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

Version 1.10.0

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

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 vistualization 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 aims is to estimate circulating tumor DNA using its chracteristical short-fragmented cfDNA. The package extracts length of each cfDNA and assist the vistuallization of fragment-length distribution. A short-fragment ratio is calculated per non-overlapping fixed-sized bins. Genome-wide copy-number alteration estimated by the short-fragmented cfDNA . The ctDNA estimation score (CES) comprehensively estimate 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 *Call Copy-number Variation from SLRatio and segmentation*

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_bambin <- readRDS(example_file)
sample_profile <- get_fragment_profile(sample_bambin,sample_id = "Patient1")
```

get_segment_bysolution *Return CNV segmentation result from given all CNV solutions*

Description

Return CNV segmentation result from given all CNV solutions

Usage

```
get_segment_bysolution(solution, sample_segmentation, SL_distance_df)
```

Arguments

solution	solution dataframe
sample_segmentation	Segmeantion dataframe
SL_distance_df	Distance matrix

Value

list of segmentation per solution

get_solution_table *Get summarised table of cnv solutions*

Description

Get summarised table of cnv solutions

Usage

```
get_solution_table(cnv_solutions)
```

Arguments

cnv_solutions	cnvcalling result from function call_cnv.R

Value

Dataframe of solution table

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)
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 *Check UCSC chromosomes format for input bam file*

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 *Make Fragment-length density table*

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-tranformed Short/Long-fragment Ratio*

Description

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

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

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

---

read_PoN_files *Read Fragment Profile from a list of rds file*

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 *Segmentation data with PSCBS*

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_isize_KolmogorovSmirnov *KolmogorovSmirnov test for insert size*

Description

KolmogorovSmirnov test for insert size

Usage

```
test_isize_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_isize_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

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