

# Package ‘cfdnakit’

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**Title** Fragmen-length analysis package from high-throughput sequencing of cell-free DNA (cfDNA)

**Version** 1.1.2

**Description** This package provides basic functions for analyzing shallow whole-genome sequencing (~0.3X or more) of cell-free DNA (cfDNA). The package basically extracts the length of cfDNA fragments and aids the visualization of fragment-length information. The package also extract fragment-length information per non-overlapping fixed-sized bins and used it for calculating ctDNA estimation score (CES).

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cfdnakit-package	<i>Fragmen-length analysis package from high-throughput sequencing of cell-free DNA (cfDNA)</i>
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## Description

This package provides basic functions for analyzing shallow whole-genome sequencing (~0.3X or more) of cell-free DNA (cfDNA). The package basically extracts the length of cfDNA fragments and aids the visualization of fragment-length information. The package also extract fragment-length information per non-overlapping fixed-sized bins and used it for calculating ctDNA estimation score (CES).

## Details

This package provides functions for analyzing using shallow whole-genome sequencing data (~0.3X or more) of circulating cell-free DNA (cfDNA). The aim is to estimate circulating tumor DNA using its characteristic short-fragmented cfDNA. The package extracts length of each cfDNA and assists the visualization of fragment-length distribution. A short-fragment ratio is calculated per non-overlapping fixed-sized bins. Genome-wide copy-number alteration is estimated by the short-fragmented cfDNA. The ctDNA estimation score (CES) comprehensively estimates the circulating tumor DNA based on the short-fragment analysis.

## Author(s)

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## Examples

```
library(cfdnakit)
## Reading in a bamfile
sample_bamfile = system.file("extdata",
                             "ex.plasma.bam",
                             package = "cfdnakit")
plasma_SampleBam = read_bamfile(sample_bamfile,
                               apply_blacklist = FALSE)

## Plot a fragment-length distribution of a sample
plot_fragment_dist(list("Plasma.Sample"=plasma_SampleBam))

## Plot a fragment-length distribution of two samples
control_RDS_file =
  system.file("extdata", "BH01_CHR15.SampleBam.rds",
             package = "cfdnakit")
### Load example SampleBam of Healthy cfDNA
control_bins =
  readRDS(control_RDS_file)

comparing_list = list("Healthy.cfDNA"=control_bins,
                     "Patient.1"=plasma_SampleBam)
plot_fragment_dist(comparing_list)

## Derived and plot genome-wide short-fragment cfDNA
patient.SampleFragment =
  get_fragment_profile(plasma_SampleBam,
                     sample_id = "Patient.1")
plot_sl_ratio(patient.SampleFragment)

## Derived and plot normalized short-fragment cfDNA
PoN_rdsfile = system.file(
  "extdata",
  "ex.PoN.rds",
  package = "cfdnakit")
```

```

## Loading example PoN data
PoN.profiles = readRDS(PoN_rdsfile)

sample_zscore =
  get_zscore_profile(patient.SampleFragment,
                    PoN.profiles)
sample_zscore_segment = segmentByPSCB(sample_zscore)
plot_transformed_sl(sample_zscore, sample_zscore_segment)

## Estimate circulating tumor DNA
calculate_CES_score(sample_zscore_segment)

```

---

calculate\_CES\_score    *Calculate CES Score from Segmentation*

---

### Description

Calculate CES Score from Segmentation

### Usage

```
calculate_CES_score(sample_segmentation)
```

### Arguments

```
sample_segmentation
  Segmentation Dataframe
```

### Value

Numeric; CES score

### Examples

```

### Loading example SampleBam file
example_file <- system.file("extdata", "example_patientcfDNA_SampleBam.RDS", package = "cfdnakit")
sample_bambin <- readRDS(example_file)
### Example PoN
PoN_rdsfile <- system.file("extdata", "ex.PoN.rds", package = "cfdnakit")
pon_profiles <- readRDS(PoN_rdsfile)
sample_profile <- get_fragment_profile(sample_bambin, sample_id = "Patient1")

sample_zscore <- get_zscore_profile(sample_profile, pon_profiles)

sample_zscore_segment <- segmentByPSCB(sample_zscore)

calculate_CES_score(sample_zscore_segment)

```

---

call_cnv	<i>Call Copy-number Variation from SLRatio and segmentation</i>
----------	---

---

## Description

Call Copy-number Variation from SLRatio and segmentation

## Usage

```
call_cnv(
  sample_segmentation,
  sample_zscore,
  callChr = seq_len(22),
  tfs = c(0, 0.7),
  ploidies = c(1.5, 3),
  MaxCN = 4
)
```

## Arguments

sample_segmentation	segmentation dataframe from segmentByPSCBS
sample_zscore	zscore dataframe
callChr	chromosome to analysis : Default c(1:22)
tfs	range of fitting tumor fraction : Default c(0,0.8)
ploidies	range of fitting chromosomal ploidy : Default c(1.5,4)
MaxCN	maximum copy-number : Default 4

## Value

List of cnvcalling solutions

## Examples

```
### Loading example SampleBam file
example_file <- system.file("extdata", "example_patientcfDNA_SampleBam.RDS", package = "cfdnakit")
sample_bambin <- readRDS(example_file)
### Example PoN
PoN_rdsfile <- system.file("extdata", "ex.PoN.rds", package = "cfdnakit")
pon_profiles <- readRDS(PoN_rdsfile)
sample_profile <- get_fragment_profile(sample_bambin, sample_id = "Patient1")

sample_zscore <- get_zscore_profile(sample_profile, pon_profiles)

sample_zscore_segment <- segmentByPSCB(sample_zscore)

sample_cnv <- call_cnv(sample_zscore_segment, sample_zscore, tfs=c(0.1,0.3), ploidies=c(1.5,2), MaxCN=3)
```

```
plot_cnv_solution(sample_cnv, selected_solution = 1)
```

---

create\_blacklist\_gr     *Create Blacklist regions GRanges object*

---

**Description**

Create Blacklist regions GRanges object

**Usage**

```
create_blacklist_gr(blacklist_files)
```

**Arguments**

blacklist\_files  
Character; Filepath to file containing blacklist regions

**Value**

GRanges object of blacklist regions

---

create\_PoN             *Create Panel-of-Normal (PoN) object*

---

**Description**

Create Panel-of-Normal (PoN) object

**Usage**

```
create_PoN(list_rdsfiles)
```

**Arguments**

list\_rdsfiles     Character; a file contains paths to Profile.Rdata per line

**Value**

Null

**Examples**

```
healthy.1 <- system.file("extdata","ex.healthy1.rds",package = "cfdnakit")
healthy.2 <- system.file("extdata","ex.healthy2.rds",package = "cfdnakit")

path_to_PoN_txt <- paste0(system.file("extdata",package = "cfdnakit"),"/temp.reference_healthy.listfile")
fileConn<-file(path_to_PoN_txt)
writeLines(c(healthy.1,healthy.2), fileConn)
close(fileConn)

PoN.profiles <- create_PoN(path_to_PoN_txt)
file.remove(path_to_PoN_txt)
```

---

extract\_insert\_size     *Extract Insert size from SampleBam*

---

**Description**

Extract Insert size from SampleBam

**Usage**

```
extract_insert_size(readbam_bin, maximum_length = 600, minimum_length = 20)
```

**Arguments**

readbam\_bin     SampleBam Object

maximum\_length     Int; Maximum length of fragment. cfDNA fragment longer than this value will not be considered; Default 600

minimum\_length     Int; Minimum length of fragment. cfDNA fragment shorter than this value will not be considered; Default 20

**Value**

Numeric Vector; Insert size of given sample

**Examples**

```
### Loading example SampleBam file
example_file <- system.file("extdata","example_patientcfDNA_SampleBam.RDS",package = "cfdnakit")
sample_bambin <- readRDS(example_file)
extract_insert_size(sample_bambin)
### Extract only insert size of fragment having specific size
extract_insert_size(sample_bambin,maximum_length=500, minimum_length = 50)
```

---

filter\_read\_on\_blacklist

*Filter out reads on blacklist regions*

---

### Description

Filter out reads on blacklist regions

### Usage

```
filter_read_on_blacklist(sample_bin, blacklist_files = NULL, genome = "hg19")
```

### Arguments

sample_bin	SampleBam; Object from function read_bamfile
blacklist_files	Character; Filepath to file containing blacklist regions
genome	Character; Abbreviation of reference genome; Either hg19 or mm10. default:hg19

### Value

SampleBam after filtering out read on balck list regions

---

fragment\_dist

*Get insert-size distribution table*

---

### Description

Get insert-size distribution table

### Usage

```
fragment_dist(readbam_bin, maximum_length = 600, minimum_length = 20)
```

### Arguments

readbam_bin	SampleBam Object from function read_bamfile
maximum_length	Int; Maximum length of fragment. cfDNA fragment longer than this value will not be considered; Default 600
minimum_length	Int; Minimum length of fragment. cfDNA fragment shorter than this value will not be considered; Default 20

### Value

Distribution table of fragment length



---

get\_fragment\_profile *Getting fragment-length information*

---

## Description

Getting fragment-length information

## Usage

```
get_fragment_profile(  
  readbam_bin,  
  sample_id,  
  genome = "hg19",  
  short_range = c(100, 150),  
  long_range = c(151, 250),  
  maximum_length = 600,  
  minimum_length = 20  
)
```

## Arguments

readbam_bin	SampleBam Object
sample_id	Character; Given sample ID
genome	abbreviation of reference genome; namely hg19, mm10. default:hg19
short_range	Vector of 2 Int; Range of fragment length to be defined as short fragment; Default c(100,150)
long_range	Vector of 2 Int; Range of fragment length to be defined as long fragment; Default c(151,250)
maximum_length	Int; Maximum length of fragment. cfDNA fragment longer than this value will not be considered; Default 600
minimum_length	Int; Minimum length of fragment. cfDNA fragment shorter than this value will not be considered; Default 20

## Value

SampleFragment Object; Fragment length information for quality check and downstream analysis per bin and summary of sample

## Examples

```
example_file <- system.file("extdata", "example_patientcfDNA_SampleBam.RDS", package = "cfDNAkit")  
sample_bam_bin <- readRDS(example_file)  
sample_profile <- get_fragment_profile(sample_bam_bin, sample_id = "Patient1")
```



**Examples**

```

#'
### Loading example SampleBam file
example_file <- system.file("extdata", "example_patientcfDNA_SampleBam.RDS", package = "cfdnakit")
sample_bambin <- readRDS(example_file)
### Example PoN
PoN_rdsfile <- system.file("extdata", "ex.PoN.rds", package = "cfdnakit")
pon_profiles <- readRDS(PoN_rdsfile)
sample_profile <- get_fragment_profile(sample_bambin, sample_id = "Patient1")

sample_zscore <- get_zscore_profile(sample_profile, pon_profiles)

sample_zscore_segment <- segmentByPSCB(sample_zscore)

sample_cnv <- call_cnv(sample_zscore_segment, sample_zscore, tfs=c(0.1,0.3), ploidy=c(1.5,2), MaxCN=3)
get_solution_table(sample_cnv)

```

---

get\_zscore\_profile      *Transform SLRatio with PoN Fragment profile*

---

**Description**

Transform SLRatio with PoN Fragment profile

**Usage**

```
get_zscore_profile(fragment_profile, pon_profile)
```

**Arguments**

```

fragment_profile
                Sample Profile
pon_profile     PoN Profiles

```

**Value**

Dataframe of robust transformed SLratio

**Examples**

```

### Loading example SampleBam file
example_file <- system.file("extdata", "example_patientcfDNA_SampleBam.RDS", package = "cfdnakit")
sample_bambin <- readRDS(example_file)

### Example PoN
PoN_rdsfile <- system.file("extdata", "ex.PoN.rds", package = "cfdnakit")
pon_profiles <- readRDS(PoN_rdsfile)
sample_profile <- get_fragment_profile(sample_bambin, sample_id = "Patient1")

```

```
sample_zscore <- get_zscore_profile(sample_profile,pon_profiles)
sample_zscore_segment <- segmentByPSCB(sample_zscore)
```

---

GRCh2UCSCGRanges      *Convert GRCh chromosome format to UCSC style*

---

**Description**

Convert GRCh chromosome format to UCSC style

**Usage**

```
GRCh2UCSCGRanges(which)
```

**Arguments**

which                  GRanges object;

**Value**

GRanges; GRanges after chromosome format conversion

---

if\_exist\_baifile      *Check if bai file exist from given bam*

---

**Description**

Check if bai file exist from given bam

**Usage**

```
if_exist_baifile(bamfile)
```

**Arguments**

bamfile                Character; Path to sample bamfile

**Value**

Boolean if the bai file exist

---

if_ucsc_chrformat	<i>Check UCSC chromosomes format for input bam file</i>
-------------------	---

---

**Description**

Check UCSC chromosomes format for input bam file

**Usage**

```
if_ucsc_chrformat(bamfile_path)
```

**Arguments**

bamfile\_path    Character; Path to sample bamfile

**Value**

Boolean; if the input bam file is UCSC format, chr prefix

---

make_density_table	<i>Make Fragment-length density table</i>
--------------------	---

---

**Description**

Make Fragment-length density table

**Usage**

```
make_density_table(readbam_bin, minimum_length, maximum_length)
```

**Arguments**

readbam\_bin    List; A list containing SampleBam object/objects from the read\_bamfile function

minimum\_length    numeric;

maximum\_length    numeric

**Value**

data.frame

---

overlap\_bin\_with\_segment  
*Overlap and merge bin data frame with segmentation dataframe*

---

**Description**

Overlap and merge bin data frame with segmentation dataframe

**Usage**

```
overlap_bin_with_segment(per_bin_profile, sample_segmentation)
```

**Arguments**

```
per_bin_profile      bin dataframe
sample_segmentation segmentation dataframe
```

**Value**

dataframe of overlapping bin and segmentation

---

plot\_cnv\_solution      *Plot Fragment-length profile with CNV calling result*

---

**Description**

Plot Fragment-length profile with CNV calling result

**Usage**

```
plot_cnv_solution(
  cnvcall,
  selected_solution = 1,
  genome = "hg19",
  ylim = c(-30, 30)
)
```

**Arguments**

```
cnvcall      solution results from call_cnv function
selected_solution
              solution rank to plot
genome       Character; version of reference genome (default hg19)
ylim        Vector of 2 Int; ylim of plot (default c(-20,20))
```

**Value**

ggplot object plot Genomics CNV profile of selected solution

**Examples**

```
### Loading example SampleBam file
example_file <- system.file("extdata", "example_patientcfDNA_SampleBam.RDS", package = "cfdnakit")
sample_bambin <- readRDS(example_file)
### Example PoN
PoN_rdsfile <- system.file("extdata", "ex.PoN.rds", package = "cfdnakit")
pon_profiles <- readRDS(PoN_rdsfile)
sample_profile <- get_fragment_profile(sample_bambin, sample_id = "Patient1")

sample_zscore <- get_zscore_profile(sample_profile, pon_profiles)
sample_zscore_segment <- segmentByPSCB(sample_zscore)

sample_cnv <- call_cnv(sample_zscore_segment, sample_zscore, tfs=c(0.1,0.3), ploidies=c(1.5,2), MaxCN=3)
plot_cnv_solution(sample_cnv, selected_solution = 1)
```

---

plot\_distance\_matrix *Plot Distance Matrix from CNVCalling*

---

**Description**

Plot Distance Matrix from CNVCalling

**Usage**

```
plot_distance_matrix(cnvcall)
```

**Arguments**

cnvcall           cnvcalling result from function call\_cnv.R

**Value**

ggplot object ; distance matrix per cnvcalling solution

**Examples**

```
### Loading example SampleBam file
example_file <- system.file("extdata", "example_patientcfDNA_SampleBam.RDS", package = "cfdnakit")
sample_bambin <- readRDS(example_file)
### Example PoN
PoN_rdsfile <- system.file("extdata", "ex.PoN.rds", package = "cfdnakit")
pon_profiles <- readRDS(PoN_rdsfile)
sample_profile <- get_fragment_profile(sample_bambin, sample_id = "Patient1")
```

```

sample_zscore <- get_zscore_profile(sample_profile,pon_profiles)
sample_zscore_segment <- segmentByPSCB(sample_zscore)

sample_cnv <- call_cnv(sample_zscore_segment,sample_zscore, tfs=c(0.1,0.3),ploidies=c(1.5,2), MaxCN=3)
plot_distance_matrix(sample_cnv)

```

---

plot\_fragment\_dist      *Plot Fragment-length Distribution*

---

### Description

Plot Fragment-length Distribution

### Usage

```
plot_fragment_dist(readbam_list, maximum_length = 550, minimum_length = 20)
```

### Arguments

readbam_list	List; A list containing SampleBam object/objects from the read_bamfile function
maximum_length	Int; Maximum length of fragment. cfDNA fragment longer than this value will not be considered; Default 550
minimum_length	Int; Minimum length of fragment. cfDNA fragment shorter than this value will not be considered; Default 20

### Value

distribution plot

### Examples

```

example_file <- system.file("extdata","example_patientcfDNA_SampleBam.RDS",package = "cfdnakit")
sample_bambin <- readRDS(example_file)

### adding more samples to the plot
example_file2 <- system.file("extdata","BH01_CHR15.SampleBam.rds",package = "cfdnakit")
control_bambin <- readRDS(example_file2)
readbam_list <- list(plasma1 = sample_bambin, Healthy.blood.plasma=control_bambin)
plot_fragment_dist(readbam_list)

```



---

plot\_sl\_ratio      *Plot Short/Long-fragment Ratio*

---

**Description**

Plot Short/Long-fragment Ratio

**Usage**

```
plot_sl_ratio(fragment_profile, ylim = c(0, 0.4), genome = "hg19")
```

**Arguments**

fragment_profile	list
ylim	plot y-axis limit
genome	Character; version of reference genome (default hg19)

**Value**

plot

**Examples**

```
example_file <- system.file("extdata", "example_patientcfDNA_SampleBam.RDS", package = "cfdnakit")
sample_bambin <- readRDS(example_file)
sample_profile <- get_fragment_profile(sample_bambin, sample_id = "Patient1")
plot_sl_ratio(fragment_profile = sample_profile)

### change plot y-axis
plot_sl_ratio(fragment_profile = sample_profile, ylim=c(0.1,0.5))

### change reference genome
plot_sl_ratio(fragment_profile = sample_profile, genome="hg38")
```

---

plot\_transformed\_sl      *Plot z-transformed Short/Long-fragment Ratio*

---

**Description**

Plot z-transformed Short/Long-fragment Ratio

**Usage**

```
plot_transformed_sl(
  sample_transformed_sl,
  sample_segment_df = NULL,
  ylim = c(-30, 30),
  genome = "hg19"
)
```

**Arguments**

```
sample_transformed_sl      Dataframe z-transformed SLRatio from get_zscore_profile
sample_segment_df         Dataframe segmenation from segmentByPSCB
ylim                       plot y-axis limit
genome                     Character; version of reference genome (default hg19)
```

**Value**

Genome-wide plot of z-transformed SLRatio

**Examples**

```
### Loading example SampleBam file
example_file <- system.file("extdata", "example_patientcfDNA_SampleBam.RDS", package = "cfdnakit")
sample_bambin <- readRDS(example_file)
### Example PoN
PoN_rdsfile <- system.file("extdata", "ex.PoN.rds", package = "cfdnakit")
pon_profiles <- readRDS(PoN_rdsfile)
sample_profile <- get_fragment_profile(sample_bambin, sample_id = "Patient1")

sample_zscore <- get_zscore_profile(sample_profile, pon_profiles)
sample_zscore_segment <- segmentByPSCB(sample_zscore)
plot_transformed_sl(sample_zscore, sample_zscore_segment)
## Change reference genome
plot_transformed_sl(sample_zscore, sample_zscore_segment, genome="hg38")
```

---

read_bamfile	<i>Read a bam file Read a bam file from give path. Alignment and sequencing read information will be binned into non-overlapping size</i>
--------------	---

---

**Description**

Read a bam file Read a bam file from give path. Alignment and sequencing read information will be binned into non-overlapping size

**Usage**

```
read_bamfile(
  bamfile_path,
  binsize = 1000,
  blacklist_files = NULL,
  genome = "hg19",
  target_bedfile = NULL,
  min_mapq = 20,
  apply_blacklist = TRUE
)
```

**Arguments**

bamfile_path	Character; Path to sample bamfile
binsize	Int; Size of non-overlapping windows in KB. Only 100,500 and 1000 is available; Default 1000
blacklist_files	Character; Filepath to file containing blacklist regions
genome	Character; abbreviation of reference genome; available genome: hg19,hg38, mm10. default:hg19
target_bedfile	Character; Path to exon/target bedfile; Default NULL
min_mapq	Int; minimum read mapping quality; Default 20
apply_blacklist	Logical; To exclude read on the blacklist regions Default TRUE

**Value**

SampleBam Object; A list object containing read information from the BAM file.

**Examples**

```
f1 <- system.file("extdata","ex.plasma.bam",package = "cfdnakit")
### read bam file with default params (hg19, 1000K binsize)
sample.bam <-read_bamfile(f1, apply_blacklist=FALSE)
```

---

read_PoN_files	<i>Read Fragment Profile from a list of rds file</i>
----------------	--

---

**Description**

Read Fragment Profile from a list of rds file

**Usage**

```
read_PoN_files(list_rdsfiles)
```

**Arguments**

list\_rdsfiles path to file containing list of rds file

**Value**

list containing content of rds file

---

segmentByPSCB	<i>Segmentation data with PSCBS</i>
---------------	-------------------------------------

---

**Description**

Segmentation data with PSCBS

**Usage**

```
segmentByPSCB(sample_transformed_sl)
```

**Arguments**

sample\_transformed\_sl  
dataframe of z-transformed SLRatio

**Value**

Dataframe of segmentation result

**Examples**

```
### Loading example SampleBam file
example_file <- system.file("extdata", "example_patientcfDNA_SampleBam.RDS", package = "cfdnakit")
sample_bambin <- readRDS(example_file)
### Example PoN
PoN_rdsfile <- system.file("extdata", "ex.PoN.rds", package = "cfdnakit")
pon_profiles <- readRDS(PoN_rdsfile)
sample_profile <- get_fragment_profile(sample_bambin, sample_id = "Patient1")

sample_zscore <- get_zscore_profile(sample_profile, pon_profiles)
sample_zscore_segment <- segmentByPSCB(sample_zscore)
```

---

```
test_ysize_KolmogorovSmirnov
      KolmogorovSmirnov test for insert size
```

---

**Description**

KolmogorovSmirnov test for insert size

**Usage**

```
test_ysize_KolmogorovSmirnov(control_insert_size, sample_insert_size)
```

**Arguments**

```
control_insert_size
      Vector of insert size of a control sample
sample_insert_size
      Vector of insert size of a testing sample
```

**Value**

KS.Test result

**Examples**

```
### Loading example SampleBam file
example_file <- system.file("extdata", "example_patientcfDNA_SampleBam.RDS", package = "cfdnakit")
sample_bambin <- readRDS(example_file)
control_rds <- "BH01_CHR15.SampleBam.rds"
control_RDS_file <- system.file("extdata", control_rds, package = "cfdnakit")
control_fragment_profile <- readRDS(control_RDS_file)
sample.isize <- extract_insert_size(sample_bambin)
healthy.isize <- extract_insert_size(control_fragment_profile)
test_ysize_KolmogorovSmirnov(sample.isize, healthy.isize)
```

---

```
UCSC2GRChSampleBam      Convert UCSC chromosome format to GRCh style from a list of alignment information
```

---

**Description**

Convert UCSC chromosome format to GRCh style from a list of alignment information

**Usage**

```
UCSC2GRChSampleBam(sample.bam)
```

**Arguments**

sample.bam      list of alignment information from function read\_bamfile

**Value**

List; list of alignment information after conversion

---

util.bias\_correct      *Correct GC Bias readcount*

---

**Description**

Correct GC Bias readcount

**Usage**

```
util.bias_correct(readcount, bias)
```

**Arguments**

readcount      numeric  
bias            numeric

**Value**

numeric

---

zscore\_transform      *zscore\_transform transforms SLRatio profile into z-score*

---

**Description**

zscore\_transform transforms SLRatio profile into z-score

**Usage**

```
zscore_transform(per_bin_profile)
```

**Arguments**

per\_bin\_profile  
SampleFragment from function get\_fragment\_profile

**Value**

dataframe of z-score per bin

---

`%>%`*Pipe operator*

---

**Description**

See `magrittr::%>%` for details.

**Arguments**

<code>lhs</code>	A value or the <code>magrittr</code> placeholder.
<code>rhs</code>	A function call using the <code>magrittr</code> semantics.

**Value**

The result of calling `rhs(lhs)`.

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