Package 'TmCalculator'

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Type Package

Title Melting Temperature of Nucleic Acid Sequences

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Description This tool is extended from methods in Bio.SeqUtils.MeltingTemp of python. The melting temperature of nucleic acid sequences can be calculated in three method, the Wallace rule (Thein & Wallace (1986) <doi:10.1016/S0140-6736(86)90739-7>), empirical formulas based on G and C content (Marmur J. (1962) <doi:10.1016/S0022-2836(62)80066-7>, Schildkraut C. (2010) <doi:10.1002/bip.360030207>, Wetmur J G (1991) <doi:10.3109/10409239109114069>, Untergasser,A. (2012) <doi:10.1093/nar/gks596>, von Ahsen N (2001) <doi:10.1093/clinchem/47.11.1956>) and nearest neighbor thermodynamics (Breslauer K J (1986) <doi:10.1073/pnas.83.11.3746>, Sugimoto N (1996) <doi:10.1093/nar/24.22.4501>, Allawi H (1998) <doi:10.1093/nar/26.11.2694>, SantaLucia J (2004) <doi:10.1146/annurev.biophys.32.110601.141800>, Freier S (1986) <doi:10.1073/pnas.83.24.9373>, Xia T (19 marito S (2000) <doi:10.1093/nar/28.9.1929>, Turner D H (2010) <doi:10.1093/nar/gkp892>, Sugimoto N (1995) <doi:10.1016/S0048-9697(98)00088-6>, Allawi H T (1997) <doi:10.1021/bi962590c>, Santalucia N (2005) <doi:10.1093/nar/gki918>), and it can also be corrected with salt ions and chemical compound (SantaLucia J (1996) <doi:10.1021/bi951907q>, SantaLucia J(1998) <doi:10.1073/pnas.95.4.1460>, Owczarzy R (2004) <doi:10.1021/bi034621r>, Owczarzy R (2008) <doi:10.102

BugReports https://github.com/JunhuiLi1017/TmCalculator/issues

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Depends R (>= 2.10)

NeedsCompilation no

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R topics documented:

`c2s` *convert a vector of characters into a string*

Description

Simply convert a vector of characters such as c("H","e","l","l","o","W","o","r","l","d") into a single string "HelloWorld".

Usage

```
c2s(characters)
```

Arguments

`characters`	A vector of characters

Value

Retrun a strings

Author(s)

Junhui Li

References

```
citation("TmCalculator")
```

Examples

```
c2s(c("H","e","l","l","o","W","o","r","l","d"))
```

check_filter *Check and filter invalid base of nucleotide sequences*

Description

In general, whitespaces and non-base characters are removed and characters are converted to uppercase in given method.

Usage

```
check_filter(ntseq, method)
```

Arguments

ntseq	Sequence (5' to 3') of one strand of the DNA nucleic acid duplex as string or vector of characters
method	TM_Wallace: check and return "A","B","C","D","G","H","I","K","M","N","R","S","T","V","W" and "Y" TM_GC: check and return "A","B","C","D","G","H","I","K","M","N","R","S","T","V","W", "X" and "Y" TM_NN: check and return "A","C","G","I" and "T"

Value

Return a sequence which fullfils the requirements of the given method.

Author(s)

Junhui Li

References

```
citation("TmCalculator")
```

Examples

```
ntseq <- c("ATCGBDHKMNRVYWSqq")
check_filter(ntseq,method='Tm_Wallace')
check_filter(ntseq,method='Tm_NN')
```

chem_correction *Corrections of melting temperature with chemical substances*

Description

Corrections coefficient of melting temperature with DMSO and formamide and these corrections are rough approximations.

Usage

```
chem_correction(
  DMSO = 0,
  fmd = 0,
  DMSOfactor = 0.75,
  fmdmethod = c("concentration", "molar"),
  fmdfactor = 0.65,
  ptGC
)
```

Arguments

DMSO	Percent DMSO
fmd	Formamide concentration in percentage (fmdmethod="concentration") or molar (fmdmethod="molar").
DMSOfactor	Coefficient of Tm decreases per percent DMSO. Default=0.75 von Ahsen N (2001) <PMID:11673362>. Other published values are 0.5, 0.6 and 0.675.
fmdmethod	"concentration" method for formamide concentration in percentage and "molar" for formamide concentration in molar
fmdfactor	Coefficient of Tm decrease per percent formamide. Default=0.65. Several papers report factors between 0.6 and 0.72.
ptGC	Percentage of GC(%).

Details

fmdmethod = "concentration"

Correction = - factor*percentage_of_formamide

fmdmethod = "molar"

Correction = (0.453*GC/100 - 2.88) x formamide

Author(s)

Junhui Li

References

von Ahsen N, Wittwer CT, Schutz E , et al. Oligonucleotide melting temperatures under PCR conditions: deoxynucleotide Triphosphate and Dimethyl sulfoxide concentrations with comparison to alternative empirical formulas. Clin Chem 2001, 47:1956-C1961.

Examples

```
chem_correction(DMSO=3)
chem_correction(fmd=1.25, fmdmethod="molar", ptGC=50)
```

`complement` *complement and reverse complement base of nucleotide sequences*

Description

get reverse complement and complement base of nucleotide sequences

Usage

```
complement(ntseq, reverse = FALSE)
```

Arguments

`ntseq`	Sequence (5' to 3') of one strand of the nucleic acid duplex as string or vector of characters
`reverse`	Logical value, TRUE is reverse complement sequence, FALSE is not.

Author(s)

Junhui Li

References

```
citation("TmCalculator")
```

Examples

```
complement("ATCGYCGYsWwsaVv")
complement("ATCGYCGYsWwsaVv",reverse=TRUE)
```

GC *Calculate G and C content of nucleotide sequences*

Description

Calculate G and C content of nucleotide sequences. The number of G and C in sequence is divided by length of sequence(when totalnt is TRUE) or the number of all A,T,C,G and ambiguous base.

Usage

```
GC(ntseq, ambiguous = FALSE, totalnt = TRUE)
```

Arguments

ntseq	Sequence (5' to 3') of one strand of the nucleic acid duplex as string or vector of characters.
ambiguous	Ambiguous bases are taken into account to compute the G and C content when ambiguous is TRUE.
totalnt	Sum of 'G' and 'C' bases divided by the length of the sequence when totalnt is TRUE.

Value

Content of G and C(range from 0 to 100

Author(s)

Junhui Li

Examples

```
GC(c("a","t","c","t","g","g","g","c","c","a","g","t","a"))#53.84615
GC("GCATSWSYK",ambiguous = TRUE)#55.55556
```

print.TmCalculator *Prints melting temperature from a* TmCalculator *object*

Description

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

Usage

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

Arguments

x	An object of class TmCalculator.
...	Unused

Value

The melting temperature value.

---

s2c *convert a string into a vector of characters*

Description

Simply convert a single string such as "HelloWorld" into a vector of characters such as c("H","e","l","l","o","W","o","r","l","d

Usage

```
s2c(strings)
```

Arguments

strings	A single string such as "HelloWorld"

Value

Retrun a vector of characters

Author(s)

Junhui Li

References

```
citation("TmCalculator")
```

Examples

```
s2c(c("HelloWorld"))
```

`salt_correction` *Corrections of melting temperature with salt ions*

Description

Corrections coefficient of melting temperature or entropy with different operations

Usage

```
salt_correction(
  Na = 0,
  K = 0,
  Tris = 0,
  Mg = 0,
  dNTPs = 0,
 method = c("Schildkraut2010", "Wetmur1991", "SantaLucia1996", "SantaLucia1998-1",
    "SantaLucia1998-2", "Owczarzy2004", "Owczarzy2008"),
  ntseq,
  ambiguous = FALSE
)
```

Arguments

`Na`	Millimolar concentration of Na
`K`	Millimolar concentration of K
`Tris`	Millimolar concentration of Tris
`Mg`	Millimolar concentration of Mg
`dNTPs`	Millimolar concentration of dNTPs
`method`	Method to be applied including "Schildkraut2010", "Wetmur1991","SantaLucia1996", "SantaLucia1998-1", "SantaLucia1998-2","Owczarzy2004","Owczarzy2008". First fourth methods correct Tm, fifth method corrects deltaS, sixth and seventh methods correct 1/Tm. See details for the method description.
`ntseq`	Sequence (5' to 3') of one strand of the nucleic acid duplex as string or vector of characters.
`ambiguous`	Ambiguous bases are taken into account to compute the G and C content when ambiguous is TRUE.

Details

The methods are:

1 Schildkraut C (2010) <doi:10.1002/bip.360030207>

2 Wetmur J G (1991) <doi:10.3109/10409239109114069>

3 SantaLucia J (1996) <doi:10.1021/bi951907q>

4 SantaLucia J (1998) <doi:10.1073/pnas.95.4.1460>

5 SantaLucia J (1998) <doi:10.1073/pnas.95.4.1460>

6 Owczarzy R (2004) <doi:10.1021/bi034621r>

7 Owczarzy R (2008) <doi:10.1021/bi702363u>

methods 1-4: Tm(new) = Tm(old) + correction

method 5: deltaS(new) = deltaS(old) + correction

methods 6+7: Tm(new) = 1/(1/Tm(old) + correction)

Author(s)

Junhui Li

References

Schildkraut C . Dependence of the melting temperature of DNA on salt concentration[J]. Biopolymers, 2010, 3(2):195-208.

Wetmur J G . DNA Probes: Applications of the Principles of Nucleic Acid Hybridization[J]. CRC Critical Reviews in Biochemistry, 1991, 26(3-4):3

Santalucia , J , Allawi H T , Seneviratne P A . Improved Nearest-Neighbor Parameters for Predicting DNA Duplex Stability, [J]. Biochemistry, 1996, 35(11):3555-3562.

SantaLucia, J. A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics[J]. Proceedings of the National Academy of Sciences, 1998, 95(4):1460-1465.

Owczarzy R , You Y , Moreira B G , et al. Effects of Sodium Ions on DNA Duplex Oligomers: Improved Predictions ofMelting Temperatures[J]. Biochemistry, 2004, 43(12):3537-3554.

Owczarzy R , Moreira B G , You Y , et al. Predicting Stability of DNA Duplexes in Solutions Containing Magnesium and Monovalent Cations[J]. Biochemistry, 2008, 47(19):5336-5353.

Examples

```
ntseq <- c("acgtTGCAATGCCGTAWSDBSYXX")
salt_correction(Na=390, K=20, Tris=0, Mg=10, dNTPs=25, method="Owczarzy2008", ntseq)
```

`Tm_GC` *Calculate the melting temperature using empirical formulas based on GC content*

Description

Calculate the melting temperature using empirical formulas based on GC content with different options

Usage

```
Tm_GC(
  ntseq,
  ambiguous = FALSE,
  userset = NULL,
  variant = c("Primer3Plus", "Chester1993", "QuikChange", "Schildkraut1965",
   "Wetmur1991_MELTING", "Wetmur1991_RNA", "Wetmur1991_RNA/DNA", "vonAhsen2001"),
  Na = 0,
  K = 0,
  Tris = 0,
  Mg = 0,
  dNTPs = 0,
 saltcorr = c("Schildkraut2010", "Wetmur1991", "SantaLucia1996", "SantaLucia1998-1",
    "Owczarzy2004", "Owczarzy2008"),
  mismatch = TRUE,
  DMSO = 0,
  fmd = 0,
  DMSOfactor = 0.75,
  fmdfactor = 0.65,
  fmdmethod = c("concentration", "molar"),
  outlist = TRUE
)
```

Arguments

ntseq	Sequence (5' to 3') of one strand of the nucleic acid duplex as string or vector of characters.
ambiguous	Ambiguous bases are taken into account to compute the G and C content when ambiguous is TRUE.
userset	A vector of four coefficient values. Usersets override value sets.
variant	Empirical constants coefficient with 8 variant: Chester1993, QuikChange, Schildkraut1965, Wetmur1991_MELTING, Wetmur1991_RNA, Wetmur1991_RNA/DNA, Primer3Plus and vonAhsen2001
Na	Millimolar concentration of Na, default is 0
K	Millimolar concentration of K, default is 0
Tris	Millimolar concentration of Tris, default is 0
Mg	Millimolar concentration of Mg, default is 0
dNTPs	Millimolar concentration of dNTPs, default is 0
saltcorr	Salt correction method should be chosen when provide 'userset'. Options are "Schildkraut2010", "Wetmur1991","SantaLucia1996","SantaLucia1998-1","Owczarzy2004","Owczarzy Note that "SantaLucia1998-2" is not available for this function.
mismatch	If 'True' (default) every 'X' in the sequence is counted as mismatch
DMSO	Percent DMSO
fmd	Formamide concentration in percentage (fmdmethod="concentration") or molar (fmdmethod="molar").

DMSOfactor	Coeffecient of Tm decreases per percent DMSO. Default=0.75 von Ahsen N (2001) <PMID:11673362>. Other published values are 0.5, 0.6 and 0.675.
fmdfactor	Coeffecient of Tm decrease per percent formamide. Default=0.65. Several papers report factors between 0.6 and 0.72.
fmdmethod	"concentration" method for formamide concentration in percentage and "molar" for formamide concentration in molar
outlist	output a list of Tm and options or only Tm value, default is TRUE.

Details

Empirical constants coefficient with 8 variant:

Chester1993: Tm = 69.3 + 0.41(Percentage_GC) - 650/N

QuikChange: Tm = 81.5 + 0.41(Percentage_GC) - 675/N - Percentage_mismatch

Schildkraut1965: Tm = 81.5 + 0.41(Percentage_GC) - 675/N + 16.6 x log[Na+]

Wetmur1991_MELTING: Tm = 81.5 + 0.41(Percentage_GC) - 500/N + 16.6 x log([Na+]/(1.0 + 0.7 x [Na+])) - Percentage_mismatch

Wetmur1991_RNA: Tm = 78 + 0.7(Percentage_GC) - 500/N + 16.6 x log([Na+]/(1.0 + 0.7 x [Na+])) - Percentage_mismatch

Wetmur1991_RNA/DNA: Tm = 67 + 0.8(Percentage_GC) - 500/N + 16.6 x log([Na+]/(1.0 + 0.7 x [Na+])) - Percentage_mismatch

Primer3Plus: Tm = 81.5 + 0.41(Percentage_GC) - 600/N + 16.6 x log[Na+]

vonAhsen2001: Tm = 77.1 + 0.41(Percentage_GC) - 528/N + 11.7 x log[Na+]

Author(s)

Junhui Li

References

Marmur J , Doty P . Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature.[J]. Journal of Molecular Biology, 1962, 5(1):109-118.

Schildkraut C . Dependence of the melting temperature of DNA on salt concentration[J]. Biopolymers, 2010, 3(2):195-208.

Wetmur J G . DNA Probes: Applications of the Principles of Nucleic Acid Hybridization[J]. CRC Critical Reviews in Biochemistry, 1991, 26(3-4):33.

Untergasser A , Cutcutache I , Koressaar T , et al. Primer3–new capabilities and interfaces[J]. Nucleic Acids Research, 2012, 40(15):e115-e115.

von Ahsen N, Wittwer CT, Schutz E , et al. Oligonucleotide melting temperatures under PCR conditions: deoxynucleotide Triphosphate and Dimethyl sulfoxide concentrations with comparison to alternative empirical formulas. Clin Chem 2001, 47:1956-1961.

Examples

```
ntseq <- c("ATCGTGCGTAGCAGTACGATCAGTAG")
out <- Tm_GC(ntseq,ambiguous=TRUE,variant="Primer3Plus",Na=50,mismatch=TRUE)
out
out$Tm
out$Options
```

Tm_NN *Calculate melting temperature using nearest neighbor thermodynamics*

Description

Calculate melting temperature using nearest neighbor thermodynamics

Usage

```
Tm_NN(
  ntseq,
  ambiguous = FALSE,
  comSeq = NULL,
  shift = 0,
  nn_table = c("DNA_NN4", "DNA_NN1", "DNA_NN2", "DNA_NN3", "RNA_NN1", "RNA_NN2",
    "RNA_NN3", "R_DNA_NN1"),
  tmm_table = "DNA_TMM1",
  imm_table = "DNA_IMM1",
  de_table = c("DNA_DE1", "RNA_DE1"),
  dnac1 = 25,
  dnac2 = 25,
  selfcomp = FALSE,
  Na = 0,
  K = 0,
  Tris = 0,
  Mg = 0,
  dNTPs = 0,
  saltcorr = c("Schildkraut2010", "Wetmur1991", "SantaLucia1996", "SantaLucia1998-1",
    "SantaLucia1998-2", "Owczarzy2004", "Owczarzy2008"),
  DMSO = 0,
  fmd = 0,
  DMSOfactor = 0.75,
  fmdfactor = 0.65,
  fmdmethod = c("concentration", "molar"),
  outlist = TRUE
)
```

Arguments

ntseq	Sequence (5' to 3') of one strand of the nucleic acid duplex as string or vector of characters.
ambiguous	Ambiguous bases are taken into account to compute the G and C content when ambiguous is TRUE.Default is FALSE.
comSeq	Complementary sequence. The sequence of the template/target in 3'->5' direction
shift	Shift of the primer/probe sequence on the template/target sequence, default=0. for example: when shift=0, the first nucleotide base at 5' end of primer align to first one at 3' end of template. When shift=-1, the second nucleotide base at 5' end of primer align to first one at 3' end of template. When shift=1, the first nucleotide base at 5' end of primer align to second one at 3' end of template. The shift parameter is necessary to align primer/probe and template/target if they have different lengths or if they should have dangling ends.
nn_table	Thermodynamic NN values, eight tables are implemented. For DNA/DNA hybridizations: DNA_NN1,DNA_NN2,DNA_NN3,DNA_NN4 For RNA/RNA hybridizations: RNA_NN1,RNA_NN2,RNA_NN3 For RNA/DNA hybridizations: R_DNA_NN1
tmm_table	Thermodynamic values for terminal mismatches. Default: DNA_TMM1
imm_table	Thermodynamic values for internal mismatches, may include insosine mismatches. Default: DNA_IMM1
de_table	Thermodynamic values for dangling ends. DNA_DE1(default) and RNA_DE1
dnac1	Concentration of the higher concentrated strand [nM]. Typically this will be the primer (for PCR) or the probe. Default=25.
dnac2	Concentration of the lower concentrated strand [nM].
selfcomp	Sequence self-complementary, default=False. If 'True' the primer is thought binding to itself, thus dnac2 is not considered.
Na	Millimolar concentration of Na, default is 0
K	Millimolar concentration of K, default is 0
Tris	Millimolar concentration of Tris, default is 0
Mg	Millimolar concentration of Mg, default is 0
dNTPs	Millimolar concentration of dNTPs, default is 0
saltcorr	Salt correction method should be chosen when provide 'userset' Options are "Schildkraut2010", "Wetmur1991","SantaLucia1996","SantaLucia1998-1", "SantaLucia1998-2","Owczarzy2004","Owczarzy2008". Note that NA means no salt correction.
DMSO	Percent DMSO
fmd	Formamide concentration in percentage (fmdmethod="concentration") or molar (fmdmethod="molar").
DMSOfactor	Coeffecient of Tm decreases per percent DMSO. Default=0.75 von Ahsen N (2001) <PMID:11673362>. Other published values are 0.5, 0.6 and 0.675.

fmdfactor	Coeffecient of Tm decrease per percent formamide. Default=0.65. Several papers report factors between 0.6 and 0.72.
fmdmethod	"concentration" method for formamide concentration in percentage and "molar" for formamide concentration in molar.
outlist	output a list of Tm and options or only Tm value, default is TRUE.

Details

DNA_NN1: Breslauer K J (1986) <doi:10.1073/pnas.83.11.3746>

DNA_NN2: Sugimoto N (1996) <doi:10.1093/nar/24.22.4501>

DNA_NN3: Allawi H (1998) <doi:10.1093/nar/26.11.2694>

DNA_NN4: SantaLucia J (2004) <doi:10.1146/annurev.biophys.32.110601.141800>

RNA_NN1: Freier S (1986) <doi:10.1073/pnas.83.24.9373>

RNA_NN2: Xia T (1998) <doi:10.1021/bi9809425>

RNA_NN3: Chen JL (2012) <doi:10.1021/bi3002709>

R_DNA_NN1: Sugimoto N (1995)<doi:10.1016/S0048-9697(98)00088-6>

DNA_TMM1: Bommarito S (2000) <doi:10.1093/nar/28.9.1929>

DNA_IMM1: Peyret N (1999) <doi:10.1021/bi9825091> & Allawi H T (1997) <doi:10.1021/bi962590c> & Santalucia N (2005) <doi:10.1093/nar/gki918>

DNA_DE1: Bommarito S (2000) <doi:10.1093/nar/28.9.1929>

RNA_DE1: Turner D H (2010) <doi:10.1093/nar/gkp892>

Author(s)

Junhui Li

References

Breslauer K J , Frank R , Blocker H , et al. Predicting DNA duplex stability from the base sequence.[J]. Proceedings of the National Academy of Sciences, 1986, 83(11):3746-3750.

Sugimoto N , Nakano S , Yoneyama M , et al. Improved Thermodynamic Parameters and Helix Initiation Factor to Predict Stability of DNA Duplexes[J]. Nucleic Acids Research, 1996, 24(22):4501-5.

Allawi, H. Thermodynamics of internal C.T mismatches in DNA[J]. Nucleic Acids Research, 1998, 26(11):2694-2701.

Hicks L D , Santalucia J . The thermodynamics of DNA structural motifs.[J]. Annual Review of Biophysics & Biomolecular Structure, 2004, 33(1):415-440.

Freier S M , Kierzek R , Jaeger J A , et al. Improved free-energy parameters for predictions of RNA duplex stability.[J]. Proceedings of the National Academy of Sciences, 1986, 83(24):9373-9377.

Xia T , Santalucia , J , Burkard M E , et al. Thermodynamic Parameters for an Expanded Nearest-Neighbor Model for Formation of RNA Duplexes with Watson-Crick Base Pairs,[J]. Biochemistry, 1998, 37(42):14719-14735.

Chen J L , Dishler A L , Kennedy S D , et al. Testing the Nearest Neighbor Model for Canonical RNA Base Pairs: Revision of GU Parameters[J]. Biochemistry, 2012, 51(16):3508-3522.

Bommarito S, Peyret N, Jr S L. Thermodynamic parameters for DNA sequences with dangling ends[J]. Nucleic Acids Research, 2000, 28(9):1929-1934.

Turner D H , Mathews D H . NNDB: the nearest neighbor parameter database for predicting stability of nucleic acid secondary structure[J]. Nucleic Acids Research, 2010, 38(Database issue):D280-D282.

Sugimoto N , Nakano S I , Katoh M , et al. Thermodynamic Parameters To Predict Stability of RNA/DNA Hybrid Duplexes[J]. Biochemistry, 1995, 34(35):11211-11216.

Allawi H, SantaLucia J: Thermodynamics and NMR of internal G-T mismatches in DNA. Biochemistry 1997, 36:10581-10594.

Santalucia N E W J . Nearest-neighbor thermodynamics of deoxyinosine pairs in DNA duplexes[J]. Nucleic Acids Research, 2005, 33(19):6258-67.

Peyret N , Seneviratne P A , Allawi H T , et al. Nearest-Neighbor Thermodynamics and NMR of DNA Sequences with Internal A-A, C-C, G-G, and T-T Mismatches, [J]. Biochemistry, 1999, 38(12):3468-3477.

Examples

```
ntseq <- c("AAAATTTTTTTCCCCCCCCCCCCCCGGGGGGGGGGGGGTGTGCGCTGC")
out <- Tm_NN(ntseq,Na=50)
out
out$Options
```

`Tm_Wallace` *Calculate the melting temperature using the 'Wallace rule'*

Description

The Wallace rule is often used as rule of thumb for approximate melting temperature calculations for primers with 14 to 20 nt length.

Usage

```
Tm_Wallace(ntseq, ambiguous = FALSE, outlist = TRUE)
```

Arguments

`ntseq`	Sequence (5' to 3') of one strand of the DNA nucleic acid duplex as string or vector of characters (**Note:** Non-DNA characters are ignored by this method).
`ambiguous`	Ambiguous bases are taken into account to compute the G and C content when ambiguous is TRUE.
`outlist`	output a list of Tm and options or only Tm value, default is TRUE.

Author(s)

Junhui Li

References

Thein S L , Lynch J R , Weatherall D J , et al. DIRECT DETECTION OF HAEMOGLOBIN E WITH SYNTHETIC OLIGONUCLEOTIDES[J]. The Lancet, 1986, 327(8472):93.

Examples

```
ntseq = c('acgtTGCAATGCCGTAWSDBSY') #for wallace rule

out <- Tm_Wallace(ntseq,ambiguous = TRUE)
out
out$Options
```

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