

# Package ‘cfTools’

February 27, 2024

**Type** Package

**Title** Informatics Tools for Cell-Free DNA Study

**Version** 1.2.0

**Description** The cfTools R package provides methods for cell-free DNA (cfDNA) methylation data analysis to facilitate cfDNA-based studies. Given the methylation sequencing data of a cfDNA sample, for each cancer marker or tissue marker, we deconvolve the tumor-derived or tissue-specific reads from all reads falling in the marker region. Our read-based deconvolution algorithm exploits the pervasiveness of DNA methylation for signal enhancement, therefore can sensitively identify a trace amount of tumor-specific or tissue-specific cfDNA in plasma. cfTools provides functions for (1) cancer detection: sensitively detect tumor-derived cfDNA and estimate the tumor-derived cfDNA fraction (tumor burden); (2) tissue deconvolution: infer the tissue type composition and the cfDNA fraction of multiple tissue types for a plasma cfDNA sample. These functions can serve as foundations for more advanced cfDNA-based studies, including cancer diagnosis and disease monitoring.

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**Encoding** UTF-8

**Suggests** BiocStyle, knitr, rmarkdown, testthat (>= 3.0.0)

**Config/testthat/edition** 3

**RoxygenNote** 7.2.3

**Imports** Rcpp, utils, GenomicRanges, basilisk, R.utils, stats,  
cfToolsData

**StagedInstall** no

**biocViews** Software, BiomedicalInformatics, Epigenetics, Sequencing,  
MethylSeq, DNAMethylation, DifferentialMethylation

**VignetteBuilder** knitr

**LinkingTo** Rcpp, BH

**URL** <https://github.com/jasminezhoulab/cfTools>

**BugReports** <https://github.com/jasminezhoulab/cfTools/issues>

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

**git\_branch** RELEASE\_3\_18

**git\_last\_commit** c33a0cc

**git\_last\_commit\_date** 2023-10-24

**Repository** Bioconductor 3.18

**Date/Publication** 2024-02-27

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beta_matrix	<i>Beta value matrix</i>
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**Description**

A list of methylation levels (e.g., beta values), where each row is a sample and each column is a marker

**Usage**

```
data("beta_matrix")
```

**Format**

A tibble with 20 rows and 3 variables

**marker1** Beta values of marker1 for all samples

**marker2** Beta values of marker2 for all samples

**marker3** Beta values of marker3 for all samples

**Value**

A tibble with 20 rows and 3 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

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CancerDetector	<i>Cancer Detector</i>
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**Description**

Detect tumor-derived cfDNA and estimate the tumor burden.

**Usage**

```
CancerDetector(  
  readsBinningFile,  
  tissueMarkersFile,  
  lambda = 0.5,  
  id = "sample"  
)
```

**Arguments**

`readsBinningFile` a file of the fragment-level methylation states of reads that mapped to the markers.

`tissueMarkersFile` a file of paired shape parameters of beta distributions for markers.

`lambda` a number controlling "confounding" markers' distance from average markers.

`id` the sample ID.

**Value**

a list containing the cfDNA tumor burden and the normal cfDNA fraction.

**Examples**

```
## input files
demo.dir <- system.file("data", package="cfTools")
readsBinningFile <- file.path(demo.dir, "CancerDetector.reads.txt.gz")
tissueMarkersFile <- file.path(demo.dir, "CancerDetector.markers.txt.gz")
lambda <- 0.5
id <- "test"

CancerDetector(readsBinningFile, tissueMarkersFile, lambda, id)
```

---

CancerDetector.markers

*Cancer-specific marker parameter*

---

**Description**

The paired shape parameters of beta distributions for cancer-specific markers

**Usage**

```
data("CancerDetector.markers")
```

**Format**

A tibble with 1266 rows and 3 variables

**markerName** Name of the marker

**tumor** Paired beta distribution shape parameters for tumor samples

**normalPlasma** Paired beta distribution shape parameters for normal plasma samples

**Value**

A tibble with 1266 rows and 3 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

---

CancerDetector.reads    *Fragment-level methylation state for cancer detection*

---

**Description**

The fragment-level methylation states of reads that mapped to the cancer-specific markers

**Usage**

```
data("CancerDetector.reads")
```

**Format**

A tibble with 9991 rows and 2 variables

**markerName** Name of the marker

**methState** Fragment-level methylation states, which are represented by a sequence of binary values (0 represents unmethylated CpG and 1 represents methylated CpG on the same fragment)

**Value**

A tibble with 9991 rows and 2 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

---

cfDeconvolve                    *cfDNA methylation read deconvolution*

---

**Description**

Infer the tissue-type composition of plasma cfDNA.

**Usage**

```
cfDeconvolve(  
  readsBinningFile,  
  tissueMarkersFile,  
  numTissues,  
  emAlgorithmType = "em.global.unknown",  
  likelihoodRatioThreshold = 2,  
  emMaxIterations = 100,  
  id = "sample"  
)
```

**Arguments**

readsBinningFile	a file of the fragment-level methylation states of reads that mapped to the markers. Either in plain text or compressed form.
tissueMarkersFile	a file of paired shape parameters of beta distributions for markers.
numTissues	a number of tissue types.
emAlgorithmType	a read-based tissue deconvolution EM algorithm type: em.global.unknown (default), em.global.known, em.local.unknown, em.local.known.
likelihoodRatioThreshold	a positive float number. Default is 2.
emMaxIterations	a number of EM algorithm maximum iteration. Default is 100.
id	the sample ID.

**Value**

a list containing the cfDNA fractions of different tissue types and an unknown class.

**Examples**

```
## input files
demo.dir <- system.file("data", package="cfTools")
readsBinningFile <- file.path(demo.dir, "cfDeconvolve.reads.txt.gz")
tissueMarkersFile <- file.path(demo.dir, "cfDeconvolve.markers.txt.gz")
numTissues <- 7
emAlgorithmType <- "em.global.unknown"
likelihoodRatioThreshold <- 2
emMaxIterations <- 100
id <- "test"

cfDeconvolve(readsBinningFile, tissueMarkersFile, numTissues,
emAlgorithmType, likelihoodRatioThreshold, emMaxIterations, id)
```

---

cfDeconvolve.markers *Tissue-specific marker parameter*

---

**Description**

The paired shape parameters of beta distributions for tissue-specific markers

**Usage**

```
data("cfDeconvolve.markers")
```

**Format**

A tibble with 10 rows and 8 variables

**markerName** Name of the marker

**tissue1** Paired beta distribution shape parameters for tissue1 samples

**tissue2** Paired beta distribution shape parameters for tissue2 samples

**tissue3** Paired beta distribution shape parameters for tissue3 samples

**tissue4** Paired beta distribution shape parameters for tissue4 samples

**tissue5** Paired beta distribution shape parameters for tissue5 samples

**tissue6** Paired beta distribution shape parameters for tissue6 samples

**tissue7** Paired beta distribution shape parameters for tissue7 samples

**Value**

A tibble with 10 rows and 8 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

---

cfDeconvolve.reads      *Fragment-level methylation state for tissue deconvolution*

---

**Description**

The fragment-level methylation states of reads that mapped to the tissue-specific markers

**Usage**

```
data("cfDeconvolve.reads")
```

**Format**

A tibble with 942 rows and 2 variables

**markerName** Name of the marker

**methState** Fragment-level methylation states, which are represented by a sequence of binary values (0 represents unmethylated CpG and 1 represents methylated CpG on the same fragment)

**Value**

A tibble with 942 rows and 2 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

cfSort

*cfSort: tissue deconvolution*

---

**Description**

Tissue deconvolution in cfDNA using DNN models.

**Usage**

```
cfSort(readsBinningFile, id = "sample")
```

**Arguments**

`readsBinningFile`  
a file of the fragment-level methylation states of reads that mapped to the cfSort markers. In compressed form.

`id`  
the sample ID.

**Value**

the tissue composition of the cfDNA sample.

**Examples**

```
## input files
demo.dir <- system.file("data", package="cfTools")
readsBinningFile <- file.path(demo.dir, "cfSort.reads.txt.gz")
id <- "test"

cfSort(readsBinningFile, id)
```

---

cfSort.reads*Fragment-level methylation state for cfSort tissue deconvolution*

---

**Description**

The fragment-level methylation states of reads that mapped to the cfSort markers

**Usage**

```
data("cfSort.reads")
```



**Format**

A tibble with 99999 rows and 6 variables

**markerName** Name of the cfSort marker

**cpgPosition** Postions of CpG sites on the fragment

**methState** Fragment-level methylation states, which are represented by a sequence of binary values (0 represents unmethylated CpG and 1 represents methylated CpG on the same fragment)

**methCount** Number of methylated CpG sites on the fragment

**unmethCount** Number of unmethylated CpG sites on the fragment

**strand** Strand

**Value**

A tibble with 99999 rows and 6 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

---

cfsort\_markers

*cfSort markers*

---

**Description**

Marker information for the cfSort function, where each row is the information about a marker

**Usage**

```
data("cfsort_markers")
```

**Format**

A tibble with 51035 rows and 4 variables

**marker\_index** The marker index used in cfSort method

**alpha\_threshold** The alpha threshold for each marker

**pair** The pair of tissues used for identifying the marker

**group** The group number for each marker

**Value**

A tibble with 51035 rows and 4 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

---

`cfTools`*cfTools: a versatile package for analyzing cell-free DNA data*

---

**Description**

Given the methylation sequencing data of a cell-free DNA (cfDNA) sample, for each cancer marker or tissue marker, we deconvolve the tumor-derived or tissue-specific reads from all reads falling in the marker region. Our read-based deconvolution algorithm exploits the pervasiveness of DNA methylation for signal enhancement, therefore can sensitively identify a trace amount of tumor-specific or tissue-specific cfDNA in plasma.

**Details**

Specifically, cfTools can deconvolve different sources of cfDNA fragments (or reads) in two contexts:

1. Cancer detection: separate cfDNA fragments into tumor-derived fragments and background normal fragments (2 classes), and estimate the tumor-derived cfDNA fraction.
2. Tissue deconvolution: separate cfDNA fragments from different tissues (> 2 classes), and estimate the cfDNA fraction of different tissue types (including an unknown type) for a plasma cfDNA sample.

These functions can serve as foundations for more advanced cfDNA-based studies, including cancer diagnosis and disease monitoring.

For an overview of the functionality provided by the package, please see the vignette: `vignette(package="cfTools")`

**Author(s)**

Ran Hu <huran@ucla.edu>, Mary Louisa Stackpole, Shuo Li, Xianghong Jasmine Zhou <XJZhou@mednet.ucla.edu>, Wenyan Li <WenyanLi@mednet.ucla.edu>

**See Also**

[CancerDetector](#), [cfDeconvolve](#), [cfSort](#), [MergeCpGs](#), [MergePEReads](#), [GenerateFragMeth](#), [GenerateMarkerParam](#)

---

`CpG_OB_demo`*Methylation information for CpG on the original bottom strand (OB)*

---

**Description**

Methylation information for CpG on the original bottom strand (OB), which is one of the outputs from 'bismark methylation extractor'

**Usage**

```
data("CpG_OB_demo")
```

**Format**

A tibble with 2224 rows and 5 variables

**sequence ID** ID of the sequence

**methylation state** Methylated or unmethylated CpG site

**chromosome name** Chromosome name

**chromosome start** Chromosome start position

**methylation call** Methylation call

**Value**

A tibble with 2224 rows and 5 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

---

CpG\_OT\_demo

*Methylation information for CpG on the original top strand (OT)*

---

**Description**

Methylation information for CpG on the original top strand (OT), which is one of the outputs from 'bismark methylation extractor'

**Usage**

```
data("CpG_OT_demo")
```

**Format**

A tibble with 2556 rows and 5 variables

**sequence ID** ID of the sequence

**methylation state** Methylated or unmethylated CpG site

**chromosome name** Chromosome name

**chromosome start** Chromosome start position

**methylation call** Methylation call

**Value**

A tibble with 2556 rows and 5 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

demo.fragment\_level.meth.bed

*Fragment-level methylation information*

---

### **Description**

A BED file of fragment-level methylation information

### **Usage**

```
data("demo.fragment_level.meth.bed")
```

### **Format**

A tibble with 552 rows and 9 variables

**chr** Chromosome

**start** Chromosome start

**end** Chromosome end

**name** ID of the sequence

**fragmentLength** Fragment length

**strand** Strand

**cpgNumber** Number of CpG sites on the fragment

**cpgPosition** Postions of CpG sites on the fragment

**methState** A string of methylation states of CpG sites on the fragment

### **Value**

A tibble with 552 rows and 9 variables

### **Author(s)**

Ran Hu <huran@ucla.edu>

---

demo.refo\_frag.bed     *Fragment-level information*

---

**Description**

A BED file of fragment-level information

**Usage**

```
data("demo.refo_frag.bed")
```

**Format**

A tibble with 559 rows and 6 variables

**chr** Chromosome

**start** Chromosome start

**end** Chromosome end

**fragmentLength** Fragment length

**strand** Strand

**name** ID of the sequence

**Value**

A tibble with 559 rows and 6 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

---

demo.refo\_meth.bed     *Methylation information on fragments*

---

**Description**

A BED file of methylation information on fragments

**Usage**

```
data("demo.refo_meth.bed")
```

**Format**

A tibble with 552 rows and 8 variables

**chr** Chromosome

**cpgStart** Start position of first CpG on the fragment

**cpgEnd** End position of first CpG on the fragment

**strand** Strand

**cpgNumber** Number of CpG sites on the fragment

**cpgPosition** Positions of CpG sites on the fragment

**methState** A string of methylation states of CpG sites on the fragment

**name** ID of the sequence

**Value**

A tibble with 552 rows and 8 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

---

demo.sorted.bed

*Paired-end sequencing reads*

---

**Description**

Paired-end sequencing reads information

**Usage**

```
data("demo.sorted.bed")
```

**Format**

A tibble with 1117 rows and 6 variables

**chr** Chromosome name

**start** Chromosome start

**end** Chromosome end

**name** Sequence ID

**score** Mapping quality score

**strand** Strand

**Value**

A tibble with 1117 rows and 6 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

---

GenerateFragMeth

*Generate fragment-level information about methylation states*

---

**Description**

Join two lists containing the fragment information and the methylation states on each fragment into one list.

**Usage**

```
GenerateFragMeth(frag_bed, meth_bed, output.dir = "", id = "")
```

**Arguments**

frag_bed	a BED file containing information for every fragment, which is the output of MergePEReads().
meth_bed	a BED file containing methylation states on every fragment, which is the output of MergeCpGs().
output.dir	a path to the output directory. Default is "", which means the output will not be written into a file.
id	an ID name for the input data. Default is "", which means the output will not be written into a file.

**Value**

a list in BED file format and/or written to an output BED file.

**Examples**

```
## input files
demo.dir <- system.file("data", package="cfTools")
frag_bed <- read.delim(file.path(demo.dir, "demo.refo_frag.bed.txt.gz"),
  colClasses = "character")
meth_bed <- read.delim(file.path(demo.dir, "demo.refo_meth.bed.txt.gz"),
  colClasses = "character")

output <- GenerateFragMeth(frag_bed, meth_bed)
```

---

GenerateMarkerParam    *Generate the methylation pattern of markers*

---

### Description

Output paired shape parameters of beta distributions for methylation markers.

### Usage

```
GenerateMarkerParam(x, sample.types, marker.names, output.file = "")
```

### Arguments

x	a list of methylation levels (e.g., beta values), where each row is a sample and each column is a marker.
sample.types	a vector of sample types (e.g., tumor or normal, tissue types) corresponding to the rows of the list.
marker.names	a vector of marker names corresponding to the columns of the list.
output.file	a character string naming the output file. Default is "", which means the output will not be written into a file.

### Value

a list containing the paired shape parameters of beta distributions for markers and/or written to an output file.

### Examples

```
## input files
demo.dir <- system.file("data", package="cfTools")
methLevel <- read.table(file.path(demo.dir, "beta_matrix.txt.gz"),
  row.names=1, header = TRUE)
sampleTypes <- read.table(file.path(demo.dir, "sample_type.txt.gz"),
  row.names=1, header = TRUE)$sampleType
markerNames <- read.table(file.path(demo.dir, "marker_index.txt.gz"),
  row.names=1, header = TRUE)$markerIndex

output <- GenerateMarkerParam(methLevel, sampleTypes, markerNames)
```



---

markers.bed	<i>Genomic positions of markers</i>
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---

**Description**

A BED file of genomic regions of markers

**Usage**

```
data("markers.bed")
```

**Format**

A tibble with 3 rows and 4 variables

**chr** Chromosome  
**start** Chromosome start  
**end** Chromosome end  
**markerName** Marker name

**Value**

A tibble with 3 rows and 4 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

---

marker_index	<i>Marker name</i>
--------------	--------------------

---

**Description**

A vector of marker names corresponding to the columns of the list of methylation levels.

**Usage**

```
data("marker_index")
```

**Format**

A tibble with 3 rows and 1 variables

**markerIndex** Marker name

**Value**

A tibble with 3 rows and 1 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

---

MergeCpGs

*Generate fragment-level methylation states of CpGs*

---

**Description**

Merge the methylation states of all CpGs corresponding to the same fragment onto one line in output.

**Usage**

```
MergeCpGs(CpG_OT, CpG_OB, output.dir = "", id = "")
```

**Arguments**

CpG_OT	a file of methylation information for CpG on the original top strand (OT), which is one of the outputs from ‘bismark methylation extractor’.
CpG_OB	a file of methylation information for CpG on the original bottom strand (OB), which is one of the outputs from ‘bismark methylation extractor’.
output.dir	a path to the output directory. Default is "", which means the output will not be written into a file.
id	an ID name for the input data. Default is "", which means the output will not be written into a file.

**Value**

a list in BED file format and/or written to an output BED file.

**Examples**

```
## input files
demo.dir <- system.file("data", package="cfTools")
CpG_OT <- file.path(demo.dir, "CpG_OT_demo.txt.gz")
CpG_OB <- file.path(demo.dir, "CpG_OB_demo.txt.gz")

output <- MergeCpGs(CpG_OT, CpG_OB)
```

---

MergePereads	<i>Generate fragment-level information for paired-end sequencing reads</i>
--------------	--

---

**Description**

Merge BED file (the output of ‘bedtools bamtobed’) to fragment-level for paired-end sequencing reads.

**Usage**

```
MergePereads.bed_file, output.dir = "", id = ""
```

**Arguments**

bed_file	a (sorted) BED file of paired-end reads.
output.dir	a path to the output directory. Default is "", which means the output will not be written into a file.
id	an ID name for the input data. Default is "", which means the output will not be written into a file.

**Value**

a list in BED file format and/or written to an output BED file.

**Examples**

```
## input files
demo.dir <- system.file("data", package="cfTools")
Pereads <- file.path(demo.dir, "demo.sorted.bed.txt.gz")

output <- MergePereads(Pereads)
```

---

sample_type	<i>Sample type</i>
-------------	--------------------

---

**Description**

A vector of sample types (e.g., tumor or normal, tissue types) corresponding to the rows of the list of methylation levels.

**Usage**

```
data("sample_type")
```

**Format**

A tibble with 20 rows and 1 variables

**sampleType** Sample type

**Value**

A tibble with 20 rows and 1 variables

**Author(s)**

Ran Hu <huran@ucla.edu>

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