

# 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

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ProbeMake

*Make probes***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.<br>For DNA/DNA hybridizations: DNA_NN1,DNA_NN2,DNA_NN3,DNA_NN4<br>For RNA/RNA hybridizations: RNA_NN1,RNA_NN2,RNA_NN3<br>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),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 | 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                             |

### Value

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

### Author(s)

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### References

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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**

```
data(samplefa)
```

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