# Package 'EpiCompare'

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Type Package

Title Comparison, Benchmarking & QC of Epigenetic Datasets

Version 1.0.0

**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 (ChromHMM, ChIPseeker and enrichment analysis) of peaks. Also includes peak enrichment around TSS.

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

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

License GPL-3

**Depends** R (>= 4.1.0)

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TxDb.Hsapiens.UCSC.hg38.knownGene

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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).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"]</pre>
```

```
\label <- c("name", "score", "strand", "signal Value", "pValue", "qValue", "peak") \\ colnames(GenomicRanges::mcols(CnR_H3K27ac)) <- my_label
```

usethis::use\_data(CnR\_H3K27ac, overwrite = TRUE)

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.

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

CnT\_H3K27ac

# **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). 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.

usethis::use\_data(CnT\_H3K27ac)

### 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</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>
```

encode\_H3K27ac

Example ChIP-seq peak file

# Description

Human H3K27ac peak file generated with ChIP-seq using K562 cell-line. Raw 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.

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### 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 epigenetic datasets

# **Description**

This function compares epigenetic datasets and performs various functional analyses. The function outputs an HTML report containing results from the analysis. The report is mainly divided into three areas: (1) Peakfile information, (2) Overlapping peaks and (3) Functional annotations.

# Usage

```
EpiCompare(
  peakfiles,
  genome_build,
  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,
  interact = TRUE,
  save_output = FALSE,
  output_filename = "EpiCompare",
  output_timestamp = FALSE,
  output_dir
)
```

### **Arguments**

peakfiles

A list of peak files as GRanges object and/or as paths to BED files. If paths are provided, EpiCompare creates GRanges object. EpiCompare also accepts a list

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containing a mix of GRanges object and paths. Files must be listed using 'list()' and named using 'names()'. If not named, default file names will be assigned.

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

blacklist A GRanges object containing blacklisted regions.

picard\_files A list of summary metrics output from Picard. Files must be in data.frame for-

mat and listed using 'list()' and named using 'names()'. To import Picard dupli-

cation metrics (.txt file) into R as data frame, use 'picard <- read.table("/path/to/picard/output",

header = TRUE, fill = TRUE)'.

reference A reference peak file as GRanges object. If a reference is specified, it en-

ables two analyses: (1) plot showing statistical significance of overlapping/non-overlapping peaks; and (2) ChromHMM of overlapping/non-overlapping peaks. Please make sure that the reference file is listed and named i.e. 'list("reference\_name"

= reference\_peak)'.

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 indi-

vidual 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 enrich-

ment analysis of peak files.

tss\_plot Default FALSE. If TRUE, the report includes peak count frequency around tran-

scriptional start site. Note that this can take awhile.

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

in the report will be static.

save\_output Default FALSE. If TRUE, all outputs (tables and plots) of the analysis will be

saved in a folder (EpiCompare\_file).

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```
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.

### Value

An HTML report

```
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") # hg19 blacklist dataset
data("hg38_blacklist") # hg38 blacklist dataset
data("CnT_H3K27ac_picard") # example Picard summary output
data("CnR_H3K27ac_picard") # example Picard summary output
# prepare input data
peaks <- list(CnR_H3K27ac, CnT_H3K27ac) # create list of peakfiles</pre>
names(peaks) <- c("CnR", "CnT") # set names</pre>
picard <- list(CnR_H3K27ac_picard, CnT_H3K27ac_picard) # create list of picard outputs
names(picard) <- c("CnR", "CnT") # set names</pre>
reference_peak <- list("ENCODE" = encode_H3K27ac) # reference peak file</pre>
EpiCompare(peakfiles = peaks,
           genome_build = "hg19",
           blacklist = hg19_blacklist,
           picard_files = picard,
           reference = reference_peak,
           upset_plot = FALSE,
           stat_plot = FALSE,
           chromHMM_plot = FALSE,
           chromHMM_annotation = "K562",
           chipseeker_plot = FALSE,
           enrichment_plot = FALSE,
           tss_plot = FALSE,
           interact = FALSE,
           save_output = FALSE,
           output_dir = tempdir())
```

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# **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 listed using 'list()'. To import Picard duplication metrics (.txt file) into R as data frame, use 'picard <- read.table("/path/to/picard/output", header = TRUE, fill = TRUE)'. The list must be named e.g. 'names(picard\_list) <- c("name1","name2")'

### Value

A table summarizing metrics on fragments.

# **Examples**

```
data(CnT_H3K27ac_picard) # load example picard output
data(CnR_H3K27ac_picard) # load example picard output

# To import Picard duplication metrics (.txt file) into R as data frame
# CnT_H3K27ac_picard <- read.table("/path/to/picard/output.txt", header = TRUE,fill = TRUE)

picard <- list(CnT_H3K27ac_picard, CnR_H3K27ac_picard) # convert into list
names(picard) <- c("CnT_H3K27ac", "CnR_H3K27ac") # set names
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.

```
gather_files(
   dir,
   type = "peaks.consensus.filtered",
   nfcore_cutandrun = FALSE,
   mc.cores = 1
)
```

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# 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 "\*.peaks.bed.stringent.bed\$"
- "peaks.consensus"Finds files ending in "\*.peaks.bed.stringent.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.

mc.cores Numb

Number of cores to parallelise importing

### 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="*.narrowPeaks.bed$")</pre>
```

hg19\_blacklist

Human genome hg19 blacklisted regions

# Description

Obtained from https://www.encodeproject.org/files/ENCFF001TD0/. The ENCODE black-list includes regions of the 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.

hg38\_blacklist

# 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

Human genome hg38 blacklisted regions

# **Description**

Obtained from https://www.encodeproject.org/files/ENCFF356LFX/. The ENCODE black-list includes regions of the 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)</pre>
```

overlap\_heatmap

Generate heatmap of percentage overlap

# **Description**

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

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

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# Arguments

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

named using 'names()' If not named, default file names will be assigned.

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

# Value

An interactive heatmap

# **Examples**

```
data("encode_H3K27ac") # example dataset as GRanges object
data("CnT_H3K27ac") # example dataset as GRanges object

peaks <- list(encode_H3K27ac, CnT_H3K27ac) # create list
names(peaks) <- c("encode", "CnT") # set names

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

overlap\_percent

Calculate percentage of overlapping peaks

# Description

Calculate percentage of overlapping peaks

# Usage

```
overlap_percent(peaklist, reference, invert = FALSE)
```

# **Arguments**

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

named using 'names()' If not named, default file names will be assigned.

reference reference peaks file invert whether to invert

# Value

data frame

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# **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 using 'list()' and

named using 'names()' 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.

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# **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 listed using 'list()' and named using 'names()'. If not named, default file names are assigned.

# Value

Upset plot of overlapping peaks

```
data("encode_H3K27ac") # load example data
data("CnT_H3K27ac") # load example data
peakfile <- list(encode_H3K27ac, CnT_H3K27ac) # create list
names(peakfile) <- c("ENCODE", "CnT") # name list
my_plot <- overlap_upset_plot(peaklist = peakfile) # run function</pre>
```

peak\_info 15

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peak	_	1	n	+	$\cap$

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(peak_list, blacklist)
```

# **Arguments**

peak\_list A named list of peak files as GRanges object. Objects listed using 'list()' and

named using 'names()'.

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 'annotatePeak' from 'ChIPseeker' package. It outputs functional annotation of each peak file in a barplot.

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

plot\_chromHMM

# Arguments

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

named using 'names()' If not named, default file names will be assigned.

annotation A TxDb annotation object from Bioconductor.

# Value

barplot

# **Examples**

```
data("CnT_H3K27ac") # example dataset as GRanges object
data("CnR_H3K27ac") # example dataset as GRanges object

peaks <- list(CnT_H3K27ac, CnR_H3K27ac) # create a list
names(peaks) <- c("CnT", "CnR") # set names

## 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.

# Usage

```
plot_chromHMM(peaklist, chromHMM_annotation, genome_build, interact = TRUE)
```

# **Arguments**

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

named using 'names()' If not named, default file names will be assigned.

chromHMM\_annotation

ChromHMM annotation list

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

interact Default TRUE. By default, the heatmaps are interactive. If set FALSE, the func-

tion generates a static ChromHMM heatmap.

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# Value

ChromHMM heatmap

# **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 using 'list()' and

named using 'names()' If not named, default file names will be assigned.

annotation A TxDb annotation object from Bioconductor.

# Value

KEGG and GO dot plots

```
data("CnT_H3K27ac") # example dataset as GRanges object
data("CnR_H3K27ac") # example dataset as GRanges object
peaks <- list(CnT_H3K27ac, CnR_H3K27ac) # create a list
names(peaks) <- c("CnT", "CnR") # set names</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>
```

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 listed using 'list()' and

named using 'names()'. If not named, default file names are assigned.

blacklist Peakfile specifying blacklisted regions as GRanges object.

# Value

list of GRanges object

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)
```

# Arguments

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

named using 'names()' If not named, default file names will be assigned.

annotation A TxDb annotation object from Bioconductor.

### Value

profile plot in a list.

# **Examples**

width\_boxplot

Peak width boxplot

# **Description**

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

```
width_boxplot(peaklist)
```

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# **Arguments**

peaklist

A list of peak files as GRanges object. Files must be listed using 'list()'. Files must be named using 'names(peaklist) <- c("sample1", "sample2)'. If not named, default file names will be assigned.

### Value

A boxplot of peak widths.

# **Examples**

```
data("encode_H3K27ac") # example dataset as GRanges object
data("CnT_H3K27ac") # example dataset as GRanges object

peaks <- list(encode_H3K27ac, CnT_H3K27ac) # create list
names(peaks) <- c("encode", "CnT") # set names

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")
)
```

# **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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