mismatch of type N/N.

**Inosine bases effect:**

| Model  | Type | Limits/Remarks   | Reference                    |
|--------|------|--|------------------------------|
| san05* | DNA  | Missing parameters for tandem base pairs containing inosine bases. | Watkins and SantaLucia, 2005 |
| zno07* | RNA  | Only IU base pairs.  | Wright et al., 2007          |

\* Default model for computation.

Inosine bases (I) are not taken into account by the approximative mode.

**Hydroxyadenine bases effect:**

| Model  | Type | Limits/Remarks                           | Reference             |
|--------|------|--|-----------------------|
| sug01* | DNA  | Only 5' GA*C 3' and 5' TA*A 3' contexts. | Kawakami et al., 2001 |

\* Default model for computation.

Hydroxyadenine bases (A\*) are not taken into account by the approximative mode.

**Azobenzenes effect effect:**

| Model  | Type | Limits/Remarks   | Reference            |
|--------|------|--|----------------------|
| asa05* | DNA  | Less reliable results when the number of cis azobenzene increases. | Asanuma et al., 2005 |

\* Default model for computation.

Azobenzenes (X\_T for trans azobenzenes and X\_C for cis azobenzenes) are not taken into account by the approximative mode.

**Single locked nucleic acids effect:**

| Model  | Type | Limits.Remarks | Reference                                |
|--------|------|----------------|--|
| mct04  | DNA  |                | McTigue, Peterson, and Kahn, 2004        |
| owc11* | DNA  |                | Owczarzy, You, Groth, and Tataurov, 2011 |

\* Default model for computation.

Locked nucleic acids (AL, GL, TL and CL) are not taken into account by the approximative mode.

**Consecutive locked nucleic acids effect:**

| Model  | Type | Limits.Remarks | Reference             |
|--------|------|----------------|-----------------------|
| owc11* | DNA  |                | Owczarzy et al., 2011 |

\* Default model for computation.

Locked nucleic acids (AL, GL, TL and CL) are not taken into account by the approximative mode.

**Consecutive locked nucleic acids with single mismatch effect:**

| Model  | Type | Limits.Remarks | Reference             |
|--------|------|----------------|-----------------------|
| owc11* | DNA  |                | Owczarzy et al., 2011 |

\* Default model for computation.

Locked nucleic acids (AL, GL, TL and CL) are not taken into account by the approximative mode.

**Ion corrections****Sodium corrections:**

| Correction | Type               | Limits.Remarks      | Reference                    |
|------------|--------------------|---------------------|------------------------------|
| ahs01      | DNA                | Na>0.               | von Ahsen et al., 2001       |
| schlif     | DNA                | Na>=0.07; Na<=0.12. | Schildkraut and Lifson, 1965 |
| tanna06    | DNA                | Na>=0.001; Na<=1.   | Tan and Chen, 2006           |
| tanna07*   | RNA                | Na>=0.003; Na<=1.   | Tan and Chen, 2007           |
|            | or                 |                     |                              |
|            | 2'-O-MeRNA/RNA     |                     |                              |
| wet91      | RNA,<br>DNA<br>and | Na>0.               | Wetmur, 1991                 |

|           | RNA/DNA |  |   |
|-----------|---------|--|---|
| kam71     | DNA     | Na>0; Na>=0.069; Na<=1.02.                                   | Frank-Kamenetskii, 1971                         |
| marschdot | DNA     | Na>=0.069; Na<=1.02.   | Marmur and Doty, 1962; Blake and Delcourt, 1998 |
| owc1904   | DNA     | Na>0. (equation 19)  | Owczarzy et al., 2004                           |
| owc2004   | DNA     | Na>0. (equation 20)  | Owczarzy et al., 2004                           |
| owc2104   | DNA     | Na>0. (equation 21)  | Owczarzy et al., 2004                           |
| owc2204*  | DNA     | Na>0. (equation 22)  | Owczarzy et al., 2004                           |
| san96     | DNA     | Na>=0.1.   | SantaLucia et al., 1996                         |
| san04     | DNA     | Na>=0.05; Na<=1.1;<br>Oligonucleotides inferior to 16 bases. | SantaLucia and Hicks, 2004;<br>SantaLucia, 1998 |

\* Default correction method for computation.

#### Magnesium corrections:

| Correction | Type | Limits/Remarks   | Reference             |
|------------|------|--|-----------------------|
| owcmg08*   | DNA  | Mg>=0.0005; Mg<=0.6.   | Owczarzy et al., 2008 |
| tanmg06    | DNA  | Mg>=0.0001; Mg<=1; Oligomer length superior to 6 base pairs. | Tan and Chen, 2006    |
| tanmg07*   | RNA  | Mg>=0.1; Mg<=0.3.  | Tan and Chen, 2007    |

\* Default correction method for computation.

#### Mixed Sodium and Magnesium corrections:

| Correction | Type                                | Limits.Remarks  | Reference             |
|------------|-------------------------------------|---|-----------------------|
| owcmix08*  | DNA                                 | Mg>=0.0005; Mg<=0.6;<br>Na+K+Tris/2>0.                      | Owczarzy et al., 2008 |
| tanmix07   | DNA,<br>RNA<br>or<br>2'-O-MeRNA/RNA | Mg>=0.1; Mg<=0.3;<br>Na+K+Tris/2>=0.1;<br>Na+K+Tris/2<=0.3. | Tan and Chen, 2007    |

\* Default correction method for computation.

The ion correction by Owczarzy et al. (2008) is used by default according to the  $\frac{[Mg^{2+}]^{0.5}}{[Mon^+]}$  ratio, where  $[Mon^+] = [Na^+] + [Tris^+] + [K^+]$ .

If,

$[Mon^+] = 0$  Default sodium correction is used.

**Ratio < 0.22**, Default sodium correction is used.

**0.22 <= Ratio < 6** Default mixed Na and Mg correction is used.

**Ratio >= 6** Default magnesium correction is used.

Note that  $[Tris^+]$  is about half of the total tris buffer concentration.

#### Sodium equivalent concentration methods:

| Correction | Type | Limits/Remarks | Reference              |
|------------|------|----------------|------------------------|
| ahs01*     | DNA  |                | von Ahsen et al., 2001 |
| mit96      | DNA  |                | Mitsuhashi, 1996       |
| pey00      | DNA  |                | Peyret, 2000           |

\* Default correction method for computation.

For the other types of hybridization, the DNA default correction is used. If there are other cations when an approximative approach is used, a sodium equivalence is automatically computed. In case of nearest neighbor approach, the sodium equivalence will be used only if a sodium correction is specified by the argument `correction.ion`.

### Denaturing agent corrections

#### DMSO corrections:

| Correction | Type | Limits/Remarks                        | Reference               |
|------------|------|---------------------------------------|-------------------------|
| ahs01*     | DNA  | Not tested with experimental results. | von Ahsen et al., 2001  |
| cul76      | DNA  | Not tested with experimental results. | Cullen and Bick, 1976   |
| esc80      | DNA  | Not tested with experimental results. | Escara and Hutton, 1980 |
| mus81      | DNA  | Not tested with experimental results. | Musielski et al., 1981  |

\* Default correction method for computation.

For the other types of hybridization, the DNA default correction is used. If there is DMSO when an approximative approach is used, a DMSO correction is automatically computed. In case of nearest neighbor approach and approximative approach, the DMSO correction will be used only if a sodium correction is specified by the argument `correction.ion`.

#### Formamide corrections:

| Correction | Type | Limits/Remarks                         | Reference   |
|------------|------|--|---|
| bla96*     | DNA  | With formamide concentration in mol/L. | Blake, 1996   |
| lincorr    | DNA  | With a formamide volume.               | McConaughy et al., 1969; Record, 1967; Casey and Davidson, 1977; Hutton, 1977 |

\* Default correction method for computation.

For the other types of hybridization, the DNA default correction is used. If there is formamide when an approximative approach is used, a formamide correction is automatically computed. In case of nearest neighbor approach and approximative approach, the formamide correction will be used only if a sodium correction is specified by the argument `correction.ion`.

### References

- Marmur J, Doty P (1962). "Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature." *Journal of Molecular Biology*, **5**(1), 109–118.
- Schildkraut C, Lifson S (1965). "Dependence of the melting temperature of DNA on salt concentration." *Biopolymers*, **3**(2), 195–208.

- Record MT (1967). "Electrostatic effects on polynucleotide transitions. I. Behavior at neutral pH." *Biopolymers*, **5**(10), 975–992.
- McConaughy BL, Laird C, McCarthy BJ (1969). "Nucleic acid reassociation in formamide." *Biochemistry*, **8**(8), 3289–3295.
- Owen RJ, Hill LR, Lapage SP (1969). "Determination of DNA base compositions from melting profiles in dilute buffers." *Biopolymers*, **7**(4), 503–516.
- Frank-Kamenetskii MD (1971). "Simplification of the empirical relationship between melting temperature of DNA, its GC content and concentration of sodium ions in solution." *Biopolymers*, **10**(12), 2623–2624.
- Britten RJ, Graham DE, Neufeld BR (1974). "Analysis of repeating DNA sequences by reassociation." *Methods in Enzymology*, **29**, 363–418.
- Cullen BR, Bick MD (1976). "Thermal denaturation of DNA from bromodeoxyuridine substituted cells." *Nucleic Acids Research*, **3**(1), 49–62.
- Hutton JR (1977). "Renaturation kinetics and thermal stability of DNA in aqueous solutions of formamide and urea." *Nucleic Acids Research*, **4**(10), 3537–3555.
- Casey J, Davidson N (1977). "Rates of formation and thermal stabilities of RNA:DNA and DNA:DNA duplexes at high concentrations of formamide." *Nucleic Acids Research*, **4**(5), 1539–1552.
- Hall TJ, Grula JW, Davidson EH, Britten RJ (1980). "Evolution of sea urchin non-repetitive DNA." *Journal of Molecular Evolution*, **16**(2), 95–110.
- Escara JF, Hutton JR (1980). "Thermal stability and renaturation of DNA in dimethyl sulfoxide solutions: Acceleration of the renaturation rate." *Biopolymers*, **19**(7), 1315–1327.
- Musielski H, Mann W, Laue R, Michel S (1981). "Influence of dimethylsulfoxide on transcription by bacteriophage T3-induced RNA polymerase." *Zeitschrift für allgemeine Mikrobiologie*, **21**(6), 447–456.
- Freier SM, Kierzek R, Jaeger JA, Sugimoto N, Caruthers MH, Neilson T, Turner DH (1986). "Improved free-energy parameters for predictions of RNA duplex stability." *Proceedings of the National Academy of Sciences*, **83**(24), 9373.
- Breslauer KJ, Frank R, Blocker H, Marky LA (1986). "Predicting DNA duplex stability from the base sequence." *Proceedings of the National Academy of Sciences*, **83**(11), 3746.
- Wahl GM, Barger SL, Kimmel AR (1987). "Molecular hybridization of immobilized nucleic acids: Theoretical concepts and practical considerations." *Methods in Enzymology*, **152**, 399–407.
- Wetmur JG (1991). "DNA probes: Applications of the principles of nucleic acid hybridization." *Critical Reviews in Biochemistry and Molecular Biology*, **26**(3-4), 227–259.
- Chester N, Marshak DR (1993). "Dimethyl sulfoxide-mediated primer T<sub>m</sub> reduction: A method for analyzing the role of renaturation temperature in the polymerase chain reaction." *Analytical Biochemistry*, **209**(2), 284–290.
- Sugimoto N, Katoh M, Nakano S, Ohmichi T, Sasaki M (1994). "RNA/DNA hybrid duplexes with identical nearest-neighbor base-pairs have identical stability." *FEBS Letters*, **354**(1), 74–78.
- Sugimoto N, Nakano S, Katoh M, Matsumura A, Nakamura H, Ohmichi T, Yoneyama M, Sasaki M (1995). "Thermodynamic parameters to predict stability of RNA/DNA hybrid duplexes." *Biochemistry*, **34**(35), 11211–11216.
- SantaLucia J, Allawi HT, Seneviratne PA (1996). "Improved nearest-neighbor parameters for predicting DNA duplex stability." *Biochemistry*, **35**(11), 3555–3562.
- Sugimoto N, Nakano S, Yoneyama M, Honda K (1996). "Improved thermodynamic parameters and helix initiation factor to predict stability of DNA duplexes." *Nucleic Acids Research*, **24**(22), 4501–4505.

- Blake RD, Delcourt SG (1996). "Thermodynamic effects of formamide on DNA stability." *Nucleic Acids Research*, **24**(11), 2095–2103.
- Blake RD (1996). "Denaturation of DNA." In Meyers RA (ed.), *Encyclopedia of molecular biology and molecular medicine*, volume 2, 1–19. VCH Verlagsgesellschaft, Weinheim, Germany.
- Mitsuhashi M (1996). "Technical report: Part 1. Basic requirements for designing optimal oligonucleotide probe sequences." *Journal of Clinical Laboratory Analysis*, **10**(5), 277–284.
- Allawi HT, SantaLucia J (1997). "Thermodynamics and NMR of internal G·T mismatches in dna." *Biochemistry*, **36**(34), 10581–10594.
- SantaLucia J (1998). "A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics." *Proceedings of the National Academy of Sciences*, **95**(4), 1460.
- Xia T, SantaLucia J, Burkard ME, Kierzek R, Schroeder SJ, Jiao X, Cox C, Turner DH (1998). "Thermodynamic parameters for an expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick base pairs." *Biochemistry*, **37**(42), 14719–14735.
- Allawi HT, SantaLucia J (1998). "Thermodynamics of internal C·T mismatches in DNA." *Nucleic Acids Research*, **26**(11), 2694–2701.
- Blake RD, Delcourt SG (1998). "Thermal stability of DNA." *Nucleic Acids Research*, **26**(14), 3323–3332.
- Allawi HT, SantaLucia J (1998). "Nearest neighbor thermodynamic parameters for internal G·A mismatches in DNA." *Biochemistry*, **37**(8), 2170–2179.
- Allawi HT, SantaLucia J (1998). "Nearest-neighbor thermodynamics of internal A·C mismatches in dna: sequence dependence and pH effects." *Biochemistry*, **37**(26), 9435–9444.
- Mathews DH, Sabina J, Zuker M, Turner DH (1999). "Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure." *Journal of Molecular Biology*, **288**(5), 911–940.
- Peyret N, Seneviratne PA, Allawi HT, SantaLucia J (1999). "Nearest-Neighbor Thermodynamics and NMR of DNA Sequences with Internal A·A, C·C, G·G, and T·T Mismatches." *Biochemistry*, **38**(12), 3468–3477.
- Peyret N (2000). *Prediction of nucleic acid hybridization: Parameters and algorithms*. Ph.D. Thesis, Wayne State University, Detroit, MI.
- Bommarito S, Peyret N, SantaLucia J (2000). "Thermodynamic parameters for DNA sequences with dangling ends." *Nucleic Acids Research*, **28**(9), 1929–1934.
- Kawakami J, Kamiya H, Yasuda K, Fujiki H, Kasai H, Sugimoto N (2001). "Thermodynamic stability of base pairs between 2-hydroxyadenine and incoming nucleotides as a determinant of nucleotide incorporation specificity during replication." *Nucleic Acids Research*, **29**(16), 3289–3296.
- von Ahsen N, Wittwer CT, Schutz E (2001). "Oligonucleotide melting temperatures under PCR conditions: Nearest-neighbor corrections for Mg<sup>2+</sup>, deoxynucleotide triphosphate, and dimethyl sulfoxide concentrations with comparison to alternative empirical formulas." *Clinical Chemistry*, **47**(11), 1956–1961.
- Le Novere N (2001). "MELTING, computing the melting temperature of nucleic acid duplex." *Bioinformatics*, **17**(12), 1226–1227.
- Ohmichi T, Nakano S, Miyoshi D, Sugimoto N (2002). "Long RNA dangling end has large energetic contribution to duplex stability." *Journal of the American Chemical Society*, **124**(35), 10367–10372.
- SantaLucia J, Hicks D (2004). "The thermodynamics of DNA structural motifs." *Annual Review of Biophysics and Biomolecular Structure*, **33**(1), 415–440.

- Tanaka F, Kameda A, Yamamoto M, Ohuchi A (2004). "Thermodynamic parameters based on a nearest-neighbor model for DNA sequences with a single-bulge loop." *Biochemistry*, **43**(22), 7143–7150.
- McTigue PM, Peterson RJ, Kahn JD (2004). "Sequence-dependent thermodynamic parameters for locked nucleic acid (LNA)-DNA duplex formation." *Biochemistry*, **43**(18), 5388–5405.
- Owczarzy R, You Y, Groth CL, Tataurov AV (2011). "Stability and mismatch discrimination of locked nucleic acid-DNA duplexes." *Biochemistry*, **50**(43), 9352–9367.
- Owczarzy R, You Y, Moreira BG, Manthey JA, Huang L, Behlke MA, Walder JA (2004). "Effects of sodium ions on DNA duplex oligomers: Improved predictions of melting temperatures." *Biochemistry*, **43**(12), 3537–3554.
- Broda M, Kierzek E, Gdaniec Z, Kulinski T, Kierzek R (2005). "Thermodynamic stability of RNA structures formed by CNG trinucleotide repeats. Implication for prediction of RNA structure." *Biochemistry*, **44**(32), 10873–10882.
- Watkins NE, SantaLucia J (2005). "Nearest-neighbor thermodynamics of deoxyinosine pairs in DNA duplexes." *Nucleic Acids Research*, **33**(19), 6258–6267.
- Asanuma H, Matsunaga D, Komiyama M (2005). "Clear-cut photo-regulation of the formation and dissociation of the DNA duplex by modified oligonucleotide involving multiple azobenzenes." *Nucleic Acids Symposium Series*, 35–36. <http://www.ncbi.nlm.nih.gov/pubmed/17150620>.
- O'Toole AS, Miller S, Serra MJ (2005). "Stability of 3' double nucleotide overhangs that model the 3' ends of siRNA." *RNA*, **11**(4), 512–516. <http://www.ncbi.nlm.nih.gov/pubmed/15769878>.
- Lu ZJ, Turner DH, Mathews DH (2006). "A set of nearest neighbor parameters for predicting the enthalpy change of RNA secondary structure formation." *Nucleic Acids Research*, **34**(17), 4912–4924.
- Kierzek E, Mathews DH, Ciesielska A, Turner DH, Kierzek R (2006). "Nearest neighbor parameters for Watson-Crick complementary heteroduplexes formed between 2'-O-methyl RNA and RNA oligonucleotides." *Nucleic Acids Research*, **34**(13), 3609–3614.
- Tan Z, Chen S (2006). "Nucleic acid helix stability: Effects of salt concentration, cation valence and size, and chain length." *Biophysical Journal*, **90**(4), 1175–1190.
- O'Toole AS, Miller S, Haines N, Zink MC, Serra MJ (2006). "Comprehensive thermodynamic analysis of 3' double-nucleotide overhangs neighboring Watson-Crick terminal base pairs." *Nucleic Acids Research*, **34**(11), 3338–3344.
- Tan Z, Chen S (2007). "RNA helix stability in mixed Na(+)/Mg(2+) solution." *Biophysical Journal*, **92**(10), 3615–3632.
- Wright DJ, Rice JL, Yanker DM, Znosko BM (2007). "Nearest neighbor parameters for inosine-uridine pairs in RNA duplexes." *Biochemistry*, **46**(15), 4625–4634.
- Davis AR, Znosko BM (2007). "Thermodynamic characterization of single mismatches found in naturally occurring RNA." *Biochemistry*, **46**(46), 13425–13436.
- Blose JM, Manni ML, Klapek KA, Stranger-Jones Y, Zyra AC, Sim V, Griffith CA, Long JD, Serra MJ (2007). "Non-nearest-neighbor dependence of stability for RNA bulge loops based on the complete set of group I single nucleotide bulge loops." *Biochemistry*, **46**(51), 15123–15135.
- Badhwar J, Karri S, Cass CK, Wunderlich EL, Znosko BM (2007). "Thermodynamic characterization of RNA duplexes containing naturally occurring 1 \* 2 nucleotide internal loops." *Biochemistry*, **46**(50), 14715–14724.
- Davis AR, Znosko BM (2008). "Thermodynamic characterization of naturally occurring RNA single mismatches with G-U nearest neighbors." *Biochemistry*, **47**(38), 10178–10187.
- Miller S, Jones LE, Giovannitti K, Piper D, Serra MJ (2008). "Thermodynamic analysis of 5' and 3' single- and 3' double-nucleotide overhangs neighboring wobble terminal base pairs." *Nucleic Acids Research*, **36**(17), 5652–5659.



Owczarzy R, Moreira BG, You Y, Behlke MA, Walder JA (2008). “Predicting stability of DNA duplexes in solutions containing magnesium and monovalent cations.” *Biochemistry*, **47**(19), 5336–5353.

Watkins NE, Kennelly WJ, Tsay MJ, Tuin A, Swenson L, Lee H, Morosyuk S, Hicks DA, SantaLucia J (2011). “Thermodynamic contributions of single internal rA·dA, rC·dC, rG·dG and rU·dT mismatches in RNA/DNA duplexes.” *Nucleic Acids Research*, **39**(5), 1894–1902.

Chen JL, Dishler AL, Kennedy SD, Yildirim I, Liu B, Turner DH, Serra MJ (2012). “Testing the nearest neighbor model for canonical rna base pairs: Revision of GU parameters.” *Biochemistry*, **51**(16), 3508–3522.

Dumousseau M, Rodriguez N, Juty N, Le Novere N (2012). “MELTING, a flexible platform to predict the melting temperatures of nucleic acids.” *BMC Bioinformatics*, **13**, 101.

### See Also

For more details about algorithm, formulae and methods, see the documentation for [MELTING 5](#).

### Examples

```
# Basic usage
melting(sequence = "CAGTGAGACAGCAATGGTCG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnadna", Na.conc = 1)

# For more detailed examples refer the vignette.
## Not run:

browseVignettes(package = 'rmelting')

## End(Not run)
```

---

|              |   |
|--------------|---|
| meltingBatch | <i>Compute melting temperature of multiple nucleic acid duplexes in batch</i> |
|--------------|---|

---

### Description

Compute the enthalpy and entropy of helix-coil transition, and then the melting temperature of multiple nucleic acid duplexes in batch.

### Usage

```
meltingBatch(
  sequence,
  comp.sequence = NULL,
  environment.out = TRUE,
  options.out = TRUE,
  message.out = TRUE,
  ...
)
```

**Arguments**

|                 |  |
|-----------------|--|
| sequence        | A character vector of 5' to 3' sequences of one strand of the nucleic acid duplex ( <b>Note:</b> Uridine and thymidine are not considered as identical).   |
| comp.sequence   | A character vector of 3' to 5' complementary sequences of the nucleic acid duplex. Complementary sequences are computed by default, but need to be specified in case of mismatches, inosine(s) or hydroxyadenine(s) between the two strands. |
| environment.out | logical. If TRUE, gives the melting temperature computation environment details in the output. Default is TRUE.  |
| options.out     | logical. If TRUE, gives the details about the options (default or user specified) used for melting temperature computation in the output. Default is TRUE.   |
| message.out     | logical. If TRUE, gives the error and/or warning messages, if any in the output. Default is TRUE.  |
| ...             | Arguments for melting temperature computation (See <a href="#">melting</a> ).  |

**Value**

A data frame of the melting temperature computation results along with the details of environment, options and messages if specified by the arguments `environment.out`, `options.out` and `message.out` respectively.

**See Also**

[melting](#)

**Examples**

```
sequence <- c("CAAAAAG", "CAAAAAG", "TTTTATAATAAA", "CCATCGCTACC",
             "CAACAAG", "CCATTGCTACC", "CAAAAAAG", "GTGAAC", "AAAAAAA",
             "CAACTTGATATTATTA", "CAAATAAAG", "GCGAGC", "GGGACC",
             "CAAAGAAAG", "CTGACAAGTGTCC", "GCGAAAAGCG")

meltingBatch(sequence, nucleic.acid.conc = 0.0004,
             hybridisation.type = "dnadna", Na.conc = 1)

seq <- c("GCAUACG", "CAGUAGGUC", "CGCUCGC", "GAGUGGAG", "GACAGGCUG",
        "CAGUACGUC", "GACAUCCUG", "GACCACCUG", "CAGAAUGUC", "GCGUCGC",
        "CGUCCGG", "GACUCUCUG", "CAGCUGGUC", "GACUAGCUG", "CUCUGCUC",
        "GCGUCCG", "GUCCGCG", "CGAUCAC", "GACUACCUG", "GACGAUCUG")

comp.seq <- c("CGUUJGC", "GUCGGCCAG", "GCGUGCG", "CUCUUCUC", "CUGUGCGAC",
             "GUCGGGCAG", "CUGUUGGAC", "CUGGGGGAC", "GUCUGGCAG", "CGCUGCG",
             "GCUGGCC", "CUGAUAGAC", "GUCGUUCAG", "CUGAGCGAC", "GAGUUGAG",
             "CGCUGGC", "CUGGCGC", "GCUUGUG", "CUGAGGGAC", "CUGCCAGAC")

meltingBatch(sequence = seq, comp.seq = comp.seq, nucleic.acid.conc = 0.0004,
             hybridisation.type = "rnarna", Na.conc = 1,
             method.singleMM = "tur06")
```

---

|               |   |
|---------------|---|
| print.melting | <i>Prints melting temperature from a melting object</i> |
|---------------|---|

---

**Description**

print.melting prints to console the melting temperature value from an object of class melting.

**Usage**

```
## S3 method for class 'melting'  
print(x, ...)
```

**Arguments**

|     |                             |
|-----|-----------------------------|
| x   | An object of class melting. |
| ... | Unused                      |

**Value**

The melting temperature value (degree Celsius) in the console.

**See Also**

[melting](#)

---

|        |  |
|--------|--|
| withWE | <i>Evaluate expression and capture all warnings and errors if any along with results</i> |
|--------|--|

---

**Description**

Not exported. Strictly internal

**Usage**

```
withWE(expr)
```

**Arguments**

|      |                                 |
|------|---------------------------------|
| expr | The expression to be evaluated. |
|------|---------------------------------|

**Value**

- In cas of Warning(s)Returns the value along with the warning message(s).
- In cas of ErrorReturns NA as the value along with the error message.

**Examples**

```
foo <- function(){  
  warning("oops")  
  1}  
}
```

```
foo <- function(){  
  warning("oops")  
  warning("again oops")  
  1}  
}
```

```
foo <- function(){  
  warning("oops")  
  log("a")  
}
```

# Index

\* **internal**

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