

Package ‘CLONETv2’

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Type Package

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Description Analyze data from next-generation sequencing experiments on genomic samples. 'CLONETv2' offers a set of functions to compute allele specific copy number and clonality from segmented data and SNPs position pileup. The package has also calculated the clonality of single nucleotide variants given read counts at mutated positions. The package has been developed at the laboratory of Computational and Functional Oncology, Department of CIBIO, University of Trento (Italy), under the supervision of prof Francesca Demichelis. References: Prandi et al. (2014) <[doi:10.1186/s13059-014-0439-6](https://doi.org/10.1186/s13059-014-0439-6)>; Carreira et al. (2014) <[doi:10.1126/scitranslmed.3009448](https://doi.org/10.1126/scitranslmed.3009448)>; Romanel et al. (2015) <[doi:10.1126/scitranslmed.aac9511](https://doi.org/10.1126/scitranslmed.aac9511)>.

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Description

This package is designed to analyze data from next-generation sequencing experiments on genomic samples. It offers a set of functions to compute allele specific copy number and clonality from segmented data and SNPs position pileup. The library also calculated the clonality of single nucleotide variants given read counts at mutated positions.

The package has been developed at the laboratory of Computational and Functional Oncology, Department of CIBIO, University of Trento (Italy), under the supervision of prof. Francesca Demichelis.

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Examples

```
#####
#####
## Diploid tumor sample

## Load example data
seg_tb <- read.table(system.file("sample1.seg", package = "CLONETv2"),header = TRUE, as.is=TRUE)
pileup_tumor <- read.table(
  gzfile(system.file("sample1_tumor_pileup.tsv.gz", package = "CLONETv2")),
  header = TRUE, as.is=TRUE)
pileup_normal <- read.table(
  gzfile(system.file("sample1_normal_pileup.tsv.gz", package = "CLONETv2")),
  header = TRUE, as.is=TRUE)
snv_reads <- read.table(system.file("sample1_snv_read_count.tsv", package = "CLONETv2"),
  header = TRUE, as.is=TRUE, comment.char = "", check.names = FALSE, na.strings = "-")

## Compute beta table with default parameters
bt <- compute_beta_table(seg_tb, pileup_tumor, pileup_normal)

## Compute ploidy table with default parameters
pl_table <- compute_ploidy(bt)

## Compute admixture table with default parameters (admixture= 1-tumor_purity)
adm_table <- compute_dna_admixture(beta_table = bt, ploidy_table = pl_table)

## Check ploidy and admixture estimates
check_plot <- check_ploidy_and_admixture(beta_table = bt, ploidy_table = pl_table,
  admixture_table = adm_table)
print(check_plot)

## Compute clonality table with default parameters
scna_clonality_table <- compute_scna_clonality_table(beta_table = bt, ploidy_table = pl_table,
  admixture_table = adm_table)

## Compute allele specific scna
allele_specific_cna_table <- compute_allele_specific_scna_table(beta_table = bt,
  ploidy_table = pl_table, admixture_table = adm_table)

## Compute snvs clonality
sample_id <- "sample1"
snv_clonality_table <- compute_snv_clonality(sample_id = sample_id, snv_read_count = snv_reads,
  beta_table = bt, ploidy_table = pl_table, admixture_table = adm_table)
```

```
#####
#####
## Aneuploid tumor sample

## Load example data
seg_tb <- read.table(system.file("sample2.seg", package = "CLONETv2"),header = TRUE, as.is=TRUE)
pileup_tumor <- read.table(
  gzfile(system.file("sample2_tumor_pileup.tsv.gz", package = "CLONETv2")),
  header = TRUE, as.is=TRUE)
pileup_normal <- read.table(
  gzfile(system.file("sample2_normal_pileup.tsv.gz", package = "CLONETv2")),
  header = TRUE, as.is=TRUE)
snv_reads <- read.table(system.file("sample2_snv_read_count.tsv", package = "CLONETv2"),
  header = TRUE, as.is=TRUE, comment.char = "", check.names = FALSE, na.strings = "-")

## Compute beta table with default parameters
bt <- compute_beta_table(seg_tb, pileup_tumor, pileup_normal)

## Compute ploidy table with default parameters
pl_table <- compute_ploidy(bt)

## Compute admixture table with default parameters (admixture= 1-tumor_purity)
adm_table <- compute_dna_admixture(beta_table = bt, ploidy_table = pl_table)

## Check ploidy and admixture estimates
check_plot <- check_ploidy_and_admixture(beta_table = bt, ploidy_table = pl_table,
  admixture_table = adm_table)
print(check_plot)

## Compute clonality table with default parameters
scna_clonality_table <- compute_scna_clonality_table(beta_table = bt, ploidy_table = pl_table,
  admixture_table = adm_table)

## Compute allele specific scna
allele_specific_cna_table <- compute_allele_specific_scna_table(beta_table = bt,
  ploidy_table = pl_table, admixture_table = adm_table)

## Compute snvs clonality
sample_id <- "sample2"
snv_clonality_table <- compute_snv_clonality(sample_id = sample_id, snv_read_count = snv_reads,
  beta_table = bt, ploidy_table = pl_table, admixture_table = adm_table)

#####
#####
## Aneuploidy tumor sample with problematic ploidy estimate

## Load example data
seg_tb <- read.table(system.file("sample3.seg", package = "CLONETv2"),header = TRUE, as.is=TRUE)
pileup_tumor <- read.table(
  gzfile(system.file("sample3_tumor_pileup.tsv.gz", package = "CLONETv2")),
  header = TRUE, as.is=TRUE)
pileup_normal <- read.table(
```

```

gzfile(system.file("sample3_normal_pileup.tsv.gz", package = "CLONETv2")),
header = TRUE, as.is=TRUE)

## Compute beta table with default parameters
bt <- compute_beta_table(seg_tb, pileup_tumor, pileup_normal)

## Compute ploidy table with default parameters
pl_table <- compute_ploidy(bt)

## Compute admixture table with default parameters (admixture= 1-tumor_purity)
adm_table <- compute_dna_admixture(beta_table = bt, ploidy_table = pl_table)

## Check ploidy and admixture estimates
check_plot <- check_ploidy_and_admixture(beta_table = bt, ploidy_table = pl_table,
  admixture_table = adm_table)
print(check_plot)
## Observed data (gray points) does not fit with expcted positions (Red circles)

#####
#####
## Tumor sample with problem in the segmented input data

## Load example data
seg_tb <- read.table(system.file("sample4_seg", package = "CLONETv2"),header = TRUE, as.is=TRUE)
pileup_tumor <- read.table(
  gzfile(system.file("sample4_tumor_pileup.tsv.gz", package = "CLONETv2")),
  header = TRUE, as.is=TRUE)
pileup_normal <- read.table(
  gzfile(system.file("sample4_normal_pileup.tsv.gz", package = "CLONETv2")),
  header = TRUE, as.is=TRUE)

## Compute beta table with default parameters
bt <- compute_beta_table(seg_tb, pileup_tumor, pileup_normal)

## Compute ploidy table with default parameters
pl_table <- compute_ploidy(bt)

## Compute admixture table with default parameters (admixture= 1-tumor_purity)
adm_table <- compute_dna_admixture(beta_table = bt, ploidy_table = pl_table)

## Check ploidy and admixture estimates
check_plot <- check_ploidy_and_admixture(beta_table = bt, ploidy_table = pl_table,
  admixture_table = adm_table)
print(check_plot)
## CLONETv2 does not provide an estimate of the DNA admixture because
## (LogR, beta) data does not fit any CLONETv2 model

#####
#####
## Diploid tumor sample with subclonal hemizygous and homozygous deletions

```

```

## Load example data
seg_tb <- read.table(system.file("sample5.seg", package = "CLONETv2"),header = TRUE, as.is=TRUE)
pileup_tumor <- read.table(
  gzfile(system.file("sample5_tumor_pileup.tsv.gz", package = "CLONETv2")),
  header = TRUE, as.is=TRUE)
pileup_normal <- read.table(
  gzfile(system.file("sample5_normal_pileup.tsv.gz", package = "CLONETv2")),
  header = TRUE, as.is=TRUE)
snv_reads <- read.table(system.file("sample5_snv_read_count.tsv", package = "CLONETv2"),
  header = TRUE, as.is=TRUE, comment.char = "", check.names = FALSE, na.strings = "-")

## Compute beta table with default parameters
bt <- compute_beta_table(seg_tb, pileup_tumor, pileup_normal)

## Compute ploidy table with default parameters
pl_table <- compute_ploidy(bt)

## Compute admixture table with default parameters (admixture= 1-tumor_purity)
adm_table <- compute_dna_admixture(beta_table = bt, ploidy_table = pl_table)

## Check ploidy and admixture estimates
check_plot <- check_ploidy_and_admixture(beta_table = bt, ploidy_table = pl_table,
  admixture_table = adm_table)
print(check_plot)

## Compute clonality table with default parameters
scna_clonality_table <- compute_scna_clonality_table(beta_table = bt, ploidy_table = pl_table,
  admixture_table = adm_table)

## Compute allele specific scna
allele_specific_cna_table <- compute_allele_specific_scna_table(beta_table = bt,
  ploidy_table = pl_table, admixture_table = adm_table)

## Compute snvs clonality
sample_id <- "sample5"
snv_clonality_table <- compute_snv_clonality(sample_id = sample_id, snv_read_count = snv_reads,
  beta_table = bt, ploidy_table = pl_table, admixture_table = adm_table)

```

adm_table_toy

Toy example of admixture table.

Description

Toy example of admixture table.

Usage

```
adm_table_toy
```

Format

An object of class `data.frame` with 1 rows and 4 columns.

`allele_specific_cna_table_toy`
Toy example of allele specific table of somatic copy number.

Description

Toy example of allele specific table of somatic copy number.

Usage

`allele_specific_cna_table_toy`

Format

An object of class `data.frame` with 4 rows and 15 columns.

`bt_toy` *Toy example of beta table.*

Description

Toy example of beta table.

Usage

`bt_toy`

Format

An object of class `data.frame` with 4 rows and 10 columns.

`check_ploidy_and_admixture`*Function to compute ploidy from a beta table.*

Description

This function takes the beta table of a tumor sample and returns its ploidy.

Usage

```
check_ploidy_and_admixture(beta_table, ploidy_table, admixture_table)
```

Arguments

<code>beta_table</code>	data.frame formatted as the output of function compute_beta_table
<code>ploidy_table</code>	data.frame formatted as the output of function compute_ploidy
<code>admixture_table</code>	data.frame formatted as the output of function compute_dna_admixture

Value

A ggplot2 plot reporting log2 on the x axis and beta and the y axis. Each dot represents a segment of the input `beta_table`. Red transparent circles corresponds to expected log2 vs beta position for different allele specific copy number combinations given ploidy and admixture reported in tables `ploidy_table` and `admixture_table`, respectively. Labels in the form (cnA, cnB) indicate respectively the major and minor allele copy number value. Labels above the plot comprises sample name and ploidy/admixture estimates.

Author(s)

Davide Prandi

Examples

```
## check ploidy and admixture estimates
check_plot_toy <- check_ploidy_and_admixture(beta_table = bt_toy, ploidy_table = pl_table_toy,
  admixture_table = adm_table_toy)
```

 compute_allele_specific_scna_table

Function to compute allele specific somatic copy number

Description

This function takes the beta table of a tumor sample together with the associated ploidy and admixtures tables and computes the allele specific copy number of each segment in the beta table.

Usage

```
compute_allele_specific_scna_table(beta_table, ploidy_table,
  admixture_table, error_tb = error_table, allelic_imbalance_th = 0.5,
  n_digits = 3, n_cores = 1, debug = F)
```

Arguments

beta_table	data.frame formatted as the output of function compute_beta_table
ploidy_table	data.frame formatted as the output of function compute_ploidy
admixture_table	data.frame formatted as the output of function compute_dna_admixture
error_tb	data.frame that reports for each combination of coverage and number informative SNPs the expected estimation error around beta. The data.frame error_tb must contains 3 columns: mean.cov mean coverage n.info.snps number of informative SNPs adm.estimation.error estimated error on computed beta on a segment with coverage mean.cov and n.info.snps informative SNPs Package CLONETv2 have built in error_tb named error_table (default=error_table)
allelic_imbalance_th	maximum distance from allele specific copy number of a segment to define integer allele specific copy number value. Value 0.5 corresponds to round cnA and cnB (default=0.5)
n_digits	number of digits in the output table (default=3)
n_cores	number of cores (default=1)
debug	return extra columns for debugging (default=F)

Value

A data.frame that extends input beta_table with columns

log2.corr log2 ratio adjusted by ploidy and admixture
cnA copy number of the major allele
cnB copy number of the minor allele
cnA.int integer copy number of the major allele
cnB.int integer copy number of the minor allele

Author(s)

Davide Prandi

Examples

```
## Compute clonality table with default parameters
allele_specific_cna_table_toy <- compute_allele_specific_scna_table(
  beta_table = bt_toy, ploidy_table = pl_table_toy,
  admixture_table = adm_table_toy)
```

compute_beta_table *Function to compute beta table*

Description

This function takes segmented data and per base pileup of tumor and matched normal of a sample as input and associates a beta value to each genomic segment.

Usage

```
compute_beta_table(seg_tb, pileup_tumor, pileup_normal,
  min_coverage = 20, min_required_snps = 10, min_af_het_snps = 0.2,
  max_af_het_snps = 0.8, n_digits = 3, n_cores = 1, plot_stats = F,
  debug = F)
```

Arguments

seg_tb	data.frame in SEG format . Rows report per segment log2 ratio numeric value. CLONETv2 interprets first column as sample name, columns two to four as genomic coordinates (chromosome, start location, and end location), column five is not used, and column six is the log2 ratio returned by segmentation algorithm.
pileup_tumor, pileup_normal	data.frame reporting pileup of SNPs in tumor and normal samples respectively. First row contains column names and subsequent rows report the pileup of a specific genomic positions. Required information for each genomic position includes chromosome, position, allelic fraction, and coverage. Required column names are chr, pos, af, and cov
min_coverage	minimum number of reads for considering a pileup position valid (default=20)
min_required_snps	minimum number of snps to call beta for a segment (default=10)
min_af_het_snps	minimum allowed allelic fraction of a SNP genomic position (default=0.2)
max_af_het_snps	maximum allowed allelic fraction of a SNP genomic position (default=0.8)
n_digits	number of digits in the output table (default=3)

n_cores	number of available cores for computation (default=1)
plot_stats	plot summary statistics of the computed beta table (default=F)
debug	return extra columns for debugging (default=F)

Value

A data.frame that extends input seg_tb with columns beta, nsnp, cov, n_beta. Moreover, CLONETv2 renames columns of seg_tb as sample, chr, start, end, XYZ, log2, with XYZ being the original name of column five. For each row of the output table represents a genomic segments. For each row, the value of beta is the proportion of neutral reads in the segment, while nsnp and cov represents respectively the number of informative SNPs and the mean coverage of the given segment. The value n_beta is the proportion of neutral reads in the normal sample. The value of n_beta should be 1 as in normal samples parental chromosomes are equally represented. Values lower than 1 of n_beta could indicate the presence of germline CNVs or sequencing errors.

Author(s)

Davide Prandi, Alessandro Romanel

Examples

```
## Compue beta table with default parameters
bt_toy <- compute_beta_table(seg_tb_toy, pileup_tumor_toy, pileup_normal_toy)
```

compute_dna_admixture *Function to compute DNA admixture of a tumor sample from the associated beta table and ploidy table*

Description

This function takes a beta table and the associated ploidy table and computes DNA admixture.

Usage

```
compute_dna_admixture(beta_table, ploidy_table, min_required_snps = 10,
  min_coverage = 20, error_tb = error_table, library_type = "WES",
  n_digits = 3, n_cores = 1, debug = F)
```

Arguments

beta_table	data.frame formatted as the output of function compute_beta_table
ploidy_table	data.frame formatted as the output of function compute_ploidy
min_required_snps	minimum number of informative snps in a segment valid for computing ploidy (default=10)
min_coverage	minimum coverage of a segment valid for computing ploidy (default=20)

error_tb	data.frame that reports for each combination of coverage and number informative SNPs the expected estimation error around beta. The data.frame error_tb must contains 3 columns: mean.cov mean coverage n.info.snps number of informative SNPs adm.estimation.error estimated error on computed beta on a segment with coverage mean.cov and n.info.snps informative SNPs Package CLONETv2 have built in error_tb named error_table (default=error_table)
library_type	WES, WGS (default=WES)
n_digits	number of digits in the output table (default=3)
n_cores	number of available cores for computation (default=1)
debug	return extra columns for debugging (default=F)

Value

A data.frame with two columns: sample that corresponds to column sample of the input beta_table, and adm that represent the fraction of estimated DNA admixture

Author(s)

Davide Prandi

Examples

```
## Compute admixture table with default parameters
adm_table_toy <- compute_dna_admixture(beta_table = bt_toy, ploidy_table = pl_table_toy)
```

compute_ploidy	<i>Function to compute ploidy from a beta table.</i>
----------------	--

Description

This function takes the beta table of a tumor sample and returns its ploidy.

Usage

```
compute_ploidy(beta_table, max_homo_dels_fraction = 0.01,
  beta_limit_for_neutral_reads = 0.9, min_coverage = 20,
  min_required_snps = 10, library_type = "WES", n_digits = 3,
  n_cores = 1)
```

Arguments

beta_table	data.frame formatted as the output of function compute_beta_table
max_homo_dels_fraction	estimated maximum proportion of genomic segments corresponding to an homozygous deletion (default=0.01)
beta_limit_for_neutral_reads	minimum beta value of a segment valid for computing ploidy (default=0.90)
min_coverage	minimum coverage of a segment valid for computing ploidy (default=20)
min_required_snps	minimum number of informative snps in a segment valid for computing ploidy (default=10)
library_type	WES, WGS (default=WES)
n_digits	number of digits in the output table (default=3)
n_cores	number of available cores for computation (default=1)

Value

A data.frame with two columns: sample that corresponds to column sample of the input beta_table, and ploidy computed

Author(s)

Davide Prandi

Examples

```
## Compute ploidy table with default parameters
pl_table_toy <- compute_ploidy(bt_toy)
```

compute_scna_clonality_table

Function to compute clonality of somatic copy number data

Description

This function takes the beta table of a tumor sample together with the associated ploidy and admixtures tables and computes the clonality of each segment in the beta table.

Usage

```
compute_scna_clonality_table(beta_table, ploidy_table, admixture_table,
  error_tb = error_table, clonality_threshold = 0.85,
  beta_threshold = 0.9, n_digits = 3, n_cores = 1, debug = F)
```

Arguments

beta_table	data.frame formatted as the output of function compute_beta_table
ploidy_table	data.frame formatted as the output of function compute_ploidy
admixture_table	data.frame formatted as the output of function compute_dna_admixture
error_tb	data.frame that reports for each combination of coverage and number informative SNPs the expected estimation error around beta. The data.frame error_tb must contains 3 columns: mean.cov mean coverage n.info.snps number of informative SNPs adm.estimation.error estimated error on computed beta on a segment with coverage mean.cov and n.info.snps informative SNPs Package CLONETv2 have built in error_tb named error_table (default=error_table)
clonality_threshold	threshold to discretize continuous clonality value (default=0.85)
beta_threshold	threshold on beta value to determine clonality direction (default=0.90)
n_digits	number of digits in the output table (default=3)
n_cores	number of cores (default=1)
debug	return extra columns for debugging (default=F)

Value

A data.frame that extends input beta_table with columns

clonality estimated fraction of tumor cell with log2 copy number

clonality.min minium estimated fraction of tumor cell with log2 copy number

clonality.max minium estimated fraction of tumor cell with log2 copy number

clonality.status discretized clonality status into five values: *clonal*, large majority of the tumor cells has the same copy number; *subclonal*, not all the tumor cells has the same copy number; *not.analysed*, is is not possible to determine clonality; *uncertain.clonal* and *uncertain.subclonal* correspond respectively to *clonal* and *subclonal* populations but with less reliable clonality estimate

Author(s)

Davide Prandi

Examples

```
## Compute clonality table with default parameters
scna_clonality_table_toy <- compute_scna_clonality_table(beta_table = bt_toy,
  ploidy_table = pl_table_toy, admixture_table = adm_table_toy)
```

compute_snv_clonality *Function to compute clonality of SNVs*

Description

This function takes as input the genomic position of a SNVs and computes the percentage of genomic homogeneous cells harboring the mutation.

Usage

```
compute_snv_clonality(sample_id, snv_read_count, beta_table, ploidy_table,
  admixture_table, error_tb = error_table, error_rate = 0.05,
  n_digits = 3, n_cores = 1, annotation_style = "VEP", debug = F)
```

Arguments

sample_id	the id of the analyzed sample. It must be the same value reported in column sample of tables beta_table, ploidy_table, and admixture_table
snv_read_count	data.frame reporting in each row the genomic coordinates of an SNV together with number of reference and alternative reads covering the position in columns rc_ref_tumor and rc_alt_tumor, respectively. See parameter annotation_style for details about column names
beta_table	data.frame formatted as the output of function compute_beta_table
ploidy_table	data.frame formatted as the output of function compute_ploidy
admixture_table	data.frame formatted as the output of function compute_dna_admixture
error_tb	data.frame that reports for each combination of coverage and number informative SNPs the expected estimation error around beta. The data.frame error_tb must contains 3 columns: mean.cov mean coverage n.info.snps number of informative SNPs adm.estimation.error estimated error on computed beta on a segment with coverage mean.cov and n.info.snps informative SNPs Package CLONETv2 have built in error_tb named error_table (default=error_table)
error_rate	expected fraction of SNV positions with outlier variant allelic fraction (default=0.05)
n_digits	number of digits in the output table (default=3)
n_cores	number of cores (default=1)
annotation_style	a string that corresponds to the format of the columns that describe the genomic coordinates of a SNV. Accepted values are VEP and MAF. VEP annotation describes genomic coordinates with a single column named Location. MAF format has columns Chromosome, Start_position, and End_position for each aberrant position
debug	return extra columns for debugging (default=F)

Value

A data.frame that extends input table `snv_read_count` with columns `sample`, `cnA`, `cnB`, `t_af`, `t_af_corr`, `SNV.clonality`, and `SNV.clonality.status`. Columns `cnA` and `cnB` report the allele specific copy number of the genomic segment containing the SNV position. Columns `t_af` and `t_af_corr` are respectively raw and ploidy/purity adjusted tumor varian allelic fractions. `SNV.clonality` reports the percentage of tumor cells harboring the SNV and with allele specific copy number `cnA` and `cnB`. `SNV.clonality.status` column lists dicretized `SNV.clonality` values. Discrete states are `clonal`, `uncertain.clonal`, `uncertain.subclonal`, and `subclonal` based in threshold automatically computed on the `SNV.clonality` values. Empty `SNV.clonality.status` of an SNV indicates that clonality cannot be assessed.

Author(s)

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Examples

```
## Compute SNVs clonality
snv_clonality_table_toy <- compute_snv_clonality("toy_sample",
  snv_reads_toy, bt_toy, pl_table_toy, adm_table_toy)
```

error_table

Beta estimation error.

Description

A precomputed table reporting for different combinations of coverage and number of informative SNPs the expected error of the beta value computed by function [compute_beta_table](#).

Usage

```
error_table
```

Format

A data frame column names `mean.cov`, `n.info.snps`, and `adm.estimation.error`

mean.cov genomic segment coverage

n.info.snps number of informative SNPs

adm.estimation.error expected error on beta estimate

pileup_normal_toy *Toy example of normal pileup data.*

Description

Toy example of normal pileup data.

Usage

pileup_normal_toy

Format

An object of class data.frame with 816 rows and 11 columns.

pileup_tumor_toy *Toy example of tumor pileup data.*

Description

Toy example of tumor pileup data.

Usage

pileup_tumor_toy

Format

An object of class data.frame with 816 rows and 11 columns.

pl_table_toy *Toy example of ploidy table.*

Description

Toy example of ploidy table.

Usage

pl_table_toy

Format

An object of class data.frame with 1 rows and 2 columns.

scna_clonality_table_toy

Toy example of clonality table of somatic copy number.

Description

Toy example of clonality table of somatic copy number.

Usage

scna_clonality_table_toy

Format

An object of class `data.frame` with 4 rows and 25 columns.

seg_tb_toy

Toy example of segmetd data.

Description

Toy example of segmetd data.

Usage

seg_tb_toy

Format

An object of class `data.frame` with 4 rows and 6 columns.

snv_clonality_table_toy

Toy example of snv clonality table.

Description

Toy example of snv clonality table.

Usage

snv_clonality_table_toy

Format

An object of class `data.frame` with 2 rows and 78 columns.

snv_reads_toy	<i>Toy example of snv data.</i>
---------------	---------------------------------

Description

Toy example of snv data.

Usage

```
snv_reads_toy
```

Format

An object of class `data.frame` with 2 rows and 71 columns.

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