Computation of melting temperature of nucleic acid duplexes with **rmelting**

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1 Introduction

The R package rmelting is an interface to the MELTING 5 program (Le Novère, 2001; Dumousseau et al., 2012) to compute melting temperatures of nucleic acid duplexes (DNA/DNA, DNA/RNA, RNA/RNA or 2'-O-MeRNA/RNA) along with other thermodynamic parameters such as hybridisation enthalpy and entropy.

Melting temperatures are computed by Nearest-neighbour methods for short sequences or approximative estimation formulae for long sequences. Apart from these, several corrections are available to take into account the presence of Cations (Na, Tris, K and Mg) or denaturing agents (DMSO and formamide).



2 Installation

The package can be installed using the following functions:

```
# Install development version from Github
if (!require('devtools')) install.packages('devtools')
devtools::install_github("aravind-j/rmelting")
```

Then the package can be loaded using the function

```
library(rmelting)
```

3 Basic usage

Melting temperatures are computed in rmelting through the core function melting which takes a number of arguments (see ?melting). The following are the essential 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 (Table 1). U and T are not considered identical.

Table 1: Recognized sequences

Code	Type
A	Adenine
\mathbf{C}	Cytosine
G	Guanine
${ m T}$	Thymine
U	Uracil
I	Inosine
X_{C}	Trans azobenzenes
X_T	Cis azobenzenes
A^*	Hydroxyadenine

Code	Type
$\overline{\mathrm{AL}}$	Locked nucleic acid
TL	"
GL	"
CL	"

- 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.
- nucleic.acid.conc
 - In molar concentration (M or mol L⁻¹).
- 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.
- hybridisation.type
 - The possible options for hybridisation type are as follows (**Table 2**).

Table 2: Hybridisation type options

Option	Sequence	Complementary sequence
dnadna	DNA	DNA
rnarna	RNA	RNA
dnarna	DNA	RNA
rnadna	RNA	DNA
mrnarna	2-o-methyl RNA	RNA
rnamrna	RNA	2-o-methyl RNA

With these arguments, the melting temperature can be computed as follows.

[1] 73.35168

Only the melting temperature is given as a console output. However, the output can be assigned to an object which contains the details of the environment, options and the thermodynamics results as a list.

```
## $Sequence
## [1] "CAGTGAGACAGCAATGGTCG"
##
## $`Complementary sequence`
## [1] "GTCACTCTGTCGTTACCAGC"
##
## $`Nucleic acid concentration (M)`
## [1] 2e-06
##
## $`Hybridization type`
```

```
## [1] "dnadna"
##
## $`Na concentration (M)`
## [1] 1
## $`Mg concentration (M)`
## [1] 0
## $`Tris concentration (M)`
## [1] 0
## $`K concentration (M)`
## [1] 0
##
## $`dNTP concentration (M)`
## [1] 0
##
## $`DMSO concentration (%)`
## [1] 0
## $`Formamide concentration (M or %)`
## [1] 0
##
## $`Self complementarity`
## [1] FALSE
## $`Correction factor`
## [1] 4
# Options used
out $ Options
## $`Approximative formula`
## [1] NA
## $`Nearest neighbour model`
## [1] "all97"
##
## $`GU model`
## [1] NA
## $`Single mismatch model`
## [1] "allsanpey"
## $`Tandem mismatch model`
## [1] "allsanpey"
## $`Single dangling end model`
## [1] "bom00"
## $`Double dangling end model`
## [1] "sugdna02"
##
## $`Long dangling end model`
## [1] "sugdna02"
```

```
##
## $`Internal loop model`
## [1] "san04"
##
## $`Single bulge loop model`
## [1] "tan04"
## $`Long bulge loop model`
## [1] "san04"
##
## $`CNG repeats model`
## [1] NA
## $`Inosine bases model`
## [1] "san05"
##
## $`Hydroxyadenine bases model`
## [1] "sug01"
## $ Azobenzenes model
## [1] "asa05"
## $`Locked nucleic acids model`
## [1] "mct04"
##
## $`Ion correction method`
## [1] NA
## $`Na equivalence correction method`
## [1] "ahs01"
##
## $`DMSO correction method`
## [1] "ahs01"
##
## $`Formamide correction method`
## [1] "bla96"
##
## $Mode
## [1] "def"
# Thermodynamics results
out$Results
## $`Enthalpy (cal)`
## [1] -159000
## $`Entropy (cal)`
## [1] -430
##
## $`Enthalpy (J)`
## [1] -664620
##
## $`Entropy (J)`
## [1] -1797.4
##
```

```
## $`Melting temperature (C)`
## [1] 73.35168
```

The command for the MELTING 5 java version is saved as an attribute in the list out and can be retrieved as follows.

```
# Command for MELTING 5
attributes(out)$command
```

[1] "-S CAGTGAGACAGCAATGGTCG -H dnadna -P 2e-06 -E Na=1 -T 60"

4 Melting temperature computation

Melting temperature is computed by either approximative or nearest neighbour methods according to the length of the oligonucleotide sequences. For longer sequences (longer than the threshold value, the threshold value set by size.threshold with the default value 60) approximative method is used, while for others, nearest neighbour method is used.

4.1 Approximative methods

The approximative method for computation can be specified by the argument method.approx. The available methods are given in **Table 3**.

 Table 3: Details of approximative methods

Formula	Type	Limits/Remarks	Reference
ahs01	DNA	No mismatch	Ahsen et al. (2001)
che93	DNA	No mismatch; Na=0,	Marmur and Doty (1962)
		Mg=0.0015, Tris=0.01, K=0.05	
che93corr	DNA	No mismatch; Na=0, Mg=0.0015, Tris=0.01,	Marmur and Doty (1962)
		K=0.05	
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), Ahsen et al. (2001)
wetdna91*	DNA		Wetmur (1991)
${\tt wetrna91}^*$	RNA		Wetmur (1991)
${\tt wetdnarna91}^*$	$\mathrm{DNA}/\mathrm{RNA}$		Wetmur (1991)

^{*} Default method for computation.

Examples

```
RNA: UUAAUCUCCGUCAUCUUUAAGCCGUGGAGAGACUGUAGACUUGAACAGGGGUAAGCGGAGGCACGUAGGAUUCACAUCAU
       RNA: AAUUAGAGGCAGUAGAAAUUCGGCACCUCUCUGACAUCUGAACUUGUCCCCAUUCGCCUCCGUGCAUCCUAAGUGUAGUA
   DNA: TCTAATGTGCTGTTAGATGTATCCAGAGATAGCCGAGCATAAACTTCAACACGAGACGTTGATTGGATTTAACCATAG
       RNA: AGAUUACACGACAAUCUACAUAGGUCUCUAUCGGCUCGUAUUUGAAGUUGUGUGCUCUGCAACUAACCUAAAUUGGUAUC
# Long Nucleotide sequence
DNAseq <- c("TCTAATGTGCTGTTAGATGTATCCAGAGATAGCCGAGCATAAACTTCAACACACGAGACGTTGATTGGATTTAACCATAG")
RNAseq <- c("UUAAUCUCCGUCAUCUUUAAGCCGUGGAGAGACUGUAGACUGAACAGGGGUAAGCGGAGGCACGUAGGAUUCACAUCAU")
# Approximative method - default (DNA/DNA)
melting(sequence = DNAseq, nucleic.acid.conc = 2e-06,
       hybridisation.type = "dnadna", Na.conc = 1)
## [1] 87.82455
# Approximative method - wetdna91 (DNA/DNA)
melting(sequence = DNAseq, nucleic.acid.conc = 2e-06,
       hybridisation.type = "dnadna", Na.conc = 1,
       method.approx = "wetdna91")
## [1] 87.82455
# Approximative method - ahs01 (DNA/DNA)
melting(sequence = DNAseq, nucleic.acid.conc = 2e-06,
       hybridisation.type = "dnadna", Na.conc = 1,
       method.approx = "ahs01")
## [1] 87.325
# Approximative method - che93 (DNA/DNA)
melting(sequence = DNAseq, nucleic.acid.conc = 2e-06,
       hybridisation.type = "dnadna", Na.conc = 1,
       method.approx = "che93")
## [1] 77.575
# Approximative method - che93corr (DNA/DNA)
melting(sequence = DNAseq, nucleic.acid.conc = 2e-06,
       hybridisation.type = "dnadna", Na.conc = 1,
       method.approx = "che93corr")
## [1] 79.0125
# Approximative method - schdot (DNA/DNA)
melting(sequence = DNAseq, nucleic.acid.conc = 2e-06,
       hybridisation.type = "dnadna", Na.conc = 1,
       method.approx = "schdot")
## [1] 89.4625
# Approximative method - owe69 (DNA/DNA)
melting(sequence = DNAseq, nucleic.acid.conc = 2e-06,
       hybridisation.type = "dnadna", Na.conc = 1,
       method.approx = "owe69")
```

```
## [1] 100.96
# Approximative method - san98 (DNA/DNA)
melting(sequence = DNAseq, nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnadna", Na.conc = 1,
        method.approx = "san98")
## [1] 86.9
# Approximative method - default (RNA/RNA)
melting(sequence = RNAseq, nucleic.acid.conc = 2e-06,
        hybridisation.type = "rnarna", Na.conc = 1)
## [1] 101.1745
# Approximative method - wetrna91 (RNA/RNA)
melting(sequence = RNAseq, nucleic.acid.conc = 2e-06,
        hybridisation.type = "rnarna", Na.conc = 1,
        method.approx = "wetrna91")
## [1] 101.1745
# Approximative method - wetdnarna91 (DNA/RNA)
melting(sequence = DNAseq, nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnarna", Na.conc = 1)
## [1] 88.92455
# Approximative method - wetdnarna91 (DNA/RNA)
melting(sequence = DNAseq, nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnarna", Na.conc = 1,
        method.approx = "wetdnarna91")
```

[1] 88.92455

4.2 Nearest neighbour methods

4.2.1 Perfectly matching sequences

The nearest neighbour model for computation in case of perfectly matching sequences can be specified by the argument method.nn. The available methods are given in Table 4.

Table 4: Details of nearest neighbour methods for perfectly matching sequences

Model	Type	Limits/Remarks	Reference
all97*	DNA		Allawi and SantaLucia (1997)
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/ RNA		SantaLucia et al. (1996)

Model	Type	Limits/Remarks	Reference
tur06*	2'-O-MeRNA/ RNA	A sodium correction (san04) is automatically applied to convert the entropy (Na = 0.1M) into the entropy (Na = 1M)	Kierzek et al. (2006)

^{*} Default method for computation.

Examples

```
## [1] 73.35168
```

```
## [1] 73.35168
```

[1] 83.2203

```
## [1] 73.30191
```

[1] 75.7102

```
# Nearest neighbour method - sug96 (DNA/DNA: No Self-Complimentarity)
melting(sequence = "CAGTGAGACAGCAATGGTCG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnadna", Na.conc = 1, method.nn = "sug96")
## [1] 78.17556
# Nearest neighbour method - tan04 (DNA/DNA: No Self-Complimentarity)
melting(sequence = "CAGTGAGACAGCAATGGTCG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnadna", Na.conc = 1, method.nn = "tan04")
## [1] 71.31413
# Nearest neighbour method - default (RNA/RNA: No Self-Complimentarity)
melting(sequence = "CAGUGAGACAGCAAUGGUCG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "rnarna", Na.conc = 1)
## [1] 86.77685
# Nearest neighbour method - xia98 (RNA/RNA: No Self-Complimentarity)
melting(sequence = "CAGUGAGACAGCAAUGGUCG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "rnarna", Na.conc = 1, method.nn = "xia98")
## [1] 86.77685
# Nearest neighbour method - fre86 (RNA/RNA: No Self-Complimentarity)
melting(sequence = "CAGUGAGACAGCAAUGGUCG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "rnarna", Na.conc = 1, method.nn = "fre86")
## [1] 83.81257
# Nearest neighbour method - default (mRNA/RNA: No Self-Complimentarity)
melting(sequence = "CAGUGAGACAGCAAUGGUCG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "mrnarna", Na.conc = 1)
## [1] 99.01986
# Nearest neighbour method - tur06 (mRNA/RNA: No Self-Complimentarity)
melting(sequence = "CAGUGAGACAGCAAUGGUCG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "mrnarna", Na.conc = 1, method.nn = "tur06")
## [1] 99.01986
# Nearest neighbour method - default (DNA/RNA: No Self-Complimentarity)
melting(sequence = "CAGTGAGACAGCAATGGTCG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnarna", Na.conc = 1)
## [1] 66.77049
# Nearest neighbour method - sug95 (DNA/RNA: No Self-Complimentarity)
melting(sequence = "CAGTGAGACAGCAATGGTCG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnarna", Na.conc = 1, method.nn = "sug95")
## [1] 66.77049
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.
Examples
```

DNA: CATATGGCCATATG

```
DNA: GTATACCGGTATAC
   RNA: AUGUACAU
        RNA: UACAUGUA
# Nearest neighbour method - default (DNA/DNA: Self-Complimentarity)
melting(sequence = "CATATGGCCATATG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnadna", Na.conc = 1)
## [1] 56.00644
# Nearest neighbour method - all97 (DNA/DNA: Self-Complimentarity)
melting(sequence = "CATATGGCCATATG", nucleic.acid.conc = 2e-06,
       hybridisation.type = "dnadna", Na.conc = 1, method.nn = "all97")
## [1] 56.00644
# Nearest neighbour method - bre86 (DNA/DNA: Self-Complimentarity)
melting(sequence = "CATATGGCCATATG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnadna", Na.conc = 1, method.nn = "bre86")
## [1] 63.44605
# Nearest neighbour method - sanO4 (DNA/DNA: Self-Complimentarity)
melting(sequence = "CATATGGCCATATG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnadna", Na.conc = 1, method.nn = "san04")
## [1] 57.80792
# Nearest neighbour method - san96 (DNA/DNA: Self-Complimentarity)
melting(sequence = "CATATGGCCATATG", nucleic.acid.conc = 2e-06,
       hybridisation.type = "dnadna", Na.conc = 1, method.nn = "san96")
## [1] 55.0921
# Nearest neighbour method - sug96 (DNA/DNA: Self-Complimentarity)
melting(sequence = "CATATGGCCATATG", nucleic.acid.conc = 2e-06,
        hybridisation.type = "dnadna", Na.conc = 1, method.nn = "sug96")
## [1] 59.06213
# Nearest neighbour method - tanO4 (DNA/DNA: Self-Complimentarity)
melting(sequence = "CATATGGCCATATG", nucleic.acid.conc = 2e-06,
       hybridisation.type = "dnadna", Na.conc = 1, method.nn = "tan04")
## [1] 55.65824
# Nearest neighbour method - default (RNA/RNA: Self-Complimentarity)
melting(sequence = "AUGUACAU", nucleic.acid.conc = 2e-06,
       hybridisation.type = "rnarna", Na.conc = 1)
## [1] 30.27015
# Nearest neighbour method - xia98 (RNA/RNA: Self-Complimentarity)
melting(sequence = "AUGUACAU", nucleic.acid.conc = 2e-06,
       hybridisation.type = "rnarna", Na.conc = 1, method.nn = "xia98")
```

[1] 30.27015

[1] 31.48175

4.2.2 GU wobble base pairs effect

The nearest neighbour model for computation in case of sequences with GU wobble base pairs can be specified by the argument method.GU. The available methods are given in **Table 5**.

Table 5: Details of methods for sequences with GU wobble base pairs

Model	Type	Limits/Remarks	Reference	
tur99 ser12*	RNA RNA		Mathews et al. (1999) Chen et al. (2012)	

^{*} Default method for computation.

Examples

```
RNA: CCAGCGUCCU
```

```
## [1] 79.46955
```

```
## [1] 79.46955
```

[1] 79.46955

4.2.3 Single mismatch effect

The nearest neighbour model for computation in case of sequences with a single mismatch can be specified by the argument method.singleMM. The available methods are given in **Table 6**.

Table 6: Details of methods for sequences with single mismatch

Model	Type	Limits/Remarks	Reference
allsanpey*	DNA		Allawi and SantaLucia (1997), Allawi and SantaLucia (1998a), Allawi and SantaLucia (1998b), Allawi and SantaLucia (1998c), Peyret et al. (1999)

Model	Type	Limits/Remarks	Reference
wat11* tur06 zno07* zno08	DNA/RNA RNA RNA RNA	At least one adjacent GU base pair.	Watkins et al. (2011) Lu et al. (2006) Davis and Znosko (2007) Davis and Znosko (2008)

^{*} Default method for computation.

```
Examples
   DNA: CAACTTGATATTAATA
       DNA: GTTGAACTCTAATTAT
   RNA: GACAGGCUG
       1111 1111
   RNA: CUGUGCGAC
   DNA: CCATAACTACC
       RNA: GGUAAUGAUGG
# Single mismatch effect - default (DNA/DNA)
melting(sequence = "CAACTTGATATTAATA", comp.sequence = "GTTGAACTCTAATTAT",
       nucleic.acid.conc = 0.0004, hybridisation.type = "dnadna", Na.conc = 1)
## [1] 51.97499
# Single mismatch effect - allsanpey (DNA/DNA)
melting(sequence = "CAACTTGATATTAATA", comp.sequence = "GTTGAACTCTAATTAT",
       nucleic.acid.conc = 0.0004, hybridisation.type = "dnadna",
       Na.conc = 1, method.singleMM = "allsanpey")
## [1] 51.97499
# Single mismatch effect - default (RNA/RNA)
melting(sequence = "GACAGGCUG", comp.sequence = "CUGUGCGAC",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna", Na.conc = 1)
## [1] 54.40363
# Single mismatch effect - zno07 (RNA/RNA)
melting(sequence = "GACAGGCUG", comp.sequence = "CUGUGCGAC",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1, method.singleMM = "zno07")
## [1] 54.40363
# Single mismatch effect - zno08 (RNA/RNA)
melting(sequence = "CAGUACGUC", comp.sequence = "GUCGGGCAG",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1, method.singleMM = "zno08")
```

[1] 38.26298

4.2.4 Tandem mismatches effect

[1] 40.32976

The nearest neighbour model for computation in case of sequences with tandem mismatches can be specified by the argument method.tandemMM. The available methods are given in **Table 7**.

Table 7: Details of methods for sequences with tandem mismatches

melting(sequence = "GACGTTGGAC", comp.sequence = "CTGCGGCCTG",

Model	Type	Limits/Remarks	Reference
allsanpey*	DNA	Only GT mismatches and TA/TG mismatches.	Allawi and SantaLucia (1997), Allawi and SantaLucia (1998a), Allawi and SantaLucia (1998b), Allawi and SantaLucia (1998c), Peyret et al. (1999)
tur99*	RNA	No adjacent GU or UG base pairs.	Mathews et al. (1999), Lu et al. (2006)

^{*} Default method for computation.

Examples

```
nucleic.acid.conc = 0.0004, hybridisation.type = "dnadna",
    Na.conc = 1, method.tandemMM = "allsanpey")

## [1] 50.20175

# Tandem mismatches effect - default (RNA/RNA)
melting(sequence = "GAGCGGAG", comp.sequence = "CUCCACUC",
    nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna", Na.conc = 1)

## [1] 21.07224

# Tandem mismatches effect - tur06 (RNA/RNA)
melting(sequence = "GAGCGGAG", comp.sequence = "CUCCACUC",
    nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
    Na.conc = 1, method.tandemMM = "tur99")
```

[1] 21.07224

4.2.5 Single dangling end effect

The nearest neighbour model for computation in case of sequences with a single dangling end can be specified by the argument method.single.dangle. The available methods are given in **Table 8**.

Table 8: Details of methods for sequences with single dangling end

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 method for computation.

Examples

```
DNA:-GTAGCTACA

|||||||
DNA:ACATCGATG-

RNA:-GGCGCUG

||||||
RNA: CCGCGAC

DNA:-GGCGCUG

|||||||
RNA: CCGCGAC

# Single dangling end effect - default (DNA/DNA)

melting(sequence = "-GTAGCTACA",

nucleic.acid.conc = 0.0004, hybridisation.type = "dnadna",
```

```
Na.conc = 1)
## [1] 52.58935
# Single dangling end effect - bom00 (DNA/DNA)
melting(sequence = "-GTAGCTACA",
        nucleic.acid.conc = 0.0004, hybridisation.type = "dnadna",
        Na.conc = 1, method.single.dangle = "bom00")
## [1] 52.58935
# Single dangling end effect - sugdna02 (DNA/DNA)
melting(sequence = "-GTAGCTACA",
        nucleic.acid.conc = 0.0004, hybridisation.type = "dnadna",
        Na.conc = 1, method.single.dangle = "sugdna02")
## [1] 50.78548
# Single dangling end effect - default (RNA/RNA)
melting(sequence = "-GGCGCUG",
        nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
        Na.conc = 1)
## [1] 65.7647
\# Single dangling end effect - ser08 (RNA/RNA)
melting(sequence = "-GGCGCUG",
        nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
        Na.conc = 1, method.single.dangle = "ser08")
## [1] 65.7647
# Single dangling end effect - sugrna02 (RNA/RNA)
melting(sequence = "-GGCGCUG",
        nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1, method.single.dangle = "sugrna02")
```

[1] 65.7647

4.2.6 Double dangling end effect

The nearest neighbour model for computation in case of sequences with a double or secondary dangling ends can be specified by the argument method.double.dangle. The available methods are given in Table 9.

Table 9: Details of methods for sequences with double dangling ends

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 method for computation.

```
Examples
   DNA: --ATGCATAA
          1111111
   DNA: AATACGTA--
   RNA: --AUGCAUAA
          RNA: AAUACGUA--
# Double dangling end effect - default (DNA/DNA)
melting(sequence = "--ATGCATAA",
        nucleic.acid.conc = 0.0004, hybridisation.type = "dnadna",
        Na.conc = 1)
## [1] 44.88615
# Double dangling end effect - sugdna02 (DNA/DNA)
melting(sequence = "--ATGCATAA",
        nucleic.acid.conc = 0.0004, hybridisation.type = "dnadna",
        Na.conc = 1, method.double.dangle = "sugdna02")
## [1] 44.88615
# Double dangling end effect - default (RNA/RNA)
melting(sequence = "--AUGCAUAA",
        nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
        Na.conc = 1)
## [1] 42.79724
# Double dangling end effect - ser06 (RNA/RNA)
melting(sequence = "--AUGCAUAA",
        nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
        Na.conc = 1, method.double.dangle = "ser06")
## [1] 42.79724
# Double dangling end effect - sugrna02 (RNA/RNA)
melting(sequence = "--AUGCAUAA",
        nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
        Na.conc = 1, method.double.dangle = "sugrna02")
## [1] 41.82788
# Double dangling end effect - ser05 (RNA/RNA)
melting(sequence = "--AUGCAUAA",
        nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
        Na.conc = 1, method.double.dangle = "ser05")
```

4.2.7 Long dangling end effect

[1] 42.78815

The nearest neighbour model for computation in case of sequences with a double or secondary dangling ends can be specified by the argument method.long.dangle. The available methods are given in **Table 10**.

Table 10: Details of methods for sequences with long dangling ends

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 method for computation.

```
Examples
```

```
DNA: ----GCATATGCAAAA
            DNA: AAAACGTATACG----
   RNA: AAAAGCAUAUGC----
            RNA: ----CGUAUACGAAAA
# Long dangling end effect - default (DNA/DNA)
melting(sequence = "----GCATATGCAAAA",
       nucleic.acid.conc = 0.0004, hybridisation.type = "dnadna",
       Na.conc = 1)
## [1] 55.69854
# Long dangling end effect - sugdna02 (DNA/DNA)
melting(sequence = "----GCATATGCAAAA",
       nucleic.acid.conc = 0.0004, hybridisation.type = "dnadna",
       Na.conc = 1, method.long.dangle = "sugdna02")
## [1] 55.69854
# Long dangling end effect - default (RNA/RNA)
melting(sequence = "AAAAGCAUAUGC----",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1)
## [1] 57.21314
# Long dangling end effect - sugrna02 (RNA/RNA)
melting(sequence = "AAAAGCAUAUGC----",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1, method.long.dangle = "sugrna02")
```

[1] 57.21314

4.2.8 Internal loop effect

The nearest neighbour model for computation in case of sequences with an internal loop (more than two adjacent mismatches) can be specified by the argument method.internal.loop. The available methods are given in Table 11.

Table 11: Details of methods for sequences with internal loops

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 method for computation.

```
Examples
   DNA: GCGATTGGCACTTTGGTGAAC
       DNA: CGCTACATATGAAACCACTTG
   RNA: GACAC-GCUG
       RNA: CUGUAUCGAC
# Internal loop effect - default (DNA/DNA)
melting(sequence = "GCGATTGGCACTTTGGTGAAC", comp.sequence = "CGCTACATATGAAACCACTTG",
       nucleic.acid.conc = 0.0001, hybridisation.type = "dnadna",
       Na.conc = 1)
## [1] 84.09052
# Internal loop effect - san04 (DNA/DNA)
melting(sequence = "GCGATTGGCACTTTGGTGAAC", comp.sequence = "CGCTACATATGAAACCACTTG",
       nucleic.acid.conc = 0.0001, hybridisation.type = "dnadna",
       Na.conc = 1, method.internal.loop = "san04")
## [1] 84.09052
# Internal loop effect - default (RNA/RNA)
melting(sequence = "GACAC-GCUG", comp.sequence = "CUGUAUCGAC",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1)
## [1] 45.98713
# Internal loop effect - zno07 (RNA/RNA)
melting(sequence = "GACAC-GCUG", comp.sequence = "CUGUAUCGAC",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1, method.internal.loop = "zno07")
## [1] 40.49012
# Internal loop effect - tur06 (RNA/RNA)
melting(sequence = "GACAC-GCUG", comp.sequence = "CUGUAUCGAC",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1, method.internal.loop = "tur06")
```

[1] 45.98713

4.2.9 Single bulge loop effect

The nearest neighbour model for computation in case of sequences with a single bulge loop can be specified by the argument method.single.bulge.loop. The available methods are given in Table 12.

Table 12: Details of methods for sequences with single bulge loop

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 method for computation.

```
Examples
   DNA: TCGATTAGCGACACAGG
        DNA: AGCTAATC-CTGTGTCC
   RNA: GACUCUGUC
        1111 1111
   RNA: CUGA-ACAG
# Single bulge loop effect - default (DNA/DNA)
melting(sequence = "TCGATTAGCGACACAGG", comp.sequence = "AGCTAATC-CTGTGTCC",
       nucleic.acid.conc = 0.0001, hybridisation.type = "dnadna",
       Na.conc = 1)
## [1] 71.12754
# Single bulge loop effect - tan04 (DNA/DNA)
melting(sequence = "TCGATTAGCGACACAGG", comp.sequence = "AGCTAATC-CTGTGTCC",
       nucleic.acid.conc = 0.0001, hybridisation.type = "dnadna",
       Na.conc = 1, method.single.bulge.loop = "tan04")
## [1] 71.12754
# Single bulge loop effect - san04 (DNA/DNA)
melting(sequence = "TCGATTAGCGACACAGG", comp.sequence = "AGCTAATC-CTGTGTCC",
        nucleic.acid.conc = 0.0001, hybridisation.type = "dnadna",
       Na.conc = 1, method.single.bulge.loop = "san04")
## [1] 62.0496
# Single bulge loop effect - default (RNA/RNA)
melting(sequence = "GACUCUGUC", comp.sequence = "CUGA-ACAG",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1)
## [1] 39.47787
# Single bulge loop effect - tur06 (RNA/RNA)
melting(sequence = "GACUCUGUC", comp.sequence = "CUGA-ACAG",
```

```
nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
    Na.conc = 1, method.single.bulge.loop = "tur06")

## [1] 39.47787

# Single bulge loop effect - ser07 (RNA/RNA)
melting(sequence = "GACUCUGUC", comp.sequence = "CUGA-ACAG",
    nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
    Na.conc = 1, method.single.bulge.loop = "ser07")
```

[1] 31.42849

4.2.10 Long bulge loop effect

The nearest neighbour model for computation in case of sequences with long bulge loop can be specified by the argument method.long.bulge.loop. The available methods are given in **Table 13**.

Table 13: Details of methods for sequences with long bulge loop

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

^{*} Default method for computation.

Examples

```
DNA: ATATGACGCCACAGCG
        DNA: TATAC---GGTGTCGC
   RNA: AUAUGACGCCACAGCG
        \Pi\Pi\Pi\Pi
               11111111
   RNA: UAUAC---GGUGUCGC
# Long bulge loop effect - default (DNA/DNA)
melting(sequence = "ATATGACGCCACAGCG", comp.sequence = "TATAC---GGTGTCGC",
        nucleic.acid.conc = 0.0001, hybridisation.type = "dnadna",
       Na.conc = 1)
## [1] 51.7104
# Long bulge loop effect - sanO4 (DNA/DNA)
melting(sequence = "ATATGACGCCACAGCG", comp.sequence = "TATAC---GGTGTCGC",
       nucleic.acid.conc = 0.0001, hybridisation.type = "dnadna",
       Na.conc = 1, method.long.bulge.loop = "san04")
## [1] 51.7104
# Long bulge loop effect - default (RNA/RNA)
melting(sequence = "AUAUGACGCCACAGCG", comp.sequence = "UAUAC---GGUGUCGC",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1)
```

[1] 66.0497

4.2.11 CNG repeats effect

The nearest neighbour model for computation in case of sequences with CNG repeats can be specified by the argument method.CNG. The available methods are given in **Table 14**.

Table 14: Details of methods for sequences with CNG repeats

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

^{*} Default method for computation.

Examples

```
RNA:GCGGCGGCGCCGCCGCCG
```

```
## [1] 94.25719
```

[1] 94.25719

4.2.12 Inosine bases effect

The nearest neighbour model for computation in case of sequences with inosine bases (I) can be specified by the argument method.inosine. The available methods are given in **Table 15**.

Table 15: Details of methods for sequences with inosine bases

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 method for computation.

```
Examples
```

```
DNA: CCGICTGTIGCG
        DNA: GGCCGACACCGC
   RNA: GCAICGC
        111 111
   RNA: CGUUGCG
# Inosine bases effect - default (DNA/DNA)
melting(sequence = "CCGICTGTIGCG", comp.sequence = "GGCCGACACCGC",
        nucleic.acid.conc = 0.0001, hybridisation.type = "dnadna",
       Na.conc = 1)
## [1] 65.36853
# Inosine bases effect - san05 (DNA/DNA)
melting(sequence = "CCGICTGTIGCG", comp.sequence = "GGCCGACACCGC",
        nucleic.acid.conc = 0.0001, hybridisation.type = "dnadna",
        Na.conc = 1, method.inosine = "san05")
## [1] 65.36853
# Inosine bases effect - default (RNA/RNA)
melting(sequence = "GCAICGC", comp.sequence = "CGUUGCG",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1)
## [1] 46.75042
# Inosine bases effect - zno07 (RNA/RNA)
melting(sequence = "GCAICGC", comp.sequence = "CGUUGCG",
       nucleic.acid.conc = 0.0001, hybridisation.type = "rnarna",
       Na.conc = 1, method.inosine = "zno07")
```

4.2.13 Hydroxyadenine bases effect

[1] 46.75042

The nearest neighbour model for computation in case of sequences with hydroxyadenine bases can be specified by the argument method.hydroxyadenine. The available methods are given in **Table 16**.

Table 16: Details of methods for sequences with hydroxyadenine bases

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

^{*} Default method for computation.

Examples

[1] 68.46041

4.2.14 Azobenzenes effect

The nearest neighbour model for computation in case of sequences with azobenzenes (X_T for trans azobenzenes and X_C for cis azobenzenes) can be specified by the argument method.azobenzenes. The available methods are given in **Table 17**.

Table 17: Details of methods for sequences with azobenzenes

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

^{*} Default method for computation.

Examples

```
## [1] 47.85385
```

4.2.15 Locked nucleic acids effect

The nearest neighbour model for computation in case of sequences with locked nucleic acids can be specified by the argument method.locked. The available methods are given in **Table 18**.

Table 18: Details of methods for sequences with locked nucleic acids

Model	Type	Limits/Remarks	Reference
mct04*	DNA		McTigue et al. (2004)

^{*} Default method for computation.

Examples

```
L
DNA: CCATTGCTACC
||||||||
DNA: GGTAACGATGG
```

```
## [1] 63.61426
```

[1] 63.61426

5 Corrections

Once the melting temperature is computed, a correction is applied to it according to the concentration of nucleic acids, cations and/or denaturing agents.

5.1 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.

5.2 Ion corrections

Melting temperature is computed initially for $[Na^+] = 1$ M, after which a correction for the presence of cations ($[Na^+]$, $[K^+]$, $[Tris^+]$ and $[Mg^+]$) is applied either directly on the computed melting temperature or on the computed entropy.

Th correction methods for cation concentration can be specified by the argument correction.ion.

5.2.1 Sodium corrections

The available correction methods for sodium concentration are given in **Table 19**.

 Table 19: Details of the corrections for sodium concentration

Correction	Type	Limits/Remarks	Reference
ahs01	DNA	Na>0.	Ahsen et al. (2001)
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; Oligonucleotides inferior to 16 bases.	SantaLucia and Hicks (2004), SantaLucia (1998)
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 or 2'-O- MeRNA/RNA	Na>=0.003; Na<=1.	Tan and Chen (2007)
wet91	RNA, DNA and RNA/DNA	Na>0.	Wetmur (1991)

^{*} Default method for computation.

```
# Na correction - default (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
       Na.conc = 0.069)
## [1] 56.70492
# Na correction - owc2204 (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, correction.ion = "owc2204")
## [1] 56.70492
# Na correction - ahs01 (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, correction.ion = "ahs01")
## [1] 54.1569
# Na correction - kam71 (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
       Na.conc = 0.069, correction.ion = "kam71")
## [1] 51.72963
# Na correction - marschdot (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
```

Na.conc = 0.069, correction.ion = "marschdot")

```
## [1] 49.18075
# Na correction - owc1904 (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, correction.ion = "owc1904")
## [1] 56.18571
# Na correction - owc2004 (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, correction.ion = "owc2004")
## [1] 56.67553
# Na correction - owc2104 (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, correction.ion = "owc2104")
## [1] 56.63967
# Na correction - san96 (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, correction.ion = "san96")
## [1] 53.01651
# Na correction - san04 (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, correction.ion = "san04")
## [1] 54.15157
# Na correction - schlif (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, correction.ion = "schlif")
## [1] 48.25579
# Na correction - tanna06 (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, correction.ion = "tanna06")
## [1] 55.26711
# Na correction - wet91 (DNA/DNA)
melting(sequence = "CCAGCCAGTCTCTCC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, correction.ion = "wet91")
```

[1] 51.74573

```
# Na correction - default (RNA/RNA)
melting(sequence = "CCAGCCAGUCUCUCC",
       nucleic.acid.conc = 0.000002, hybridisation.type = "rnarna",
       Na.conc = 0.069
## [1] 75.1552
# Na correction - tanna07 (RNA/RNA)
melting(sequence = "CCAGCCAGUCUCUCC",
       nucleic.acid.conc = 0.000002, hybridisation.type = "rnarna",
       Na.conc = 0.069, correction.ion = "tanna07")
## [1] 75.1552
# Na correction - wet91 (RNA/RNA)
melting(sequence = "CCAGCCAGUCUCUCC",
       nucleic.acid.conc = 0.000002, hybridisation.type = "rnarna",
       Na.conc = 0.069, correction.ion = "wet91")
## [1] 69.55572
# Na correction - default (mRNA/RNA)
melting(sequence = "UACGCGUCAAUAACGCUA",
       nucleic.acid.conc = 0.000002, hybridisation.type = "mrnarna",
       Na.conc = 0.069
## [1] 81.57763
# Na correction - tanna07 (mRNA/RNA)
melting(sequence = "UACGCGUCAAUAACGCUA",
       nucleic.acid.conc = 0.000002, hybridisation.type = "mrnarna",
       Na.conc = 0.069, correction.ion = "tanna07")
## [1] 81.57763
# Na correction - default (DNA/RNA)
melting(sequence = "CCAGCCAGTCTCTCC",
       nucleic.acid.conc = 0.000002, hybridisation.type = "dnarna",
       Na.conc = 0.069
## [1] 62.08869
# Na correction - wet91 (DNA/RNA)
melting(sequence = "CCAGCCAGTCTCTCC",
       nucleic.acid.conc = 0.000002, hybridisation.type = "dnarna",
       Na.conc = 0.069, correction.ion = "wet91")
## [1] 62.08869
```

5.2.2 Magnesium corrections

The available correction methods for magnesium concentration are given in **Table 20**.

Table 20: Details of the corrections for magnesium concentration

Correction	Type	Limits/Remarks	Reference
owcmg08*	DNA	Mg > = 0.0005; Mg < = 0.6.	Owczarzy et al. (2008)

Correction	Type	Limits/Remarks	Reference
tanmg06	DNA	Mg>=0.0001; Mg<=1; Oligomer length superior to 6 base pairs.	Tan and Chen (2006)
tanmg07*	RNA or 2'-O-MeRNA/RNA	Mg>=0.1; Mg<=0.3.	Tan and Chen (2007)

^{*} Default method for computation.

```
# Mg correction - default (DNA/DNA)
melting(sequence = "CAGCCTCGTCGCAGC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Mg.conc = 0.0015)
## [1] 65.52043
# Mg correction - owcmg08 (DNA/DNA)
melting(sequence = "CAGCCTCGTCGCAGC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Mg.conc = 0.0015, correction.ion = "owcmg08")
## [1] 65.52043
# Mg correction - tanmg06 (DNA/DNA)
melting(sequence = "CAGCCTCGTCGCAGC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Mg.conc = 0.0015, correction.ion = "tanmg06")
## [1] 64.88082
# Mg correction - default (RNA/RNA)
melting(sequence = "CAGCCUCGUCGCAGC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "rnarna",
        Mg.conc = 0.0015)
## [1] 82.0796
# Mg correction - tanmg07 (RNA/RNA)
melting(sequence = "CAGCCUCGUCGCAGC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "rnarna",
       Mg.conc = 0.0015, correction.ion = "tanmg07")
## [1] 82.0796
\# Mg correction - default (mRNA/RNA)
melting(sequence = "UACGCGUCAAUAACGCUA",
        nucleic.acid.conc = 0.000002, hybridisation.type = "mrnarna",
        Mg.conc = 0.0015)
## [1] 90.06842
# Mg correction - tanmg07 (mRNA/RNA)
melting(sequence = "UACGCGUCAAUAACGCUA",
        nucleic.acid.conc = 0.000002, hybridisation.type = "mrnarna",
        Mg.conc = 0.0015, correction.ion = "tanmg07")
```

[1] 90.06842

5.2.3 Mixed Sodium and Magnesium corrections

The available correction methods for mixed sodium magnesium concentration are given in **Table 21**.

Table 21: Details of the corrections for mixed sodium and magnesium concentration

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

^{*} Default method for computation.

```
# Mixed Na & Mg correction - default (DNA/DNA)
melting(sequence = "CAGCCTCGTCGCAGC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, Mg.conc = 0.0015)
## [1] 65.83371
# Mixed Na & Mg correction - owcmix08 (DNA/DNA)
melting(sequence = "CAGCCTCGTCGCAGC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
       Na.conc = 0.069, Mg.conc = 0.0015, correction.ion = "owcmix08")
## [1] 65.83371
# Mixed Na & Mg correction - tanmix07 (DNA/DNA)
melting(sequence = "CAGCCTCGTCGCAGC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "dnadna",
        Na.conc = 0.069, Mg.conc = 0.0015, correction.ion = "tanmix07")
## [1] 63.21723
# Mixed Na & Mg correction - default (RNA/RNA)
melting(sequence = "CAGCCUCGUCGCAGC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "rnarna",
        Na.conc = 0.069, Mg.conc = 0.0015)
## [1] 79.40119
# Mixed Na & Mg correction - tanmix07 (RNA/RNA)
melting(sequence = "CAGCCUCGUCGCAGC",
        nucleic.acid.conc = 0.000002, hybridisation.type = "rnarna",
        Na.conc = 0.069, Mg.conc = 0.0015, correction.ion = "tanmix07")
## [1] 79.40119
# Mixed Na & Mg correction - default (mRNA/RNA)
melting(sequence = "UACGCGUCAAUAACGCUA",
        nucleic.acid.conc = 0.000002, hybridisation.type = "mrnarna",
        Na.conc = 0.069, Mg.conc = 0.0015)
```

[1] 96.46186

[1] 96.46186

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,

- $[K^+] = 0$, default sodium correction is used;
- Ratio < 0.22, default sodium correction is used;
- $0.22 \le \text{Ratio} < 6$, default mixed Na and Mg correction is used and
- Ratio \geq 6,default magnesium correction is used.

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

5.2.4 Sodium equivalent concentration methods

The available correction methods for mixed sodium magnesium concentration are given in **Table 22**.

Table 22: Details of the methods for computation of sodium equivalent concentration in the presence of other ions

Correction	Type	Limits/Remarks	Reference	
ahs01*	DNA		Ahsen et al. (2001)	
mit96	DNA		Mitsuhashi (1996)	
pey00	DNA		Peyret (2000)	

^{*} Default method for computation.

```
## [1] 65.83371
```

```
## [1] 65.83371
```

```
## [1] 65.83371
```

[1] 65.83371

5.3 Denaturing agent corrections

These include melting temperature corrections for concentration of formamide and DMSO.

5.3.1 DMSO corrections

The available correction methods for DMSO concentration are given in **Table 23**.

Table 23: Details of the corrections for DMSO concentration

Correction	Type	Limits/Remarks	Reference
ahs01*	DNA	Not tested with experimental results.	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 method for computation.

[1] 65.40154

[1] 65.40154

[1] 67.90154

[1] 66.15154

[1] 66.90154

5.3.2 Formamide corrections

The available correction methods for formamide concentration are given in Table 24.

Table 24: Details of the corrections for formamide concentration

Correction	Type	Limits/Remarks	Reference
bla96*	DNA	With formamide concentration in mol/L.	Blake (1996)
lincorr	DNA	With a $\%$ of formamide volume.	McConaughy et al. (1969), Record (1967), Casey and Davidson (1977), Hutton (1977)

^{*} Default method for computation.

[1] 66.40154

6 Equivalent options in MELTING 5

The options in MELTING 5 command line equivalent to the arguments in rmelting are given in Table 25.

Table 24: Arguments in rmelting and their equivalent options in MELTING 5 command line.

rmelting	MELTING 5 (command line)
sequence	-S
comp.sequence	-C

rmelting	MELTING 5 (command line)
nucleic.acid.conc	-P
hybridisation.type	-H
Na.conc	-E
Mg.conc	-E
Tris.conc	-E
K.conc	-E
dNTP.conc	-E
DMSO.conc	-E
formamide.conc	-E
size.threshold	-T
self	-self
correction.factor	-F
method.approx	-am
method.nn	-nn
method.GU	-GU
method.singleMM	$-\sin MM$
${\tt method.tandemMM}$	-tanMM
method.single.dangle	-sinDE
method.double.dangle	$-\mathrm{secDE}$
method.long.dangle	-lonDE
method.internal.loop	-intLP
method.single.bulge.loop	-sinBU
method.long.bulge.loop	-lonBU
method.CNG	-CNG
method.inosine	-ino
method.hydroxyadenine	-ha
method.azobenzenes	-azo
method.locked	-lck
correction.ion	-ion
method.Naeq	-naeq
correction.DMSO	-DMSO
correction.formamide	-for

7 Batch computation

Melting temperature for multiple nucleic acid duplexes can be computed using the meltingBatch function.

```
##
         Environment.Sequence Environment.Complementary sequence
   [1,] "CAAAAAG"
                              "GTTTTTC"
   [2,] "CAAAAAG"
                              "GTTTTTTC"
##
##
    [3,] "TTTTATAATAAA"
                              "AAAATATTATTT"
  [4,] "CCATCGCTACC"
                              "GGTAGCGATGG"
##
## [5,] "CAAACAAAG"
                              "GTTTGTTTC"
## [6,] "CCATTGCTACC"
                              "GGTAACGATGG"
```

```
[7,] "CAAAAAAG"
                               "GTTTTTTTC"
##
    [8,] "GTGAAC"
                               "CACTTG"
   [9,] "AAAAAAA"
                               "TTTTTTTT"
## [10,] "CAACTTGATATTATTA"
                               "GTTGAACTATAATAAT"
## [11,] "CAAATAAAG"
                               "GTTTATTTC"
## [12,] "GCGAGC"
                               "CGCTCG"
## [13.] "GGGACC"
                               "CCCTGG"
## [14,] "CAAAGAAAG"
                               "GTTTCTTTC"
## [15,] "CTGACAAGTGTC"
                               "GACTGTTCACAG"
## [16,] "GCGAAAAGCG"
                               "CGCTTTTCGC"
         Environment. Nucleic acid concentration (M)
##
   [1,] "4e-04"
   [2,] "4e-04"
##
   [3,] "4e-04"
##
##
  [4,] "4e-04"
##
   [5,] "4e-04"
##
   [6,] "4e-04"
##
   [7,] "4e-04"
##
   [8,] "4e-04"
##
   [9,] "4e-04"
## [10,] "4e-04"
## [11,] "4e-04"
## [12,] "4e-04"
## [13.] "4e-04"
## [14,] "4e-04"
## [15,] "4e-04"
## [16,] "4e-04"
         Environment. Hybridization type Environment. Na concentration (M)
##
                                          "1"
##
   [1,] "dnadna"
                                          "1"
  [2,] "dnadna"
##
                                          "1"
##
   [3,] "dnadna"
##
   [4,] "dnadna"
                                          "1"
                                          "1"
##
   [5,] "dnadna"
                                          "1"
##
   [6,] "dnadna"
                                          "1"
##
    [7,] "dnadna"
                                          "1"
##
   [8,] "dnadna"
                                          "1"
##
  [9,] "dnadna"
## [10,] "dnadna"
                                          "1"
                                          "1"
## [11,] "dnadna"
                                          "1"
## [12,] "dnadna"
                                          "1"
## [13,] "dnadna"
                                          "1"
## [14,] "dnadna"
## [15,] "dnadna"
                                          "1"
## [16,] "dnadna"
                                          "1"
         Environment.Mg concentration (M) Environment.Tris concentration (M)
                                            "0"
   [1,] "0"
##
                                            "0"
   [2,] "0"
##
                                            "0"
   [3,] "0"
##
                                            "0"
##
   [4,] "0"
   [5,] "0"
                                            "0"
##
                                            "0"
##
   [6,] "0"
  [7,] "0"
                                            "0"
##
                                            "0"
## [8,] "0"
   [9,] "0"
                                            "0"
##
```

```
"0"
## [10,] "0"
## [11,] "0"
                                             "0"
                                            "0"
## [12,] "0"
## [13,] "0"
                                             "0"
                                             "0"
## [14,] "0"
                                             "0"
## [15,] "0"
                                            "0"
## [16,] "0"
         Environment.K concentration (M) Environment.dNTP concentration (M)
##
##
    [1,] "0"
                                           "0"
                                           "0"
##
    [2,] "0"
                                           "0"
##
   [3,] "0"
                                           "0"
    [4,] "0"
##
                                           "0"
##
    [5,] "0"
                                           "0"
##
   [6,] "0"
##
   [7,] "0"
                                           "0"
    [8,] "0"
                                           "0"
##
##
   [9,] "0"
                                            "0"
                                           "0"
## [10,] "0"
## [11,] "0"
                                           "0"
                                           "0"
## [12,] "0"
                                           "0"
## [13,] "0"
                                           "0"
## [14,] "0"
## [15,] "0"
                                            "0"
                                            "0"
## [16,] "0"
##
         Environment.DMSO concentration (%)
##
    [1,] "0"
##
    [2,] "0"
##
    [3,] "0"
##
   [4,] "0"
##
   [5,] "0"
    [6,] "0"
##
##
    [7,] "0"
##
   [8,] "0"
   [9,] "0"
##
## [10,] "0"
## [11,] "0"
## [12,] "0"
## [13,] "0"
## [14,] "0"
## [15,] "0"
## [16,] "0"
##
         Environment.Formamide concentration (M or %)
##
    [1,] "0"
##
   [2,] "0"
##
   [3,] "0"
    [4,] "0"
##
##
    [5,] "0"
   [6,] "0"
##
   [7,] "0"
##
##
   [8,] "0"
   [9,] "0"
##
## [10,] "0"
## [11,] "0"
## [12,] "0"
```

```
## [13,] "0"
## [14,] "0"
## [15,] "0"
  [16,] "0"
##
         Environment.Self complementarity Environment.Correction factor
##
   [1,] "FALSE"
                                           "4"
   [2,] "FALSE"
   [3,] "FALSE"
                                           "4"
##
                                           "4"
##
    [4,] "FALSE"
                                           "4"
##
   [5,] "FALSE"
                                           "4"
   [6,] "FALSE"
   [7,] "FALSE"
                                           "4"
##
   [8,] "FALSE"
                                           "4"
##
                                           "4"
##
  [9,] "FALSE"
                                           "4"
## [10,] "FALSE"
                                           "4"
## [11,] "FALSE"
## [12,] "FALSE"
                                           "4"
                                           "4"
## [13,] "FALSE"
                                           "4"
## [14,] "FALSE"
                                           "4"
## [15,] "FALSE"
                                           "4"
## [16,] "FALSE"
         Options. Approximative formula Options. Nearest neighbour model
                                        "all97"
##
  [1,] NA
##
   [2,] NA
                                        "all97"
## [3,] NA
                                        "all97"
## [4,] NA
                                        "all97"
                                        "all97"
## [5,] NA
##
  [6,] NA
                                        "all97"
##
                                        "all97"
  [7,] NA
  [8,] NA
##
                                        "all97"
## [9,] NA
                                        "all97"
## [10,] NA
                                        "all97"
## [11,] NA
                                        "all97"
## [12,] NA
                                        "all97"
## [13,] NA
                                        "all97"
## [14,] NA
                                        "all97"
## [15,] NA
                                        "all97"
## [16,] NA
                                        "all97"
##
         Options.GU model Options.Single mismatch model
                           "allsanpey"
##
  [1,] NA
  [2,] NA
                           "allsanpey"
##
  [3,] NA
                           "allsanpey"
##
  [4,] NA
                           "allsanpey"
## [5,] NA
                           "allsanpey"
## [6,] NA
                           "allsanpey"
## [7,] NA
                           "allsanpey"
## [8,] NA
                           "allsanpey"
##
  [9,] NA
                           "allsanpey"
## [10,] NA
                           "allsanpey"
## [11,] NA
                           "allsanpey"
## [12,] NA
                           "allsanpey"
## [13,] NA
                           "allsanpey"
## [14,] NA
                           "allsanpey"
## [15,] NA
                           "allsanpey"
```

```
## [16,] NA
                           "allsanpey"
##
         Options. Tandem mismatch model Options. Single dangling end model
                                         "bom00"
##
    [1,] "allsanpey"
   [2,] "allsanpey"
                                         "bom00"
##
                                         "bom00"
##
    [3,] "allsanpey"
##
   [4,] "allsanpey"
                                         "bom00"
   [5,] "allsanpey"
                                         "bom00"
    [6,] "allsanpey"
                                         "bom00"
##
##
    [7,] "allsanpey"
                                         "bom00"
##
                                         "bom00"
    [8,] "allsanpey"
   [9,] "allsanpey"
                                         "bom00"
## [10,] "allsanpey"
                                         "bom00"
                                         "bom00"
  [11,] "allsanpey"
  [12,] "allsanpey"
                                         "bom00"
## [13,] "allsanpey"
                                         "bom00"
## [14,] "allsanpey"
                                         "bom00"
   [15,] "allsanpey"
                                         "bom00"
                                         "bom00"
   [16,] "allsanpey"
##
         Options. Double dangling end model Options. Long dangling end model
##
   [1,] "sugdna02"
                                             "sugdna02"
##
   [2,] "sugdna02"
                                             "sugdna02"
   [3,] "sugdna02"
                                             "sugdna02"
##
   [4,] "sugdna02"
                                             "sugdna02"
##
    [5,] "sugdna02"
                                             "sugdna02"
##
   [6,] "sugdna02"
                                             "sugdna02"
   [7,] "sugdna02"
                                             "sugdna02"
##
   [8,] "sugdna02"
                                             "sugdna02"
    [9,] "sugdna02"
                                             "sugdna02"
##
## [10,] "sugdna02"
                                             "sugdna02"
  [11,] "sugdna02"
                                             "sugdna02"
## [12,] "sugdna02"
                                             "sugdna02"
   [13,] "sugdna02"
                                             "sugdna02"
   [14,] "sugdna02"
                                             "sugdna02"
   [15,] "sugdna02"
                                             "sugdna02"
                                             "sugdna02"
   [16,] "sugdna02"
##
         Options.Internal loop model Options.Single bulge loop model
##
   [1,] "san04"
                                       "tan04"
##
   [2,] "san04"
                                       "tan04"
    [3,] "san04"
##
                                       "tan04"
   [4,] "san04"
                                       "tan04"
##
   [5,] "san04"
                                       "tan04"
##
   [6,] "san04"
                                       "tan04"
    [7,] "san04"
                                       "tan04"
##
##
   [8,] "san04"
                                       "tan04"
   [9,] "san04"
                                       "tan04"
## [10,] "san04"
                                       "tan04"
   [11,] "san04"
                                       "tan04"
  [12,] "san04"
                                       "tan04"
  [13,] "san04"
                                       "tan04"
## [14,] "san04"
                                       "tan04"
##
  [15,] "san04"
                                       "tan04"
## [16,] "san04"
                                       "tan04"
##
         Options.Long bulge loop model Options.CNG repeats model
##
   [1,] "san04"
                                         NA
```

```
[2,] "san04"
##
                                          NA
##
    [3,] "san04"
                                          NA
    [4,] "san04"
##
                                          NA
    [5,] "san04"
##
                                          NA
##
    [6,] "san04"
                                          NA
##
    [7,] "san04"
                                          NA
##
    [8,] "san04"
                                          NA
    [9,] "san04"
##
                                          NA
## [10,] "san04"
                                          NA
##
                                          NA
  [11,] "san04"
## [12,] "san04"
                                          NA
   [13,] "san04"
                                          NA
##
##
   [14,] "san04"
                                          NA
  [15,] "san04"
                                          NA
##
##
   [16,] "san04"
                                          NA
##
         Options. Inosine bases model Options. Hydroxyadenine bases model
##
    [1,] "san05"
                                        "sug01"
##
    [2,] "san05"
                                        "sug01"
##
    [3,] "san05"
                                        "sug01"
##
    [4,] "san05"
                                        "sug01"
##
    [5,] "san05"
                                        "sug01"
##
    [6,] "san05"
                                        "sug01"
##
    [7,] "san05"
                                        "sug01"
##
    [8,] "san05"
                                        "sug01"
##
    [9,] "san05"
                                        "sug01"
## [10,] "san05"
                                        "sug01"
##
   [11,] "san05"
                                        "sug01"
   [12,] "san05"
                                        "sug01"
##
## [13,] "san05"
                                        "sug01"
## [14,] "san05"
                                        "sug01"
##
  [15,] "san05"
                                        "sug01"
##
   [16,] "san05"
                                        "sug01"
##
         Options. Azobenzenes model Options. Locked nucleic acids model
    [1,] "asa05"
##
                                      "mct04"
    [2,] "asa05"
##
                                     "mct04"
##
    [3,] "asa05"
                                     "mct04"
##
    [4,] "asa05"
                                     "mct04"
##
    [5,] "asa05"
                                     "mct04"
##
    [6,] "asa05"
                                     "mct04"
##
    [7,] "asa05"
                                     "mct04"
    [8,] "asa05"
                                     "mct04"
##
    [9,] "asa05"
                                     "mct04"
## [10,] "asa05"
                                     "mct04"
   [11,] "asa05"
                                     "mct04"
##
## [12,] "asa05"
                                     "mct04"
## [13,] "asa05"
                                     "mct04"
   [14,] "asa05"
                                     "mct04"
##
   [15,] "asa05"
                                     "mct04"
##
##
   [16,] "asa05"
                                     "mct04"
##
         Options. Ion correction method
##
    [1,] NA
   [2,] NA
##
##
   [3,] NA
    [4,] NA
##
```

```
[5,] NA
##
##
   [6,] NA
##
   [7,] NA
   [8,] NA
##
##
   [9,] NA
## [10,] NA
## [11,] NA
## [12,] NA
## [13,] NA
## [14,] NA
## [15,] NA
## [16,] NA
         Options.Na equivalence correction method
##
   [1,] "ahs01"
##
   [2,] "ahs01"
##
    [3,] "ahs01"
##
   [4,] "ahs01"
##
   [5,] "ahs01"
##
   [6,] "ahs01"
##
   [7,] "ahs01"
##
   [8,] "ahs01"
  [9,] "ahs01"
## [10,] "ahs01"
## [11,] "ahs01"
## [12,] "ahs01"
## [13,] "ahs01"
## [14,] "ahs01"
## [15,] "ahs01"
## [16,] "ahs01"
##
         Options.DMSO correction method Options.Formamide correction method
##
    [1,] "ahs01"
                                          "bla96"
   [2,] "ahs01"
##
                                          "bla96"
   [3,] "ahs01"
                                          "bla96"
##
##
   [4,] "ahs01"
                                          "bla96"
##
    [5,] "ahs01"
                                          "bla96"
##
   [6,] "ahs01"
                                          "bla96"
##
   [7,] "ahs01"
                                          "bla96"
##
   [8,] "ahs01"
                                          "bla96"
##
   [9,] "ahs01"
                                          "bla96"
## [10,] "ahs01"
                                          "bla96"
## [11,] "ahs01"
                                          "bla96"
## [12,] "ahs01"
                                          "bla96"
## [13,] "ahs01"
                                          "bla96"
## [14,] "ahs01"
                                          "bla96"
## [15,] "ahs01"
                                          "bla96"
## [16,] "ahs01"
                                          "bla96"
##
         Options. Mode Results. Enthalpy (cal) Results. Entropy (cal)
##
   [1,] "def"
                       "-47700"
                                               "-138.1"
   [2,] "def"
                       "-55600"
                                               "-160.3"
##
                                               "-229.7"
   [3,] "def"
                       "-78800"
##
                                               "-227"
##
   [4,] "def"
                       "-83500"
                       "-64600"
  [5,] "def"
                                               "-183.2"
##
## [6,] "def"
                       "-81100"
                                               "-222.5"
## [7,] "def"
                       "-63500"
                                               "-182.5"
```

```
##
    [8,] "def"
                      "-41200"
                                              "-117.5"
                                              "-147.2"
   [9,] "def"
                      "-50700"
##
## [10,] "def"
                      "-113800"
                                              "-323.6"
## [11,] "def"
                      "-62100"
                                              "-179.8"
## [12,] "def"
                      "-46000"
                                              "-124.8"
## [13,] "def"
                      "-40400"
                                              "-109.9"
## [14.] "def"
                      "-63700"
                                              "-181.3"
## [15,] "def"
                      "-90400"
                                              "-249.5"
## [16,] "def"
                      "-80300"
                                              "-218.6"
##
         Results.Enthalpy (J) Results.Entropy (J)
  [1,] "-199386"
                              "-577.258"
   [2,] "-232408"
                              "-670.054"
##
##
   [3,] "-329384"
                              "-960.146"
  [4,] "-349030"
                              "-948.86"
##
  [5,] "-270028"
                              "-765.776"
##
##
   [6,] "-338998"
                              "-930.05"
  [7,] "-265430"
                              "-762.85"
##
  [8,] "-172216"
                              "-491.15"
   [9,] "-211926"
                              "-615.296"
##
## [10,] "-475684"
                              "-1352.648"
## [11,] "-259578"
                              "-751.564"
## [12,] "-192280"
                              "-521.664"
## [13,] "-168872"
                              "-459.382"
## [14.] "-266266"
                              "-757.834"
                              "-1042.91"
## [15,] "-377872"
## [16,] "-335654"
                              "-913.748"
         Results.Melting temperature (C) Message
##
  [1,] "31.7814953255144"
                                          NA
  [2,] "38.1103863719918"
                                          NA
## [3,] "44.5553259145469"
                                          NA
   [4,] "67.2098590318261"
##
                                          NA
##
  [5,] "47.400072116762"
                                          NA
  [6,] "63.6040550863501"
                                          NA
  [7,] "43.0400604037136"
##
                                          NA
##
   [8,] "30.1735038367475"
                                          NA
## [9,] "33.1415226764116"
                                          NA
## [10,] "59.6680431282422"
                                          NA
## [11,] "40.2828264688437"
                                          NA
## [12,] "48.2393469411973"
                                          NA
## [13,] "41.9123740666287"
                                          NA
## [14,] "45.9425910944819"
                                          NA
## [15,] "64.379329012421"
                                          NA
## [16,] "65.7707030297917"
                                          NA
```

Complementary sequences are computed by default, but need to be specified in case of mismatches, inosine(s) or hydroxyadenine(s) between the two strands.

```
"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")
##
         Environment.Sequence Environment.Complementary sequence
                               "CGUUUGC"
   [1,] "GCAUACG"
    [2,] "CAGUAGGUC"
                               "GUCGGCCAG"
##
##
   [3,] "CGCUCGC"
                               "GCGUGCG"
   [4,] "GAGUGGAG"
                               "CUCUUCUC"
##
##
   [5,] "GACAGGCUG"
                               "CUGUGCGAC"
    [6,] "CAGUACGUC"
##
                               "GUCGGGCAG"
##
   [7,] "GACAUCCUG"
                               "CUGUUGGAC"
   [8,] "GACCACCUG"
                               "CUGGGGGAC"
##
   [9,] "CAGAAUGUC"
                               "GUCUGGCAG"
## [10,] "GCGUCGC"
                               "CGCUGCG"
## [11,] "CGUCCGG"
                               "GCUGGCC"
## [12,] "GACUCUCUG"
                               "CUGAUAGAC"
## [13,] "CAGCUGGUC"
                               "GUCGUUCAG"
## [14,] "GACUAGCUG"
                               "CUGAGCGAC"
## [15,] "CUCUGCUC"
                               "GAGUUGAG"
## [16,] "GCGUCCG"
                               "CGCUGGC"
## [17,] "GUCCGCG"
                               "CUGGCGC"
## [18,] "CGAUCAC"
                               "GCUUGUG"
## [19,] "GACUACCUG"
                               "CUGAGGGAC"
## [20,] "GACGAUCUG"
                               "CUGCCAGAC"
##
         Environment. Nucleic acid concentration (M)
##
   [1,] "4e-04"
   [2,] "4e-04"
##
   [3,] "4e-04"
##
   [4,] "4e-04"
  [5,] "4e-04"
##
##
  [6,] "4e-04"
  [7,] "4e-04"
##
##
   [8,] "4e-04"
##
  [9,] "4e-04"
## [10,] "4e-04"
## [11,] "4e-04"
## [12,] "4e-04"
## [13,] "4e-04"
## [14,] "4e-04"
## [15,] "4e-04"
## [16,] "4e-04"
## [17,] "4e-04"
## [18,] "4e-04"
## [19,] "4e-04"
## [20,] "4e-04"
##
         Environment. Hybridization type Environment. Na concentration (M)
  [1,] "rnarna"
                                         "1"
##
##
   [2,] "rnarna"
                                         "1"
                                         "1"
## [3,] "rnarna"
## [4,] "rnarna"
                                         "1"
## [5,] "rnarna"
                                         "1"
```

```
"1"
    [6,] "rnarna"
                                           "1"
##
    [7,] "rnarna"
                                           "1"
    [8,] "rnarna"
##
   [9,] "rnarna"
                                           "1"
##
                                           "1"
## [10,] "rnarna"
                                           "1"
## [11,] "rnarna"
                                           "1"
## [12,] "rnarna"
## [13,] "rnarna"
                                           "1"
## [14,] "rnarna"
                                           "1"
                                           "1"
## [15,] "rnarna"
                                           "1"
## [16,] "rnarna"
                                           "1"
## [17,] "rnarna"
## [18,] "rnarna"
                                           "1"
                                           "1"
## [19,] "rnarna"
## [20,] "rnarna"
                                           "1"
##
         Environment.Mg concentration (M) Environment.Tris concentration (M)
##
   [1,] "0"
                                             "0"
   [2,] "0"
##
   [3,] "0"
                                             "0"
##
                                             "0"
   [4,] "0"
##
                                             "0"
##
   [5,] "0"
##
   [6,] "0"
                                             "0"
   [7,] "0"
                                             "0"
##
                                             "0"
##
    [8.] "0"
                                             "0"
##
   [9,] "0"
## [10,] "0"
                                             "0"
                                             "0"
## [11,] "0"
## [12,] "0"
                                             "0"
                                             "0"
## [13,] "0"
## [14,]
         "0"
                                             "0"
                                             "0"
## [15,]
         "0"
## [16,]
         "0"
                                             "0"
## [17,] "0"
                                             "0"
                                             "0"
## [18,] "0"
                                             "0"
## [19,] "0"
                                             "0"
## [20,] "0"
##
         Environment.K concentration (M) Environment.dNTP concentration (M)
##
   [1,] "0"
                                            "0"
                                            "0"
    [2,] "0"
##
                                            "0"
   [3,] "0"
##
                                            "0"
##
   [4,] "0"
   [5,] "0"
                                            "0"
##
##
    [6,] "0"
                                            "0"
##
   [7,] "0"
                                            "0"
                                            "0"
##
   [8,] "0"
                                            "0"
   [9,] "0"
##
                                            "0"
## [10,]
         "0"
                                            "0"
## [11,] "0"
## [12,] "0"
                                            "0"
                                            "0"
## [13,] "0"
                                            "0"
## [14,] "0"
                                            "0"
## [15,] "0"
                                            "0"
## [16,] "0"
## [17,] "0"
                                            "0"
```

```
"0"
## [18,] "0"
## [19,] "0"
                                           "0"
                                           "0"
   [20,] "0"
##
         Environment.DMSO concentration (%)
##
    [1,] "0"
##
    [2,] "0"
##
   [3,] "0"
    [4,] "0"
##
##
    [5,] "0"
    [6,] "0"
##
##
   [7,] "0"
    [8,] "0"
##
##
   [9,] "0"
## [10,] "0"
## [11,] "0"
         "0"
## [12,]
## [13,] "0"
## [14,] "0"
## [15,] "0"
## [16,] "0"
## [17,] "0"
## [18,] "0"
## [19,] "0"
## [20,] "0"
##
         Environment.Formamide concentration (M or %)
##
    [1,] "0"
##
    [2,] "0"
##
    [3,] "0"
##
   [4,] "0"
   [5,] "0"
##
    [6,] "0"
##
##
    [7,] "0"
   [8,] "0"
##
   [9,] "0"
##
## [10,] "0"
## [11,] "0"
## [12,] "0"
## [13,] "0"
## [14,] "0"
## [15,] "0"
## [16,] "0"
## [17,] "0"
## [18,] "0"
## [19,] "0"
## [20,] "0"
##
         Environment. Self complementarity Environment. Correction factor
    [1,] "FALSE"
                                            "4"
##
                                            "4"
##
    [2,] "FALSE"
    [3,] "FALSE"
                                            "4"
##
                                            "4"
    [4,] "FALSE"
##
                                            "4"
##
   [5,] "FALSE"
                                            "4"
##
   [6,] "FALSE"
   [7,] "FALSE"
                                            "4"
##
## [8,] "FALSE"
                                            "4"
```

```
[9,] "FALSE"
                                             "4"
                                             "4"
## [10,] "FALSE"
                                             "4"
## [11,] "FALSE"
## [12,] "FALSE"
                                             "4"
                                             "4"
## [13,] "FALSE"
## [14,] "FALSE"
                                             "4"
## [15,] "FALSE"
                                             "4"
                                             "4"
## [16,] "FALSE"
## [17,] "FALSE"
                                             "4"
                                             "4"
## [18,] "FALSE"
                                             "4"
## [19,] "FALSE"
   [20,] "FALSE"
                                             "4"
##
##
         Options. Approximative formula Options. Nearest neighbour model
##
                                         "xia98"
   [1,] NA
##
   [2,] NA
                                         "xia98"
    [3,] NA
##
                                         "xia98"
##
   [4,] NA
                                         "xia98"
##
   [5,] NA
                                         "xia98"
##
   [6,] NA
                                         "xia98"
   [7,] NA
##
                                         "xia98"
##
   [8,] NA
                                         "xia98"
##
   [9,] NA
                                         "xia98"
## [10,] NA
                                         "xia98"
## [11,] NA
                                         "xia98"
## [12,] NA
                                         "xia98"
## [13,] NA
                                         "xia98"
                                         "xia98"
## [14,] NA
## [15,] NA
                                         "xia98"
## [16,] NA
                                         "xia98"
## [17,] NA
                                         "xia98"
## [18,] NA
                                         "xia98"
## [19,] NA
                                         "xia98"
                                         "xia98"
## [20,] NA
##
         Options.GU model Options.Single mismatch model
                           "tur06"
##
    [1,] "ser12"
##
    [2,] "ser12"
                           "tur06"
##
   [3,] "ser12"
                           "tur06"
##
   [4,] "ser12"
                           "tur06"
##
    [5,] "ser12"
                           "tur06"
##
   [6,] "ser12"
                           "tur06"
   [7,] "ser12"
                           "tur06"
##
    [8,] "ser12"
                           "tur06"
    [9,] "ser12"
                           "tur06"
##
## [10,] "ser12"
                           "tur06"
## [11,] "ser12"
                           "tur06"
## [12,] "ser12"
                           "tur06"
## [13,] "ser12"
                           "tur06"
## [14,] "ser12"
                           "tur06"
## [15,] "ser12"
                           "tur06"
## [16,] "ser12"
                           "tur06"
## [17,] "ser12"
                           "tur06"
                           "tur06"
## [18,] "ser12"
## [19,] "ser12"
                           "tur06"
## [20,] "ser12"
                           "tur06"
```

```
##
         Options. Tandem mismatch model Options. Single dangling end model
##
    [1,] "tur06"
                                         "ser08"
    [2,] "tur06"
                                         "ser08"
##
    [3,] "tur06"
                                         "ser08"
##
##
    [4,] "tur06"
                                         "ser08"
##
    [5,] "tur06"
                                         "ser08"
##
    [6.] "tur06"
                                         "ser08"
    [7,] "tur06"
                                         "ser08"
##
##
    [8,] "tur06"
                                         "ser08"
##
   [9,] "tur06"
                                         "ser08"
## [10,] "tur06"
                                         "ser08"
## [11,] "tur06"
                                         "ser08"
   [12,] "tur06"
                                         "ser08"
##
## [13,] "tur06"
                                         "ser08"
## [14,] "tur06"
                                         "ser08"
## [15,] "tur06"
                                         "ser08"
##
   [16,] "tur06"
                                         "ser08"
                                         "ser08"
  [17,] "tur06"
  [18,] "tur06"
                                         "ser08"
   [19,] "tur06"
                                         "ser08"
##
##
  [20,] "tur06"
                                         "ser08"
##
         Options. Double dangling end model Options. Long dangling end model
   [1,] "ser06"
##
                                              "sugrna02"
##
    [2.] "ser06"
                                              "sugrna02"
##
   [3,] "ser06"
                                              "sugrna02"
##
   [4,] "ser06"
                                              "sugrna02"
##
   [5,] "ser06"
                                              "sugrna02"
##
    [6,] "ser06"
                                              "sugrna02"
##
   [7,] "ser06"
                                              "sugrna02"
   [8,] "ser06"
##
                                              "sugrna02"
   [9,] "ser06"
##
                                              "sugrna02"
## [10,] "ser06"
                                              "sugrna02"
## [11,] "ser06"
                                              "sugrna02"
## [12,] "ser06"
                                              "sugrna02"
## [13,] "ser06"
                                              "sugrna02"
## [14,] "ser06"
                                              "sugrna02"
## [15,] "ser06"
                                              "sugrna02"
## [16,] "ser06"
                                              "sugrna02"
## [17,] "ser06"
                                              "sugrna02"
## [18,] "ser06"
                                              "sugrna02"
  [19,] "ser06"
                                              "sugrna02"
##
   [20,] "ser06"
                                              "sugrna02"
         Options.Internal loop model Options.Single bulge loop model
##
##
    [1,] "tur06"
                                       "tur06"
   [2,] "tur06"
                                       "tur06"
##
   [3,] "tur06"
                                       "tur06"
##
    [4,] "tur06"
                                       "tur06"
##
##
   [5,] "tur06"
                                       "tur06"
##
   [6,] "tur06"
                                       "tur06"
    [7,] "tur06"
                                       "tur06"
##
##
    [8,] "tur06"
                                       "tur06"
   [9,] "tur06"
                                       "tur06"
##
## [10,] "tur06"
                                       "tur06"
## [11,] "tur06"
                                       "tur06"
```

```
## [12,] "tur06"
                                       "tur06"
## [13,] "tur06"
                                       "tur06"
## [14,] "tur06"
                                       "tur06"
## [15,] "tur06"
                                       "tur06"
## [16,] "tur06"
                                       "tur06"
## [17,] "tur06"
                                       "tur06"
## [18,] "tur06"
                                       "tur06"
## [19,] "tur06"
                                       "tur06"
## [20,] "tur06"
                                       "tur06"
##
         Options.Long bulge loop model Options.CNG repeats model
   [1,] "tur06"
                                         NA
   [2,] "tur06"
##
                                         NA
   [3,] "tur06"
##
                                         NA
   [4,] "tur06"
                                         NA
##
##
   [5,] "tur06"
                                         NA
##
    [6,] "tur06"
                                         NA
##
   [7,] "tur06"
                                         NA
##
   [8,] "tur06"
                                         NA
##
   [9,] "tur06"
                                         NA
## [10,] "tur06"
                                         NA
## [11,] "tur06"
                                         NA
## [12,] "tur06"
                                         NA
## [13,] "tur06"
                                         NA
## [14,] "tur06"
                                         NA
## [15,] "tur06"
                                         NA
## [16,] "tur06"
                                         NA
## [17,] "tur06"
                                         NA
## [18,] "tur06"
                                         NA
## [19,] "tur06"
                                         NA
## [20,] "tur06"
                                         NA
##
         Options. Inosine bases model Options. Hydroxyadenine bases model
##
    [1,] "zno07"
                                       NA
##
   [2,] "zno07"
                                       NA
   [3,] "zno07"
                                       NA
##
##
    [4,] "zno07"
                                       NA
##
   [5,] "zno07"
                                       NA
##
   [6,] "zno07"
                                       NA
##
   [7,] "zno07"
                                       NA
##
    [8,] "zno07"
                                       NA
##
   [9,] "zno07"
                                       NA
## [10,] "zno07"
                                       NA
## [11,] "zno07"
                                       NA
## [12,] "zno07"
                                       NA
## [13,] "zno07"
                                       NA
## [14,] "zno07"
                                       NA
## [15,] "zno07"
                                       NA
## [16,] "zno07"
                                       NA
## [17,] "zno07"
                                       NA
## [18,] "zno07"
                                       NA
## [19,] "zno07"
                                       NA
## [20,] "zno07"
                                       NA
##
         Options. Azobenzenes model Options. Locked nucleic acids model
##
   [1,] NA
                                     NA
##
   [2,] NA
                                     NA
```

```
## [3,] NA
                                    NA
   [4,] NA
##
                                   NA
   [5,] NA
                                   NA
   [6,] NA
                                   NA
##
   [7,] NA
##
                                   NA
##
  [8,] NA
                                   NA
## [9,] NA
                                   NA
## [10,] NA
                                   NA
## [11,] NA
                                   NA
## [12,] NA
                                   NA
## [13,] NA
                                    NA
## [14,] NA
                                    NA
## [15,] NA
                                   NA
## [16,] NA
                                    NA
## [17,] NA
                                    NA
## [18,] NA
                                    NA
## [19,] NA
                                    NA
## [20,] NA
##
         Options. Ion correction method
    [1,] NA
##
##
   [2,] NA
##
  [3,] NA
## [4,] NA
## [5,] NA
## [6,] NA
## [7,] NA
## [8,] NA
## [9,] NA
## [10,] NA
## [11,] NA
## [12,] NA
## [13,] NA
## [14,] NA
## [15,] NA
## [16,] NA
## [17,] NA
## [18,] NA
## [19,] NA
## [20,] NA
##
         Options. Na equivalence correction method
   [1,] "ahs01"
   [2,] "ahs01"
##
##
   [3,] "ahs01"
##
   [4,] "ahs01"
   [5,] "ahs01"
   [6,] "ahs01"
##
##
   [7,] "ahs01"
##
   [8,] "ahs01"
  [9,] "ahs01"
## [10,] "ahs01"
## [11,] "ahs01"
## [12,] "ahs01"
## [13,] "ahs01"
## [14,] "ahs01"
```

```
## [15,] "ahs01"
## [16,] "ahs01"
## [17,] "ahs01"
## [18,] "ahs01"
## [19,] "ahs01"
## [20,] "ahs01"
         Options.DMSO correction method Options.Formamide correction method
  [1,] "ahs01"
                                         "bla96"
##
##
   [2,] "ahs01"
                                         "bla96"
  [3,] "ahs01"
                                         "bla96"
##
  [4,] "ahs01"
                                         "bla96"
##
   [5,] "ahs01"
                                         "bla96"
##
  [6,] "ahs01"
                                         "bla96"
##
  [7,] "ahs01"
                                         "bla96"
## [8,] "ahs01"
                                         "bla96"
## [9,] "ahs01"
                                         "bla96"
## [10,] "ahs01"
                                         "bla96"
## [11,] "ahs01"
                                         "bla96"
## [12,] "ahs01"
                                         "bla96"
## [13,] "ahs01"
                                         "bla96"
## [14,] "ahs01"
                                         "bla96"
## [15,] "ahs01"
                                         "bla96"
## [16,] "ahs01"
                                         "bla96"
## [17,] "ahs01"
                                         "bla96"
## [18,] "ahs01"
                                         "bla96"
## [19,] "ahs01"
                                         "bla96"
## [20,] "ahs01"
                                         "bla96"
##
         Options. Mode Results. Enthalpy (cal) Results. Entropy (cal)
                       "-47650"
##
  [1,] "def"
                                               "-138.8"
                       "-71130"
                                               "-200.5"
## [2,] "def"
##
   [3,] "def"
                       "-57930"
                                               "-164.1"
##
  [4,] "def"
                       "-60570"
                                               "-176.6"
                       "-79870"
                                               "-219.9"
##
  [5,] "def"
  [6,] "def"
                       "-68380"
                                               "-194.5"
##
##
   [7,] "def"
                       "-73880"
                                               "-208.3"
##
  [8,] "def"
                       "-78430"
                                              "-218.3"
## [9,] "def"
                       "-59650"
                                               "-171.5"
## [10,] "def"
                       "-61330"
                                               "-173.8"
## [11,] "def"
                       "-58350"
                                               "-165.4"
## [12,] "def"
                       "-64570"
                                               "-184.7"
## [13,] "def"
                       "-70970"
                                               "-200.6"
## [14,] "def"
                       "-72010"
                                               "-203"
## [15,] "def"
                                               "-171"
                       "-58820"
## [16,] "def"
                       "-59840"
                                               "-169.6"
## [17,] "def"
                       "-59840"
                                               "-169.6"
## [18,] "def"
                       "-50210"
                                               "-148.3"
## [19,] "def"
                                               "-198.8"
                       "-70520"
## [20,] "def"
                       "-69730"
                                               "-198.2"
##
         Results.Enthalpy (J) Results.Entropy (J)
## [1,] "-199177"
                               "-580.184"
## [2,] "-297323.4"
                               "-838.09"
                               "-685.938"
## [3,] "-242147.4"
## [4,] "-253182.6"
                               "-738.188"
## [5,] "-333856.6"
                               "-919.182"
```

```
[6,] "-285828.4"
                               "-813.01"
    [7,] "-308818.4"
##
                               "-870.694"
    [8,] "-327837.4"
                               "-912.494"
    [9,] "-249337"
                               "-716.87"
## [10,] "-256359.4"
                               "-726.484"
## [11,] "-243903"
                               "-691.372"
## [12,] "-269902.6"
                               "-772.046"
## [13,] "-296654.6"
                               "-838.508"
  [14,] "-301001.8"
                               "-848.54"
  [15,] "-245867.6"
                               "-714.78"
## [16,] "-250131.2"
                               "-708.928"
  [17,] "-250131.2"
                               "-708.928"
  [18,] "-209877.8"
                               "-619.894"
                               "-830.984"
  [19,] "-294773.6"
## [20,] "-291471.4"
                               "-828.476"
##
         Results. Melting temperature (C) Message
##
    [1,] "30.1048299398322"
                                          NA
   [2,] "51.8989532242754"
                                          NA
   [3,] "44.3989325444856"
                                          NA
   [4,] "37.5791954133529"
                                          NA
##
  [5,] "62.1162425798375"
                                          NA
  [6,] "48.141439592185"
                                          NA
  [7,] "52.845957204839"
##
                                          NA
    [8,] "58.2977096620104"
                                          NA
  [9,] "41.08087522322"
                                          NA
## [10,] "46.0633145887674"
                                          NA
## [11,] "44.4380466975271"
                                          NA
## [12,] "44.8840464672343"
                                          NA
## [13,] "51.0196486690585"
                                          NA
## [14,] "52.203376709706"
                                          NA
## [15,] "37.5268181873443"
                                          NA
## [16,] "45.2688421309843"
                                          NA
## [17,] "45.2688421309843"
                                          NA
## [18,] "28.1788644808993"
                                          NA
## [19,] "51.6345164549562"
                                          NA
  [20,] "48.8860141674642"
                                          NA
```

8 Further reading

Further details about algorithm, formulae and methods are available in the MELTING 5 documentation.

9 Citing rmelting

```
##
## To cite the R package 'rmelting' in publications use:
##
## Aravind, J. and Krishna, G. K. (2019). rmelting: R Interface to
## MELTING 5. R package version 0.99.1,
## https://aravind-j.github.io/rmelting/.
##
## A BibTeX entry for LaTeX users is
##
## @Manual{,
```

```
## title = {rmelting: R Interface to MELTING 5},
## author = {J. Aravind and G. K. Krishna},
## year = {2019},
## note = {R package version 0.99.1},
## note = {https://aravind-j.github.io/rmelting/},
## }
##
## This free and open-source software implements academic research by
## the authors and co-workers. If you use it, please support the
## project by citing the package.
```

10 Session Info

sessionInfo()

```
## R Under development (unstable) (2018-10-27 r75507)
## Platform: x86 64-w64-mingw32/x64 (64-bit)
## Running under: Windows >= 8 x64 (build 9200)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_India.1252 LC_CTYPE=English_India.1252
## [3] LC_MONETARY=English_India.1252 LC_NUMERIC=C
## [5] LC_TIME=English_India.1252
##
## attached base packages:
                graphics grDevices utils
## [1] stats
                                              datasets methods
                                                                  base
## other attached packages:
## [1] rmelting 0.99.1 readxl 1.1.0
                                      usethis_1.4.0
                                                     devtools 2.0.1
## [5] testthat_2.0.1 rJava_0.9-10
##
## loaded via a namespace (and not attached):
## [1] Rcpp 1.0.0
                         highr 0.7
                                           pillar 1.3.0
## [4] cellranger_1.1.0 compiler_3.6.0
                                           prettyunits_1.0.2
## [7] base64enc 0.1-3 remotes 2.0.2
                                           tools 3.6.0
## [10] digest_0.6.18
                         pkgbuild_1.0.2
                                           pkgload_1.0.2
## [13] tibble_1.4.2
                         evaluate_0.12
                                           memoise_1.1.0
## [16] debugme_1.1.0
                         rlang_0.3.0.1
                                           bibtex_0.4.2
## [19] cli_1.0.1
                         rstudioapi_0.8
                                           commonmark_1.7
## [22] yaml_2.2.0
                         knitr_1.20
                                           withr_2.1.2
## [25] stringr_1.3.1
                         roxygen2_6.1.1
                                           xm12_1.2.0
## [28] desc_1.2.0
                         fs_1.2.6
                                           gbRd_0.4-11
                         glue_1.3.0
## [31] rprojroot_1.3-2
                                           R6_2.3.0
## [34] processx_3.2.0
                         Rdpack_0.10-3
                                           rmarkdown_1.10
                                           callr_3.0.0
## [37] sessioninfo_1.1.1 pander_0.6.3
## [40] purrr 0.2.5
                         magrittr_1.5
                                           htmltools_0.3.6
## [43] backports_1.1.2
                         ps_1.2.1
                                           assertthat_0.2.0
## [46] stringi_1.2.4 crayon_1.3.4
```

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