

# Package ‘ProbeDeveloper’

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**Type** Package

**Title** Develop Hybridization Probes

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**Description** Hybridization probes for target sequences can be made based on melting temperature value calculated by R package "TmCalculator" <<https://CRAN.R-project.org/package=TmCalculator>> and methods extended from Beliveau, B. J.,(2018) <[doi:10.1073/pnas.1714530115](https://doi.org/10.1073/pnas.1714530115)>, and those hybridization probes can be used to capture specific target regions in fluorescence in situ hybridization and next generation sequence experiments.

**License** GPL (>= 2)

**Imports** TmCalculator (>= 1.0.2),Biostrings(>= 2.12.0)

**Depends** R (>= 2.10)

**RoxygenNote** 7.1.2

**NeedsCompilation** no

**Repository** CRAN

**Date/Publication** 2022-01-31 00:10:02 UTC

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 ProbeMake

 Make probes
 

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### Description

Probes are made with a FASTA-formatted input file containing the target sequence. User can specify the allowable ranges of probe length, percent GC content, and adjust melting temperature calculated using nearest neighbor thermodynamics or empirical formulas based on GC content. Candidate probe sequences passing all checks output in BED format.

### Usage

```

ProbeMake(
  fafile,
  LN = 90,
  ln = 60,
  TM = 80,
  tm = 60,
  CG = 70,
  cg = 30,
  gap = 0,
  method = c("S2L", "L2S"),
  direction = c("3to5", "5to3"),
  prohibitseq = NULL,
  TmMethod = c("Tm_GC", "Tm_NN"),
  variant = c("Primer3Plus", "Chester1993", "QuikChange", "Schildkraut1965",
    "Wetmur1991_MELTING", "Wetmur1991_RNA", "Wetmur1991_RNA/DNA", "vonAhsen2001"),
  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,
  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")
)

```

**Arguments**

fafile	Input file with a FASTA format read by function readDNAStrngSet in R package 'Biostrings'
LN	The maximum allowed probe length, default is 90
ln	The minimum allowed probe length, default is 60
TM	The maximum allowed melting temperature, default is 80
tm	The minimum allowed melting temperature, default is 60
CG	The maximum allowed percent GC content, default is 70
cg	The minimum allowed percent GC content, default is 30
gap	The minimum gap between adjacent probes, default is 0
method	'S2L' is used to design probe extending from minimal length to the maximum until passing all checks, conversely 'L2S' make probe from maximal length to the minimum. Default is 'S2L'
direction	Design probes from 3 to 5 end or from 5 to 3 end of target sequence, default is '3to5'
prohibitseq	Prohibited sequence list, e.g prohibitseq=c("GGGGG","CCCCC"), default is NULL
TmMethod	The method used to calculate Tm, 'Tm_NN' and 'Tm_GC' can be selected
variant	Empirical constants coefficient with 8 variant for 'Tm_GC' method: Chester1993, QuikChange, Schildkraut1965, Wetmur1991_MELTING, Wetmur1991_RNA, Wetmur1991_RNA/DNA, Primer3Plus and vonAhsen2001
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 inosine mismatches. Default: DNA_IMM1
de_table	Thermodynamic values for dangling ends: DNA_DE1(default),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]. Default: 25
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. Options are "Schildkraut2010", "Wetmur1991", "SantaLucia1996", "SantaLucia1998-1", "Owczarzy2004", "Owczarzy2008". Note that "SantaLucia1998-2" is not available for this function.

DMSO	Percent of 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.
fmdfactor	Coefficient 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

### Value

Returns a bed file in the format TargetID <tab> Chr <tab> Start <tab> End <tab> Sequence <tab> Tm <tab> GC

### Author(s)

Junhui Li

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## Examples

```
data(samplefa)
ProbeMake(samplefa, LN=90, ln=60, TM=80, tm=70, CG=80, cg=20, TmMethod="Tm_NN", Na=50)
```

---

```
samplefa          sample data for target sequence region with class 'DNAStrngSet'
```

---

## Description

sample data read by function readDNAStrngSet in R package 'Biostrings' from fasta format file, there are two target sequence resion in this data

## Usage

```
data("samplefa")
```

## Format

Formal class 'DNAStrngSet' [package "Biostrings"] with 5 slots

sample data read by function readDNAStrngSet in R package 'Biostrings' from fasta format file, which is from ncbiRefSeq database for Homo Sapiens with referece genome version hg19

**Examples**

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data(samplefa)
```

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