

Package ‘recoup’

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Type Package

Title An R package for the creation of complex genomic profile plots

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Depends GenomicRanges, GenomicAlignments, ggplot2, ComplexHeatmap

Imports BiocGenerics, biomaRt, circlize, graphics, grDevices, methods, rtracklayer, plyr, stats, utils

Suggests grid, GenomeInfoDb, Rsamtools, BiocStyle, knitr, rmarkdown, zoo, RUnit, BiocInstaller, BSgenome, RSQLite, RMySQL

Enhances parallel

Description recoup calculates and plots signal profiles created from short sequence reads derived from Next Generation Sequencing technologies. The profiles provided are either summarized curve profiles or heatmap profiles. Currently, recoup supports genomic profile plots for reads derived from ChIP-Seq and RNA-Seq experiments. The package uses ggplot2 and ComplexHeatmap graphics facilities for curve and heatmap coverage profiles respectively.

License GPL (>= 3)

Encoding UTF-8

LazyLoad yes

LazyData yes

URL <https://github.com/pmoulos/recoup>

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R topics documented:

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buildAnnotationStore *Build a local annotation database for recoup*

Description

This function creates a local annotation database to be used with recoup so as to avoid long time on the fly annotation downloads and formatting.

Usage

```
buildAnnotationStore(organisms, sources,
  home = file.path(path.expand("~"), ".recoup"),
  forceDownload = TRUE, rc = NULL)
```

Arguments

| | |
|---------------|---|
| organisms | a character vector of organisms for which to download and build annotations. Check the main recoup help page for details on supported organisms. |
| sources | a character vector of public sources from which to download and build annotations. Check the main recoup help page for details on supported annotation sources. |
| home | a valid path (accessible at least by the current user) where the annotation database will be set up. It defaults to ".recoup" inside the current user's home directory. |
| forceDownload | by default, buildAnnotationStore will not download an existing annotation again (FALSE). Set to TRUE if you wish to update the annotation database. |
| rc | fraction (0-1) of cores to use in a multicore system. It defaults to NULL (no parallelization). It is used in the case of type="exon" to process the return value of the query to the UCSC Genome Browser database. |

Value

The function does not return anything. Only the annotation directory and contents are created.

Author(s)

Panagiotis Moulos

Examples

```
buildAnnotationStore("mm9","ensembl")
```

calcCoverage

Calculate coverages over a genomic region

Description

This function returns a coverage list for the genomic regions in mask argument. Generally it should not be used alone and is intended for internal use, although it is useful for calculating stand-alone coverages.

Usage

```
calcCoverage(input, mask, strand = NULL,  
            ignore.strand = TRUE, rc = NULL)
```

Arguments

| | |
|---------------|---|
| input | a GRanges object or a list of GRanges (not a GRangesList!) or the path to a BAM or BigWig file. |
| mask | a GRanges or GRangesList object. |
| strand | see the strandedParams in the main recoup function. |
| ignore.strand | see the strandedParams in the main recoup function. |
| rc | fraction (0-1) of cores to use in a multicore system. It defaults to NULL (no parallelization). |

Details

input contains the short reads in one of the formats described in the arguments section. When input is a list, this list should contain one member per chromosome of the organism of interest.

mask contains the genomic regions over which the coverage will be calculated from the input reads. When calculating RNA-Seq profiles, mask must be a named GRangesList where each member represents the exons of the respective gene.

Value

A list of R1e objects representing the genomic coverages of interest.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data", package="recoup")

# Calculate coverage Rle
mask <- makeGRangesFromDataFrame(df=test.genome,
  keep.extra.columns=TRUE)
small.cov <- calcCoverage(test.input[[1]]$ranges, mask)
```

| | |
|-------------|--|
| coverageRef | <i>Calculate coverage in a set of reference genomic regions (ChIP-Seq or unspliced mode)</i> |
|-------------|--|

Description

This function fills the coverage field in the main input argument in [recoup](#) function.

Usage

```
coverageRef(
  input,
  mainRanges,
  strandedParams = list(strand=NULL, ignoreStrand=TRUE),
  rc = NULL
)
```

Arguments

| | |
|----------------|--|
| input | an input list as in recoup but with the ranges field of each member filled (e.g. after using preprocessRanges). |
| mainRanges | the genome from recoup as a GRanges object (e.g. the output from makeGRangesFromDataFrame). |
| strandedParams | see the strandedParams argument in the main recoup function. |
| rc | fraction (0-1) of cores to use in a multicore system. It defaults to NULL (no parallelization). |

Value

Same as input with the ranges fields filled.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data", package="recoup")

# Calculate coverages
testGenomeRanges <- makeGRangesFromDataFrame(df=test.genome,
  keep.extra.columns=TRUE)
test.input <- coverageRef(
  test.input,
  mainRanges=testGenomeRanges
)
```

| | |
|----------------|---|
| coverageRnaRef | <i>Calculate coverage in a set of reference genomic regions (RNA-Seq or spliced mode)</i> |
|----------------|---|

Description

This function fills the coverage field in the main input argument in [recoup](#) function.

Usage

```
coverageRnaRef(
  input,
  mainRanges,
  strandedParams = list(strand=NULL, ignoreStrand=TRUE),
  rc = NULL
)
```

Arguments

| | |
|----------------|--|
| input | an input list as in recoup but with the ranges field of each member filled (e.g. after using preprocessRanges). |
| mainRanges | a named GRangesList where list member names are genes and list members are GRanges representing each gene's exons. |
| strandedParams | see the strandedParams argument in the main recoup function. |
| rc | fraction (0-1) of cores to use in a multicore system. It defaults to NULL (no parallelization). |

Value

Same as input with the ranges fields filled.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data",package="recoup")

# Note: the figures that will be produced will not look
# realistic or pretty and will be "bumpy". This is because
# package size limitations posed by Bioconductor guidelines
# do not allow for a full test dataset. As a result, the input
# below is not an RNA-Seq dataset. Have a look at the
# vignette on how to test with more realistic data.

# Calculate coverages
testGenomeRanges <- makeGRangesFromDataFrame(df=test.genome,
  keep.extra.columns=TRUE)
test.input <- coverageRnaRef(
  test.input,
  mainRanges=test.exons
)
```

getAnnotation

Annotation downloader

Description

This function connects to the EBI's Biomart service using the package `biomaRt` and downloads annotation elements (gene co-ordinates, exon co-ordinates, gene identifications, biotypes etc.) for each of the supported organisms. See the help page of [recoup](#) for a list of supported organisms. The function downloads annotation for an organism genes or exons. It also uses the UCSC public database connection API to download UCSC and RefSeq annotations.

Usage

```
getAnnotation(org, type, refdb="ensembl", rc=NULL)
```

Arguments

| | |
|--------------------|--|
| <code>org</code> | the organism for which to download annotation. Check the main recoup help page for details on supported organisms. |
| <code>type</code> | either "gene" or "exon". |
| <code>refdb</code> | the online source to use to fetch annotation. It can be "ensembl" (default), "ucsc" or "refseq". In the later two cases, an SQL connection is opened with the UCSC public databases. |
| <code>rc</code> | fraction (0-1) of cores to use in a multicore system. It defaults to NULL (no parallelization). It is used in the case of <code>type="exon"</code> to process the return value of the query to the UCSC Genome Browser database. |

Value

A data frame with the canonical (not isoforms!) genes or exons of the requested organism. When `type="genes"`, the data frame has the following columns: chromosome, start, end, gene_id, gc_content,

strand, gene_name, biotype. When type="exon" the data frame has the following columns: chromosome, start, end, exon_id, gene_id, strand, gene_name, biotype. The gene_id and exon_id correspond to Ensembl gene and exon accessions respectively. The gene_name corresponds to HUGO nomenclature gene names.

Note

The data frame that is returned contains only "canonical" chromosomes for each organism. It does not contain haplotypes or random locations and does not contain chromosome M.

Author(s)

Panagiotis Moulos

Examples

```
mm9.genes <- getAnnotation("mm9", "gene")
```

getBiotypes

List default Ensembl biotypes

Description

This function returns a character vector of Ensembl biotypes for each supported organism. Mostly for internal use, but can also be used to list the biotypes and use some of them to subset initial genomic regions to be profiled.

Usage

```
getBiotypes(org)
```

Arguments

org One of the supported recoup organisms See [recoup](#) for further information.

Value

A character vector of biotypes.

Author(s)

Panagiotis Moulos

Examples

```
hg18.bt <- getBiotypes("hg18")
```

`kmeansDesign`*Apply k-means clustering to profile data*

Description

This function performs k-means clustering on [recoup](#) generated profile matrices and stores the result as a factor in the design element. If no design is present, then one is created from the k-means result.

Usage

```
kmeansDesign(input, design = NULL, kmParams)
```

Arguments

| | |
|-----------------------|--|
| <code>input</code> | a list object created from recoup or partially processed by recoup or its data member. See the main input to recoup for further information. |
| <code>design</code> | See the respective argument in recoup for further information |
| <code>kmParams</code> | Contains parameters for k-means clustering on profiles. See the respective argument in recoup for further information. |

Value

The design data frame, either created from scratch or augmented by k-means clustering.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data", package="recoup")

# Calculate coverages
test.tss <- recoup(
  test.input,
  design=NULL,
  region="tss",
  type="chipseq",
  genome=test.genome,
  flank=c(1000,1000),
  selector=NULL,
  plotParams=list(plot=FALSE,profile=TRUE,
    heatmap=TRUE,device="x11"),
  rc=0.5
)

# Re-design based on k-means
kmParams=list(k=2,nstart=20,algorithm="MacQueen",iterMax=20,
  reference=NULL,seed=42)
design <- kmeansDesign(test.tss$data,kmParams=kmParams)
```

`mergeRuns`*Merge recoup outputs of same type*

Description

This function accepts two or more `recoup` output objects holding single samples to a merged object so that all samples can be used together. This is useful when many coverages must be calculated/plotted and memory issues do not allow effective parallelization.

Usage

```
mergeRuns(..., withDesign = c("auto", "drop"),
dropPlots = TRUE)
```

Arguments

| | |
|-------------------------|--|
| <code>...</code> | one or more <code>recoup</code> output objects. |
| <code>withDesign</code> | one of "auto" (default) or "drop". Determines how to merge designs. See details for further information. |
| <code>dropPlots</code> | if profile and/or heatmap plots are attached to the input object(s), they will be recalculated if <code>dropPlots=="TRUE"</code> (default) or dropped otherwise. |

Details

The `withDesign` argument controls what should be done if any input has an attached design. The default behavior ("auto") will try to do its best to preserve compatible designs. If one or more inputs have the same design, it will be applied to the rest of the samples. If there is only one design, it will be applied to all samples (if you don't want this to happen, choose "drop"). If more than one sample has an attached design but these are incompatible (different numbers of rows/rownames, columns/columnnames), then all designs are dropped. Obviously, `withDesign="drop"` drops all attached designs and the output object is free of a design data frame.

Value

A `recoup` output object with as many samples as in ...

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data", package="recoup")

test.input.shift <- test.input
names(test.input.shift) <- paste(names(test.input.shift), "_1", sep="")
test.input.shift[[1]]$id <- paste0(test.input.shift[[1]]$id, "_1")
test.input.shift[[1]]$ranges <-
  shift(test.input.shift[[1]]$ranges, 100)
test.input.shift[[2]]$id <- paste0(test.input.shift[[2]]$id, "_1")
test.input.shift[[2]]$ranges <-
```

```

shift(test.input.shift[[2]]$ranges,100)

test.tss.1 <- recoup(
  test.input,
  design=NULL,
  region="tss",
  type="chipseq",
  genome=test.genome,
  flank=c(2000,2000),
  selector=NULL,
  rc=0.5
)

test.tss.2 <- recoup(
  test.input.shift,
  design=NULL,
  region="tss",
  type="chipseq",
  genome=test.genome,
  flank=c(2000,2000),
  selector=NULL,
  rc=0.5
)

test.tss <- mergeRuns(test.tss.1,test.tss.2)

```

```
preprocessRanges
```

Read and preprocess BAM/BED files to GRanges

Description

This function reads the BAM/BED files present in the input list object and fills the ranges field of the latter. At the same time it takes care of certain preprocessing steps like normalization.

Usage

```
preprocessRanges(input, preprocessParams, genome,
bamRanges=NULL, bamParams = NULL, rc = NULL)
```

Arguments

| | |
|------------------|--|
| input | an input list as in recoup but with the ranges field of each member filled (e.g. after using preprocessRanges). |
| preprocessParams | see the preprocessParams argument in the main recoup function. |
| genome | see the genome argument in the main recoup function. |
| bamRanges | a GRanges object to mask the BAM/BED files to save time and space. If NULL, the whole file is read. |
| bamParams | see the bamParams argument in the main recoup function. |
| rc | fraction (0-1) of cores to use in a multicore system. It defaults to NULL (no parallelization). |

Value

This function fills the ranges field in the main input argument in [recoup](#) function.

Author(s)

Panagiotis Moulos

Examples

```
# This example only demonstrates the usage of the
# preprocessRanges function. The input BAM files
# included with the package will not produce
# realistic plots as they contain only a very small
# subset of the original data presented in the
# vignettes (50k reads). Please see recoup vignettes
# for further demonstrations.
test.in <- list(
  WT_H4K20me1=list(
    id="WT_H4K20me1",
    name="WT H4K20me1",
    file=system.file("extdata",
      "WT_H4K20me1_50kr.bam",
      package="recoup"),
    format="bam",
    color="#EE0000"
  ),
  Set8KO_H4K20me1=list(
    id="Set8KO_H4K20me1",
    name="Set8KO H4K20me1",
    file=system.file("extdata",
      "Set8KO_H4K20me1_50kr.bam",
      package="recoup"),
    format="bam",
    color="#00BB00"
  )
)

pp=list(
  normalize="none",
  spliceAction="split",
  spliceRemoveQ=0.75
)

test.in <- preprocessRanges(test.in,pp)
```

profileMatrix

Calculate final profile matrices for plotting

Description

This function fills the profile field in the main input argument in [recoup](#) function by calculating profile matrices from coverages which will be used for plotting.

Usage

```
profileMatrix(input, flank, binParams, rc = NULL)
```

Arguments

| | |
|-----------|--|
| input | an input list as in recoup but with the ranges the coverage fields of each member filled (e.g. after using preprocessRanges and coverageRef). |
| flank | see the flank argument in the main recoup function. |
| binParams | see the binParams argument in the main recoup function. |
| rc | fraction (0-1) of cores to use in a multicore system. It defaults to NULL (no parallelization). |

Value

Same as input with the profile fields filled.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data", package="recoup")
# Do some work
testGenomeRanges <- makeGRangesFromDataFrame(df=test.genome,
  keep.extra.columns=TRUE)
w <- width(testGenomeRanges)
testGenomeRanges <- promoters(testGenomeRanges, upstream=2000, downstream=0)
testGenomeRanges <- resize(testGenomeRanges, width=w+4000)
test.input <- coverageRef(
  test.input,
  mainRanges=testGenomeRanges
)
test.input <- profileMatrix(
  test.input,
  flank=c(2000, 2000),
  binParams=list(flankBinSize=50, regionBinSize=150,
    sumStat="mean", interpolation="auto"),
  rc=0.5
)
```

Description

This function calculates and plots signal profiles created from short sequence reads derived from Next Generation Sequencing technologies. The profiles provided are either summarized curve profiles or heatmap profiles. Currently, recoup supports genomic profile plots for reads derived from ChIP-Seq and RNA-Seq experiments. The function uses ggplot2 and ComplexHeatmap graphics facilities for curve and heatmap coverage profiles respectively. The output list object can be reused as input to this function which will automatically recognize which profile elements need to be recalculated, saving time.

Usage

```
recoup(
  input,
  design = NULL,
  region = c("genebody", "tss", "tes", "custom"),
  type = c("chipseq", "rnaseq"),
  genome = c("hg18", "hg19", "hg38", "mm9", "mm10",
             "rn5", "dm3", "danrer7", "pantro4", "susscr3"),
  refdb = c("ensembl", "ucsc", "refseq"),
  flank = c(2000, 2000),
  fraction = 1,
  orderBy = list(
    what = c("none", "suma", "sumn",
             "maxa", "maxn", "avga", "avgn", "hcn"),
    order = c("descending", "ascending"),
    custom = NULL
  ),
  binParams = list(
    flankBinSize = 0,
    regionBinSize = 0,
    sumStat = c("mean", "median"),
    interpolation = c("auto", "spline", "linear",
                    "neighborhood"),
    forceHeatmapBinning = TRUE,
    forcedBinSize = c(50, 200),
    chunking = FALSE
  ),
  selector = NULL,
  preprocessParams = list(
    fragLen = NA,
    cleanLevel = c(0, 1, 2, 3),
    normalize = c("none", "linear",
                 "downsample", "sampleto"),
    sampleTo = 1e+6,
    spliceAction = c("split", "keep", "remove"),
    spliceRemoveQ = 0.75,
    bedGenome = NA,
    seed = 42
  ),
  plotParams = list(
    plot = TRUE,
    profile = TRUE,
```

```

heatmap = TRUE,
correlation = TRUE,
signalScale = c("natural", "log2"),
heatmapScale = c("common", "each" ),
heatmapFactor = 1,
corrScale = c("normalized", "each"),
sumStat = c("mean", "median"),
smooth = TRUE,
corrSmoothPar = ifelse(is.null(design), 0.1,
  0.5),
singleFacet = c("none", "wrap", "grid"),
multiFacet = c("wrap", "grid"),
conf = TRUE,
device = c("x11", "png", "jpg", "tiff", "bmp",
  "pdf", "ps"),
outputDir = ".",
outputBase = NULL
),
saveParams = list(
  ranges = TRUE,
  coverage = TRUE,
  profile = TRUE,
  profilePlot = TRUE,
  heatmapPlot = TRUE,
  correlationPlot = TRUE
),
kmParams = list(
  k = 0,
  nstart = 20,
  algorithm = c("Hartigan-Wong",
    "Lloyd", "Forgy", "MacQueen"),
  iterMax = 20,
  reference = NULL,
  seed = 42
),
strandedParams = list(
  strand = NULL,
  ignoreStrand = TRUE
),
ggplotParams = list(
  title = element_text(size = 12),
  axis.title.x = element_text(size = 10,
    face = "bold"),
  axis.title.y = element_text(size = 10,
    face = "bold"),
  axis.text.x = element_text(size = 9,
    face = "bold"),
  axis.text.y = element_text(size = 10,
    face = "bold"),
  strip.text.x = element_text(size = 10,
    face = "bold"),
  strip.text.y = element_text(size = 10,

```

```

        face = "bold"),
        legend.position = "bottom",
        panel.margin = grid::unit(1, "lines")
    ),
    complexHeatmapParams = list(
      main = list(
        cluster_rows = ifelse(length(grep(
          "hc", orderBy$what)) > 0, TRUE, FALSE),
        cluster_columns = FALSE,
        column_title_gp = grid::gpar(fontsize = 10,
          font = 2),
        show_row_names = FALSE,
        show_column_names = FALSE,
        heatmap_legend_param = list(
          color_bar = "continuous"
        )
      ),
      group=list(
        cluster_rows = ifelse(length(grep(
          "hc", orderBy$what)) > 0, TRUE, FALSE),
        cluster_columns = FALSE,
        column_title_gp = grid::gpar(fontsize = 10,
          font = 2),
        show_row_names = FALSE,
        show_column_names = FALSE,
        row_title_gp = grid::gpar(fontsize = 8,
          font = 2),
        gap = unit(5, "mm"),
        heatmap_legend_param = list(
          color_bar = "continuous"
        )
      )
    ),
    bamParams = NULL,
    onTheFly = FALSE,
    localDbHome = file.path(path.expand("~"), ".recoup"),
    rc = NULL
  )

```

Arguments

- | | |
|--------|--|
| input | the main input to recoup can be either a list or a configuration file (with essentially the same contents as the list). In case of list input, it is a list of n lists, where n the number of samples. See Details for the inner list contents. Alternatively, input can be a text tab delimited file with a specific header (the same fields as each inner list when input is a list) and one row for each sample. Again, see Details section for the field specifications. |
| design | either a data frame with grouping factors as columns (e.g. two grouping factors can be strand, and Ensembl biotype) or a tab delimited text file with the same content (grouping factors in columns). If a data frame, the row.names attribute must correspond to the names (e.g. rownames) of the genome argument, or be a superset or subset of them. If a file, the first column must correspond to the |

| | |
|-----------|--|
| | names (e.g. rownames) of the genome argument or be a superset or subset of them. |
| region | one of "tss", "tes", "genebody", "custom". |
| type | one of "chipseq", "rnaseq". |
| genome | when region is "tss", "tes" or "genebody", genome can be one of "hg38", "hg19", "hg18", "mm10", "mm9", "dm3", "rn5", "danrer7", "pantro4", "susscr3" for human, mouse, fruitfly, rat, zebrafish and chimpanzee genomes respectively. When when region is one of the above or "custom", genome can be a tab delimited BED-like text file or a data frame. |
| refdb | one of "ensembl", "ucsc" or "refseq". It will be used to retrieve genomic reference regions when genome argument is one of the supported organisms. |
| flank | a vector of length two with the number of base pairs to flank upstream and downstream the region. Minimum flank is 0bp and maximum is 50kb. It is always expressed in bp. |
| fraction | a number from 0 to 1 (default) denoting the fraction of total data to be used. See Details for further information. |
| orderBy | a named list whose members control the order of the genomic regions (related to the genome and region arguments as they appear in heatmap profiles. The list has the following fields: <ul style="list-style-type: none"> • what: one of "none" (default), "suma", "sumn", "maxa", "maxn", "avga", "avgn", "hcn", where n in "sumn", "maxn", "hcn" is the index of the profile which should be used as reference. See Details for further information. • order: either "descending" (default) for ordering coverages from highest to lowest, or "ascending" for the opposite. • custom: a numeric vector of custom values (e.g. RNA abundance) that will be used to sort all the profiles. If provided, what will be ignored. Defaults to NULL. |
| binParams | a named list whose members control the resolution of the coverage profiles. The list has the following fields: <ul style="list-style-type: none"> • flankBinSize: the number of intervals (bins) into which the upstream and downstream regions are split and the per-base coverage is averaged across. If 0 (default), no binning is performed and the profiles are calculated based at the base-pair level (the highest possible resolution). • regionBinSize: the number of intervals (bins) into which the main region is split and the per-base coverage is averaged across. If 0 (default), no binning is performed and the profiles are calculated based at the base-pair level (the highest possible resolution). • sumStat: the statistic which is used to summarize the bin coverage. Can be "mean" (default) or "median". • interpolation: the interpolation method to be used for coverage interpolation when the reference regions are of unequal lengths (e.g. gene bodies) and the regionBinSize is larger than some of the former. Can be "auto" (default), "spline", "linear" or "neighborhood". See Details for further explanations of each option. • forceHeatmapBinning: if TRUE (default) and the profile resolution is very high (see flankBinSize and regionBinSize above), binning is applied prior to heatmap profile generation, otherwise the heatmaps will be oversized and will take a lot of time to render. Set to FALSE if both flankBinSize and regionBinSize are not zero so as to avoid unnecessary profile recalculations. |

- `forcedBinSize`: a vector with two integers representing the `flankBinSize` and `regionBinSize` to be used with `forceHeatmapBinning` above.
- `chunking`: TRUE (default) or FALSE. When TRUE, recoup will try to chunk the data for profile matrix calculation.

See Details for a few further notes in the usage of `binParams`.

selector

a named list whose members control some subsetting abilities regarding the input reference genomic regions (`genome` argument) or NULL (default) when the `genome` argument is/may be custom. When list, it has the following fields:

- `id`: a vector of ids of the same type as those present in the genome file or organism type/version.
- `bioype`: a vector of Ensembl biotypes that will be used to filter the genome when the latter is one of the supported organisms. Not used when genome is a custom file.
- `exonType`: currently not used.

preprocessParams

a named list whose members control certain preprocessing steps applied to the `GRanges` objects obtained while or after reading the input BAM/BED files with short reads or BigWig files with processed signals. The list has the following fields:

- `fragLen`: the expected DNA fragment length. Reads will be extended (or truncated) to this size. Not used for RNA-Seq type plots.
- `cleanLevel`: integer from 0 (default) to 3, controlling read filtering level prior to profile generation. See details for further information.
- `normalize`: one of "none" (default), "linear", "downsample", "sampleto". Controls how the coverages are normalized across samples. See Details for explanation of these options.
- `sampleTo`: a fixed library size for downsampling to be used with "sampleto" option above. If a sample has less reads than this fixed size, it is silently reported as is.
- `spliceAction`: one of "split" (default), "keep", "remove". Controls the action to be performed with spliced reads in the case of RNA-Seq samples. See Details for explanation of these options.
- `spliceRemoveQ`: the quantile of putative joint spliced read length to be used for read filtering when `spliceAction` is "remove". See Details for further explanations.
- `bedGenome`: one of the supported genomes, as when reading from bed files, chromosomal lengths are not available and must be retrieved with another way.
- `seed`: seed for random number generation to be used when `spliceAction` is "downsample" or "sampleto". See Details for further explanations. Defaults to 42.

plotParams

a named list whose members control profile (curve and heatmap) plotting parameters. The list has the following fields:

- `plot`: if set to TRUE (default), the plots created with the calculated profiles with recoup are displayed. Set to FALSE to plot later using the output object.
- `profile`: if set to TRUE (default), the average coverage profile across the genomic regions of preference is calculated. Set to FALSE to suppress this.

- `heatmap`: if set to TRUE (default), the coverage heatmap profile across the genomic regions of preference is calculated. Set to FALSE to suppress this.
- `correlation`: if set to TRUE (default), the plots created with the calculated coverage correlations are displayed. Set to FALSE to plot later using the output object.
- `signalScale`: one of "natural" (default) or "log2" to control the signal scale of the final coverage plots. Hint: use log2 scale for RNA-Seq profiles as it produces much smoother plots.
- `heatmapScale`: one of "common" (default) or "each". When "common", a common heatmap color scale is calculated for all samples. When "each", each heatmap has its own color scale.
- `heatmapFactor`: a positive numeric value by which the upper color scale limit of the heatmap profile is multiplied. Defaults to 1. See Details for further information.
- `corrScale`: either "normalized" (default) or "each". Controls the scale display in coverage correlation plots. See Details for further information.
- `sumStat`: the statistic which is used to summarize coverage matrices. Can be "mean" (default) or "median".
- `smooth`: if TRUE (default), the final curve profiles are smoothed using splines. Set to FALSE for no smoothing. If the reference genomic regions are many, the differences are minimal.
- `corrSmoothPar`: a numeric value between 0 and 1 which controls the smoothing of correlation plots. Its default value is controlled by the presence of design. See Details for further information.
- `corrScale`: either "normalized" (default) or "each". See Details for further information.
- `singleFacet`: how should ggplot2 should facet the profiles with 1-factor design and only one sample whose profile will be plotted. Can be "none" (default), "wrap" or "grid". When "none", no gridding is applied and design factors are distinguished by colour. With more than one design factors, the `multiFacet` option below is used.
- `multiFacet`: how should ggplot2 should facet the profiles with 1-factor design and more than one samples whose profile will be plotted. Can be "wrap" (default) or "grid". 2 or 3 (3rd would be colour) factor designs are faceted with "grid".
- `conf`: plot also confidence intervals using `geom_ribbon` in profile or correlation plots.
- `device`: the R plotting device to redirect the plots to. Can be "x11" (default), "png", "jpg", "tiff", "bmp", "pdf", "ps".
- `outputDir`: the directory to place profiles when the plotting device is not "x11". Defaults to ".".
- `outputBase`: the naming template for output files when the plotting device is not "x11". The extensions "_profile" and "_heatmap" will be appended to distinguish each plot type. Leave NULL (default) for automatic filename generation.

`saveParams`

a named list which controls the information to be stored in the recoup output list object. The list has the following fields:

- `ranges`: set to TRUE (default) to store the GRanges object obtained from the BAM/BED files. Set to FALSE for not saving. Not applicable when input is of type BigWig.

- `coverage`: set to TRUE (default) to store the Rle list object obtained from the coverage calculations. Set to FALSE for not saving.
- `profile`: set to TRUE (default) to store the profile matrices extracted from coverage summarizations. Set to FALSE for not saving. It must be present when using the recoup output in the plotting functions `recoupProfile` and `recoupHeatmap`.
- `profilePlot`: set to TRUE (default) to store ggplot object containing the average coverage plot. Set to FALSE for not saving. Must be TRUE if you wish to use the recoup output later with `recoupPlot`.
- `heatmapPlot`: set to TRUE (default) to store ComplexHeatmap object containing the coverage heatmap plot. Set to FALSE for not saving. Must be TRUE if you wish to use the recoup output later with `recoupPlot`.
- `correlationPlot`: set to TRUE (default) to store ggplot object containing coverage correlation plot. Set to FALSE for not saving. Must be TRUE if you wish to use the recoup output later with `recoupPlot`.

See the Details section for some additional information.

`kmParams` a named list which controls the execution of k-means clustering using standard R base function `kmeans` otherwise. The list has the following fields:

- `k`: the number of clusters for k-means clustering. When 0 (default), no k-means clustering is performed.
- `nstart`: See `kmeans`.
- `algorithm`: See `kmeans`.
- `iterMax`: See the `iter.max` parameter in `kmeans`.
- `reference`: which profile to use as reference for the determination of clusters and ordering. The rest of the heatmaps will be ordered according to the reference clustering. It can be either a sample id or NULL (default). If the latter, all the profile matrices are merged into one big matrix and k-means clustering is performed on that matrix.
- `seed`: See `kmeans`.

The result of k-means clustering will be appended to `design` as an additional field. If `design` is NULL, it will be created and passed to the plotting functions.

`strandedParams` a named list which controls how strand information will be treated (if present). The list has the following fields:

- `strand`: if set to NULL (default) then reads from both strands are used from the input BAM/BED files. If "+" or "-", then only the respective strands are used. Not applicable for input of type BigWig.
- `ignoreStrand`: TRUE (default) or FALSE. Passed to the `ignore.strand` argument in the `findOverlaps` function used during coverage calculations.

`ggplotParams` a named list with theme parameters passed to the `ggplot` function of the `ggplot2` package. See the documentation of `ggplot2` for further details. Only the parameters mentioned in the function call are used.

`complexHeatmapParams`

a named list with groups of parameters passed to the `Heatmap` function of the `ComplexHeatmap` package. The list has the following fields:

- `main`: `Heatmap` parameters applied to each non-split (according to `design`) heatmap. See the recoup function call for supported parameters and `Heatmap` for further details.

- main: [Heatmap](#) parameters applied to each split (according to design) heatmap. See the `recoup` function call for supported parameters and [Heatmap](#) for further details.

| | |
|-------------|---|
| bamParams | BAM file read parameters passed to <code>BamFile</code> . See the related function. Currently this is not used. |
| onTheFly | Read short reads directly from BAM files when input contains paths to BAM files. In this case the storage of short reads in the output list object as a <code>GRanges</code> object is not possible. This argument is not used in this release and is a placeholder for the next release. |
| localDbHome | local path to retrieve annotation from when genome is one of the supported organisms. See also buildAnnotationStore . |
| rc | fraction (0-1) of cores to use in a multicore system. It defaults to <code>NULL</code> (no parallelization). |

Details

When input is a list, it should contain as many sublists as the number of samples. Each sublist must have at least the following fields:

- `id`: a unique identifier for each sample which should not contain whitespaces and preferably no special characters.
- `file`: the full path to the BAM, BED or BigWig file. If the path to the BAM is a hyperlink, the BAM file must be indexed. BigWigs are already indexed.
- `format`: one of "bam", "bed" or "bigwig".

Additionally, each sublist may also contain the following fields:

- `name`: a sample name which will appear in plots.
- `color`: either an R color (see the [colors](#) function) or a hexadecimal color (e.g. "#FF0000").

When input is a text file, this should be strictly tab-delimited (no other delimiters like comma), should contain a header with the same names (case sensitive) as the sublist fields above (`id`, `file`, `format` are mandatory and `name`, `color` are optional).

When genome is not one of the supported organisms, it should be a text tab delimited file (only tabs supported) with a header line, or a data frame, where the basic BED field must be present, that means that at least "chromosome", "start", "end", a unique identifier column and "strand" must be present, \ preferably in this order. This file is read in a data frame and then passed to the [makeGRangesFromDataFrame](#) function from the `GenomicRanges` package which takes care of the rest. See also the [makeGRangesFromDataFrame](#)'s documentation. When genome is one of the supported organisms, `recoup` takes care of the rest.

When `region` is "tss", the curve and heatmap profiles are centered around the TSS of the (gene) regions provided with the genome argument, flanked according to the `flank` argument. The same is applied for `region="tes"` where the plots are centered around the transcription end site. When `region` is "genebody", the profiles consist of two flanking parts (upstream of the TSS and downstream of the TES) and a middle part consisting of the gene body coverage profile. The latter is constructed by creating a fixed number of intervals (bins) along each gene and averaging the coverage of each interval. In some extreme cases (e.g. for small genes), the number of bins may be larger than the gene length. In these cases, a few zeros are distributed randomly across the number of bins to reach the predefined number of gene body intervals. When `region` is "custom" the behavior depends on the custom regions length. If it contains single-base intervals (e.g. ChIP-Seq peak centers), then the behavior is similar to the TSS behavior above. If it contains genomic intervals of equal or unequal size, the behavior is similar to the gene body case.

The `fraction` parameter controls the total fraction of both total reads and genomic regions to be used for profile creation. This means that the total reads for each sample are randomly downsampled to $\text{fraction} \times 100\%$ of the original reads and the same applied to the input genomic regions. This practice is followed by similar packages (like `ngs.plot`) and serves the purpose of a quick overview of how the actual profiles look before profiling the whole genome.

Regarding the `orderBy` parameters, for the options of the `what` parameter "sum" type of options order profiles according to i) the sum of coverages of all samples in each genomic region when `orderBy$what="suma"` or ii) the sum of coverages of sample `n` (e.g. 2) in each genomic region when `orderBy$what="sumn"` (e.g. `orderBy$what="sum1"`). The same apply for the "max" type of options but this time the ordering is performed according to the position of the highest coverage in each genomic profile. Ties in the position of highest coverage are broken randomly and sorting is performed with the default R `sort`. Similarly for "avg" type of options, the ordering is performed according to the average total coverage of a reference region. For the "hc" type of options, hierarchical clustering is performed on the selected (`n`) reference profile (e.g. `orderBy$what="hc1"`) and this ordering is applied to the rest of the sample profiles. When `what="none"`, no ordering is performed and the input order is used (genome argument). If any design is present through the `design` argument or k-means clustering is also performed (through the `kmParams` argument), the `orderBy` directives are applied to each sub-profile created by design or k-means clustering.

Regarding the `flankBinSize` field of `binParams`, it is used only when `region="genebody"` or `region="custom"` and the custom regions are not single-base regions. This happens as when the genomic regions to be profiled are single-base regions (e.g. TSSs or ChIP-Seq peak centers), these regions are merged with the flanking areas and altogether form the main genomic region. In these cases, only the `regionBinSize` field value is used. Note that when `type="rnaseq"` or `region="genebody"` or `region="custom"` with non single-base regions the values of `flankBinSize` and `regionBinSize` offer a fine control over how the flanking and the main regions are presented in the profiles. For example, when `flankBinSize=100` and `regionBinSize=100` with a gene body profile plot, the outcome will look kind of "unrealistic" as the e.g. 2kb flanking regions will look very similar to the usually larger gene bodies. On the other hand if `flankBinSize=50` and `regionBinSize=200`, this setting will create more "realistic" gene body profiles as the flanking regions will be squished and the gene body area will look expanded. Within the same parameter group is also interpolation. When working with reference regions of different lengths (e.g. gene bodies), it happens very often that their lengths are a little to a lot smaller than the number of bins into which they should be split and averaged in order to be able to create the average curve and heatmap profiles. `recoup` allows for dynamic resolutions by permitting to the user to set the number of bins into which genomic areas will be binned or by allowing a per-base resolution where possible. The interpolation parameter controls what happens in such cases. When "spline", the R function `spline` is used, with the default method, to produce a spline interpolation of the same size as the `regionBinSize` option and is used as the coverage for that region. When "linear", the procedure is the same as above but using `approx`. When "neighborhood", a number of NA values are distributed randomly across the small area coverage vector, excluding the first and the last two positions, in order to reach `regionBinSize`. Then, each NA position is filled with the mean value of the two values before and the two values after the NA position, with `na.rm=TRUE`. This method should be avoided when >20% of the values of the extended vector are NA's as it may cause a crash. However, it should be the most accurate one in the opposite case (few NA's). When "auto" (the default), a hybrid of "spline" and "neighborhood" is applied. If the NA's constitute more than 20% of the extended vector, "spline" is used, otherwise "neighborhood". None of the above is applied to regions of equal length as there is no need for that.

Regarding the usage of `selector$id` field, this requires some careful usage, as if the ids present there and the ids of the genome areas do not match, there will be no genomic regions left to calculate coverage profiles on and the program will crash.

Regarding the usage of the `preprocessParams` argument, the `normalize` field controls how the

GRanges representing the reads extracted from BAM/BED files or the signals extracted from BigWig files will be normalized. When "none", no normalization is applied and external normalization is assumed. When "linear", all the library sizes are divided by the maximum one and a normalization factor is calculated for each sample. The coverage of this sample across the input genomic regions is then multiplied by this factor. When "downsample", all libraries are downsampled to the minimum library size among samples. When "sampleTo", all libraries are downsampled to a fixed number of reads. The "seed" field is used to define a seed for the generation of the random indices of reads to be removed for downsampling. It is required for reproducible results. The sampleTo field of preprocessParams tells recoup the fixed number of reads to downsample all libraries when preprocessParams\$normalize="sampleTo". It defaults to 1 million reads (1e+6). The spliceAction field of preprocessParams is used to control the action to be taken in the presence of RNA-Seq spliced reads (implies type="rnaseq"). When "keep", no action is performed regarding the spliced reads (represented as very long reads spanning intronic regions in the GRanges object). When "remove", these reads are excluded from coverage calculations according to their length as follows: firstly the length distribution of all reads lengths (using the width function for GRanges) is calculated. Then the quantile defined by the field spliceRemoveQ of preprocessParams is calculated and reads above the length corresponding to this quantile are excluded. When "splice", then splice junction information inferred from CIGAR strings (if) present in the BAM files is used to splice the longer reads and calculate real coverages. This option is not available with BED files, however, BED files can contain pre-spliced reads using for example BEDTools for conversion. It should also be noted that in the case of BigWig files, only linear normalization is supported as there is no information on raw reads. The cleanLevel field controls what filtering will be applied to the raw reads read from BAM/BED files prior to producing the signal track. It can have four values: 0 for no read processing/filtering, (use reads as they are, no uniqueness and no removal of unlocalized regions and mitochondrial DNA reads, unless filtered by the user before using recoup), 1 for removing unlocalized regions (e.g. chrU, hap, random etc.), 2 for removing reads of level 1 plus mitochondrial reads (chrM) and 3 for removing reads of level 2 plus using unique reads only. The default is level 0 (no filtering).

Regarding the heatmapFactor option of plotParams, it controls the color scale of the heatmap as follows: the default value (1) causes the extremes of the heatmap colors to be linearly and equally distributed across the actual coverage profile values. If set smaller than 1, the the upper extreme of the coverage values (which by default maps to the upper color point) is multiplied by this factor and this new value is set as the upper color break (limit). This has the effect of decreasing the brightness of the heatmap as color is saturated before reaching the maximum coverage value. If set greater than 1, then the heatmap brightness is increased. Regarding the correlation option of plotParams, if TRUE then recoup calculates average coverage values for each reference region (row-wise in the profile matrices) instead of the average coverage in each base of the reference regions (column-wise in the profile matrices). This is particularly useful for checking whether total genome profiles for some biological factor/condition correlate with each other. This potential correlation is becoming even clearer when orderBy\$what is not "none". Regarding the corrScale option of plotParams, it controls whether the average coverage curves over the set of reference genomic regions (one average coverage vale per genomic region, note the difference with the profiles where the coverage is calculated over the genomic locations themselves) should be normalized to a 0-1 scale or not. This is particularly useful when plotting data from different libraries (e.g. PolII and H3K27me1 occupancy over gene bodies) where other types of normalization (e.g. read downsampling cannot be applied). Regarding the corrSmoothPar option of plotParams, it controls the smoothing parameter for coverage correlation curves. If design is present, spline smoothing is applied ([smooth.spline](#)) with spar=0.5 else lowess smoothing is applied ([lowess](#)) with f=0.1. corrSmoothPar controls the spar and f respectively.

Regarding the usage of saveParams argument, this is useful for several purposes: one is for re-using recoup without re-reading BAM/BED/BigWig files. If the ranges are present in the input object to recoup, they are not re-calculated. If not stored, the memory/storage usage is reduced but the object

can be used only for simply replotting the profiles using `recoupProfile` and/or `recoupHeatmap` functions.

As a note regarding parallel calculations, the number of cores assigned to recoup depends both on the number of cores and the available RAM in your system. The most RAM expensive part of recoup is currently the construction of binned profile matrices. If you have a lot of cores (e.g. 16) but less than 128Gb of RAM for this number of cores, you should avoid using all cores, especially with large BAM files. Half of them would be more appropriate.

Finally, the output list of recoup can be provided as input again to recoup with some input parameters changed. recoup will then automatically recognize what has been changed and recalculate some, all or none of the genomic region profiles, depending on what input parameters have changed. For example, if any of the ordering options change (e.g. from no profile ordering to k-means clustering), then no recalculations are performed and the process is very fast. If region binning is changed (`binParams$flankBinSize` or `binParams$regionBinSize`), then only profile matrices are recalculated and coverages are maintained. If any of the `preprocessParams` changes, this causes all object including the short reads to be reimported and profiles recalculated from the beginning.

Value

a named list with five members:

- `data`: the input argument if it was a list or the resulting list from the unexported internal `readConfig` function, with the `ranges`, `coverage` and `profile` fields filled according to `saveParams`. This data member can be used again as an argument to `recoup`. The `coverage` and `profile` fields will be recalculated according to recoup parameters but the `ranges` will be reused if the input files are not changed.
- `design`: the design data frame which is used to facet the profiles.
- `plots`: the `ggplot2` and/or `Heatmap` objects created by recoup.
- `callopts`: the majority of recoup call parameters. Their storage serves the reuse of a recoup list object so that only certain elements of plots are recalculated.

Author(s)

Panagiotis Moulos

Examples

```
# Load some sample data
data("recoup_test_data", package="recoup")

# Note: the figures that will be produced will not look
# realistic and will be "bumpy". This is because package
# size limitations posed by Bioconductor guidelines do not
# allow for a full test dataset. Have a look at the
# vignette on how to test with more realistic data.

# TSS high resolution profile with no design
test.tss <- recoup(
  test.input,
  design=NULL,
  region="tss",
  type="chipseq",
  genome=test.genome,
```

```

    flank=c(2000,2000),
    selector=NULL,
    rc=0.5
)

# Genebody low resolution profile with 2-factor design,
# wide genebody and more narrow flanking
test.gb <- recoup(
  test.input,
  design=test.design,
  region="genebody",
  type="chipseq",
  genome=test.genome,
  flank=c(2000,2000),
  binParams=list(flankBinSize=50,regionBinSize=150),
  orderBy=list(what="hc1"),
  selector=NULL,
  rc=0.5
)

```

recoupCorrelation *Plot (faceted) average genomic coverage correlations*

Description

This function takes as input argument and output object from [recoup](#) and creates the average genomic curve correlations according to the options present in the input object. It can be used with saved recoup outputs so as to recreate the plots without re-reading BAM/BED files and re-calculating coverages.

Usage

```
recoupCorrelation(recoupObj, samples = NULL, rc = NULL)
```

Arguments

| | |
|-----------|--|
| recoupObj | a list object created from recoup . |
| samples | which samples to plot. Either numeric (denoting the sample indices) or sample ids. Defaults to NULL for all samples. |
| rc | fraction (0-1) of cores to use in a multicore system. It defaults to NULL (no parallelization). |

Value

The function returns the recoupObj with the slot for the correlation plot filled. See also the recoupPlot, getr and setr function.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data", package="recoup")

# Calculate coverages
test.tss <- recoup(
  test.input,
  design=NULL,
  region="tss",
  type="chipseq",
  genome=test.genome,
  flank=c(2000, 2000),
  selector=NULL,
  plotParams=list(profile=FALSE, correlation=TRUE,
    heatmap=FALSE),
  rc=0.5
)

# Plot coverage correlations
recoupCorrelation(test.tss, rc=0.5)
```

recoupHeatmap

Plot genomic coverage heatmaps

Description

This function takes as input argument and output object from [recoup](#) and creates heatmaps depicting genomic coverages using the `ComplexHeatmap` package and the options present in the input object. It can be used with saved recoup outputs so as to recreate the plots without re-reading BAM/BED files and re-calculating coverages.

Usage

```
recoupHeatmap(recoupObj, samples = NULL, rc = NULL)
```

Arguments

| | |
|-----------|--|
| recoupObj | a list object created from recoup . |
| samples | which samples to plot. Either numeric (denoting the sample indices) or sample ids. Defaults to NULL for all samples. |
| rc | fraction (0-1) of cores to use in a multicore system. It defaults to NULL (no parallelization). |

Value

The function returns the recoupObj with the slot for the profile plot filled. See also the `recoupPlot`, `getr` and `setr` function.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data", package="recoup")

# Calculate coverages
test.tss <- recoup(
  test.input,
  design=NULL,
  region="tss",
  type="chipseq",
  genome=test.genome,
  flank=c(2000, 2000),
  selector=NULL,
  plotParams=list(profile=FALSE, heatmap=FALSE),
  rc=0.5
)

# Plot coverage profiles
recoupHeatmap(test.tss, rc=0.5)
```

recoupPlot

Plot list objects returned by recoup

Description

This function takes as input argument an output object from [recoup](#) and plots the ggplot2 and ComplexHeatmap objects stored there.

Usage

```
recoupPlot(recoupObj, what = c("profile", "heatmap",
  "correlation"), device = c("x11", "png", "jpg", "tiff",
  "bmp", "pdf", "ps"), outputDir = ".",
  outputBase = paste(sapply(recoupObj, function(x)
    return(x$data$id)), sep = "_"), mainh = 1, ...)
```

Arguments

| | |
|------------|--|
| recoupObj | a list object created from recoup . |
| what | one or more of "profile", "heatmap" or "correlation". See the plotParams in the main recoup function. A minimum valid version is provided for default plotting. |
| device | a valid R graphics device. See the plotParams in the main recoup function. |
| outputDir | a valid directory when device is not "x11". See the plotParams in the main recoup function. |
| outputBase | a valid file name to be used as basis when device is not "x11". See the plotParams in the main recoup function. Defaults to a concatenation of sample ids. |
| mainh | the reference heatmap for ordering operations. Normally, calculated in recoup . See also the draw function in the ComplexHeatmap package. Defaults to the first heatmap. |
| ... | further parameters passed either to ggsave or the base graphics devices of R. |

Value

This function does not returns anything, just plots the recoup plots.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data", package="recoup")

# Calculate coverages
test.tss <- recoup(
  test.input,
  design=NULL,
  region="tss",
  type="chipseq",
  genome=test.genome,
  flank=c(2000, 2000),
  selector=NULL,
  plotParams=list(plot=FALSE, profile=TRUE,
    heatmap=TRUE, device="x11"),
  rc=0.5
)

# Plot coverage profiles
recoupPlot(test.tss)
```

recoupProfile

Plot (faceted) average genomic coverage profiles

Description

This function takes as input argument and output object from [recoup](#) and creates the average genomic curve profiles according to the options present in the input object. It can be used with saved recoup outputs so as to recreate the plots without re-reading BAM/BED files and re-calculating coverages.

Usage

```
recoupProfile(recoupObj, samples = NULL, rc = NULL)
```

Arguments

| | |
|-----------|--|
| recoupObj | a list object created from recoup . |
| samples | which samples to plot. Either numeric (denoting the sample indices) or sample ids. Defaults to NULL for all samples. |
| rc | fraction (0-1) of cores to use in a multicore system. It defaults to NULL (no parallelization). |

Value

The function returns the `recoupObj` with the slot for the profile plot filled. See also the `recoupPlot`, `getr` and `setr` function.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data", package="recoup")

# Calculate coverages
test.tss <- recoup(
  test.input,
  design=NULL,
  region="tss",
  type="chipseq",
  genome=test.genome,
  flank=c(2000, 2000),
  selector=NULL,
  plotParams=list(profile=FALSE, heatmap=FALSE),
  rc=0.5
)

# Plot coverage profiles
recoupProfile(test.tss, rc=0.5)
```

recoup_test_data

Reference and genomic sample regions for recoup testing

Description

The testing data package contains a small gene set, a design data frame, some genomic regions and an input object for testing of recoup with ChIP-Seq and RNA-Seq data. Specifically:

- `test.input`: A small data set which contains 10000 reads from H4K20me1 ChIP-Seq data from WT adult mice and Set8 (Pr-Set7) KO mice. The tissue is liver.
- `test.genome`: A small gene set (100 genes) and their coordinates from mouse mm9 chromosome 12.
- `test.design`: A data frame containing the 100 above genes categorized according to expression and strand.
- `test.exons`: A `GRangesList` containing the exons of the 100 above genes for use with recoup RNA-Seq mode.

Format

data.frame and list objects whose format is accepted by recoup.

Author(s)

Panagiotis Moulos

Source

Personal communication with the Talianidis lab at BSRC 'Alexander Fleming'. Unpublished data.

| | |
|------------|--|
| removeData | <i>Remove data from recoup list object</i> |
|------------|--|

Description

This function clears members of the `recoup` output object that must be cleared in order to apply a new set of parameters without completely rerunning `recoup`.

Usage

```
removeData(input, type = c("ranges", "coverage",  
                           "profile"))
```

Arguments

| | |
|-------|--|
| input | a list object created from <code>recoup</code> or its data member. |
| type | one of "ranges", "coverage", "profile". |

Details

This function clears members of the `recoup` output object which typically take some time to be calculated but it is necessary to clean them if the user wants to change input parameters that cause recalculations of these members. For example, if the user changes the `binParams`, the profile matrices ("profile" object member) have to be recalculated.

`type` controls what data will be removed. "ranges" removes the reads imported from BAM/BED files. This is useful when for example the normalization method is changed. "coverage" removes the calculated coverages over the reference genomic regions. This is required again when the normalization method changes. "profile" removes the profile matrices derived from coverages. This is required for example when the `binParams` main argument changes.

Value

A list which is normally the output of `recoup` without the members that have been removed from it.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data  
data("recoup_test_data", package="recoup")  
  
# Before removing  
names(test.input)  
  
# Remove a member
```

```
test.input <- removeData(test.input,"ranges")

# Removed
names(test.input)
```

simpleGetSet

Get and set some reusable objects from a recoup object

Description

The `getr` and `setr` functions are used to get several reusable/changeable objects of [recoup](#) or replace them (e.g. when the user wishes to change some `ggplot` or `ComplexHeatmap` parameters manually in a plot, or change the heatmap profile ordering mode).

Usage

```
getr(obj, key = c("design", "profile", "heatmap",
                 "correlation", "orderBy", "kmParams", "plotParams"))
setr(obj, key, value = NULL)
```

Arguments

| | |
|--------------------|--|
| <code>obj</code> | a list object created from recoup . |
| <code>key</code> | one of "design", "profile", "heatmap", "correlation", "orderBy", "kmParams", "plotParams". For "profile", "heatmap", the respective plots are retrieved or changed according to which function is called. For <code>setr</code> it can (and preferably) be a named list of arguments to be changed in the <code>recoup</code> list object. The list names are the same as above. For the rest, see the main recoup man page. |
| <code>value</code> | a valid <code>ggplot</code> or <code>HeatmapList</code> object created from recoupProfile or recoupHeatmap recoupCorrelation when changing plots. Values for all other types are also checked for validity. |

Value

For `getr`, the object asked to be retrieved. For `setr`, the `obj` with the respective slots filled or replaced with `value`.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data", package="recoup")

# Calculate coverages
test.tss <- recoup(
  test.input,
  design=NULL,
  region="tss",
  type="chipseq",
```

```

    genome=test.genome,
    flank=c(2000,2000),
    selector=NULL,
    plotParams=list(plot=FALSE,profile=TRUE,
                    heatmap=TRUE,device="x11"),
    rc=0.5
)

# Plot coverage profiles

# Get the curve profile plot
pp <- getr(test.tss,"profile")

# Change some ggplot parameter
pp <- pp +
  theme(axis.title.x=element_text(size=14))

# Store the new plot
test.tss <- setr(test.tss,"profile",pp)
## or even better
# test.tss <- setr(test.tss,list(profile=pp))

```

 sliceObj

Subset recoup output list objects

Description

This function takes as input argument an output object from [recoup](#) and subsets it according to the inputs *i*, *j*, *k*. The attached plots may or may not be recalculated. Other input parameters stored in `obj$callopts` are not changed apart from any selector option which is dropped. Note that when slicing vertically (by *j*), the `$coverage` member of the input data (if present) is **not** sliced, but remains as is. You can drop it using [removeData](#) as it is used to recalculate profile matrices only if bin sizes are changed in a [recoup](#) call.

Usage

```

sliceObj(obj, i = NULL, j = NULL, k = NULL,
         dropPlots = FALSE, rc = NULL)

```

Arguments

| | |
|------------------|--|
| <code>obj</code> | a list object created from recoup . |
| <code>i</code> | vector of numeric or character indices, corresponding to the index or rownames or names of reference genomic regions. The design object member will also be subset. If there is a selector attached to the input object (see recoup arguments) it will be dropped. |
| <code>j</code> | vector of numeric indices corresponding to the profile matrix vertical index (or base pair position or bin of base pairs) so as to subset the profile. The function will do its best to "guess" new plotting x-axis labels. |
| <code>k</code> | vector of numeric or character indices corresponding to sample index or sample names. These will be returned. |

`dropPlots` if profile and/or heatmap plots are attached to the input object, they will be recalculated if `dropPlots=="TRUE"` (default) or dropped otherwise.

`rc` fraction (0-1) of cores to use in a multicore system. It defaults to NULL (no parallelization).

Value

A recoup list object, subset according to `i`, `j`.

Author(s)

Panagiotis Moulos

Examples

```
# Load some data
data("recoup_test_data", package="recoup")

# Calculate coverages
test.tss <- recoup(
  test.input,
  design=NULL,
  region="tss",
  type="chipseq",
  genome=test.genome,
  flank=c(2000, 2000),
  selector=NULL,
  plotParams=list(plot=FALSE, profile=TRUE,
    heatmap=TRUE, device="x11"),
  rc=0.5
)

# Plot coverage profiles
o <- sliceObj(test.tss, i=1:10, k=1)
```

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