# Package 'EpiCompare'

April 10, 2023

Type Package

**Title** Comparison, Benchmarking & QC of Epigenomic Datasets

Version 1.2.0

**Description** EpiCompare is used to compare and analyse epigenetic datasets for quality control and benchmarking purposes.

The package outputs an HTML report consisting of three sections:

- (1. General metrics) Metrics on peaks (percentage of blacklisted and non-standard peaks, and peak widths) and fragments (duplication rate) of samples,
- (2. Peak overlap) Percentage and statistical significance of overlapping and non-overlapping peaks. Also includes upset plot and
- (3. Functional annotation) functional annotation

 $(Chrom HMM,\, ChIP seeker\, and\, enrichment\, analysis)\, of\, peaks.$ 

Also includes peak enrichment around TSS.

License GPL-3

URL https://github.com/neurogenomics/EpiCompare

BugReports https://github.com/neurogenomics/EpiCompare/issues

**Depends** R (>= 4.1.0)

**Imports** AnnotationHub, BRGenomics, ChIPseeker, data.table, genomation, GenomicRanges, IRanges, GenomeInfoDb, ggplot2, htmltools, methods, plotly, reshape2, rmarkdown, rtracklayer, stats, stringr, utils, BiocGenerics

Suggests badger, BiocFileCache, BiocParallel, parallel, BiocStyle,

clusterProfiler, GenomicAlignments, grDevices, htmlwidgets,

knitr, org.Hs.eg.db, testthat (>= 3.0.0), tidyr,

TxDb.Hsapiens.UCSC.hg19.knownGene,

TxDb.Hsapiens.UCSC.hg38.knownGene,

TxDb.Mmusculus.UCSC.mm9.knownGene,

TxDb.Mmusculus.UCSC.mm10.knownGene,

BSgenome. Hsapiens. UCSC. hg19, BSgenome. Hsapiens. UCSC. hg38,

BSgenome.Mmusculus.UCSC.mm9, BSgenome.Mmusculus.UCSC.mm10,

UpSetR, plyranges, scales, Matrix, consensusSeekeR

VignetteBuilder knitr

```
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Encoding UTF-8
LazyData FALSE
RoxygenNote 7.2.1.9000
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bpplapply

Wrapper for bplapply

## Description

Wrapper function for bplapply that automatically handles issues with **BiocParallel** related to different OS platforms.

## Usage

```
bpplapply(
   X,
   FUN,
   apply_fun = parallel::mclapply,
   workers = 1,
   progressbar = workers > 1,
   verbose = workers == 1,
   use_snowparam = TRUE,
   register_now = FALSE,
   ...
)
```

## Arguments

Χ	Any object for which methods length, [, and [[ are implemented.
FUN	The function to be applied to each element of X.
apply_fun	Iterator function to use.
workers	Number of threads to parallelize across.
progressbar	logical(1) Enable progress bar (based on plyr:::progress_text).
verbose	Print messages.
use_snowparam	Whether to use SnowParam (default: TRUE) or MulticoreParam (FALSE) when parallelising across multiple workers.
register_now	Register the cores now with register (TRUE), or simply return the BPPARAM object (default: FALSE).
	Arguments passed on to BiocParallel::bplapply

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BPPARAM An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation, or a list of BiocParallelParam instances, to be applied in sequence for nested calls to **BiocParallel** functions.

BPREDO A list of output from bplapply with one or more failed elements. When a list is given in BPREDO, bpok is used to identify errors, tasks are rerun and inserted into the original results.

BPOPTIONS Additional options to control the behavior of the parallel evaluation, see bpoptions.

#### Value

(Named) list.

## **Examples**

```
X <- stats::setNames(seq_len(length(letters)), letters)
out <- bpplapply(X, print)</pre>
```

CnR\_H3K27ac

Example CUT&Run peak file

#### Description

Human H3K27ac peak file generated with CUT&Run using K562 cell-line from Meers et al., (2019). Human genome build hg19 was used. Raw peak file (.BED) was obtained from GEO (https://trace.ncbi.nlm.nih.gov/Traces/sra/?run=SRR8581604). Peak calling was performed by Leyla Abbasova using MACS2. The peak file was then processed into GRanges object. Peaks located on chromosome 1 were subsetted to reduce the dataset size.

## Usage

```
data("CnR_H3K27ac")
```

#### **Format**

An object of class GRanges of length 2707.

#### Source

The code to prepare the .Rda file from the raw peak file is:

```
# sequences were directly downloaded from https://trace.ncbi.nlm.nih.gov/Traces/sra/?run=SRR8581604
# and peaks (BED file) were generated by Leyla Abbasova (Neurogenomics Lab, Imperial College
London)
CnR_H3K27ac <- ChIPseeker::readPeakFile("path", as = "GRanges")
CnR_H3K27ac <- CnR_H3K27ac[seqnames(CnR_H3K27ac)== "chr1"]
my_label <- c("name", "score", "strand", "signalValue", "pValue", "qValue", "peak")</pre>
```

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```
colnames(GenomicRanges::mcols(CnR_H3K27ac)) <- my_label
usethis::use_data(CnR_H3K27ac, overwrite = TRUE)</pre>
```

CnR\_H3K27ac\_picard

Example Picard duplication metrics file 2

## **Description**

Duplication metrics output on CUT&Run H3K27ac file (sample accession: SRR8581604). Raw sequences were aligned to hg19 genome and after, Picard was performed by Leyla Abbasova. The duplication summary output generated by Picard was processed to reduce the size of data.

## Usage

```
data("CnR_H3K27ac_picard")
```

#### **Format**

An object of class data. frame with 1 rows and 10 columns.

#### Source

```
The code to prepare the .Rda file is:
```

```
picard <- read.table("path/to/picard/duplication/output", header = TRUE, fill = TRUE)
CnR_H3K27ac_picard <- picard[1,]
usethis::use_data(CnR_H3K27ac_picard, overwrite = TRUE)</pre>
```

CnT\_H3K27ac

Example CUT&Tag peak file

## **Description**

Human H3K27ac peak file generated with CUT&Tag using K562 cell-line from Kaya-Okur et al., (2019). Human genome build hg19 was used. Raw peak file (.BED) was obtained from GEO (https://trace.ncbi.nlm.nih.gov/Traces/sra/?run=SRR8383507). Peak calling was performed by Leyla Abbasova using MACS2. The peak file was then imported as an GRanges object. Peaks located on chromosome 1 were subsetted to reduce the dataset size.

## Usage

```
data("CnT_H3K27ac")
```

## **Format**

An object of class GRanges of length 1670.

#### Source

The code to prepare the .Rda file from the raw peak file is:

```
# sequences were directly downloaded from https://trace.ncbi.nlm.nih.gov/Traces/sra/?run=SRR8383507
# and peaks (BED file) were generated by Leyla Abbasova (Neurogenomics Lab, Imperial College
London)
CnT_H3K27ac <- ChIPseeker::readPeakFile("path", as = "GRanges")
CnT_H3K27ac <- CnT_H3K27ac[seqnames(CnT_H3K27ac)== "chr1"]
my_label <- c("name", "score", "strand", "signalValue", "pValue", "qValue", "peak")
colnames(GenomicRanges::mcols(CnT_H3K27ac)) <- my_label
usethis::use_data(CnT_H3K27ac)</pre>
```

CnT\_H3K27ac\_picard

Example Picard duplication metrics file 1

## **Description**

Duplication metrics output of CUT&Tag H3K27ac file (sample accession: SRR8581604). Raw sequences were aligned to hg19 genome and Picard was performed by Leyla Abbasova. The duplication summary output generated by Picard was processed to reduce the size of data.

## Usage

```
data("CnT_H3K27ac_picard")
```

#### **Format**

An object of class data. frame with 1 rows and 10 columns.

## **Source**

The code to prepare the .Rda file is:

```
picard <- read.table("path/to/picard/duplication/output", header = TRUE, fill = TRUE)]
CnT_H3K27ac_picard <- picard[1,]
usethis::use_data(CnT_H3K27ac_picard, overwrite = TRUE)</pre>
```

compute\_consensus\_peaks

Compute consensus peaks

## Description

Compute consensus peaks from a list of GRanges.

## Usage

```
compute_consensus_peaks(
  grlist,
  groups = NULL,
  genome_build,
  lower = 2,
  upper = Inf,
  min.gapwidth = 1L,
  method = c("granges", "consensusseeker"),
  ...
)
```

#### **Arguments**

grlist Named list of GRanges objects.

groups A character vector of the same length as grlist defining how to group GRanges

objects when computing consensus peaks.

genome\_build Genome build name.

lower, upper The lower and upper bounds for the slice.

min.gapwidth Ranges separated by a gap of at least min.gapwidth positions are not merged.

method Method to call peaks with:

• "granges" : Simple overlap procedure using GRanges functions. Faster but less accurate.

• "consensusseeker": Uses findConsensusPeakRegions to compute consensus peaks. Slower but more accurate.

Arguments passed on to consensusSeekeR::findConsensusPeakRegions

narrowPeaks a GRanges containing called peak regions of signal enrichment based on pooled, normalized data for all analyzed experiments. All GRanges entries must have a metadata field called "name" which identifies the region to the called peak. All GRanges entries must also have a row name which identifies the experiment of origin. Each peaks entry must have an associated narrowPeaks entry. A GRanges entry is associated to a narrowPeaks entry by having a identical metadata "name" field and a identical row name.

- peaks a GRanges containing called peaks of signal enrichment based on pooled, normalized data for all analyzed experiments. All GRanges entries must have a metadata field called "name" which identifies the called peak. All GRanges entries must have a row name which identifies the experiment of origin. Each peaks entry must have an associated narrowPeaks entry. A GRanges entry is associated to a narrowPeaks entry by having a identical metadata "name" field and a identical row name.
- chrInfo a Seqinfo containing the name and the length of the chromosomes to analyze. Only the chomosomes contained in this Seqinfo will be analyzed.
- extendingSize a numeric value indicating the size of padding on both sides of the position of the peaks median to create the consensus region. The minimum size of the consensus region is equal to twice the value of the extendingSize parameter. The size of the extendingSize must be a positive integer. Default = 250.
- expandToFitPeakRegion a logical indicating if the region size, which is set by the extendingSize parameter is extended to include the entire narrow peak regions of all peaks included in the unextended consensus region. The narrow peak regions of the peaks added because of the extension are not considered for the extension. Default: FALSE.
- shrinkToFitPeakRegion a logical indicating if the region size, which is set by the extendingSize parameter is shrinked to fit the narrow peak regions of the peaks when all those regions are smaller than the consensus region. Default: FALSE.
- minNbrExp a positive numeric or a positive integer indicating the minimum number of experiments in which at least one peak must be present for a potential consensus region. The numeric must be a positive integer inferior or equal to the number of experiments present in the narrowPeaks and peaks parameters. Default = 1.
- nbrThreads a numeric or a integer indicating the number of threads to use in parallel. The nbrThreads must be a positive integer. Default = 1.

#### **Details**

*NOTE:* If you get the error "Error in serialize(data, node\$con): error writing to connection", try running closeAllConnections and rerun compute\_consensus\_peaks. This error can sometimes occur when compute\_consensus\_peaks has been disrupted partway through.

## Value

Named list of consensus peak GRanges.

## Source

GenomicRanges tutorial consensusSeekeR

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#### **Examples**

compute\_corr

Compute correlation matrix

#### **Description**

Compute correlation matrix on all peak files.

## Usage

```
compute_corr(
  peakfiles,
  reference = NULL,
  genome_build,
  keep_chr = NULL,
  drop_empty_chr = FALSE,
  bin_size = 5000,
  method = "spearman",
  intensity_cols = c("total_signal", "qValue", "Peak Score", "score"),
  return_bins = FALSE,
  workers = 1
)
```

## **Arguments**

peakfiles

A list of peak files as GRanges object and/or as paths to BED files. If paths are provided, EpiCompare imports the file as GRanges object. EpiCompare also accepts a list containing a mix of GRanges objects and paths. Files must be listed and named using list(). E.g. list("name1"=file1, "name2"=file2). If no names are specified, default file names will be assigned.

reference

A named list containing reference peak file(s) as GRanges object. Please ensure that the reference file is listed and named i.e. list("reference\_name" = reference\_peak). If more than one reference is specified, individual reports for each reference will be generated. However, please note that specifying more than one reference can take awhile. If a reference is specified, it enables two analyses: (1) plot showing statistical significance of overlapping/non-overlapping peaks; and (2) ChromHMM of overlapping/non-overlapping peaks.

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genome\_build The build of \*\*all\*\* peak and reference files to calculate the correlation matrix

on. If all peak and reference files are not of the same build use liftover\_grlist to convert them all before running. Genome build should be one of hg19, hg38,

mm9, mm10.

keep\_chr Which chromosomes to keep.

drop\_empty\_chr Drop chromosomes that are not present in any of the peakfiles (default: FALSE).

bin\_size Default of 100. Base-pair size of the bins created to measure correlation. Use

smaller value for higher resolution but longer run time and larger memory usage.

Default spearman (i.e. non-parametric). A character string indicating which correlation coefficient (or covariance) is to be computed. One of "pearson",

"kendall", or "spearman": can be abbreviated.

intensity\_cols Depending on which columns are present, this value will be used to get quantiles and ultimately calculate the correlations:

- "total\_signal": Used by the peak calling software SEACR. NOTE: Another SEACR column (e.g. "max\_signal") can be used together or instead of "total\_signal".
- "qValue"Used by the peak calling software MACS2/3. Should contain the negative log of the p-values after multiple testing correction.
- "Peak Score": Used by the peak calling software HOMER.

return\_bins If TRUE, returns a named list with both the rebinned (standardised) peaks ("bin")

and the correlation matrix ("cor"). If FALSE (default), returns only the correlation

matrix (unlisted).

workers Number of cores to parallelise across (in applicable functions).

#### Value

method

correlation matrix

encode\_H3K27ac

encode\_H3K27ac

Example ChIP-seq peak file

## **Description**

Human H3K27ac peak file generated with ChIP-seq using K562 cell-line. Human genome build hg19 was used. The peak file (.BED) was obtained from ENCODE project (https://www.encodeproject.org/files/ENCFF044JNJ/). The BED file was then imported as an GRanges object. Peaks located on chromosome 1 were subsetted to reduce the dataset size.

## Usage

```
data("encode_H3K27ac")
```

#### **Format**

An object of class GRanges of length 5142.

#### Source

The code to prepare the .Rda file from the raw peak file is:

```
# dataset was directly downloaded from
# https://www.encodeproject.org/files/ENCFF044JNJ/encode_H3K27ac <- ChIPseeker::readPeakFile("path",
as = "GRanges")
encode_H3K27ac <- encode_H3K27ac[seqnames(encode_H3K27ac) == "chr1"]
my_label <- c("name", "score", "strand", "signalValue", "pValue", "qValue", "peak")
colnames(GenomicRanges::mcols(encode_H3K27ac)) <- my_label
usethis::use_data(encode_H3K27ac, overwrite = TRUE)</pre>
```

EpiCompare

Compare epigenomic datasets

## Description

This function compares and analyses multiple epigenomic datasets and outputs an HTML report containing all results of the analysis. The report is mainly divided into three sections: (1) General Metrics on Peakfiles, (2) Peak Overlaps and (3) Functional Annotation of Peaks.

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## Usage

```
EpiCompare(
  peakfiles,
  genome_build,
  genome_build_output = "hg19",
 blacklist,
  picard_files = NULL,
  reference = NULL,
  upset_plot = FALSE,
  stat_plot = FALSE,
  chromHMM_plot = FALSE,
  chromHMM_annotation = "K562",
  chipseeker_plot = FALSE,
  enrichment_plot = FALSE,
  tss_plot = FALSE,
  precision_recall_plot = FALSE,
  n_{threshold} = 15,
  corr_plot = FALSE,
  bin_size = 5000,
  interact = TRUE,
  save_output = FALSE,
  output_filename = "EpiCompare",
  output_timestamp = FALSE,
  output_dir,
  display = NULL,
 workers = 1
)
```

## **Arguments**

peakfiles

A list of peak files as GRanges object and/or as paths to BED files. If paths are provided, EpiCompare imports the file as GRanges object. EpiCompare also accepts a list containing a mix of GRanges objects and paths. Files must be listed and named using list(). E.g. list("name1"=file1, "name2"=file2). If no names are specified, default file names will be assigned.

genome\_build

A named list indicating the human genome build used to generate each of the following inputs:

- "peakfiles": Genome build for the peakfiles input. Assumes genome build is the same for each element in the peakfiles list.
- "reference": Genome build for the reference input.
- "blacklist": Genome build for the blacklist input.

Example input list:

```
genome_build = list(peakfiles="hg38", reference="hg19", blacklist="hg19")
```

Alternatively, you can supply a single character string instead of a list. This should *only* be done in situations where all three inputs (peakfiles, reference,

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blacklist) are of the same genome build. For example: genome\_build = "hg19"

genome\_build\_output

Genome build to standardise all inputs to. Liftovers will be performed automatically as needed. Default: "hg19".

blacklist

A GRanges object containing blacklisted regions.

picard\_files

A list of summary metrics output from Picard. Files must be in data.frame format and listed using list() and named using names(). To import Picard duplication metrics (.txt file) into R as data frame, use:

picard <- read.table("/path/to/picard/output", header = TRUE, fill =
TRUE).</pre>

reference

A named list containing reference peak file(s) as GRanges object. Please ensure that the reference file is listed and named i.e. list("reference\_name" = reference\_peak). If more than one reference is specified, individual reports for each reference will be generated. However, please note that specifying more than one reference can take awhile. If a reference is specified, it enables two analyses: (1) plot showing statistical significance of overlapping/non-overlapping peaks; and (2) ChromHMM of overlapping/non-overlapping peaks.

upset\_plot

Default FALSE. If TRUE, the report includes upset plot of overlapping peaks.

stat\_plot

Default FALSE. If TRUE, the function creates a plot showing the statistical significance of overlapping/non-overlapping peaks. Reference peak file must be provided.

chromHMM\_plot

Default FALSE. If TRUE, the function outputs ChromHMM heatmap of individual peak files. If a reference peak file is provided, ChromHMM annotation of overlapping and non-overlapping peaks is also provided.

chromHMM\_annotation

ChromHMM annotation for ChromHMM plots. Default K562 cell-line. Cell-line options are:

- "K562" = K-562 cells
- "Gm12878" = Cellosaurus cell-line GM12878
- "H1hesc" = H1 Human Embryonic Stem Cell
- "Hepg2" = Hep G2 cell
- "Hmec" = Human Mammary Epithelial Cell
- "Hsmm" = Human Skeletal Muscle Myoblasts
- "Huvec" = Human Umbilical Vein Endothelial Cells
- "Nhek" = Normal Human Epidermal Keratinocytes
- "Nhlf" = Normal Human Lung Fibroblasts

chipseeker\_plot

Default FALSE. If TRUE, the report includes a barplot of ChIPseeker annotation of peak files.

enrichment\_plot

Default FALSE. If TRUE, the report includes dotplots of KEGG and GO enrichment analysis of peak files.

tss\_plot

Default FALSE. If TRUE, the report includes peak count frequency around transcriptional start site. Note that this can take awhile.

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precision\_recall\_plot Default is FALSE. If TRUE, creates a precision-recall curve plot and an F1 plot using plot\_precision\_recall. n\_threshold Number of thresholds to test. corr\_plot Default is FALSE. If TRUE, creates a correlation plot across all peak files using plot\_corr. Default of 100. Base-pair size of the bins created to measure correlation. Use bin\_size smaller value for higher resolution but longer run time and larger memory usage. interact Default TRUE. By default, all heatmaps are interactive. If set FALSE, all heatmaps in the report will be static. Default FALSE. If TRUE, all outputs (tables and plots) of the analysis will be save\_output saved in a folder (EpiCompare file). output\_filename Default EpiCompare.html. If otherwise, the html report will be saved in the specified name. output\_timestamp Default FALSE. If TRUE, date will be included in the file name. output\_dir Path to where output HTML file should be saved. After completion, automatically display the HTML report file in one of the foldisplay lowing ways: • "browser" : Display the report in your default web browser.

• "rsstudio": Display the report in Rstudio.

• NULL (default): Do not display the report.

workers Number of cores to parallelise across (in applicable functions).

#### Value

Path to one or more HTML report files.

```
### Load Data ###
data("encode_H3K27ac") # example dataset as GRanges object
data("CnT_H3K27ac") # example dataset as GRanges object
data("CnR_H3K27ac") # example dataset as GRanges object
data("hg19_blacklist") # hg38 blacklist dataset
data("CnT_H3K27ac_picard") # example Picard summary output
data("CnR_H3K27ac_picard") # example Picard summary output
#### Prepare Input ####
# create named list of peakfiles
peaks <- list(CnR=CnR_H3K27ac, CnT=CnT_H3K27ac)</pre>
# create named list of picard outputs
picard <- list(CnR=CnR_H3K27ac_picard, CnT=CnT_H3K27ac_picard)</pre>
# reference peak file
reference_peak <- list("ENCODE" = encode_H3K27ac)</pre>
```

fragment\_info 15

fragment\_info

Summary on fragments

## **Description**

This function outputs a summary on fragments using metrics generated by Picard. Provides the number of mapped fragments, duplication rate and number of unique fragments.

## Usage

```
fragment_info(picard_list)
```

## **Arguments**

picard\_list

Named list of duplication metrics generated by Picard as data frame. Data frames must be named and listed using list(). e.g. list("name1"=file1, "name2"=file2). To import Picard duplication metrics (.txt file) into R as data frame, use picard <- read.table("/path/to/picard/output", header = TRUE, fill = TRUE).

## Value

A table summarizing metrics on fragments.

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```
### Run ###
df <- fragment_info(picard_list = picard)</pre>
```

gather\_files

Gather files

## Description

Recursively find peak/picard files stored within subdirectories and import them as a list of GRanges objects.

#### **Usage**

```
gather_files(
  dir,
  type = "peaks.stringent",
  nfcore_cutandrun = FALSE,
  return_paths = FALSE,
  rbind_list = FALSE,
  workers = 1,
  verbose = TRUE
)
```

## **Arguments**

dir

Directory to search within.

type

File type to search for. Options include:

- "<pattern>"Finds files matching an arbitrary regex pattern specified by user.
- "peaks.stringent"Finds files ending in "\*.stringent.bed\$"
- "peaks.consensus"Finds files ending in "\*.consensus.peaks.bed\$"
- "peaks.consensus.filtered" Finds files ending in"\*.consensus.peaks.filtered.awk.bed\$"
- "picard"Finds files ending in "\*.target.markdup.MarkDuplicates.metrics.txt\$"

nfcore\_cutandrun

Whether the files were generated by the nf-core/cutandrun Nextflow pipeline. If TRUE, can use the standardised folder structure to automatically generate more

descriptive file names with sample IDs.

Return only the file paths without actually reading them in as GRanges. return\_paths

rbind\_list Bind all objects into one.

workers integer(1) Number of workers. Defaults to the maximum of 1 or the num-

> ber of cores determined by detectCores minus 2 unless environment variables R\_PARALLELLY\_AVAILABLECORES\_FALLBACK or BIOCPARALLEL\_WORKER\_NUMBER

are set otherwise.

verbose Print messages. group\_files 17

#### **Details**

For "peaks.stringent" files called with SEACR, column names will be automatically added:

- total\_signal: Total signal contained within denoted coordinates.
- max\_signalMaximum bedgraph signal attained at any base pair within denoted coordinates.
- max\_signal\_region Region representing the farthest upstream and farthest downstream bases within the denoted coordinates that are represented by the maximum bedgraph signal.

#### Value

A named list of GRanges objects.

## **Examples**

```
#### Make example files ####
save_paths <- EpiCompare::write_example_peaks()
dir <- unique(dirname(save_paths))
#### Gather/import files ####
peaks <- EpiCompare::gather_files(dir=dir, type="peaks.narrow")</pre>
```

group\_files

Group files

#### **Description**

Assign group names to each file in a named list based on a series of string searches based on combinations of relevant metadata factors.

#### Usage

```
group_files(peakfiles, searches)
```

## **Arguments**

peakfiles

A list of peak files as GRanges object and/or as paths to BED files. If paths are provided, EpiCompare imports the file as GRanges object. EpiCompare also accepts a list containing a mix of GRanges objects and paths. Files must be listed and named using list(). E.g. list("name1"=file1, "name2"=file2). If no names are specified, default file names will be assigned.

searches

A named list of substrings to group peakfiles by.

hg19\_blacklist

## **Examples**

hg19\_blacklist

Human genome hg19 blacklisted regions

## **Description**

Obtained from https://www.encodeproject.org/files/ENCFF001TDO/. The ENCODE black-list includes regions of the hg19 genome that have anomalous and/or unstructured signals independent of the cell-line or experiment. Removal of ENCODE blacklist is recommended for quality measure.

## Usage

```
data("hg19_blacklist")
```

#### **Format**

An object of class GRanges of length 411.

## Source

The code to prepare the .Rda file is:

```
# blacklisted regions were directly downloaded
# from https://www.encodeproject.org/files/ENCFF001TDO/
hg19_blacklist <- ChIPseeker::readPeakFile(file.path(path), as = "GRanges")
usethis::use_data(hg19_blacklist, overwrite = TRUE)</pre>
```

hg38\_blacklist

hg38\_blacklist

Human genome hg38 blacklisted regions

## **Description**

Obtained from https://www.encodeproject.org/files/ENCFF356LFX/. The ENCODE black-list includes regions of the hg38 genome that have anomalous and/ or unstructured signals independent of the cell-line or experiment. Removal of ENCODE blacklist is recommended for quality measure.

## Usage

```
data("hg38_blacklist")
```

#### **Format**

An object of class GRanges of length 910.

#### **Source**

```
The code to prepare the .Rda file is:

## blacklisted regions were directly downloaded

## from https://www.encodeproject.org/files/ENCFF356LFX/
hg38_blacklist <- ChIPseeker::readPeakFile(file.path(path), as = "GRanges")
usethis::use_data(hg38_blacklist, overwrite = TRUE)
```

liftover\_grlist

Liftover peak list

## Description

Perform genome build liftover to one or more GRanges objects at once.

## Usage

```
liftover_grlist(
  grlist,
  input_build,
  output_build = "hg19",
  style = "UCSC",
  keep_chr = paste0("chr", c(seq_len(22), "X", "Y")),
  as_grangeslist = FALSE,
  merge_all = FALSE,
  verbose = TRUE
)
```

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## **Arguments**

grlist A named list of GRanges objects, or simply a single unlisted GRanges object.

Can perform liftover within species or across species.

input\_build The genome build of grlist.

output\_build Desired genome build for grlist to be lifted over to.

style Chromosome style, set by seqlevelsStyle.

"UCSC": Uses the chromosome style "chr1"."NCBI": Uses the chromosome style "1"

keep\_chr Which chromosomes to keep.
as\_grangeslist Return as a GRangesList.

merge\_all Merge all GRanges into a single GRanges object.

verbose Print messages.

#### Value

Named list of lifted GRanges objects.

## **Examples**

overlap\_heatmap General

Generate heatmap of percentage overlap

## **Description**

This function generates a heatmap showing percentage of overlapping peaks between peak files.

## Usage

```
overlap_heatmap(peaklist, interact = TRUE)
```

## **Arguments**

peaklist A list of peak files as GRanges object. Files must be listed and named using

list(). e.g. list("name1"=file1, "name2"=file2). If not named, default

file names will be assigned.

interact Default TRUE. By default heatmap is interactive. If FALSE, heatmap is static.

overlap\_percent 21

## Value

An interactive heatmap

## **Examples**

```
### Load Data ###
data("encode_H3K27ac") # example peakfile GRanges object
data("CnT_H3K27ac") # example peakfile GRanges object

### Create Named List ###
peaks <- list("encode"=encode_H3K27ac, "CnT"=CnT_H3K27ac)

### Run ###
my_heatmap <- overlap_heatmap(peaklist = peaks)</pre>
```

overlap\_percent

Calculate percentage of overlapping peaks

## **Description**

This function calculates the percentage of overlapping peaks and outputs a table or matrix of results.

## Usage

```
overlap_percent(
  peaklist1,
  peaklist2,
  invert = FALSE,
  precision_recall = TRUE,
  suppress_messages = TRUE)
```

## **Arguments**

A list of peak files as GRanges object. Files must be listed and named using list(). e.g. list("name1"=file1, "name2"=file2). If not named, default file names will be assigned.

peaklist2 peaklist1 A list of peak files as GRanges object. Files must be listed and named using list(). e.g. list("name1"=file1, "name2"=file2).

invert If TRUE, keep only the ranges in x that do not overlap ranges.

precision\_recall

Return percision-recall results for all combinations of peaklist1 (the "query") and peaklist2 (the "subject"). See subsetByOverlaps for more details on this terminology.

suppress\_messages

Suppress messages.

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#### Value

data frame

## **Examples**

overlap\_stat\_plot

Statistical significance of overlapping peaks

## **Description**

This function calculates the statistical significance of overlapping/ non-overlapping peaks against a reference peak file. If the reference peak file has the BED6+4 format (peak called by MACS2), the function generates a series of boxplots showing the distribution of q-values for sample peaks that are overlapping and non-overlapping with the reference. If the reference peak file does not have the BED6+4 format, the function uses 'enrichPeakOverlap()' from 'ChIPseeker' package to calculate the statistical significance of overlapping peaks only. In this case, please provide an annotation file as TxDb object.

#### Usage

```
overlap_stat_plot(reference, peaklist, annotation = NULL)
```

## **Arguments**

reference A reference peak file as GRanges object.

peaklist A list of peak files as GRanges object. Files must be listed and named using

list(). E.g. list("name1"=file1, "name2"=file2). If not named, default

file names will be assigned.

annotation A TxDb annotation object from Bioconductor. This is required only if the refer-

ence file does not have BED6+4 format.

## Value

A boxplot or barplot showing the statistical significance of overlapping/non-overlapping peaks.

overlap\_upset\_plot 23

## **Examples**

overlap\_upset\_plot

Generate Upset plot for overlapping peaks

## **Description**

This function generates upset plot (UpSetR package) of overlapping peaks.

## Usage

```
overlap_upset_plot(peaklist)
```

## Arguments

peaklist

A named list of peak files as GRanges object. Objects must be listed and named using list(). e.g. list("name1"=file1, "name2"=file2). If not named, default file names are assigned.

## Value

Upset plot of overlapping peaks

```
### Load Data ###
data("encode_H3K27ac") # load example data
data("CnT_H3K27ac") # load example data

### Create Named List ###
peakfile <- list("encode"=encode_H3K27ac, "CnT"=CnT_H3K27ac)

### Run ###
my_plot <- overlap_upset_plot(peaklist = peakfile)</pre>
```

peak\_info

Summary of Peak Information

## **Description**

This function outputs a table summarizing information on the peak files. Provides the total number of peaks and the percentage of peaks in blacklisted regions.

## Usage

```
peak_info(peaklist, blacklist)
```

## **Arguments**

peaklist A named list of peak files as GRanges object. Objects listed using list("name1"

= peak, "name2" = peak2).

blacklist A GRanges object containing blacklisted regions.

#### Value

A summary table of peak information

## **Examples**

```
plot_ChIPseeker_annotation
```

Create ChIPseeker annotation plot

## **Description**

This function annotates peaks using ChIPseeker::annotatePeak. It outputs functional annotation of each peak file in a barplot.

plot\_chromHMM 25

## Usage

```
plot_ChIPseeker_annotation(peaklist, annotation)
```

## **Arguments**

peaklist A list of peak files as GRanges object. Files must be listed and named using

list(). e.g. list("name1"=file1, "name2"=file2). If not named, default

file names will be assigned.

annotation A TxDb annotation object from Bioconductor.

#### Value

barplot

## **Examples**

```
### Load Data ###
data("CnT_H3K27ac") # example peakfile GRanges object
data("CnR_H3K27ac") # example peakfile GRanges object

### Create Named Peaklist ###
peaks <- list("CnT"=CnT_H3K27ac, "CnR"=CnR_H3K27ac)

## not run
# txdb<-TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene
# my_plot <- plot_ChIPseeker_annotation(peaklist = peaks
# annotation = txdb)</pre>
```

plot\_chromHMM

Plot ChromHMM heatmap

## Description

Creates a heatmap using outputs from ChromHMM using ggplot2. The function takes a list of peakfiles, performs ChromHMM and outputs a heatmap. ChromHMM annotation file must be loaded prior to using this function. ChromHMM annotations are aligned to hg19, and will be automatically lifted over to the genome\_build to match the build of the peaklist.

## Usage

```
plot_chromHMM(
   peaklist,
   chromHMM_annotation,
   cell_line = NULL,
   genome_build,
   interact = TRUE,
   return_data = FALSE
)
```

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## **Arguments**

A named list of peak files as GRanges object. If list is not named, default names will be assigned.

chromHMM\_annotation
ChromHMM annotation list.

cell\_line
If not cell\_line, will replace chromHMM\_annotation by importing chromHMM data for a given cell line using get\_chromHMM\_annotation.

genome\_build
The human genome reference build used to generate peakfiles. "hg19" or "hg38".

interact
Default TRUE. By default, the heatmaps are interactive. IfFALSE, the function generates a static ChromHMM heatmap.

return\_data Return the plot data as in addition to the plot itself.

#### Value

ChromHMM heatmap, or a named list.

## **Examples**

plot\_corr

Plot correlation of peak files

## **Description**

Plot correlation by binning genome and measuring correlation of peak quantile ranking. This ranking is based on p-value or other peak intensity measure dependent on the peak calling approach.

## Usage

```
plot_corr(
  peakfiles,
  reference,
  genome_build,
  bin_size = 5000,
```

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```
keep\_chr = NULL,
  drop_empty_chr = FALSE,
 method = "spearman",
  intensity_cols = c("total_signal", "qValue", "Peak Score", "score"),
  interact = FALSE,
 workers = 1,
  show_plot = TRUE
)
```

#### Arguments

peakfiles

A list of peak files as GRanges object and/or as paths to BED files. If paths are provided, EpiCompare imports the file as GRanges object. EpiCompare also accepts a list containing a mix of GRanges objects and paths. Files must be listed and named using list(). E.g. list("name1"=file1, "name2"=file2). If no names are specified, default file names will be assigned.

reference

A named list containing reference peak file(s) as GRanges object. Please ensure that the reference file is listed and named i.e. list("reference\_name" = reference\_peak). If more than one reference is specified, individual reports for each reference will be generated. However, please note that specifying more than one reference can take awhile. If a reference is specified, it enables two analyses: (1) plot showing statistical significance of overlapping/nonoverlapping peaks; and (2) ChromHMM of overlapping/non-overlapping peaks.

genome\_build

The build of \*\*all\*\* peak and reference files to calculate the correlation matrix on. If all peak and reference files are not of the same build use liftover\_grlist to convert them all before running. Genome build should be one of hg19, hg38, mm9, mm10.

bin\_size

Default of 100. Base-pair size of the bins created to measure correlation. Use smaller value for higher resolution but longer run time and larger memory usage.

keep\_chr

Which chromosomes to keep.

method

drop\_empty\_chr Drop chromosomes that are not present in any of the peakfiles (default: FALSE). Default spearman (i.e. non-parametric). A character string indicating which correlation coefficient (or covariance) is to be computed. One of "pearson", "kendall", or "spearman": can be abbreviated.

intensity\_cols Depending on which columns are present, this value will be used to get quantiles and ultimately calculate the correlations:

- "total signal": Used by the peak calling software SEACR. NOTE: Another SEACR column (e.g. "max\_signal") can be used together or instead of "total signal".
- "qValue"Used by the peak calling software MACS2/3. Should contain the negative log of the p-values after multiple testing correction.
- "Peak Score": Used by the peak calling software HOMER.

interact

Default TRUE. By default, all heatmaps are interactive. If set FALSE, all heatmaps in the report will be static.

workers

Number of cores to parallelise across (in applicable functions).

show\_plot

Show the plot.

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#### Value

list with correlation plot (corr\_plot) and correlation matrix (data)

## **Examples**

plot\_enrichment

Generate enrichment analysis plots

## **Description**

This function runs KEGG and GO enrichment analysis of peak files and generates dot plots.

## Usage

```
plot_enrichment(peaklist, annotation)
```

## **Arguments**

peaklist A list of peak files as GRanges object. Files must be listed and named using

list(). e.g. list("name1"=file1, "name2"=file2). If not named, default

file names will be assigned.

annotation A TxDb annotation object from Bioconductor.

#### Value

KEGG and GO dot plots

```
### Load Data ###
data("CnT_H3K27ac") # example peakfile GRanges object
data("CnR_H3K27ac") # example peakfile GRanges object
### Create Named Peaklist ###
peaks <- list("CnT"=CnT_H3K27ac, "CnR"=CnR_H3K27ac)</pre>
```

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```
## not run
# txdb<-TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene
# my_plot <- plot_enrichment(peaklist = peaks,
# annotation = txdb)</pre>
```

plot\_precision\_recall Plot precision-recall curves

## **Description**

Plot precision-recall curves (and optionally F1 plots) by iteratively testing for peak overlap across a series of thresholds used to filter peakfiles. Each GRanges object in peakfiles will be used as the "query" against each GRanges object in reference as the subject. Will automatically use any columns that are specified with thresholding\_cols and present within each GRanges object to create percentiles for thresholding. *NOTE*: Assumes that all GRanges in peakfiles and reference are already aligned to the same genome build.

## Usage

```
plot_precision_recall(
  peakfiles,
  reference,
  thresholding_cols = c("total_signal", "qValue", "Peak Score"),
  initial_threshold = 0,
  n_{threshold} = 10,
 max_threshold = 1,
 workers = 1,
  plot_f1 = TRUE,
  subtitle = NULL,
  color = "peaklist1",
  shape = color,
  facets = "peaklist2 ~ .",
  interact = FALSE,
  show_plot = TRUE
)
```

#### Arguments

peakfiles

A list of peak files as GRanges object and/or as paths to BED files. If paths are provided, EpiCompare imports the file as GRanges object. EpiCompare also accepts a list containing a mix of GRanges objects and paths. Files must be listed and named using list(). E.g. list("name1"=file1, "name2"=file2). If no names are specified, default file names will be assigned.

reference

A named list containing reference peak file(s) as GRanges object. Please ensure that the reference file is listed and named i.e. list("reference\_name"

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= reference\_peak). If more than one reference is specified, individual reports for each reference will be generated. However, please note that specifying more than one reference can take awhile. If a reference is specified, it enables two analyses: (1) plot showing statistical significance of overlapping/non-overlapping peaks; and (2) ChromHMM of overlapping/non-overlapping peaks.

## thresholding\_cols

Depending on which columns are present, GRanges will be filtered at each threshold according to one or more of the following:

- "total\_signal": Used by the peak calling software SEACR. NOTE: Another SEACR column (e.g. "max\_signal") can be used together or instead of "total signal".
- "qValue"Used by the peak calling software MACS2/3. Should contain the negative log of the p-values after multiple testing correction.
- "Peak Score": Used by the peak calling software HOMER.

#### initial\_threshold

Numeric threshold that was provided to SEACR (via the parameter --ctrl) when calling peaks without an IgG control.

n\_threshold Number of thresholds to test.

max\_threshold Maximum threshold to test.

workers Number of cores to parallelise across (in applicable functions).

plot\_f1 Generate a plot with the F1 score vs. threshold as well.

subtitle Plot subtitle.

color Variable to color data points by.
shape Variable to set data point shapes by.

facets This argument is soft-deprecated, please use rows and cols instead.

interact Default TRUE. By default, all heatmaps are interactive. If set FALSE, all heatmaps

in the report will be static.

show\_plot Show the plot.

## Value

list with data and precision recall and F1 plots

precision\_recall 31

precision\_recall

Compute precision-recall

#### **Description**

Compute precision and recall using each GRanges object in peakfiles as the "query" against each GRanges object in reference as the subject.

## Usage

```
precision_recall(
  peakfiles,
  reference,
  thresholding_cols = c("total_signal", "qValue", "Peak Score"),
  initial_threshold = 0,
  n_threshold = 15,
  max_threshold = 1,
  workers = 1,
  ...
)
```

#### **Arguments**

peakfiles

A list of peak files as GRanges object and/or as paths to BED files. If paths are provided, EpiCompare imports the file as GRanges object. EpiCompare also accepts a list containing a mix of GRanges objects and paths. Files must be listed and named using list(). E.g. list("name1"=file1, "name2"=file2). If no names are specified, default file names will be assigned.

reference

A named list containing reference peak file(s) as GRanges object. Please ensure that the reference file is listed and named i.e. list("reference\_name" = reference\_peak). If more than one reference is specified, individual reports for each reference will be generated. However, please note that specifying more than one reference can take awhile. If a reference is specified, it enables two analyses: (1) plot showing statistical significance of overlapping/non-overlapping peaks; and (2) ChromHMM of overlapping/non-overlapping peaks.

thresholding\_cols

Depending on which columns are present, GRanges will be filtered at each threshold according to one or more of the following:

- "total\_signal": Used by the peak calling software SEACR. NOTE: Another SEACR column (e.g. "max\_signal") can be used together or instead of "total\_signal".
- "qValue"Used by the peak calling software MACS2/3. Should contain the negative log of the p-values after multiple testing correction.
- "Peak Score": Used by the peak calling software HOMER.

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initial\_threshold

Numeric threshold that was provided to SEACR (via the parameter --ctrl)

when calling peaks without an IgG control.

n\_threshold Number of thresholds to test.
max\_threshold Maximum threshold to test.

workers Number of cores to parallelise across (in applicable functions).

... Arguments passed on to bpplapply apply\_fun Iterator function to use.

verbose Print messages.

register\_now Register the cores now with register (TRUE), or simply return the BPPARAM object (default: FALSE).

use\_snowparam Whether to use SnowParam (default: TRUE) or MulticoreParam

(FALSE) when parallelising across multiple workers.

progressbar logical(1) Enable progress bar (based on plyr:::progress\_text).

X Any object for which methods length, [, and [[ are implemented.

FUN The function to be applied to each element of X.

#### Value

Overlap

## **Examples**

rebin\_peaks

Rebin peaks

## **Description**

Standardise a list of peak files by rebinning them into fixd-width tiles across the genome.

## Usage

```
rebin_peaks(
  peakfiles,
  genome_build,
  intensity_cols = c("total_signal", "qValue", "Peak Score", "score"),
  bin_size = 5000,
```

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```
keep\_chr = NULL,
  drop_empty_chr = FALSE,
  as\_sparse = TRUE,
 workers = 1,
  verbose = TRUE,
)
```

#### **Arguments**

peakfiles

A list of peak files as GRanges object and/or as paths to BED files. If paths are provided, EpiCompare imports the file as GRanges object. EpiCompare also accepts a list containing a mix of GRanges objects and paths. Files must be listed and named using list(). E.g. list("name1"=file1, "name2"=file2). If no names are specified, default file names will be assigned.

genome\_build

The build of \*\*all\*\* peak and reference files to calculate the correlation matrix on. If all peak and reference files are not of the same build use liftover\_grlist to convert them all before running. Genome build should be one of hg19, hg38, mm9, mm10.

intensity\_cols Depending on which columns are present, this value will be used to get quantiles and ultimately calculate the correlations:

- "total signal": Used by the peak calling software SEACR. NOTE: Another SEACR column (e.g. "max\_signal") can be used together or instead of "total signal".
- "qValue"Used by the peak calling software MACS2/3. Should contain the negative log of the p-values after multiple testing correction.
- "Peak Score": Used by the peak calling software HOMER.

bin\_size

Default of 100. Base-pair size of the bins created to measure correlation. Use smaller value for higher resolution but longer run time and larger memory usage.

keep\_chr

Which chromosomes to keep.

drop\_empty\_chr

Drop chromosomes that are not present in any of the peakfiles (default: FALSE).

as\_sparse

Return the rebinned peaks as a sparse matrix (default: TRUE), which is more efficiently stored than a dense matrix (FALSE).

workers

Number of cores to parallelise across (in applicable functions).

verbose

Print messages.

. . .

Arguments passed on to bpplapply

apply\_fun Iterator function to use.

register\_now Register the cores now with register (TRUE), or simply return the BPPARAM object (default: FALSE).

use\_snowparam Whether to use SnowParam (default: TRUE) or MulticoreParam (FALSE) when parallelising across multiple workers.

progressbar logical(1) Enable progress bar (based on plyr:::progress\_text).

X Any object for which methods length, [, and [[ are implemented.

FUN The function to be applied to each element of X.

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#### Value

Binned peaks matrix

## **Examples**

tidy\_peakfile

Tidy peakfiles in GRanges

## Description

This function filters peak files by removing peaks in blacklisted regions and in non-standard chromosomes. It also checks that the input list of peakfiles is named. If no names are provided, default file names will be used.

## Usage

```
tidy_peakfile(peaklist, blacklist)
```

## **Arguments**

peaklist A named list of peak files as GRanges object. Objects must be named and listed

using list(). e.g. list("name1"=file1, "name2"=file2) If not named, de-

fault names are assigned.

blacklist Peakfile specifying blacklisted regions as GRanges object.

#### Value

list of GRanges object

```
### Load Data ###
data("encode_H3K27ac") # example peakfile GRanges object
data("CnT_H3K27ac") # example peakfile GRanges object
data("hg19_blacklist") # blacklist region for hg19 genome

### Create Named Peaklist ###
peaklist <- list("encode"=encode_H3K27ac, "CnT"=CnT_H3K27ac)</pre>
```

translate\_genome 35

translate\_genome

Translate genome

## Description

Translate the name of a genome build from one format to another.

## Usage

```
translate_genome(
  genome,
  style = c("UCSC", "Ensembl", "NCBI"),
  default_genome = NULL,
  omit_subversion = TRUE
)
```

## **Arguments**

genome A character vector of genomes equivalent to UCSC version or Ensembl Assemblies

style A single value equivalent to "UCSC" or "Ensembl" specifying the output genome default\_genome Default genome build when genome is NULL.

omit\_subversion

Omit any subversion suffixes after the ".".

## Value

Standardized genome build name as a character string.

```
genome <- translate_genome(genome="hg38", style="Ensembl")
genome2 <- translate_genome(genome="mm10", style="UCSC")</pre>
```

36 tss\_plot

tss\_plot

Read count frequency around TSS

## **Description**

This function generates a plot of read count frequency around TSS.

## Usage

```
tss_plot(
  peaklist,
  annotation,
  upstream = 3000,
  downstream = upstream,
  conf = 0.95,
  resample = 500,
  workers = 1
)
```

## **Arguments**

A list of peak files as GRanges object. Files must be listed and named using peaklist list(). e.g. list("name1"=file1, "name2"=file2) If not named, default file names will be assigned. A TxDb annotation object from Bioconductor. annotation upstream upstream from TSS site downstream downstream from TSS site conf Confidence interval threshold estimated by bootstrapping (0.95 means 95 Argument passed to plotAvgProf. resample Number of bootstrapped iterations to run. Argument passed to plotAvgProf. Number of cores to parallelise bootstrapping across. Argument passed to plotAvgworkers

#### Value

profile plot in a list.

Prof.

width\_boxplot 37

width\_boxplot

Peak width boxplot

## **Description**

This function creates boxplots showing the distribution of widths in each peak file.

## Usage

```
width_boxplot(peaklist)
```

## **Arguments**

peaklist

A list of peak files as GRanges object. Files must be named and listed using list(). e.g. list("name1"=file1, "name2"=file2)

#### Value

A boxplot of peak widths.

## **Examples**

```
### Load Data ###
data("encode_H3K27ac") # example peaklist GRanges object
data("CnT_H3K27ac") # example peaklist GRanges object
### Create Named Peaklist ###
peaks <- list("encode"=encode_H3K27ac, "CnT"=CnT_H3K27ac)
### Run ###
my_plot <- width_boxplot(peaklist = peaks)</pre>
```

write\_example\_peaks

Write example peaks

## **Description**

Write example peaks datasets to disk.

## Usage

```
write_example_peaks(
  dir = file.path(tempdir(), "processed_results"),
  datasets = c("encode_H3K27ac", "CnT_H3K27ac", "CnR_H3K27ac")
)
```

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## Arguments

dir Directory to save peak files to.

datasets Example datasets from **EpiCompare** to write.

## Value

Named vector of paths to saved peak files.

```
save_paths <- EpiCompare::write_example_peaks()</pre>
```

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