# Package 'cinaR'

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**Type** Package

**Title** A Computational Pipeline for Bulk 'ATAC-Seq' Profiles

**Version** 0.2.3

**Description** Differential analyses and Enrichment pipeline for bulk 'ATAC-seq' data analyses. This package combines different packages to have an ultimate package for both data analyses and visualization of 'ATAC-seq' data. Methods are described in 'Karakaslar et al.' (2021) <doi:10.1101/2021.03.05.434143>.

**License** GPL-3

**Encoding** UTF-8

**LazyData** true

**URL** https://github.com/eonurk/cinaR/

**BugReports** https://github.com/eonurk/cinaR/issues/

**Depends** R (>= 4.0.0)

**Imports** ChIPseeker, DESeq2, dplyr, edgeR, fgsea, GenomicRanges, TxDb.Hsapiens.UCSC.hg38.knownGene, TxDb.Hsapiens.UCSC.hg19.knownGene, TxDb.Mmusculus.UCSC.mm10.knownGene, ggplot2, ggrepel, grDevices, limma, utils, pheatmap, preprocessCore, RColorBrewer, sva, writexl

**RoxygenNote** 7.1.2

**Suggests** knitr, rmarkdown, markdown

**VignetteBuilder** knitr

**NeedsCompilation** no

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**Repository** CRAN

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# Contents



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`annotatePeaks` *annotatePeaks*

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## Description

Runs DA pipeline and makes it ready for enrichment analyses

## Usage

```
annotatePeaks(cp, reference.genome, show.annotation.pie = FALSE, verbose)
```

## Arguments

| | |
|---|---|
| `cp` | bed formatted consensus peak matrix: CHR, START, STOP and raw peak counts (peaks by 3+samples) |
| `reference.genome` | genome of interested species. It should be 'hg38', 'hg19' or 'mm10'. |
| `show.annotation.pie` | shows the annotation pie chart produced with ChipSeeker |
| `verbose` | prints messages through running the pipeline |

## Value

DApeaks returns DA peaks

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bed *Example peaks from bone marrow of B6 mice*

---

## Description

Example peaks from bone marrow of B6 mice

## Usage

```
data(atac_seq_consensus_bm)
```

## Format

An object of class `data.frame` with 1000 rows and 25 columns.

## Examples

```
data(atac_seq_consensus_bm)
```

---

cinaR *cinaR*

---

## Description

Runs differential analyses and enrichment pipelines

## Usage

```
cinaR(
  matrix,
  contrasts,
  experiment.type = "ATAC-Seq",
  DA.choice = 1,
  DA.fdr.threshold = 0.05,
  DA.lfc.threshold = 0,
  comparison.scheme = "OVO",
  save.DA.peaks = FALSE,
  DA.peaks.path = NULL,
  norm.method = "cpm",
  filter.method = "custom",
  library.threshold = 2,
  cpm.threshold = 1,
  TSS.threshold = 50000,
```

```
  show.annotation.pie = FALSE,
  reference.genome = NULL,
  batch.correction = FALSE,
  batch.information = NULL,
  additional.covariates = NULL,
  sv.number = NULL,
  run.enrichment = TRUE,
  enrichment.method = NULL,
  enrichment.FDR.cutoff = 1,
  background.genes.size = 20000,
  geneset = NULL,
  verbose = TRUE
)
```

## Arguments

| | |
|---|---|
| matrix | either bed formatted consensus peak matrix (peaks by 3+samples) CHR, START, STOP and raw peak counts OR count matrix (genes by 1+samples). |
| contrasts | user-defined contrasts for comparing samples |
| experiment.type | The type of experiment either set to "ATAC-Seq" or "RNA-Seq" |
| DA.choice | determines which pipeline to run: (1) edgeR, (2) limma-voom, (3) limma-trend, (4) DEseq2. Note: Use limma-trend if consensus peaks are already normalized, otherwise use other methods. |
| DA.fdr.threshold | fdr cut-off for differential analyses |
| DA.lfc.threshold | log-fold change cutoff for differential analyses |
| comparison.scheme | either one-vs-one (OVO) or one-vs-all (OVA) comparisons. |
| save.DA.peaks | saves differentially accessible peaks to an excel file |
| DA.peaks.path | the path which the excel file of the DA peaks will be saved, if not set it will be saved to current directory. |
| norm.method | normalization method for consensus peaks |
| filter.method | filtering method for low expressed peaks |
| library.threshold | number of libraries a peak occurs so that it is not filtered default set to 2 |
| cpm.threshold | count per million threshold for not to filter a peak |
| TSS.threshold | Distance to transcription start site in base-pairs. Default set to 50,000. |
| show.annotation.pie | shows the annotation pie chart produced with ChipSeeker |
| reference.genome | genome of interested species. It should be 'hg38', 'hg19' or 'mm10'. |

batch.correction
: logical, if set will run unsupervised batch correction via sva (default) or if the batch information is known 'batch.information' argument should be provided by user.

batch.information
: character vector, given by user.

additional.covariates
: vector or data.frame, this parameter will be directly added to design matrix before running the differential analyses, therefore won't affect the batch corrections but adjust the results in down-stream analyses.

sv.number
: number of surrogate variables to be calculated using SVA, best left untouched.

run.enrichment
: logical, turns off enrichment pipeline

enrichment.method
: There are two methodologies for enrichment analyses, Hyper-geometric p-value (HPEA) or Geneset Enrichment Analyses (GSEA).

enrichment.FDR.cutoff
: FDR cut-off for enriched terms, p-values are corrected by Benjamini-Hochberg procedure

background.genes.size
: number of background genes for hyper-geometric p-value calculations. Default is 20,000.

geneset
: Pathways to be used in enrichment analyses. If not set vp2008 (Chaussabel, 2008) immune modules will be used. This can be set to any geneset using 'read.gmt' function from 'qusage' package. Different modules are available: https://www.gsea-msigdb.org/gsea/downloads.jsp.

verbose
: prints messages through running the pipeline

## Value

returns differentially accessible peaks

## Examples

```
data(atac_seq_consensus_bm) # calls 'bed'

# a vector for comparing the examples
contrasts <- sapply(strsplit(colnames(bed), split = "-", fixed = TRUE),
                    function(x){x[1]})[4:25]

results <- cinaR(bed, contrasts, reference.genome = "mm10")
```

## color_values *color values*

### Description

color values

### Usage

```
color_values
```

### Format

An object of class `character` of length 8.

## differentialAnalyses *Differential Analyses*

### Description

Runs differential analyses pipeline of choice on consensus peaks

### Usage

```
differentialAnalyses(
  final.matrix,
  contrasts,
  experiment.type,
  DA.choice,
  DA.fdr.threshold,
  DA.lfc.threshold,
  comparison.scheme,
  save.DA.peaks,
  DA.peaks.path,
  batch.correction,
  batch.information,
  additional.covariates,
  sv.number,
  verbose
)
```

### Arguments

| | |
|---|---|
| final.matrix | Annotated Consensus peaks |
| contrasts | user-defined contrasts for comparing samples |
| experiment.type | The type of experiment either set to "ATAC-Seq" or "RNA-Seq" |
| DA.choice | determines which pipeline to run: (1) edgeR, (2) limma-voom, (3) limma-trend, (4) DEseq2 |
| DA.fdr.threshold | fdr cut-off for differential analyses |
| DA.lfc.threshold | log-fold change cutoff for differential analyses |
| comparison.scheme | either one-vs-one (OVO) or one-vs-all (OVA) comparisons. |
| save.DA.peaks | logical, saves differentially accessible peaks to an excel file |
| DA.peaks.path | the path which the excel file of the DA peaks will be saved, if not set it will be saved to current directory. |
| batch.correction | logical, if set will run unsupervised batch correction via sva (default) or if the batch information is known 'batch.information' argument should be provided by user. |
| batch.information | character vector, given by user. |
| additional.covariates | vector or data.frame, this parameter will be directly added to design matrix before running the differential analyses, therefore won't affect the batch corrections but adjust the results in down-stream analyses. |
| sv.number | number of surrogate variables to be calculated using SVA, best left untouched. |
| verbose | prints messages through running the pipeline |

### Value

returns consensus peaks (batch corrected version if enabled) and DA peaks

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## dot_plot *dot_plot*

---

### Description

Given the results from 'cinaR' it produces dot plots for enrichment analyses.

### Usage

```
dot_plot(results, fdr.cutoff = 0.1, filter.pathways = FALSE)
```

### Arguments

| | |
|---|---|
| results | cinaR result object |
| fdr.cutoff | Pathways with smaller fdr values than the cut-off will be shown as dots. |
| filter.pathways | logical, it will filter the pathways from dot plot with fdr values less than 'fdr.cutoff'. |

### Value

ggplot object

### Examples

```
library(cinaR)
data(atac_seq_consensus_bm) # calls 'bed'

# a vector for comparing the examples
contrasts <- sapply(strsplit(colnames(bed), split = "-", fixed = TRUE),
                    function(x){x[1]})[4:25]

results <- cinaR(bed, contrasts, reference.genome = "mm10")

dot_plot(results)
```

---

## filterConsensus *filterConsensus*

---

### Description

Filters lowly expressed peaks from down-stream analyses

### Usage

```
filterConsensus(
  cp,
  filter.method = "custom",
  library.threshold = 2,
  cpm.threshold = 1
)
```

### Arguments

| | |
|---|---|
| cp | consensus peak matrix, with unique ids at rownames. |
| filter.method | filtering method for low expressed peaks |
| library.threshold | number of libraries a peak occurs so that it is not filtered default set to 2 |
| cpm.threshold | count per million threshold for not to filter a peak |

## Value

returns differentially accessible peaks

## Examples

```
set.seed(123)
cp <- matrix(rexp(200, rate=.1), ncol=20)

## using cpm function from `edgeR` package
cp.filtered <- filterConsensus(cp)
```

---

grch37 *Grch37*

---

## Description

Grch37

## Usage

```
data(grch37)
```

## Format

An object of class `tbl_df` (inherits from `tbl`, `data.frame`) with 66978 rows and 3 columns.

---

grch38 *Grch38*

---

## Description

Grch38

## Usage

```
data(grch38)
```

## Format

An object of class `tbl_df` (inherits from `tbl`, `data.frame`) with 67495 rows and 3 columns.

grcm38 *Grcm38*

## Description

Grcm38

## Usage

```
data(grcm38)
```

## Format

An object of class `data.frame` with 25350 rows and 4 columns.

GSEA *GSEA*

## Description

Gene set enrichment analyses, runs 'fgsea' package implementation with preset values.

## Usage

```
GSEA(genes, geneset)
```

## Arguments

| | |
|---|---|
| genes | DA gene names to be checked if they are over-represented or not. |
| geneset | Pathways to be used in enrichment analyses. If not set vp2008 (Chaussabel, 2008) immune modules will be used. This can be set to any geneset using 'read.gmt' function from 'qusage' package. Different modules are available: https://www.gsea-msigdb.org/gsea/downloads.jsp. |

## Value

data.frame, list of pathways and their enrichment (adjusted) p-values.

## References

G. Korotkevich, V. Sukhov, A. Sergushichev. Fast gene set enrichment analysis. bioRxiv (2019), doi:10.1101/060012

## Examples

```
library(cinaR)
library(fgsea)
data(examplePathways)
data(exampleRanks)
GSEA(exampleRanks, examplePathways)
```

---

heatmap_differential *heatmap_differential*

---

## Description

plot differentially accessible peaks for a given comparison

## Usage

```
heatmap_differential(results, comparison = NULL, ...)
```

## Arguments

| | |
|---|---|
| results | cinaR result object |
| comparison | these are created by cinaR from 'contrasts' user provided. If not selected the first comparison will be shown! |
| ... | additional arguments for heatmap function, for more info '?pheatmap' |

## Value

ggplot object

## Examples

```
library(cinaR)
data(atac_seq_consensus_bm) # calls 'bed'

# a vector for comparing the examples
contrasts <- sapply(strsplit(colnames(bed), split = "-", fixed = TRUE),
                    function(x){x[1]})[4:25]

results <- cinaR(bed, contrasts, reference.genome = "mm10")

heatmap_differential(results)
```

heatmap_var_peaks *heatmap_var_peaks*

## Description

plot most variable k peaks (default k = 100) among all samples

## Usage

```
heatmap_var_peaks(results, heatmap.peak.count = 100, ...)
```

## Arguments

| | |
|---|---|
| results | cinaR result object |
| heatmap.peak.count | number of peaks to be plotted. If number of peaks are less than k then all peaks will be used. |
| ... | additional arguments for heatmap function, for more info '?pheatmap' |

## Value

ggplot object

## Examples

```
library(cinaR)
data(atac_seq_consensus_bm) # calls 'bed'

# creating dummy results
results <- NULL
results[["cp"]] <- bed[,c(4:25)]

heatmap_var_peaks(results)
```

HPEA *HPEA*

## Description

Hyper-geometric p-value enrichment analyses, looking for over-representation of a set of genes on given pathways.

## Usage

```
HPEA(genes, geneset, background.genes.size)
```

### Arguments

| | |
|---|---|
| genes | DA gene names to be checked if they are over-represented or not. |
| geneset | Pathways to be used in enrichment analyses. If not set vp2008 (Chaussabel, 2008) immune modules will be used. This can be set to any geneset using 'read.gmt' function from 'qusage' package. Different modules are available: https://www.gsea-msigdb.org/gsea/downloads.jsp. |
| background.genes.size | number of background genes for hyper-geometric p-value calculations. Default is 20,000. |

### Value

data.frame, list of pathways and their enrichment (adjusted) p-values.

### Examples

```
library(cinaR)

data("VP2008")
genes.to.test <- vp2008[[1]][1:10]
HPEA(genes.to.test,vp2008, background.genes.size = 20e3)
```

---

## normalizeConsensus *normalizeConsensus*

---

### Description

Normalizes consensus peak using different methods

### Usage

```
normalizeConsensus(cp, norm.method = "cpm", log.option = FALSE)
```

### Arguments

| | |
|---|---|
| cp | bed formatted consensus peak matrix: CHR, START, STOP and raw peak counts (peaks by 3+samples) |
| norm.method | normalization method for consensus peaks |
| log.option | logical, log option for cpm function in edgeR |

### Value

Normalized consensus peaks

## Examples

```
set.seed(123)
cp <- matrix(rexp(200, rate=.1), ncol=20)

## using cpm function from `edgeR` package
cp.normalized <- normalizeConsensus(cp)

## quantile normalization option
cp.normalized <- normalizeConsensus(cp, norm.method = "quantile")
```

---

pca_plot *pca_plot*

---

## Description

pca_plot

## Usage

```
pca_plot(results, overlaid.info, sample.names = NULL, show.names = TRUE)
```

## Arguments

| | |
|---|---|
| results | cinaR result object |
| overlaid.info | overlaid information onto the samples |
| sample.names | names of the samples shown on pca plot |
| show.names | logical, if set FALSE sample names will be hidden |

## Value

ggplot object

## Examples

```
#' library(cinaR)
data(atac_seq_consensus_bm) # calls 'bed'

# creating dummy results
results <- NULL
results[["cp"]] <- bed[,c(4:25)]

# a vector for comparing the examples
contrasts <- sapply(strsplit(colnames(bed), split = "-", fixed = TRUE),
                    function(x){x[1]})[4:25]

## overlays the contrasts info onto PCA plots
pca_plot(results, contrasts)
```

```
## you can overlay other information as well,
## as long as it is the same length with the
## number of samples.

sample.info <- c(rep("Group A", 11), rep("Group B", 11))
pca_plot(results, sample.info, show.names = FALSE)
```

---

`run_enrichment` *run_enrichment*

---

## Description

This function is run, if the enrichment pipeline wants to be called afterwards. Setting reference genome to the same genome which cinaR was run should be given to this function!

## Usage

```
run_enrichment(
  results,
  geneset = NULL,
  experiment.type = "ATAC-Seq",
  reference.genome = NULL,
  enrichment.method = NULL,
  enrichment.FDR.cutoff = 1,
  background.genes.size = 20000,
  verbose = TRUE
)
```

## Arguments

| | |
|---|---|
| `results` | list, DA peaks list for different contrasts |
| `geneset` | Pathways to be used in enrichment analyses. If not set vp2008 (Chaussabel, 2008) immune modules will be used. This can be set to any geneset using 'read.gmt' function from 'qusage' package. Different modules are available: https://www.gsea-msigdb.org/gsea/downloads.jsp. |
| `experiment.type` | The type of experiment either set to "ATAC-Seq" or "RNA-Seq" |
| `reference.genome` | genome of interested species. It should be 'hg38', 'hg19' or 'mm10'. |
| `enrichment.method` | There are two methodologies for enrichment analyses, Hyper-geometric p-value (HPEA) or Geneset Enrichment Analyses (GSEA). |
| `enrichment.FDR.cutoff` | FDR cut-off for enriched terms, p-values are corrected by Benjamini-Hochberg procedure |

background.genes.size
: number of background genes for hyper-geometric p-value calculations. Default is 20,000.

verbose
: prints messages through running the pipeline

## Value

list, enrichment analyses results along with corresponding differential analyses outcomes

## Examples

```
library(cinaR)
data(atac_seq_consensus_bm) # calls 'bed'

# a vector for comparing the examples
contrasts <- sapply(strsplit(colnames(bed), split = "-", fixed = TRUE),
                    function(x){x[1]})[4:25]

results <- cinaR(bed, contrasts, reference.genome = "mm10", run.enrichment = FALSE)

results_with_enrichment <- run_enrichment(results, reference.genome = "mm10")
```

---

# scale_rows *scale_rows*

---

## Description

Normalize (z-score) rows of a matrix

## Usage

```
scale_rows(x)
```

## Arguments

x
: a matrix, possibly containing gene by samples

## Value

Row-normalized matrix

## Examples

```
library(cinaR)
data(atac_seq_consensus_bm) # calls 'bed'
bed.row.normalized <- scale_rows(bed[,c(4:25)])
head(bed.row.normalized)
```

show_comparisons *show_comparisons*

## Description

returns the names of the created comparisons

## Usage

```
show_comparisons(results)
```

## Arguments

| | |
|---|---|
| results | output of the cinaR |

## Value

comparisons created

verboseFn *verboseFn*

## Description

returns a printing function to be used with in the script

## Usage

```
verboseFn(verbose)
```

## Arguments

| | |
|---|---|
| verbose | boolean, determines whether the output going be printed or not |

## Value

print function

vp2008 *Immune modules*

## Description

Immune modules

## Usage

```
data(VP2008)
```

## Format

An object of class GMT; see `read.gmt` from qusage package.

## References

Chaussabel et al. (2008) Immunity 29:150-164 (PubMed)

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