Package ‘ChromSCape’

January 2, 2024

**Title**  Analysis of single-cell epigenomics datasets with a Shiny App

**Version**  1.12.0

**Description**  ChromSCape - Chromatin landscape profiling for Single Cells - is a ready-to-launch user-friendly Shiny Application for the analysis of single-cell epigenomics datasets (scChIP-seq, scATAC-seq, scCUT&Tag, ...) from aligned data to differential analysis & gene set enrichment analysis. It is highly interactive, enables users to save their analysis and covers a wide range of analytical steps: QC, preprocessing, filtering, batch correction, dimensionality reduction, visualisation, clustering, differential analysis and gene set analysis.

**License**  GPL-3

**biocViews**  ShinyApps, Software, SingleCell, ChIPSeq, ATACSeq, MethylSeq, Classification, Clustering, Epigenetics, PrincipalComponent, SingleCell, ATACSeq, ChIPSeq, Annotation, BatchEffect, MultipleComparison, Normalization, Pathways, Preprocessing, QualityControl, ReportWriting, Visualization, GeneSetEnrichment, DifferentialPeakCalling

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annotation_from_merged_peaks

Find nearest peaks of each gene and return refined annotation

Description

Find nearest peaks of each gene and return refined annotation

Usage

annotation_from_merged_peaks(scExp, odir, merged_peaks, geneTSS_annotation)

Arguments

- scExp: A SingleCellExperiment object
- odir: An output directory where to write the mergedpeaks BED file
- merged_peaks: A list of GRanges object containing the merged peaks
- geneTSS_annotation: A GRanges object with reference genes

Value

A data.frame with refined annotation
Description

annotToCol2

Usage

annotToCol2(
    annotS = NULL,
    annotT = NULL,
    missing = c("", NA),
    anotype = NULL,
    maxnumcateg = 2,
    categCol = NULL,
    quantitCol = NULL,
    plotLegend = TRUE,
    plotLegendFile = NULL
)

Arguments

annotS    A color matrix
annotT    A color matrix
missing   Convert missing to NA
anotype   Annotation type
maxnumcateg Maximum number of categories
categCol  Categorical columns
quantitCol Quantitative columns
plotLegend Plot legend ?
plotLegendFile Which file to plot legend ?

Value

A matrix of continuous or discrete colors

Examples

data("scExp")
annotToCol2(SingleCellExperiment::colData(scExp), plotLegend = FALSE)
**anocol_binary**

**Description**
Helper binary column for anocol function

**Usage**

```r
anocol_binary(anocol, anotype, plotLegend, annotS)
```

**Arguments**

- `anocol`: The color feature matrix
- `anotype`: The feature types
- `plotLegend`: Plot legend?
- `annotS`: A color matrix

**Value**
A color matrix similar to anocol with binary columns colored

---

**anocol_categorical**

**Description**
Helper binary column for anocol function

**Usage**

```r
anocol_categorical(anocol, categCol, anotype, plotLegend, annotS)
```

**Arguments**

- `anocol`: The color feature matrix
- `categCol`: Colors for categorical features
- `anotype`: The feature types
- `plotLegend`: Plot legend?
- `annotS`: A color matrix

**Value**
A color matrix similar to anocol with binary columns colored
bams_to_matrix_indexes

Count bam files on interval to create count indexes

Description

Count bam files on interval to create count indexes

Usage

bams_to_matrix_indexes(dir, which, BPPARAM = BiocParallel::bpparam())

Arguments

dir A directory containing single cell BAM files and BAI files
which Genomic Range on which to count
BPPARAM BPPARAM object for multiprocessing. See bpparam for more informations. Will take the default BPPARAM set in your R session.

Value

A list containing a "feature index" data.frame and a count vector for non 0 entries, both used to form the sparse matrix

beds_to_matrix_indexes

Count bed files on interval to create count indexes

Description

Count bed files on interval to create count indexes

Usage

beds_to_matrix_indexes(dir, which, BPPARAM = BiocParallel::bpparam())

Arguments

dir A directory containing the single cell BED files
which Genomic Range on which to count
BPPARAM BPPARAM object for multiprocessing. See bpparam for more informations. Will take the default BPPARAM set in your R session.

Value

A list containing a "feature index" data.frame and a names of cells as vector both used to form the sparse matrix
**calculate_CNA**

*Estimate copy number alterations in cytobands*

**Description**

Cytobands are considered large enough in order that a variation at the cytoband level is not considered as an epigenetic event but as a genetic event, e.g. Copy Number Alterations. The function successively:

- Calculates the fraction of reads in each cytoband (FrCyto). See `calculate_cyto_mat`
- Calculates the log2-ratio FrCyto of each cell by the average FrCyto in normal cells. See `calculate_logRatio_CNA`
- Estimates if there was a gain or a loss of copy in each cytoband. See `calculate_gain_or_loss`

The corresponding matrices are accessibles in the reducedDim slots "cytoBands", "logRatio_cytoBands" and "gainOrLoss_cytoBands" respectively.

**Usage**

```r
calculate_CNA(
  scExp,
  control_samples = unique(scExp$sample_id)[1],
  ref_genome = c("hg38", "mm10")[1],
  quantiles_to_define_gol = c(0.05, 0.95)
)
```

**Arguments**

- `scExp` A SingleCellExperiment with "logRatio_cytoBand" reducedDim slot filled. See `calculate_logRatio_CNA`
- `control_samples` Sample IDs of the normal sample to take as reference.
- `ref_genome` Reference genome ("hg38" or "mm10")
- `quantiles_to_define_gol` Quantiles of normal log2-ratio distribution below/above which cytoband is considered to be a loss/gain. (c(0.05,0.95)). See `calculate_gain_or_loss`

**Value**

The SCE with the fraction of reads, log2-ratio and gain or loss in each cytobands in each cells (of dimension cell x cytoband) in the reducedDim slots.
**Examples**

data("scExp")
scExp = calculate_CNA(scExp, control_samples = unique(scExp$sample_id)[1],
ref_genome="hg38", quantiles_to_define_gol = c(0.05,0.95))
SingleCellExperiment::reducedDim(scExp, "cytoBand")
SingleCellExperiment::reducedDim(scExp, "logRatio_cytoBand")
SingleCellExperiment::reducedDim(scExp, "gainOrLoss_cytoBand")

---

**calculate_cyto_mat**  
*Calculate Fraction of reads in each cytobands*

**Description**

Re-Count binned reads onto cytobands and calculate the fraction of reads in each of the cytoband in each cell. For each cell, the fraction of reads in any given cytoband is calculated. Cytobands are considered large enough in order that a variation at the cytoband level is not considered as an epigenetic event but as a genetic event, e.g. Copy Number Alterations.

**Usage**

calculate_cyto_mat(scExp, ref_genome = c("hg38", "mm10")[1])

**Arguments**

- **scExp**: A SingleCellExperiment with genomic coordinate as features (peaks or bins)
- **ref_genome**: Reference genome ('hg38' or 'mm10')

**Value**

The SCE with the fraction of reads in each cytobands in each cells (of dimension cell x cytoband) in the reducedDim slot "cytoBand".

**Examples**

data("scExp")
scExp = calculate_cyto_mat(scExp, ref_genome="hg38")
SingleCellExperiment::reducedDim(scExp, "cytoBand")
**calculate_gain_or_loss**

*Estimate the copy gains/loss of tumor vs normal based on log2-ratio of fraction of reads*

**Description**

Given a SingleCellExperiment object with the slot "logRatio_cytoBand" containing the log2-ratio of the fraction of reads in each cytoband, estimate if the cytoband was lost or acquired a gain in a non-quantitative way. To do so, the quantiles distribution of the normal cells are calculated, and any cytoband below or above will be considered as a loss/gain. The False Discovery Rate is directly proportional to the quantiles.

**Usage**

```r
calculate_gain_or_loss(scExp, controls, quantiles = c(0.05, 0.95))
```

**Arguments**

- `scExp`: A SingleCellExperiment with "logRatio_cytoBand" reducedDim slot filled. See `calculate_logRatio_CNA`
- `controls`: Sample IDs or Cell IDs of the normal sample to take as reference.
- `quantiles`: Quantiles of normal log2-ratio distribution below/above which cytoband is considered to be a loss/gain. (c(0.05,0.95))

**Value**

The SCE with the gain or loss in each cytobands in each cells (of dimension cell x cytoband) in the reducedDim slot "gainOrLoss_cytoBand".

**Examples**

```r
data("scExp")
scExp = calculate_cyto_mat(scExp, ref_genome="hg38")
scExp = calculate_logRatio_CNA(scExp, controls=unique(scExp$sample_id)[1])
scExp = calculate_gain_or_loss(scExp, controls=unique(scExp$sample_id)[1])
SingleCellExperiment::reducedDim(scExp, "gainOrLoss_cytoBand")
```
calculate_logRatio_CNA

*Calculate the log2-ratio of tumor vs normal fraction of reads in cytobands*

**Description**

Given a SingleCellExperiment object with the slot "cytoBand" containing the fraction of reads in each cytoband, calculates the log2-ratio of tumor vs normal fraction of reads in cytobands, cell by cell. If the average signal in normal sample in a cytoband is 0, set this value to 1 so that the ratio won’t affect the fraction of read value.

**Usage**

```r
calculate_logRatio_CNA(scExp, controls)
```

**Arguments**

- `scExp` A SingleCellExperiment with "cytoBand" reducedDim slot filled.
  - see `calculate_cyto_mat`
- `controls` Sample IDs or Cell IDs of the normal sample to take as reference.

**Value**

The SCE with the log2-ratio of fraction of reads in each cytobands in each cells (of dimension cell x cytoband) in the reducedDim slot "logRatio_cytoBand".

**Examples**

```r
data("scExp")
scExp = calculate_cyto_mat(scExp, ref_genome="hg38")
scExp = calculate_logRatio_CNA(scExp, controls=unique(scExp$sample_id)[1])
SingleCellExperiment::reducedDim(scExp, "logRatio_cytoBand")
```

---

**call_macs2_merge_peaks**

*Calling MACS2 peak caller and merging resulting peaks*

**Description**

Calling MACS2 peak caller and merging resulting peaks
changeRange

Usage

call_macs2_merge_peaks(
  affectation,
  odir,
  p.value,
  format = c("scBED", "BAM")[1],
  ref,
  peak_distance_to_merge
)

Arguments

  affectation Annotation data.frame with cell cluster and cell id information
  odir Output directory to write MACS2 output
  p.value P value to detect peaks, passed to MACS2
  format File format, either "BAM" or "scBED"
  ref Reference genome to get chromosome information from.
  peak_distance_to_merge Distance to merge peaks

Value

  A list of merged GRanges peaks

changeRange

Description

changeRange

Usage

changeRange(v, newmin = 1, newmax = 10)

Arguments

  v A numeric vector
  newmin New min
  newmax New max

Value

  A matrix with values scaled between newmin and newmax
CheA3_TF_nTargets

A data.frame with the number of targets of each TF in ChEA3

Description

This data.frame was obtained by downloading datasets from ChEA3 database (https://maayanlab.cloud/chea3/) and merging targets for:

- ARCHS4_Coexpression
- ENCODE_ChIP-seq
- Enrichr_Queries
- GTEx_Coexpression
- Literature_ChIP-seq
- ReMap_ChIP-seq

Usage

```r
data("CheA3_TF_nTargets")
```

Format

CheA3_TF_nTargets - a data.frame with 1632 rows (unique TFs) and 2 columns

References


The data.frame is composed of two columns:

- TF column containing the TF gene names (human)
- nTargets_TF containing the number of targets for this TF in the combined database.

Examples

```r
data("CheA3_TF_nTargets")
head(CheA3_TF_nTargets)
```
check_correct_datamatrix

检查矩阵列名是否格式正确，必要时进行修正

Description

检查矩阵列名是否格式正确，必要时进行修正

Usage

check_correct_datamatrix(datamatrix_single, sample_name = "")

Arguments

datamatrix_single
  A sparse matrix

sample_name
  矩阵样本名称用于警告

Value

一个正确的sparseMatrix

choose_cluster_scExp

选择数量的簇

Description

此函数接受一个SingleCellExperiment对象和一个簇的数量作为输入，输出一个SingleCellExperiment对象，其中每个细胞被分配到一个相关性簇在colData。同时计算用ConsensusClusterPlus计算的共识关联的层次聚类。

Usage

choose_cluster_scExp(
  scExp,
  nclust = 3,
  consensus = FALSE,
  hc_linkage = "ward.D"
)

Arguments

scExp
  A SingleCellExperiment对象，包含consclust在元数据。

nclust
  选择的簇数（3）

consensus
  使用共识聚类结果而不是简单的层次聚类？（FALSE）

hc_linkage
  层次聚类的关联方法。见cor.（‘ward.D’）
Value

Returns a SingleCellExperiment object with each cell assigned to a correlation cluster in colData.

Examples

data("scExp")
scExp_cf = correlation_and_hierarchical_clust_scExp(scExp)
scExp_cf = choose_cluster_scExp(scExp_cf,nclust=3,consensus=FALSE)
table(scExp_cf$cell_cluster)

scExp_cf = consensus_clustering_scExp(scExp)
scExp_cf_consensus = choose_cluster_scExp(scExp_cf,nclust=3,consensus=TRUE)
table(scExp_cf_consensus$cell_cluster)

choose_perplexity  

Choose perplexity depending on number of cells for Tsne

Description

Choose perplexity depending on number of cells for Tsne

Usage

choose_perplexity(dataset)

Arguments

dataset A matrix of features x cells (rows x columns)

Value

A number between 5 and 30 to use in Rtsne function

col2hex  

Col2Hex

Description

Transform character color to hexadecimal color code.

Usage

col2hex(cname)
colors_scExp

Arguments

cname            Color name

Value

The HEX color code of a particular color

---

colors_scExp    Adding colors to cells & features

Description

Adding colors to cells & features

Usage

colors_scExp(
  scExp,
  annotCol = "sample_id",
  color_by = "sample_id",
  color_df = NULL
)

Arguments

scExp            A SingleCellExperiment Object
annotCol         Column names to color
color_by         If specifying color_df, column names to color
color_df         Color data.frame to specify which color for which condition

Value

A SingleCellExperiment with additionnal "color" columns in colData

Examples

data("scExp")
scExp = colors_scExp(scExp,annotCol = c("sample_id",
"total_counts"),
color_by = c("sample_id","total_counts"))

#Specific colors using a manually created data.frame :
color_df = data.frame(sample_id=unique(scExp$sample_id),
sample_id_color=c("red","blue","green","yellow"))
scExp = colors_scExp(scExp,annotCol="sample_id",
color_by="sample_id",color_df=color_df)
**combine_datamatrix**

Combine two matrices and emit warning if no regions are in common

**Description**

Combine two matrices and emit warning if no regions are in common

**Usage**

```r
combine_datamatrix(datamatrix, datamatrix_single, file_names, i)
```

**Arguments**

- `datamatrix`: A sparse matrix or NULL if empty
- `datamatrix_single`: Another sparse matrix
- `file_names`: File name corresponding to the matrix for warnings
- `i`: file number

**Value**

A combined sparse matrix

**combine_enrichmentTests**

Run enrichment tests and combine into list

**Description**

Run enrichment tests and combine into list

**Usage**

```r
combine_enrichmentTests(
  diff,
  enrichment_qval,
  qval.th,
  logFC.th,
  min.percent,
  annotFeat_long,
  peak_distance,
  refined_annotation,
  GeneSets,
  GeneSetsDf,
  GenePool,
  progress = NULL
)
```
comparable_variables

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>diff</td>
<td>Differential list</td>
</tr>
<tr>
<td>enrichment_qval</td>
<td>Adjusted p-value threshold above which a pathway is considered significative list</td>
</tr>
<tr>
<td>qval.th</td>
<td>Differential analysis adjusted p.value threshold</td>
</tr>
<tr>
<td>logFC.th</td>
<td>Differential analysis log-fold change threshold</td>
</tr>
<tr>
<td>min.percent</td>
<td>Minimum fraction of cells having the feature active to consider it as significantly differential. (0.01)</td>
</tr>
<tr>
<td>annotFeat_long</td>
<td>Long annotation</td>
</tr>
<tr>
<td>peak_distance</td>
<td>Maximum gene to peak distance</td>
</tr>
<tr>
<td>refined_annotation</td>
<td>Refined annotation data.frame if peak calling is done</td>
</tr>
<tr>
<td>GeneSets</td>
<td>List of pathways</td>
</tr>
<tr>
<td>GeneSetsDf</td>
<td>Data.frame of pathways</td>
</tr>
<tr>
<td>GenePool</td>
<td>Pool of possible genes for testing</td>
</tr>
<tr>
<td>progress</td>
<td>A shiny Progress instance to display progress bar.</td>
</tr>
</tbody>
</table>

Value

A list of list of pathway enrichment data.frames for Both / Over / Under and for each cluster

comparable_variables

Find comparable variable scExp

Description

Find comparable variable scExp

Usage

comparable_variables(scExp, allExp = TRUE)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>scExp</td>
<td>A SingleCellExperiment</td>
</tr>
<tr>
<td>allExp</td>
<td>A logical indicating wether alternative experiments comparable variables should also be fetch.</td>
</tr>
</tbody>
</table>

Value

A character vector with the comparable variable names
CompareedgeRGLM

Creates a summary table with the number of genes under- or overexpressed in each group and outputs several graphical representations

**Description**

Creates a summary table with the number of genes under- or overexpressed in each group and outputs several graphical representations

**Usage**

```r
CompareedgeRGLM(
  dataMat = NULL,
  annot = NULL,
  ref_group = NULL,
  groups = NULL,
  featureTab = NULL,
  norm_method = "TMMwsp"
)
```

**Arguments**

- `dataMat`: reads matrix
- `annot`: selected annotation of interest
- `ref_group`: List containing one or more vectors of reference samples. Name of the vectors will be used in the results table. The length of this list should be 1 or the same length as the `groups` list
- `groups`: List containing the IDs of groups to be compared with the reference samples. Names of the vectors will be used in the results table
- `featureTab`: Feature annotations to be added to the results table
- `norm_method`: Which method to use for normalizing ("upperquantile")

**Value**

A dataframe containing the foldchange and p.value of each feature

**Author(s)**

Eric Letouze & Celine Vallot

**Examples**

```r
data("scExp")
scExp_cf = correlation_and_hierarchical_clust_scExp(scExp)
scExp_cf = choose_cluster_scExp(scExp_cf,nclust=2,consensus=FALSE)
featureTab = as.data.frame(SummarizedExperiment::rowRanges(scExp_cf))
```
rownames(featureTab) = featureTab$ID
gerf_group = list("C1"=scExp_cf$cell_id[which(scExp_cf$cell_cluster=="C1")])
groups = list("C2"=scExp_cf$cell_id[which(scExp_cf$cell_cluster=="C2")])
myres = CompareedgeRGLM(as.matrix(SingleCellExperiment::counts(scExp_cf)),
annot=as.data.frame(SingleCellExperiment::colData(scExp_cf)),
ref_group=ref_group, groups=groups, featureTab=featureTab)

Description

CompareWilcox

Usage

CompareWilcox(
  dataMat = NULL,
  annot = NULL,
  ref_group = NULL,
  groups = NULL,
  featureTab = NULL,
  block = NULL,
  BPPARAM = BiocParallel::bpparam()
)

Arguments

dataMat A raw count matrix
annot A cell annotation data.frame
ref_group List with cells in reference group(s)
groups List with cells in group(s) to test
featureTab data.frame with feature annotation
block Use a blocking factor to counteract batch effect?
BPPARAM BPPARAM object for multiprocessing. See bpparam for more informations.
Will take the default BPPARAM set in your R session.

Value

A dataframe containing the foldchange and p.value of each feature

Author(s)

Eric Letouze & Celine Vallot & Pacome Prompsy
**concatenate_scBed_into_clusters**

*Concatenate single-cell BED into clusters*

**Description**

Concatenate single-cell BED into clusters

**Usage**

`concatenate_scBed_into_clusters(affectation, files_list, odir)`

**Arguments**

- `affectation` : Annotation data.frame containing cluster information
- `files_list` : Named list of scBED file paths to concatenate. List Names must match affectation$sample_id and basenames must match affectation$barcode.
- `odir` : Output directory to write concatenate pseudo-bulk BEDs.

**Value**

Merge single-cell BED files into cluster BED files. Ungzip file if BED is gzipped.
**consensus_clustering_scExp**

Wrapper to apply ConsensusClusterPlus to scExp object

**Description**


**Usage**

```r
consensus_clustering_scExp(
  scExp,
  prefix = NULL,
  maxK = 10,
  reps = 100,
  pItem = 0.8,
  pFeature = 1,
  distance = "pearson",
  clusterAlg = "hc",
  innerLinkage = "ward.D",
  finalLinkage = "ward.D",
  plot_consclust = "pdf",
  plot_icl = "png"
)
```

**Arguments**

- **scExp**: A SingleCellExperiment object containing 'PCA' in reducedDims.
- **prefix**: character value for output directory. Directory is created only if plot_consclust is not NULL. This title can be an absolute or relative path.
- **maxK**: integer value. maximum cluster number to evaluate. (10)
- **reps**: integer value. number of subsamples. (100)
- **pItem**: numerical value. proportion of items to sample. (0.8)
- **pFeature**: numerical value. proportion of features to sample. (1)
- **distance**: character value. 'pearson': (1 - Pearson correlation), 'spearman' (1 - Spearman correlation), 'euclidean', 'binary', 'maximum', 'canberra', 'minkowski' or custom distance function. ('pearson')
- **clusterAlg**: character value. cluster algorithm. 'hc' hierarchical (hclust), 'pam' for partitioning around medoids, 'km' for k-means upon data matrix, 'kmdist' ('hc') for k-means upon distance matrices (former km option), or a function that returns a clustering. ('hc')
correlation_and_hierarchical_clust_scExp

innerLinkage  hierarchical linkage method for subsampling. ('ward.D')
finalLinkage  hierarchical linkage method for consensus matrix. ('ward.D')
plot_consclust character value. NULL - print to screen, 'pdf', 'png', 'pngBMP' for bitmap png, helpful for large datasets. ('pdf')
plot_icl      same as above for item consensus plot. ('png')

Details

This function takes as input a SingleCellExperiment object that must have 'PCA' in reducedDims and outputs a SingleCellExperiment object containing consclust list calculated cluster consensus and item consensus scores in metadata.

Value

Returns a SingleCellExperiment object containing consclust list, calculated cluster consensus and item consensus scores in metadata.

References


Examples

data("scExp")
scExp_cf = correlation_and_hierarchical_clust_scExp(scExp)
scExp_cf = consensus_clustering_scExp(scExp)

Description

Calculates cell to cell correlation matrix based on the PCA feature space and runs hierarchical clustering taking 1 - correlation scores as distance.

Usage

correlation_and_hierarchical_clust_scExp(scExp, hc_linkage = "ward.D")

Arguments

scExp        A SingleCellExperiment object, containing 'PCA' in reducedDims.
hc_linkage   A linkage method for hierarchical clustering. See cor. ('ward.D')
count_coverage

Details
This function takes as input a SingleCellExperiment object that must have PCA calculated and outputs a SingleCellExperiment object with correlation matrix and hierarchical clustering.

Value
Return a SingleCellExperiment object with correlation matrix & hierarchical clustering.

Examples
```r
data("scExp")
scExp_cf = correlation_and_hierarchical_clust_scExp(scExp)
```

count_coverage

Create a smoothed and normalized coverage track from a BAM file and given a bin GenomicRanges object (same as deepTools bamCoverage)

Description
Normalization is CPM, smoothing is done by averaging on n_smoothBin regions left and right of any given region.

Usage
```r
count_coverage(
  input,
  format = "BAM",
  bins,
  canonical_chr,
  norm_factor,
  n_smoothBin = 5,
  ref = "hg38",
  read_size = 101,
  original_bins = NULL
)
```

Arguments
- **input**: Either a named list of character vector of path towards single-cell BED files or a sparse raw matrix of small bins (<500bp). If a named list specifying scBEDn the names MUST correspond to the 'sample_id' column in your SingleCellExperiment object. The single-cell BED files names MUST match the barcode names in your SingleCellExperiment (column 'barcode'). The scBED files can be gzipped or not.
- **format**: File format, either "BAM" or "BED"
- **bins**: A GenomicRanges object of binned genome
**create_project_folder**  

Create ChromSCape project folder

**Description**  

Creates a project folder that will be recognizable by ChromSCape Shiny application.

**Usage**  

```r
create_project_folder(
  output_directory,
  analysis_name = "Analysis_1",
  ref_genome = c("hg38", "mm10")[[1]]
)
```

**Arguments**

- `output_directory`  
  Path towards the directory to create the 'ChromSCape_Analyses' folder and the analysis subfolder. If this path already contains the 'ChromSCape_Analyses' folder, will only create the analysis subfolder.

- `analysis_name`  
  Name of the analysis. Must only contain alphanumerical characters or '_'.

- `ref_genome`  
  Reference genome, either 'hg38' or 'mm10'.

**Value**  

Creates the project folder and returns the root of the project.

**Examples**  

dir = tempdir()
create_project_folder(output_directory = dir,
  analysis_name = "Analysis_1")
list.dirs(file.path(dir))
```
create_sample_name_mat

Create a sample name matrix

Description
Create a sample name matrix

Usage
create_sample_name_mat(nb_samples, samples_names)

Arguments
- nb_samples: Number of samples
- samples_names: Character vector of sample names

Value
A matrix

create_scDataset_raw
Create a simulated single cell datamatrix & cell annotation

Description
Create a simulated single cell datamatrix & cell annotation

Usage
create_scDataset_raw(
cells = 300,
features = 600,
featureType = c("window", "peak", "gene"),
sparse = TRUE,
nsamp = 4,
ref = "hg38",
batch_id = factor(rep(1, nsamp))
)
create_scExp

Wrapper to create the single cell experiment from count matrix and feature dataframe

## Arguments

cells Number of cells (300)
features Number of features (600)
featureType Type of feature (window)
sparse Is matrix sparse? (TRUE)
nsamp Number of samples (4)
ref Reference genome (‘hg38’)
batch_id Batch origin (factor((1,1,1,1)))

## Value

A list composed of
* mat : a sparse matrix following an approximation of the negative binomial law (adapted to scChIPseq)
* annot : a data.frame of cell annotation
* batches : an integer vector with the batch number for each cell

## Examples

```r
# Creating a basic sparse 600 genomic bins x 300 cells matrix and annotation
dl = create_scDataset_raw()
head(l(mat))
head(l(annot))
head(l(batches))

# Specifying number of cells, features and samples
l2 = create_scDataset_raw(cells = 500, features = 500, nsamp=2)

# Specifying species
mouse_l = create_scDataset_raw(ref="mm10")

# Specifying batches
batch_l = create_scDataset_raw(nsamp=4, batch_id = factor(c(1,1,2,2)))

# Peaks of different size as features
peak_l = create_scDataset_raw(featureType="peak")
head(peak_l(mat))

# Genes as features
gene_l = create_scDataset_raw(featureType="gene")
head(gene_l(mat))
```
create_scExp

Description
Create the single cell experiment from (sparse) datamatrix and feature dataframe containing feature names and location. Also optionally removes zero count Features, zero count Cells, non canonical chromosomes, and chromosome M. Calculates QC Metrics (scran).

Usage
create_scExp(
  datamatrix,
  annot,
  remove_zero_cells = TRUE,
  remove_zero_features = TRUE,
  remove_non_canonical = TRUE,
  remove_chr_M = TRUE,
  mainExpName = "main",
  verbose = TRUE
)

Arguments
  datamatrix       A matrix or sparseMatrix of raw counts. Features x Cells (rows x columns).
  annot           A data.frame containing informations on cells. Should have the same number of rows as the number of columns in datamatrix.
  remove_zero_cells remove cells with zero counts ? (TRUE)
  remove_zero_features remove cells with zero counts ? (TRUE)
  remove_non_canonical remove non canonical chromosomes ?(TRUE)
  remove_chr_M    remove chromosomes M ? (TRUE)
  mainExpName     Name of the mainExpName e.g. 'bins', 'peaks'... ("default")
  verbose         (TRUE)

Value
Returns a SingleCellExperiment object.

Examples
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp
Differential Analysis Custom in 'One vs One' mode

**Description**
Differential Analysis Custom in 'One vs One' mode

**Usage**
```r
DA_custom(
  affectation,
  by,
  counts,
  method,
  feature,
  block,
  ref,
  group,
  progress = NULL,
  BPPARAM = BiocParallel::bpparam()
)
```

**Arguments**
- `affectation`: An annotation data.frame with cell_id and
- `by`: A character specifying the column of the object containing the groups of cells to compare.
- `counts`: Count matrix
- `method`: DA method: Wilcoxon or EdgeR
- `feature`: Feature tables
- `block`: Blocking feature
- `ref`: If de_type is custom, the reference to compare (data.frame), must be a one-column data.frame with cell_clusters or sample_id as character in rows
- `group`: If de_type is custom, the group to compare (data.frame), must be a one-column data.frame with cell_clusters or sample_id as character in rows
- `progress`: A shiny Progress instance to display progress bar.
- `BPPARAM`: BPPARAM object for multiprocessing. See bpparam for more informations. Will take the default BPPARAM set in your R session.

**Value**
A list of results, groups compared and references
Differential Analysis in 'One vs Rest' mode

Description

Differential Analysis in 'One vs Rest' mode

Usage

DA_one_vs_rest(
  affectation,
  by, 
  counts,
  method,
  feature,
  block,
  progress = NULL, 
  BPPARAM = BiocParallel::bpparam()
)

Arguments

affectation An annotation data.frame with cell_id and cell_cluster columns

by = A character specifying the column of the object containing the groups of cells to compare.

counts Count matrix

method DA method : Wilcoxon or EdgeR

feature Feature tables

block Blocking feature

progress A shiny Progress instance to display progress bar.

BPPARAM BPPARAM object for multiprocessing. See bpparam for more informations. Will take the default BPPARAM set in your R session.

Value

A list of results, groups compared and references
DA_pairwise  

**Run differential analysis in Pairwise mode**

**Description**

Run differential analysis in Pairwise mode

**Usage**

```r
DA_pairwise(
  affectation,
  by,
  counts,
  method,
  feature,
  block,
  progress = NULL,
  BPPARAM = BiocParallel::bpparam()
)
```

**Arguments**

- **affectation**: An annotation data.frame with cell_cluster and cell_id columns
- **by**: A character specifying the column of the object containing the groups of cells to compare.
- **counts**: Count matrix
- **method**: DA method, Wilcoxon or edgeR
- **feature**: Feature data.frame
- **block**: Blocking feature
- **progress**: A shiny Progress instance to display progress bar.
- **BPPARAM**: BPPARAM object for multiprocessing. See bpparam for more informations. Will take the default BPPARAM set in your R session.

**Value**

A list of results, groups compared and references
**define_feature**

*Define the features on which reads will be counted*

**Description**

Define the features on which reads will be counted

**Usage**

```r
define_feature(ref = c("hg38","mm10")[1],
               peak_file = NULL,
               bin_width = NULL,
               genebody = FALSE,
               extendPromoter = 2500)
```

**Arguments**

- `ref` Reference genome
- `peak_file` A bed file if counting on peaks
- `bin_width` A number of bins if dividing genome into fixed width bins
- `genebody` A logical indicating if feature should be counted in genebodies and promoter.
- `extendPromoter` Extension length before TSS (2500).

**Value**

A GRanges object

**Examples**

```r
gr_bins = define_feature("hg38", bin_width = 50000)
gr_genes = define_feature("hg38", genebody = TRUE, extendPromoter = 5000)
```

**detect_samples**

*Heuristic discovery of samples based on cell labels*

**Description**

Identify a fixed number of common string (samples) in a set of varying strings (cells). E.g. in the set "Sample1_cell1","Sample1_cell2","Sample2_cell1","Sample2_cell2" and with `nb_samples`=2, the function returns "Sample1","Sample1","Sample2","Sample2".

**Usage**

```r
detect_samples(barcodes, nb_samples = 1)
```
**differential_activation**

**Arguments**

- **barcodes**  
  Vector of cell barcode names (e.g., Sample1_cell1, Sample1_cell2...)

- **nb_samples**  
  Number of samples to find

**Value**

character vector of sample names the same length as cell labels

**Examples**

```r
barcodes = c(paste0("HBCx22_BC_.", seq_len(100)),
paste0("mouse_sample_XX",208:397))
samples = detect_samples(barcodes, nb_samples=2)
```

**Description**

Based on the statement that single-cell epigenomic datasets are very sparse, specifically when analyzing small bins or peaks, we can define each feature as being 'active' or not simply by the presence or the absence of reads in this feature. This is the equivalent of binarize the data. When trying to find differences in signal for a feature between multiple cell groups, this function simply compare the percentage of cells 'activating' the feature in each of the group. The p.values are then calculated using a Pearson’s Chi-squared Test for Count Data (comparing the number of active cells in one group vs the other) and corrected using Benjamini-Hochberg correction for multiple testing.

**Usage**

```r
differential_activation(
  scExp,
  by = c("cell_cluster", "sample_id")[1],
  verbose = TRUE,
  progress = NULL
)
```

**Arguments**

- **scExp**  
  A SingleCellExperiment object containing consclus with selected number of cluster.

- **by**  
  Which grouping to run the marker enrichment?

- **verbose**  
  Print?

- **progress**  
  A shiny Progress instance to display progress bar.
differential_analysis_scExp

Details

To calculate the logFC, the percentage of activation of the features are corrected for total number of
reads to correct for library size bias. For each cluster (‘group’) the function consider the rest of the
cells as the reference.

Value

Returns a dataframe of differential activation results that contains the rowData of the SingleCellEx-
periment with additional logFC, q.value, group activation (fraction of cells active for each feature in
the group cells), reference activation (fraction of cells active for each feature in the reference cells).

See Also

For Pearson’s Chi-squared Test for Count Data chisq.test. For other differential analysis see differential_analysis_scExp.

Examples

data("scExp")
res = differential_activation(scExp, by = "cell_cluster")
res = differential_activation(scExp, by = "sample_id")

---

differential_analysis_scExp

Runs differential analysis between cell clusters

Description

Based on clusters of cell defined previously, runs non-parametric Wilcoxon Rank Sum test to find
significantly depleted or enriched features, in ‘one_vs_rest’ mode or ‘pairwise’ mode. In pairwise
mode, each cluster is compared to all other cluster individually, and then pairwise comparisons
between clusters are combined to find overall differential features using combineMarkers function
from scran.

Usage

differential_analysis_scExp(
  scExp,
  de_type = c("one_vs_rest_fast", "one_vs_rest", "pairwise", "custom")[[1]],
  by = "cell_cluster",
  method = "wilcox",
  block = NULL,
  group = NULL,
  ref = NULL,
  prioritize_genes = nrow(scExp) > 20000,
  max_distanceToTSS = 1000,
  progress = NULL,
  BPPARAM = BiocParallel::bpparam()
)
differential_analysis_scExp

Arguments

scExp A SingleCellExperiment object containing consclust with selected number of cluster.

de_type Type of comparisons. Either 'one_vs_rest', to compare each cluster against all others, or 'pairwise' to make 1 to 1 comparisons. ('one_vs_rest')

by = A character specifying the column of the object containing the groups of cells to compare. Exclusive with de_type == custom

method Differential testing method, either 'wilcox' for Wilcoxon non-parametric testing or 'neg.binomial' for edgerGLM based testing. ('wilcox')

block Use batches as blocking factors ? If TRUE, block will be taken as the column "batch_id" from the SCE. Cells will be compared only within samples belonging to the same batch.

group If de_type = "custom", the sample / cluster of interest as a one-column data.frame. The name of the column is the group name and the values are character either cluster ("C1", "C2", ...) or sample_id.

ref If de_type = "custom", the sample / cluster of reference as a one-column data.frame. The name of the column is the group name and the values are character either cluster ("C1", "C2", ...) or sample_id.

prioritize_genes First filter by loci being close to genes ? E.g. for differential analysis, it is more relevant to keep features close to genes

max_distanceToTSS If prioritize_genes is TRUE, the maximum distance to consider a feature close to a gene.

progress A shiny Progress instance to display progress bar.

BPPARAM BPPARAM object for multiprocessing. See bpparam for more informations. Will take the default BPPARAM set in your R session.

Details

This functions takes as input a SingleCellExperiment object with consclust, the type of comparison, either 'one_vs_rest' or 'pairwise', the adjusted p-value threshold (qval.th) and the fold-change threshold (logFC.th). It outputs a SingleCellExperiment object containing a differential list.

Value

Returns a SingleCellExperiment object containing a differential list.

Examples

data("scExp")
scExp_cf = differential_analysis_scExp(scExp)
distPearson

Description
distPearson

Usage
distPearson(m)

Arguments
m A matrix

Value
A dist object

enrichmentTest

Description
enrichmentTest

Usage
enrichmentTest(gene.sets, mylist, possibleIds, sep = ";", silent = FALSE)

Arguments
gene.sets A list of reference gene sets
mylist A list of genes to test
possibleIds All existing genes
sep Separator used to collapse genes
silent Silent mode ?

Value
A dataframe with the gene sets and their enrichment p.value
enrich_TF_ChEA3_genes   Find the TF that are enriched in the differential genes using ChEA3 API

Description
Find the TF that are enriched in the differential genes using ChEA3 API

Usage
enrich_TF_ChEA3_genes(genes)

Arguments

genes        A character vector with the name of genes to enrich for TF.

Value
Returns a SingleCellExperiment object containing list of enriched Gene Sets for each cluster, either in depleted features, enriched features or simply differential features (both).

References

Examples

data(scExp)
enrich_TF_ChEA3_genes(head(unlist(strsplit(SummarizedExperiment::rowData(scExp)$Gene, split = ",", fixed = TRUE)))

enrich_TF_ChEA3_scExp   Find the TF that are enriched in the differential genes using ChEA3 database

Description
Find the TF that are enriched in the differential genes using ChEA3 database
Usage

```r
enrich_TF_ChEA3_scExp(
  scExp,  
  ref = "hg38",  
  qval.th = 0.01,  
  logFC.th = 1,  
  min.percent = 0.01,  
  peak_distance = 1000,  
  use_peaks = FALSE,  
  progress = NULL,  
  verbose = TRUE
)
```

Arguments

- `scExp`: A SingleCellExperiment object containing list of differential features.
- `ref`: A reference annotation, either 'hg38' or 'mm10'. ('hg38')
- `qval.th`: Adjusted p-value threshold to define differential features. (0.01)
- `logFC.th`: Fold change threshold to define differential features. (1)
- `min.percent`: Minimum fraction of cells having the feature active to consider it as significantly differential. (0.01)
- `peak_distance`: Maximum distanceToTSS of feature to gene TSS to consider associated, in bp. (1000)
- `use_peaks`: Use peak calling method (must be calculated beforehand). (FALSE)
- `progress`: A shiny Progress instance to display progress bar.
- `verbose`: A logical to print message or not. (TRUE)

Value

Returns a SingleCellExperiment object containing list of enriched Gene Sets for each cluster, either in depleted features, enriched features or simply differential features (both).

Examples

```r
data("scExp")

scExp = enrich_TF_ChEA3_scExp(
  scExp,  
  ref = "hg38",  
  qval.th = 0.01,  
  logFC.th = 1,  
  min.percent = 0.01)```
exclude_features_scExp

Remove specific features (CNA, repeats)

Description

Remove specific features (CNA, repeats)

Usage

```
exclude_features_scExp(
  scExp,
  features_to_exclude,
  by = "region",
  verbose = TRUE
)
```

Arguments

- `scExp` A SingleCellExperiment object.
- `features_to_exclude` A GenomicRanges object or data.frame containing genomic regions or features to exclude or path towards a BED file containing the features to exclude.
- `by` Type of features. Either 'region' or 'feature_name'. If 'region', will look for genomic coordinates in columns 1-3 (chr,start,stop). If 'feature_name', will look for a genes in first column. ('region')
- `verbose` (TRUE)

Value

A SingleCellExperiment object without features to exclude.

Examples

```
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
features_to_exclude = data.frame(chr=c("chr4","chr7","chr17"),
  start=c(50000,8000000,2000000),
  end=c(100000,16000000,2500000))
features_to_exclude = as(features_to_exclude,"GRanges")
scExp = exclude_features_scExp(scExp,features_to_exclude)
scExp
```
feature_annotation_scExp

Add gene annotations to features

Description

Add gene annotations to features

Usage

feature_annotation_scExp(scExp, ref = "hg38", reference_annotation = NULL)

Arguments

scExp A SingleCellExperiment object.
ref Reference genome. Either 'hg38' or 'mm10'. ('hg38')
reference_annotation A data.frame containing gene (or else) annotation with genomic coordinates.

Value

A SingleCellExperiment object with annotated rowData.

Examples

raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp = feature_annotation_scExp(scExp)
head(SummarizedExperiment::rowRanges(scExp))

# Mouse
raw = create_scDataset_raw(ref = "mm10")
scExp = create_scExp(raw$mat, raw$annot)
scExp = feature_annotation_scExp(scExp,ref="mm10")
head(SummarizedExperiment::rowRanges(scExp))

filter_correlated_cell_scExp

Filter lowly correlated cells

Description

Remove cells that have a correlation score lower than what would be expected by chance with other cells.
filter_correlated_cell_scExp

Usage

```r
filter_correlated_cell_scExp(scExp, random_iter = 5,
corr_threshold = 99, percent_correlation = 1,
downsamle = 2500, verbose = TRUE, n_process = 250,
BPPARAM = BiocParallel::bpparam())
```

Arguments

- `scExp`: A SingleCellExperiment object containing 'Cor', a correlation matrix, in reducedDims.
- `random_iter`: Number of random matrices to create to calculate random correlation scores. (50)
- `corr_threshold`: Quantile of random correlation score above which a cell is considered to be 'correlated' with another cell. (99)
- `percent_correlation`: Percentage of the cells that any cell must be 'correlated' to in order to not be filtered. (1)
- `downsample`: Number of cells to calculate correlation filtering threshold. (2500)
- `verbose`: Print messages. (TRUE)
- `n_process`: Number of cell to proceed at a time. Increase this number to increase speed at memory cost
- `BPPARAM`: BPPARAM object for multiprocessing. See bpparam for more informations. Will take the default BPPARAM set in your R session.

Details

This function takes as input a SingleCellExperiment object that must have correlation matrix calculated and outputs a SingleCellExperiment object without lowly correlated cells. TSNE is recalculated.

Value

Returns a SingleCellExperiment object without lowly correlated cells. The calculated correlation score limit threshold is saved in metadata.

Examples

```r
data("scExp")
dim(scExp)
scExp_cf = filter_correlated_cell_scExp(scExp,
corr_threshold = 99, percent_correlation = 1)
dim(scExp_cf)
```
filter_genes_with_refined_peak_annotation

Filter genes based on peak calling refined annotation

Description

Filter genes based on peak calling refined annotation

Usage

filter_genes_with_refined_peak_annotation(
  refined_annotation,
  peak_distance,
  signific,
  over,
  under
)

Arguments

refined_annotation
  A data.frame containing each gene distance to real peak
peak_distance
  Minimum distance to an existing peak to accept a given gene
signific
  Indexes of all significantly differential genes
over
  Indexes of all significantly overexpressed genes
under
  Indexes of all significantly underexpressed genes

Value

List of significantly differential, overexpressed and underexpressed genes close enough to existing peaks

filter_scExp

Filter cells and features

Description

Function to filter out cells & features from SingleCellExperiment based on total count per cell, number of cells 'ON' in features and top covered cells that might be doublets.
find_clusters_louvain_scExp

Usage

filter_scExp(
  scExp,
  min_cov_cell = 1600,
  quant_removal = 95,
  min_count_per_feature = 10,
  verbose = TRUE
)

Arguments

scExp A SingleCellExperiment object.
min_cov_cell Minimum counts for each cell. (1600)
quant_removal Centile of cell counts above which cells are removed. (95)
min_count_per_feature Minimum number of reads per feature (10).
verbose (TRUE)

Value

Returns a filtered SingleCellExperiment object.

Examples

raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp. = filter_scExp(scExp)

# No feature filtering (all features are valuable)
scExp. = filter_scExp(scExp,min_count_per_feature=30)

# No cell filtering (all features are valuable)
scExp. = filter_scExp(scExp,min_cov_cell=0,quant_removal=100)

find_clusters_louvain_scExp

Build SNN graph and find cluster using Louvain Algorithm

Description

Build SNN graph and find cluster using Louvain Algorithm
find_top_features

Usage

```r
find_clusters_louvain_scExp(
  scExp,
  k = 10,
  resolution = 1,
  use.dimred = "PCA",
  type = c("rank", "number", "jaccard")[3],
  BPPARAM = BiocParallel::bpparam()
)
```

Arguments

- `scExp`: A SingleCellExperiment with PCA calculated
- `k`: An integer scalar specifying the number of nearest neighbors to consider during graph construction.
- `resolution`: A numeric specifying the resolution of clustering to pass to igraph::cluster_louvain function.
- `use.dimred`: A string specifying the dimensionality reduction to use.
- `type`: A string specifying the type of weighting scheme to use for shared neighbors.
- `BPPARAM`: BPPARAM object for multiprocessing. See bpparam for more informations. Will take the default BPPARAM set in your R session.

Value

A SingleCellExperiment containing the vector of clusters (named C1, C2 ....)

Examples

```r
data('scExp')

scExp = find_clusters_louvain_scExp(scExp, k = 10)
```

find_top_features

Find most covered features

Description

Find the top most covered features that will be used for dimensionality reduction. Optionally remove non-top features.
Usage

find_top_features(
    scExp,
    n = 20000,
    keep_others = FALSE,
    prioritize_genes = FALSE,
    max_distanceToTSS = 10000,
    verbose = TRUE
)

Arguments

scExp A SingleCellExperiment.

n Either an integer indicating the number of top covered regions to find or a character vector of the top percentile of features to keep (e.g. 'q20' to keep top 20% features).

keep_others Logical indicating if non-top regions are to be removed from the SCE or not (FALSE).

prioritize_genes First filter by loci being close to genes? E.g. for differential analysis, it is more relevant to keep features close to genes.

max_distanceToTSS If prioritize_genes is TRUE, the maximum distance to consider a feature close to a gene.

verbose Print?

Value

A SCE with top features

Examples

data(scExp)
scExp_top = find_top_features(scExp, n = 4000, keep_others = FALSE)

generate_analysis Generate a complete ChromSCape analysis

Description

Generate a complete ChromSCape analysis
generate_analysis

Usage

```r
generate_analysis(input_data_folder, 
                  analysis_name = "Analysis_1", 
                  output_directory = "/", 
                  input_data_type = c("scBED", "DenseMatrix", "SparseMatrix", "scBAM")[[1]], 
                  rebin_sparse_matrix = FALSE, 
                  feature_count_on = c("bins", "genebody", "peaks")[[1]], 
                  feature_count_parameter = 50000, 
                  ref_genome = c("hg38", "mm10")[[1]], 
                  run = c("filter", "CNA", "cluster", "consensus", "peak_call", "coverage", 
                          "DA", "GSA", "report")[[1,3,6,7,8,9]], 
                  min_reads_per_cell = 1000, 
                  max_quantile_read_per_cell = 99, 
                  n_top_features = 40000, 
                  norm_type = "CPM", 
                  subsample_n = NULL, 
                  exclude_regions = NULL, 
                  n_clust = NULL, 
                  corr_threshold = 99, 
                  percent_correlation = 1, 
                  maxK = 10, 
                  qval.th = 0.1, 
                  logFC.th = 1, 
                  enrichment_qval = 0.1, 
                  doBatchCorr = FALSE, 
                  batch_sels = NULL, 
                  control_samples_CNA = NULL, 
                  genes_to_plot = c("Krt8", "Krt5", "Tgfb1", "Foxq1", "Cdkn2b", 
                                    "Cdkn2a", "chr7:15000000-20000000")
)
```

Arguments

- **input_data_folder**: Directory containing the input data.
- **analysis_name**: Name given to the analysis.
- **output_directory**: Directory where to create the analysis and the HTML report.
- **input_data_type**: The type of input data.
- **feature_count_on**: For raw data type, on which features to count the cells.
- **feature_count_parameter**: Additional parameter corresponding to the 'feature_count_on' parameter. E.g. for 'bins' must be a numeric, e.g. 50000, for 'peaks' must be a character containing path towards a BED peak file.
generate_analysis

rebin_sparse_matrix
A boolean specifying if the SparseMatrix should be rebinned on features (see feature_count_on and feature_count_parameter).

ref_genome The genome of reference.

run What steps to run. By default runs everything. Some steps are required in order to run downstream steps.

min_reads_per_cell Minimum number of reads per cell.

max_quantile_read_per_cell Upper quantile above which to consider cells doublets.

n_top_features Number of features to keep in the analysis.

norm_type Normalization type.

subsample_n Number of cells per condition to downsample to, for performance principally.

exclude_regions Path towards a BED file containing CNA to exclude from the analysis (optional).

n_clust Number of clusters to force choice of clusters.

corr_threshold Quantile of correlation above which two cells are considered as correlated.

percent_correlation Percentage of the total cells that a cell must be correlated with in order to be kept in the analysis.

maxK Upper cluster number to rest for ConsensusClusterPlus.

qval.th Adjusted p-value below which to consider features differential.

logFC.th Log2-fold-change above/below which to consider a feature depleted/enriched.

enrichment_qval Adjusted p-value below which to consider a gene set as significantly enriched in differential features.

doBatchCorr Logical indicating if batch correction using fastMNN should be run.

batch_sels If doBatchCorr is TRUE, a named list containing the samples in each batch.

control_samples_CNA If running CopyNumber Analysis, a character vector of the sample names that are 'normal'.

genes_to_plot A character vector containing genes of interest of which to plot the coverage.

Value

Creates a ChromSCape-readable directory and saved objects, as well as a multi-tabbed HTML report resuming the analysis.

Examples

## Not run:
generate_analysis("/path/to/data/", "Analysis_1")

## End(Not run)
generate_count_matrix  Generate count matrix

Description
Generate count matrix

Usage

generate_count_matrix(cells, features, sparse, cell_names, feature_names)

Arguments

cells  Number of cells
features  Number of features
sparse  Is matrix sparse ?
cell_names  Cell names
feature_names  Feature names

Value
A matrix or a sparse matrix

generate_coverage_tracks  Generate cell cluster pseudo-bulk coverage tracks

Description
Generate cell cluster pseudo-bulk coverage tracks. First, scBED files are concatenated into cell clusters contained in the 'by' column of your SingleCellExperiment object. To do so, for each sample in the given list, the barcodes of each cluster are grepped and BED files are merged into pseudo-bulk of clusters (C1,C2,...). Two cells from different can have the same barcode ID as cell affectation is done sample by sample. Then coverage of pseudo-bulk BED files is calculated by averaging & smoothing reads on small genomic window (150bp per default). The pseudo bulk BED and BigWigs coverage tracks are writtend to the output directory. This functionality is not available on Windows as it uses the 'cat' and 'gzip' utilities from Unix OS.
Usage

```r
generate_coverage_tracks(
  scExp_cf,
  input,
  odir,
  format = "scBED",
  ref_genome = c("hg38", "mm10")[1],
  bin_width = 150,
  n_smoothBin = 5,
  read_size = 101,
  quantile_for_peak_calling = 0.85,
  by = "cell_cluster",
  progress = NULL
)
```

Arguments

- `scExp_cf`: A SingleCellExperiment with cluster selected. (see `choose_cluster_scExp`). It is recommended having a minimum of ~100 cells per cluster in order to obtain smooth tracks.
- `input`: Either a named list of character vector of path towards single-cell BED files or a sparse raw matrix of small bins (~500bp). If a named list specifying scBED the names MUST correspond to the 'sample_id' column in your SingleCellExperiment object. The single-cell BED files names MUST match the barcode names in your SingleCellExperiment (column 'barcode'). The scBED files can be gzipped or not.
- `odir`: The output directory to write the cumulative BED and BigWig files.
- `format`: File format, either "raw_mat", "BED" or "BAM"
- `ref_genome`: The genome of reference, used to constrain to canonical chromosomes. Either 'hg38' or 'mm10'. 'hg38' per default.
- `bin_width`: The width of the bin to create the coverage track. The smaller the greater the resolution & runtime. Default to 150.
- `n_smoothBin`: Number of bins left & right to average ('smooth') the signal on. Default to 5.
- `read_size`: The estimated size of reads. Default to 101.
- `quantile_for_peak_calling`: The quantile to define the threshold above which signal is considered as a peak.
- `by`: A character specifying a categorical column of scExp_cf metadata by which to group cells and generate coverage tracks and peaks.
- `progress`: A Progress object for Shiny. Default to NULL.

Value

Generate coverage tracks (.bigwig) for each group in the SingleCellExperiment "by" column.
Examples

```r
## Not run:
data(scExp)
input_files_coverage = list(
    "scChIP_Jurkat_K4me3" = paste0("/path/to/",scExp$barcode[1:51],".bed"),
    "scChIP_Ramos_K4me3" = paste0("/path/to/",scExp$barcode[52:106],".bed")
)
generate_coverage_tracks(scExp, input_files_coverage, "/path/to/output", ref_genome = "hg38")

## End(Not run)
```

---

### generate_feature_names

**Generate feature names**

**Usage**

```r
generate_feature_names(featureType, ref, features)
```

**Arguments**

- `featureType`: Type of feature
- `ref`: Reference genome
- `features`: Number of features to generate

**Value**

A character vector of feature names

---

### generate_report

From a ChromSCape analysis directory, generate an HTML report.

**Description**

From a ChromSCape analysis directory, generate an HTML report.
gene_set_enrichment_analysis_scExp

Usage

generate_report(
  ChromSCape_directory,
  prefix = NULL,
  run = c("filter", "CNA", "cluster", "consensus", "peak_call", "coverage", "DA",
          "GSA", "report")[c(1, 3, 6, 7, 8, 9)],
  genes_to_plot = c("Krt8", "Krt5", "Tgfb1", "Foxq1", "Cdkn2b", "Cdkn2a",
                    "chr7:15000000-20000000"),
  control_samples_CNA = NULL
)

Arguments

  ChromSCape_directory
    Path towards the ChromSCape directory of which you want to create the report.
    The report will be created at the root of this directory.

  prefix
    Name of the analysis with the filtering parameters (e.g. Analysis_3000_100000_99_uncorrected).
    You will find the prefix in the Filtering_Normalize_Reduce subfolder.

  run
    Which steps to report ("filter", "CNA", "cluster", "consensus", "peak_call", "coverage", "DA", "GSA", "report"). Only indicate steps that were done in the analysis. By default do not report CNA, consensus and peak calling.

  genes_to_plot
    For the UMAP, which genes do you want to see in the report.

  control_samples_CNA
    If running the Copy Number Alteration (CNA) part, which samples are the controls

Value

  Generate an HTML report at the root of the analysis directory.

Examples

  ## Not run:
  generate_analysis("/path/to/data/", "Analysis_1")

  ## End(Not run)
** gene_set_enrichment_analysis_scExp 53  

**Description**

This function takes previously calculated differential features and runs hypergeometric test to look for enriched gene sets in the genes associated with differential features, for each cell cluster. This functions takes as input a SingleCellExperiment object with consclust, the type of comparison, either ‘one_vs_rest’ or ‘pairwise’, the adjusted p-value threshold (qval.th) and the fold-change threshold (logFC.th). It outputs a SingleCellExperiment object containing a differential list.

**Usage**

gene_set_enrichment_analysis_scExp(  
  scExp,  
enrichment_qval = 0.1,  
  ref = "hg38",  
  GeneSets = NULL,  
  GeneSetsDf = NULL,  
  GenePool = NULL,  
  qval.th = 0.01,  
  logFC.th = 1,  
  min.percent = 0.01,  
  peak_distance = 1000,  
  use_peaks = FALSE,  
  GeneSetClasses = c("c1_positional", "c2_curated", "c3_motif", "c4_computational",  
    "c5_GO", "c6_oncogenic", "c7_immunologic", "hallmark"),  
  progress = NULL
)

**Arguments**

- **scExp**  
  A SingleCellExperiment object containing list of differential features.

- **enrichment_qval**  
  Adjusted p-value threshold for gene set enrichment. (0.1)

- **ref**  
  A reference annotation, either ‘hg38’ or ‘mm10’. (‘hg38’)

- **GeneSets**  
  A named list of gene sets. If NULL will automatically load MSigDB list of gene sets for specified reference genome. (NULL)

- **GeneSetsDf**  
  A dataframe containing gene sets & class of gene sets. If NULL will automatically load MSigDB dataframe of gene sets for specified reference genome. (NULL)

- **GenePool**  
  The pool of genes to run enrichment in. If NULL will automatically load Gen-  
  code list of genes fro specified reference genome. (NULL)

- **qval.th**  
  Adjusted p-value threshold to define differential features. (0.01)

- **logFC.th**  
  Fold change threshold to define differential features. (1)

- **min.percent**  
  Minimum fraction of cells having the feature active to consider it as significantly differential. (0.01)

- **peak_distance**  
  Maximum distanceToTSS of feature to gene TSS to consider associated, in bp. (1000)

- **use_peaks**  
  Use peak calling method (must be calculated beforehand). (FALSE)
getExperimentNames

GeneSetClasses  Which classes of MSIGdb to look for.
progress       A shiny Progress instance to display progress bar.

Value
Returns a SingleCellExperiment object containing list of enriched Gene Sets for each cluster, either in depleted features, enriched features or simply differential features (both).

Examples

data("scExp")

# Usually recommending qval.th = 0.01 & logFC.th = 1 or 2
## Not run: scExp_cf = gene_set_enrichment_analysis_scExp(scExp, 
  qval.th = 0.4, logFC.th = 0.3)
## End(Not run)

gene_set_enrichment_analysis

getExperimentNames  Get experiment names from a SingleCellExperiment

Description
Get experiment names from a SingleCellExperiment

Usage
gene_set_enrichment_analysis(scExp)

Arguments
scExp       A SingleCellExperiment with named mainExp and altExps.

Value
Character vector of unique experiment names

Examples

data(scExp)
gene_set_enrichment_analysis(scExp)
getMainExperiment

*Get Main experiment of a SingleCellExperiment*

**Description**

Get Main experiment of a SingleCellExperiment

**Usage**

```r
getMainExperiment(scExp)
```

**Arguments**

- `scExp`: A SingleCellExperiment with named mainExp and altExps.

**Value**

The swapped SingleCellExperiment towards "main" experiment

**Examples**

```r
data(scExp)
getMainExperiment(scExp)
```

---

get_color_dataframe_from_input

*Get color dataframe from shiny::colorInput*

**Description**

Get color dataframe from shiny::colorInput

**Usage**

```r
get_color_dataframe_from_input(
  input,
  levels_selected,
  color_by = c("sample_id", "total_counts"),
  input_id_prefix = "color_
)
```
get_cyto_features

Arguments

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>input</td>
<td>Shiny input object</td>
</tr>
<tr>
<td>levels_selected</td>
<td>Names of the features</td>
</tr>
<tr>
<td>color_by</td>
<td>Which feature color to retrieve</td>
</tr>
<tr>
<td>input_id_prefix</td>
<td>Prefix in front of the feature names</td>
</tr>
</tbody>
</table>

Value

A data.frame with the feature levels and the colors of each level of this feature.

Description

Map features of a SingleCellExperiment onto the cytobands of a given genome. Some features might not be mapped to any cytobands (e.g. if they are not in the canonical chromosomes), and are removed from the returned object.

Usage

```r
get_cyto_features(scExp, ref_genome = c("hg38", "mm10")[1])
```

Arguments

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>scExp</td>
<td>A SingleCellExperiment with genomic coordinate as features (peaks or bins)</td>
</tr>
<tr>
<td>ref_genome</td>
<td>Reference genome ('hg38' or 'mm10')</td>
</tr>
</tbody>
</table>

Details

The cytobands are an arbitrary cutting of the genome that dates back to staining metaphase chromosomes with Giemsa.

Value

A data.frame of the SCE features with their corresponding cytoband name

Examples

```r
data("scExp")
matching_cyto = get_cyto_features(scExp, ref_genome="hg38")
```
get_genomic_coordinates

Get SingleCellExperiment’s genomic coordinates

Description

Get SingleCellExperiment’s genomic coordinates

Usage

get_genomic_coordinates(scExp)

Arguments

scExp A SingleCellExperiment object.

Value

A GRanges object of genomic coordinates.

Examples

raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
feature_GRanges = get_genomic_coordinates(scExp)

get_most_variable_cyto

Retrieve the cytobands with the most variable fraction of reads

Description

Given a SingleCellExperiment object with the slot "cytoBand" containing the fraction of reads in each cytoband, calculates the variance of each cytoband and returns a data.frame with the top variables cytobands. Most cytobands are expected to be unchanged between normal and tumor samples, therefore focusing on the top variable cytobands enable to focus on the most intereseting regions.

Usage

get_most_variable_cyto(scExp, top = 50)

Arguments

scExp A SingleCellExperiment with "cytoBand" reducedDim slot filled.
top Number of cytobands to return (50).
get_pathway_mat_scExp

Value
A data.frame of the top variable cytoBands and their variance

Examples

data("scExp")
scExp = calculate_cyto_mat(scExp, ref_genome="hg38")
get_most_variable_cyto(scExp, top=50)

get_pathway_mat_scExp  Get pathway matrix

Description
Get pathway matrix

Usage
get_pathway_mat_scExp(
  scExp,
  pathways,
  max_distanceToTSS = 1000,
  ref = "hg38",
  GeneSetClasses = c("c1_positional", "c2_curated", "c3_motif", "c4_computational",
                     "c5_GO", "c6_oncogenic", "c7_immunologic", "hallmark"),
  progress = NULL
)

Arguments

scExp A SingleCellExperiment
pathways A character vector specifying the pathways to retrieve the cell count for.
max_distanceToTSS Numeric. Maximum distance to a gene’s TSS to consider a region linked to a gene. (1000)
ref Reference genome, either mm10 or hg38
GeneSetClasses Which classes of MSIGdb to load
progress A shiny Progress instance to display progress bar.

Value
A matrix of cell to pathway
Examples

data(scExp)
mat = get_pathway_mat_scExp(scExp, pathways = "KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY")

Description

gg_fill_hue

Usage

gg_fill_hue(n)

Arguments

n                  num hues

Value

A color in HEX format

Description

groupMat

Usage

groupMat(mat = NA, margin = 1, groups = NA, method = "mean")

Arguments

mat                A matrix
margin             By row or columns ?
groups             Groups
method             Method to group

Value

A grouped matrix
H1proportion

**Description**

H1proportion

**Usage**

H1proportion(pv = NA, lambda = 0.5)

**Arguments**

- pv: P.value vector
- lambda: Lambda value

**Value**

H1 proportion value

---

**has_genomic_coordinates**

*Does SingleCellExperiment has genomic coordinates in features?*

**Description**

Does SingleCellExperiment has genomic coordinates in features?

**Usage**

has_genomic_coordinates(scExp)

**Arguments**

- scExp: A SingleCellExperiment object

**Value**

TRUE or FALSE
**Examples**

```r
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
has_genomic_coordinates(scExp)
raw_genes = create_scDataset_raw(featureType="gene")
scExp_gene = create_scExp(raw_genes$mat, raw_genes$annot)
has_genomic_coordinates(scExp_gene)
```

---

**Description**

hclustAnnotHeatmapPlot

**Usage**

```r
hclustAnnotHeatmapPlot(
  x = NULL,
  hc = NULL,
  hmColors = NULL,
  anocol = NULL,
  xpos = c(0.1, 0.9, 0.114, 0.885),
  ypos = c(0.1, 0.5, 0.5, 0.6, 0.62, 0.95),
  dendro.cex = 1,
  xlab.cex = 0.8,
  hmRowNames = FALSE,
  hmRowNames.cex = 0.5
)
```

**Arguments**

- `x` A correlation matrix
- `hc` An hclust object
- `hmColors` A color palette
- `anocol` A matrix of colors
- `xpos` Xpos
- `ypos` Ypos
- `dendro.cex` Size of denro names
- `xlab.cex` Size of x label
- `hmRowNames` Write rownames ?
- `hmRowNames.cex` Size of rownames ?
Value

A heatmap

--

hg38.chromosomes  Data.frame of chromosome length - hg38

Description

This data frame provides the length of each "canonical" chromosomes of Homo Sapiens genome build hg38.

Usage

data("hg38.chromosomes")

Format

hg38.chromosomes - a data frame with 24 rows and 3 variables:

- **chr**  Chromosome - character
- **start**  Start of the chromosome (bp) - integer
- **end**  End of the chromosome (bp) - integer

--

hg38.cytoBand  Data.frame of cytoBand location - hg38

Description

This data frame provides the location of each cytoBands of Homo Sapiens genome build hg38.

Usage

data("hg38.cytoBand")

Format

hg38.cytoBand - a data frame with 862 rows and 4 variables:

- **chr**  Chromosome - character
- **start**  Start of the chromosome (bp) - integer
- **end**  End of the chromosome (bp) - integer
- **cytoBand**  Name of the cytoBand - character
**Description**

This dataframe was extracted from Gencode v25 and report the Transcription Start Site of each gene in the Homo Sapiens genome build hg38.

**Usage**

```r
data("hg38.GeneTSS")
```

**Format**

hg38.GeneTSS - a data frame with 24 rows and 3 variables:

- `chr` Chromosome - character
- `start` Start of the gene (TSS) - integer
- `end` End of the gene - integer
- `gene` Gene symbol - character

**Description**

imageCol

**Usage**

```r
imageCol(
    matcol = NULL,
    strat = NULL,
    xlab.cex = 0.5,
    ylab.cex = 0.5,
    drawLines = c("none", "h", "v", "b")[1],
    ...
)
```

**Arguments**

- `matcol` A matrix of colors
- `strat` Strat
- `xlab.cex` X label size
- `ylab.cex` Y label size
- `drawLines` Draw lines ?
- `...` Additional parameters
import_count_input_files

*Import and count input files depending on their format*

**Description**

Import and count input files depending on their format

**Usage**

```r
import_count_input_files(
  files_dir_list,
  file_type,
  which,
  ref,
  verbose,
  progress,
  BPPARAM = BiocParallel::bpparam()
)
```

**Arguments**

- `files_dir_list`: A named list of directories containing the input files.
- `file_type`: Input file type.
- `which`: A GRanges object of features.
- `ref`: Reference genome.
- `verbose`: Print ?
- `progress`: A progress object for Shiny.
- `BPPARAM`: BPPARAM object for multiprocessing. See `bpparam` for more informations. Will take the default BPPARAM set in your R session.

**Value**

A list with the feature indexes data.frame containing non-zeroes entries in the count matrix and the cell names
**Description**

Combine one or multiple matrices together to create a sparse matrix and cell annotation data.frame.

**Usage**

```r
import_scExp(file_paths, remove_pattern = "", temp_path = NULL)
```

**Arguments**

- `file_paths`: A character vector of file names towards single cell epigenomic matrices (features x cells) (must be .txt / .tsv)
- `remove_pattern`: A string pattern to remove from the sample names. Can be a regexp.
- `temp_path`: In case matrices are stored in temporary folder, a character vector of path towards temporary files. (NULL)

**Value**

A list containing:

- `datamatrix`: a sparseMatrix of features x cells
- `annot_raw`: an annotation of cells as data.frame

**Examples**

```r
mat1 = mat2 = create_scDataset_raw()$mat
tmp1 = tempfile(fileext = ".tsv")
tmp2 = tempfile(fileext = ".tsv")
write.table(as.matrix(mat1), file=tmp1, sep = "\t",
row.names = TRUE, col.names = TRUE, quote = FALSE)
write.table(as.matrix(mat2), file=tmp2, sep = "\t",
row.names = TRUE, col.names = TRUE, quote = FALSE)
file_paths = c(tmp1, tmp2)
out = import_scExp(file_paths)
```
index_peaks_barcodes_to_matrix_indexes

Read index-peaks-barcodes trio files on interval to create count indexes

Description
Read index-peaks-barcodes trio files on interval to create count indexes

Usage

index_peaks_barcodes_to_matrix_indexes(
  feature_file,
  matrix_file,
  barcode_file,
  binarize = FALSE
)

Arguments

feature_file      A file containing the features genomic locations
matrix_file       A file containing the indexes of non-zeroes values and their value (respectively
                  i,j,x,see sparseMatrix)
barcode_file      A file containing the barcode ids
binarize          Binarize matrix ?

Value
A list containing a "feature index" data.frame, name_cells, and a region GenomicRange object used to form the sparse matrix

inter_correlation_scExp

Calculate inter correlation between cluster or samples

Description
Calculate inter correlation between cluster or samples
intra_correlation_scExp

Usage

\[
\text{intra\_correlation\_scExp}(\text{scExp\_cf}, \text{by} = c("sample\_id", "cell\_cluster")[1], \text{reference\_group} = \text{unique(scExp\_cf[[by]]})[1], \text{other\_groups} = \text{unique(scExp\_cf[[by]])}, \text{fullCor} = \text{TRUE})
\]

Arguments

- **scExp_cf**: A SingleCellExperiment
- **by**: On which feature to calculate correlation ("sample_id" or "cell_cluster")
- **reference_group**: Reference group to calculate correlation with. Must be in accordance with "by".
- **other_groups**: Groups on which to calculate correlation (can contain multiple groups, and also reference_group). Must be in accordance with "by".
- **fullCor**: A logical specifying if the correlation matrix was calculated on the entire set of cells (TRUE).

Value

A data.frame of average inter-correlation of cells in other_groups with cells in reference_group

Examples

\[
\text{data(scExp)} \\
\text{intra\_correlation\_scExp(scExp)}
\]

\[
\text{intra\_correlation\_scExp}
\]

*Calculate intra correlation between cluster or samples*

Description

Calculate intra correlation between cluster or samples

Usage

\[
\text{intra\_correlation\_scExp}(\text{scExp\_cf}, \text{by} = c("sample\_id", "cell\_cluster")[1], \text{fullCor} = \text{TRUE})
\]
Arguments

- **scExp_cf** A SingleCellExperiment
- **by** On which feature to calculate correlation ("sample_id" or "cell_cluster")
- **fullCor** Logical specifying if the correlation matrix was run on the entire number of cells or on a subset.

Value

A data.frame of cell average intra-correlation

Examples

```r
data(scExp)
intra_correlation_scExp(scExp, by = "sample_id")
intra_correlation_scExp(scExp, by = "cell_cluster")
```

launchApp

Launch ChromSCape

Description

Main function to launch ChromSCape in your favorite browser. You can pass additional parameters that you would pass to shiny::runApp (runApp)

Usage

```r
launchApp(launch.browser = TRUE, ...)
```

Arguments

- **launch.browser** Wether to launch browser or not
- **...** Additional parameters passed to runApp

Value

Launches the shiny application

Examples

```r
## Not run:
launchApp()
```

## End(Not run)
### load_MSIGdb

**Load and format MSIGdb pathways using msigdb package**

**Description**

Load and format MSIGdb pathways using msigdb package

**Usage**

```r
load_MSIGdb(
  ref,
  GeneSetClasses = c("c1_positional", "c2_curated", "c3_motif", "c4_computational",
                     "c5_GO", "c6_oncogenic", "c7_immunologic", "hallmark")
)
```

**Arguments**

- `ref` : Reference genome, either mm10 or hg38
- `GeneSetClasses` : Which classes of MSIGdb to load

**Value**

A list containing the GeneSet (list), GeneSetDf (data.frame) and GenePool character vector of all possible genes

---

### merge_MACS2_peaks

**Merge peak files from MACS2 peak caller**

**Description**

Merge peak files from MACS2 peak caller

**Usage**

```r
merge_MACS2_peaks(peak_file, peak_distance_to_merge, min_peak_size = 200, ref)
```

**Arguments**

- `peak_file` : A character specifying the path towards the peak file (BED or bedGraph format)
- `peak_distance_to_merge` : Maximum distance to merge two peaks
- `min_peak_size` : An integer specifying the minimum size of peaks
- `ref` : Reference genome

**Value**

Peaks as GRanges
mm10.chromosomes  

Data.frame of chromosome length - mm10

Description

This data frame provides the length of each "canonical" chromosomes of Mus Musculus (Mouse) genome build mm10.

Usage

data("mm10.chromosomes")

Format

mm10.chromosomes - a data frame with 24 rows and 3 variables:

  chr  Chromosome - character
  start  Start of the chromosome (bp) - integer
  end  End of the chromosome (bp) - integer

mm10.cytoBand  

Data.frame of cytoBandlocation - mm10

Description

This data frame provides the location of each cytoBands of Homo Sapiens genome build mm10.

Usage

data("mm10.cytoBand")

Format

mm10.cytoBand - a data frame with 862 rows and 4 variables:

  chr  Chromosome - character
  start  Start of the chromosome (bp) - integer
  end  End of the chromosome (bp) - integer
  cytoBand  Name of the cytoBand - character
Description

This dataframe was extracted from Gencode v25 and report the Transcription Start Site of each gene in the Mus Musculus genome build mm10 (Mouse).

Usage

data("mm10.GeneTSS")

Format

mm10.GeneTSS - a data frame with 24 rows and 3 variables:

- chr  Chromosome name - character
- start  Start of the gene (TSS) - integer
- end  End of the gene - integer
- gene  Gene symbol - character

normalize_scExp  Normalize counts

Description

Normalize counts

Usage

normalize_scExp(
  scExp,
  type = c("CPM", "TFIDF", "RPKM", "TPM", "feature_size_only")
)

Arguments

scExp  A SingleCellExperiment object.

Arguments

A SingleCellExperiment object containing normalized counts. (See ?normcounts())
**Examples**

```r
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp = normalize_scExp(scExp)
head(SingleCellExperiment::normcounts(scExp))
```

---

**num_cell_after_cor_filt_scExp**

*Number of cells before & after correlation filtering*

**Description**

Number of cells before & after correlation filtering

**Usage**

```r
num_cell_after_cor_filt_scExp(scExp, scExp_cf)
```

**Arguments**

- `scExp` SingleCellExperiment object before correlation filtering.
- `scExp_cf` SingleCellExperiment object after correlation filtering.

**Value**

A colored kable with the number of cells per sample before and after filtering for display

**Examples**

```r
data("scExp")
scExp_cf = correlation_and_hierarchical_clust_scExp(scExp)
scExp_cf = filter_correlated_cell_scExp(scExp_cf,
corr_threshold = 99, percent_correlation = 1)
## Not run: num_cell_after_cor_filt_scExp(scExp,scExp_cf)
```
num_cell_after_QC_filt_scExp

Table of cells before / after QC

**Description**

Table of cells before / after QC

**Usage**

```r
num_cell_after_QC_filt_scExp(scExp, annot, datamatrix)
```

**Arguments**

- **scExp**: A SingleCellExperiment object.
- **annot**: A raw annotation data.frame of cells before filtering.
- **datamatrix**: A matrix of cells per regions before filtering.

**Value**

A formatted kable in HTML.

**Examples**

```r
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp_filtered = filter_scExp(scExp)
## Not run: num_cell_after_QC_filt_scExp(
##     scExp_filtered, SingleCellExperiment::colData(scExp))
## End(Not run)
```

---

num_cell_before_cor_filt_scExp

Table of number of cells before correlation filtering

**Description**

Table of number of cells before correlation filtering

**Usage**

```r
num_cell_before_cor_filt_scExp(scExp)
```
Arguments

scExp  A SingleCellExperiment Object

Value

A colored kable with the number of cells per sample for display

Examples

data("scExp")
## Not run: num_cell_before_cor_filt_scExp(scExp)

data("scExp")
scExp_cf = correlation_and_hierarchical_clust_scExp(scExp)
scExp_cf = choose_cluster_scExp(scExp Cf, nclust=3, consensus=FALSE)
## Not run: num_cell_in_cluster_scExp(scExp Cf)
num_cell_scExp

Table of cells

Description

Table of cells

Usage

num_cell_scExp(annot, datamatrix)

Arguments

annot An annotation of cells. Can be obtain through 'colData(scExp)'.
datamatrix A matrix of cells per regions before filtering.

Value

A formatted kable in HTML.

Examples

raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
## Not run: num_cell_scExp(SingleCellExperiment::colData(scExp))

pca_irlba_for_sparseMatrix

Run sparse PCA using irlba SVD

Description

This function allows to run a PCA using IRLBA Singular Value Decomposition in a fast & memory efficient way. The incremental Lanczos bidiagonalisation algorithm allows to keep the matrix sparse as the "loci" centering is implicit. The function then multiplies by the approximate singular values (svd$d) in order to get more importance to the first PCs proportionnally to their singular values. This step is crucial for downstream approaches, e.g. UMAP or T-SNE.

Usage

pca_irlba_for_sparseMatrix(x, n_comp, work = 3 * n_comp)
plot_cluster_consensus_scExp

Arguments

x A sparse normalized matrix (features x cells)
n_comp The number of principal components to keep
work Working subspace dimension, larger values can speed convergence at the cost of more memory use.

Value
The rotated data, e.g. the cells x PC column in case of sc data.

Description
Plot cluster consensus score for each k as a bargraph.

Usage
plot_cluster_consensus_scExp(scExp)

Arguments
scExp A SingleCellExperiment

Value
The consensus score for each cluster for each k as a barplot

Examples
data("scExp")
plot_cluster_consensus_scExp(scExp)
**plot_correlation_PCA_scExp**

*Plotting correlation of PCs with a variable of interest*

**Description**

Plotting correlation of PCs with a variable of interest

**Usage**

```r
plot_correlation_PCA_scExp(
  scExp,
  correlation_var = "total_counts",
  color_by = NULL,
  topPC = 10
)
```

**Arguments**

- `scExp` A SingleCellExperiment Object
- `correlation_var` A string specifying with which numeric variable from colData of scExp to calculate and plot the correlation of each PC with. ('total_counts')
- `color_by` A string specifying with which categorical variable to color the plot. ('NULL')
- `topPC` An integer specifying the number of PCs to plot correlation with 10

**Value**

A ggplot histogram representing the distribution of count per cell

**Examples**

```r
data("scExp")
plot_correlation_PCA_scExp(scExp, topPC = 25)
plot_correlation_PCA_scExp(scExp, color_by = "cell_cluster")
plot_correlation_PCA_scExp(scExp, color_by = "sample_id")
```
plot_coverage_BigWig  Coverage plot

**Description**

Coverage plot

**Usage**

```r
plot_coverage_BigWig(
  coverages,
  label_color_list,
  peaks = NULL,
  chrom,
  start,
  end,
  ref = "hg38"
)
```

**Arguments**

- `coverages`: A list containing sample coverage as GenomicRanges
- `label_color_list`: List of colors, list names are labels
- `peaks`: A GRanges object containing peaks location to plot (optional)
- `chrom`: Chromosome
- `start`: Start
- `end`: End
- `ref`: Genomic Reference

**Value**

A coverage plot annotated with genes

**Examples**

```r
data(scExp)
```
plot_differential_summary_scExp

Differential summary barplot

Description

Differential summary barplot

Usage

plot_differential_summary_scExp(
  scExp_cf,
  qval.th = 0.01,
  logFC.th = 1,
  min.percent = 0.01
)

Arguments

scExp_cf A SingleCellExperiment object
qval.th Adjusted p-value threshold. (0.01)
logFC.th Fold change threshold. (1)
min.percent Minimum fraction of cells having the feature active to consider it as significantly differential. (0.01)

Value

A barplot summary of differential analysis

Examples

data("scExp")
plot_differential_summary_scExp(scExp)

plot_differential_volcano_scExp

Volcano plot of differential features

Description

Volcano plot of differential features
plot_distribution_scExp

Usage

plot_distribution_scExp(
    scExp,  
    raw = TRUE, 
    log10 = FALSE, 
    pseudo_counts = 1, 
    bins = 150 
)

Description

Plotting distribution of signal

plot_differential_volcano_scExp

Usage

plot_differential_volcano_scExp(
    scExp_cf,  
    group = "C1", 
    logFC.th = 1, 
    qval.th = 0.01, 
    min.percent = 0.01 
)

Arguments

scExp_cf A SingleCellExperiment object

group A character indicating the group for which to plot the differential volcano plot. ("C1")

logFC.th Fold change threshold. (1)

qval.th Adjusted p-value threshold. (0.01)

min.percent Minimum fraction of cells having the feature active to consider it as significantly differential. (0.01)

Value

A volcano plot of differential analysis of a specific cluster

Examples

data("scExp")
plot_differential_volcano_scExp(scExp,"C1")
Arguments

- `scExp`: A SingleCellExperiment Object
- `raw`: Use raw counts?
- `log10`: Transform using log10?
- `pseudo_counts`: Pseudo-count to add if using log10
- `bins`: Number of bins in the histogram

Value

A ggplot histogram representing the distribution of count per cell

Examples

```r
data("scExp")
plot_distribution_scExp(scExp)
```

plot_gain_or_loss_barplots

Plot Gain or Loss of cytobands of the most variables cytobands

Description

Plot Gain or Loss of cytobands of the most variables cytobands

Usage

```r
plot_gain_or_loss_barplots(scExp, cells = NULL, top = 20)
```

Arguments

- `scExp`: A SingleCellExperiment with "logRatio_cytoBand" reducedDim slot filled. See `calculate_logRatio_CNA`
- `cells`: Cell IDs of the tumor samples to
- `top`: Number of most variables cytobands to plot

Value

Plot the gains/lost in the selected cells of interest as multiple barplots

Plot the gains/lost in the selected cells of interest as multiple barplots
Examples

data("scExp")
scExp = calculate_CNA(scExp, control_samples = unique(scExp$sample_id)[1],
ref_genome="hg38", quantiles_to_define_gol = c(0.05,0.95))
plot_gain_or_loss_barplots(scExp, cells = scExp$cell_id[which(
scExp$sample_id %in% unique(scExp$sample_id)[2])])

plot_heatmap_scExp

Description

Plot cell correlation heatmap with annotations

Usage

plot_heatmap_scExp(
scExp,
name_hc = "hc_cor",
corColors = (grDevices::colorRampPalette(c("royalblue", "white", "indianred1"))(256),
color_by = NULL,
downsamp = 1000,
hc_linkage = "ward.D"
)

Arguments

scExp A SingleCellExperiment Object
name_hc Name of the hclust contained in the SingleCellExperiment object
corColors A palette of colors for the heatmap
color_by Which features to add as additional bands on top of plot
downsamp Number of cells to downsample
hc_linkage A linkage method for hierarchical clustering. See cor. (‘ward.D’)

Value

A heatmap of cell to cell correlation, grouping cells by hierarchical clustering.
Examples

```r
data("scExp")
plot_heatmap_scExp(scExp)
```

Description

Violin plot of inter-correlation distribution between one or multiple groups and one reference group

Usage

```r
plot_inter_correlation_scExp(
  scExp_cf,
  by = c("sample_id", "cell_cluster")[1],
  jitter_by = NULL,
  reference_group = unique(scExp_cf[[by]])[1],
  other_groups = unique(scExp_cf[[by]]),
  downsample = 5000
)
```

Arguments

- **scExp_cf**  A SingleCellExperiment
- **by**  Color by sample_id or cell_cluster
- **jitter_by**  Add jitter points of another layer (cell_cluster or sample_id)
- **reference_group**  Character containing the reference group name to calculate correlation from.
- **other_groups**  Character vector of the other groups for which to calculate correlation with the reference group.
- **downsample**  Downsampling for plotting

Value

A violin plot of inter-correlation

Examples

```r
data(scExp)
plot_intra_correlation_scExp(scExp)
```
plot_intra_correlation_scExp

Violin plot of intra-correlation distribution

Description

Violin plot of intra-correlation distribution

Usage

plot_intra_correlation_scExp(
    scExp_cf,
    by = c("sample_id", "cell_cluster")[1],
    jitter_by = NULL,
    downsample = 5000
)

Arguments

scExp_cf A SingleCellExperiment
by Color by sample_id or cell_cluster
jitter_by Add jitter points of another layer (cell_cluster or sample_id)
downsample Downsample for plotting

Value

A violin plot of intra-correlation

Examples

data(scExp)
plot_intra_correlation_scExp(scExp)

plot_most_contributing_features

Plot Top/Bottom most contributing features to PCA

Description

Plot Top/Bottom most contributing features to PCA
plot_percent_active_feature_scExp

Usage

plot_percent_active_feature_scExp(
  scExp,
  gene,
  by = c("cell_cluster", "sample_id") [1],
  highlight = NULL,
  downsample = 5000,
  max_distanceToTSS = 1000
)

Description

Barplot of the % of active cells for a given features

Usage

plot_percent_active_feature_scExp(
  scExp,
  component = "Component_1",
  n_top_bot = 10
)
plot_pie_most_contributing_chr

Pie chart of top contribution of chromosomes in the 100 most contributing features to PCA #'

Usage

plot_pie_most_contributing_chr(
    scExp,
    component = "Component_1",
    n_top_bot = 100
)

Arguments

scExp A SingleCellExperiment containing "PCA" in reducedDims and gene annotation in rowRanges
component The name of the component of interest
n_top_bot An integer number of top and bot regions to plot (100)
Value

A pie chart showing the distribution of chromosomes in the top features with the largest absolute value in the component of interest

Examples

data(scExp)
plot_pie_most_contributing_chr(scExp, component = "Component_1")

plot_reduced_dim_scExp

Plot reduced dimensions (PCA, TSNE, UMAP)

Description

Plot reduced dimensions (PCA, TSNE, UMAP)

Usage

plot_reduced_dim_scExp(
  scExp, 
  color_by = "sample_id", 
  reduced_dim = c("PCA", "TSNE", "UMAP"), 
  select_x = NULL, 
  select_y = NULL, 
  downsample = 5000, 
  transparency = 0.6, 
  size = 1, 
  max_distanceToTSS = 1000, 
  annotate_clusters = "cell_cluster" %in% colnames(colData(scExp)), 
  min_quantile = 0.01, 
  max_quantile = 0.99 
)

Arguments

scExp A SingleCellExperiment Object
color_by Character of feature used for coloration. Can be cell metadata ("total_counts", 
"sample_id", ...) or a gene name.
reduced_dim Reduced Dimension used for plotting
select_x Which variable to select for x axis
select_y Which variable to select for y axis
downsample Number of cells to downsample
transparency Alpha parameter, between 0 and 1
size Size of the points.
plot_reduced_dim_scExp_CNA

Plot UMAP colored by Gain or Loss of cytobands

Description

Plot UMAP colored by Gain or Loss of cytobands

Usage

plot_reduced_dim_scExp_CNA(scExp, cytoBand)

Arguments

scExp : A SingleCellExperiment with "gainOrLoss_cytoBand" reducedDim slot filled. See calculate_gain_or_loss

cytoBand : Which cytoBand to color cells by

Value

Plot the gains/lost of the cytoband overlayed on the epigenetic UMAP.

max_distanceToTSS

The maximum distance to TSS to consider a gene linked to a region. Used only if "color_by" is a gene name.

annotate_clusters

A logical indicating if clusters should be labelled. The 'cell_cluster' column should be present in metadata.

min_quantile

The lower threshold to remove outlier cells, as quantile of cell embeddings (between 0 and 0.5).

max_quantile

The upper threshold to remove outlier cells, as quantile of cell embeddings (between 0.5 and 1).

Value

A ggplot geom_point plot of reduced dimension 2D representation

Examples

data("scExp")
plot_reduced_dim_scExp(scExp, color_by = "sample_id")
plot_reduced_dim_scExp(scExp, color_by = "total_counts")
plot_reduced_dim_scExp(scExp, reduced_dim = "UMAP")
plot_reduced_dim_scExp(scExp, color_by = "CD52", reduced_dim = "UMAP")
Examples

```r
data("scExp")
scExp = calculate_CNA(scExp, control_samples = unique(scExp$sample_id)[1], ref_genome="hg38", quantiles_to_define_gol = c(0.05,0.95))
plot_reduced_dim_scExp_CNA(scExp, get_most_variable_cyto(scExp)$cytoBand[1])
```

---

**plot_top_TF_scExp**  
*Barplot of top TFs from ChEA3 TF enrichment analysis*

Description

Barplot of top TFs from ChEA3 TF enrichment analysis

Usage

```r
plot_top_TF_scExp(
  scExp,
  group = unique(scExp$cell_cluster)[1],
  set = c("Differential", "Enriched", "Depleted")[1],
  type = c("Score", "nTargets", "nTargets_over_TF", "nTargets_over_genes")[1],
  n_top = 25
)
```

Arguments

- `scExp`  
  A SingleCellExperiment

- `group`  
  A character string specifying the differential group to display the top TFs

- `set`  
  A character string specifying the set of genes in which the TF were enriched, either 'Differential', 'Enriched' or 'Depleted'.

- `type`  
  A character string specifying the Y axis of the plot, either the number of differential targets or the ChEA3 integrated mean score. E.g. either "Score", "nTargets", "nTargets_over_TF" for the number of target genes over the total number of genes targeted by the TF or "nTargets_over_genes" for the number of target genes over the number of genes in the gene set.

- `n_top`  
  An integer specifying the number of top TF to display

Value

A bar plot of top TFs from ChEA3 TF enrichment analysis
Examples

```r
data("scExp")

plot_top_TF_scExp(
  scExp,
  group = "C1",
  set = "Differential",
  type = "Score",
  n_top = 10)

plot_top_TF_scExp(
  scExp,
  group = "C1",
  set = "Enriched",
  type = "nTargets_over_genes",
  n_top = 20)
```

**plot_violin_feature_scExp**

*Violin plot of features*

### Description

Violin plot of features

### Usage

```r
plot_violin_feature_scExp(
  scExp,
  gene,
  by = c("cell_cluster", "sample_id")[1],
  downsample = 5000,
  max_distanceToTSS = 1000
)
```

### Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>scExp</code></td>
<td>A SingleCellExperiment</td>
</tr>
<tr>
<td><code>gene</code></td>
<td>A character specifying the gene to plot</td>
</tr>
<tr>
<td><code>by</code></td>
<td>Color violin by cell_cluster or sample_id (&quot;cell_cluster&quot;)</td>
</tr>
<tr>
<td><code>downsample</code></td>
<td>Downsample for plotting (5000)</td>
</tr>
<tr>
<td><code>max_distanceToTSS</code></td>
<td>Numeric. Maximum distance to a gene’s TSS to consider a region linked to a gene. (1000)</td>
</tr>
</tbody>
</table>
Value

A violin plot of intra-correlation

Examples

data(scExp)
plot_violin_feature_scExp(scExp, "UBXN10")

Description

Preprocess and filter matrix annotation data project folder to SCE

Usage

preprocessing_filtering_and_reduction(
  datamatrix,
  annot_raw,
  min_reads_per_cell = 1600,
  max_quantile_read_per_cell = 95,
  n_top_features = 40000,
  norm_type = "CPM",
  n_dims = 10,
  remove_PC = NULL,
  subsample_n = NULL,
  ref_genome = "hg38",
  exclude_regions = NULL,
  doBatchCorr = FALSE,
  batch_sels = NULL
)

Arguments

datamatrix A sparse count matrix of features x cells.
annot_raw A data.frame with barcode, cell_id, sample_id, batch_id, total_counts
min_reads_per_cell Minimum read per cell to keep the cell
max_quantile_read_per_cell Upper count quantile threshold above which cells are removed
n_top_features Number of features to keep
norm_type Normalization type c("CPM", "TFIDF", "RPKM", "TPM", "feature_size_only")
n_dims An integer specifying the number of dimensions to keep for PCA
remove_PC A vector of string indicating which principal components to remove before
downstream analysis as probably correlated to library size. Should be under the
form: 'Component_1', 'Component_2', ... Recommended when using 'TFIDF'
normalization method. (NULL)
subsample_n Number of cells to subsample.
ref_genome Reference genome ("hg38" or "mm10").
exclude_regions GenomicRanges with regions to remove from the object.
doBatchCorr Run batch correction? TRUE or FALSE
batch_sels If doBatchCorr is TRUE, List of characters. Names are batch names, characters
are sample names.

Value
A SingleCellExperiment object containing feature spaces.

Examples

```r
raw <- create_scDataset_raw()
scExp = preprocessing_filtering_and_reduction(raw$mat, raw$annot)
```

---

preprocess_CPM Preprocess scExp - Counts Per Million (CPM)

Description
Preprocess scExp - Counts Per Million (CPM)

Usage
```
preprocess_CPM(scExp)
```

Arguments
scExp A SingleCellExperiment Object

Value
A SingleCellExperiment object.

Examples
```r
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp = preprocess_CPM(scExp)
head(SingleCellExperiment::normcounts(scExp))
```
**preprocess_feature_size_only**

*Preprocess scExp - size only*

**Description**

Preprocess scExp - size only

**Usage**

```r
preprocess_feature_size_only(scExp)
```

**Arguments**

- `scExp` A SingleCellExperiment Object

**Value**

A SingleCellExperiment object.

**Examples**

```r
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp = preprocess_feature_size_only(scExp)
head(SingleCellExperiment::normcounts(scExp))
```

---

**preprocess_RPKM**

*Preprocess scExp - Read per Kilobase Per Million (RPKM)*

**Description**

Preprocess scExp - Read per Kilobase Per Million (RPKM)

**Usage**

```r
preprocess_RPKM(scExp)
```

**Arguments**

- `scExp` A SingleCellExperiment Object

**Value**

A SingleCellExperiment object.
Examples

```r
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp = preprocess_RPKM(scExp)
head(SingleCellExperiment::normcounts(scExp))
```

---

**preprocess_TFIDF**  
*Preprocess scExp - TF-IDF*

**Description**

Preprocess scExp - TF-IDF

**Usage**

```r
preprocess_TFIDF(scExp, scale = 10000, log = TRUE)
```

**Arguments**

- `scExp`  
  A SingleCellExperiment Object

- `scale`  
  A numeric to multiply the matrix in order to have human readable numbers.  
  Has no impact on the downstream analysis

- `log`  
  Wether to use neperian log on the TF-IDF normalized data or not.

**Value**

A SingleCellExperiment object.

**Examples**

```r
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp = preprocess_TFIDF(scExp)
head(SingleCellExperiment::normcounts(scExp))
```
**preprocess_TPM**

*Preprocess scExp - Transcripts per Million (TPM)*

---

**Description**

Preprocess scExp - Transcripts per Million (TPM)

**Usage**

```r
preprocess_TPM(scExp)
```

**Arguments**

- `scExp` A SingleCellExperiment Object

**Value**

A SingleCellExperiment object.

**Examples**

```r
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp = preprocess_TPM(scExp)
head(SingleCellExperiment::normcounts(scExp))
```

---

**rawfile_ToBigWig**

*rawfile_ToBigWig: reads in BAM file and write out BigWig coverage file, normalized and smoothed*

---

**Description**

rawfile_ToBigWig: reads in BAM file and write out BigWig coverage file, normalized and smoothed

**Usage**

```r
rawfile_ToBigWig(
  input,
  BigWig_filename,
  format = "BAM",
  bin_width = 150,
  norm_factor,
  n_smoothBin = 5,
  ref = "hg38",
  read_size = 101,
```
raw_counts_to_sparse_matrix

Create a sparse count matrix from various format of input data.

Description

This function takes three different type of single-cell input: - Single cell BAM files (sorted) - Single cell BED files (gzipped) - A combination of an index file, a peak file and cell barcode file (The index file is composed of three column: index i, index j and value x for the non zeroes entries in the sparse matrix.)
raw_counts_to_sparse_matrix

Usage

raw_counts_to_sparse_matrix(
  files_dir_list,  
  file_type = c("scBED", "scBAM", "FragmentFile"),
  use_Signac = TRUE,  
  peak_file = NULL,  
  n_bins = NULL,  
  bin_width = NULL,  
  genebody = NULL,  
  extendPromoter = 2500,  
  verbose = TRUE,  
  ref = c("hg38", "mm10")[1],  
  progress = NULL,  
  BPPARAM = BiocParallel::bpparam()
)

Arguments

  files_dir_list A named character vector of directories containing the files. The names correspond to sample names.
  file_type Input file(s) type(s) ("scBED","scBAM","FragmentFile")
  use_Signac Use Signac wrapper function 'FeatureMatrix' if the Signac package is installed (TRUE).
  peak_file A file containing genomic location of peaks (NULL)
  n_bins The number of bins to tile the genome (NULL)
  bin_width The size of bins to tile the genome (NULL)
  genebody Count on genes (body + promoter) ? (NULL)
  extendPromoter If counting on genes, number of base pairs to extend up or downstream of TSS (2500).
  verbose Verbose (TRUE)
  ref reference genome to use (hg38)
  progress Progress object for Shiny
  BPPARAM BPPARAM object for multiprocessing. See bpparam for more informations. Will take the default BPPARAM set in your R session.

Details

This functions re-counts signal on either fixed genomic bins, a set of user-defined peaks or around the TSS of genes.

Value

A sparse matrix of features x cells
read_sparse_matrix

Read in one or multiple sparse matrices (10X format)

Description

Given one or multiple directories, look in each directory for a combination of the following files:

- A 'features' file containing unique feature genomic locations -in tab separated format ( *_features.bed / .txt / .tsv / .gz), e.g. chr, start and end
- A 'barcodes' file containing unique barcode names (_barcode.txt / .tsv / .gz)
- A 'matrix' A file containing indexes of non zero entries (_matrix.mtx / .gz)

Usage

read_sparse_matrix(files_dir_list, ref = c("hg38", "mm10")[1], verbose = TRUE)

read_count_mat_with_separated_chr_start_end

Read a count matrix with three first columns (chr,start,end)

Description

Read a count matrix with three first columns (chr,start,end)

Usage

read_count_mat_with_separated_chr_start_end(
  path_to_matrix,
  format_test,
  separator
)

Arguments

path_to_matrix Path to the count matrix
format_test Sample of the read.table
separator Separator character

Value

A sparseMatrix with rownames in the form "chr1:1222-55555"

References

Stuart el al., Multimodal single-cell chromatin analysis with Signac bioRxiv https://doi.org/10.1101/2020.11.09.373613
Arguments

files_dir_list  A named character vector containing the full path towards folders. Each folder should contain only the Feature file, the Barcode file and the Matrix file (see description).

ref Reference genome (used to filter non-canonical chromosomes).

verbose Print ?

Value

Returns a list containing a datamatrix and cell annotation

Examples

## Not run:
sample_dirs = c("/path/to/folder1/", "/path/to/folder2/")
names(sample_dirs) = c("sample_1", "sample_2")
out <- read_sparse_matrix(sample_dirs, ref = "hg38")
head(out$datamatrix)
head(out$annot_raw)

## End(Not run)
rebin_matrix

Transforms a bins x cells count matrix into a larger bins x cells count matrix.

Description

This function is best used to re-count large number of small bins or peaks (e.g. <= 5000bp) into equal or larger sized bins. The genome is either cut in fixed bins (e.g. 50,000bp) or into an user defined number of bins. Bins are calculated based on the canonical chromosomes. Note that if peaks are larger than bins, or if peaks are overlapping multiple bins, the signal is added to each bin. Users can increase the minimum overlap to consider peaks overlapping bins (by default 150bp, size of a nucleosome) to diminish the number of peaks overlapping multiple region. Any peak smaller than the minimum overlap threshold will be dismissed. Therefore, library size might be slightly different from peaks to bins if signal was duplicated into multiple bins or omitted due to peaks smaller than minimum overlap.

Usage

rebin_matrix(
  mat,
  bin_width = 50000,
  custom_annotation = NULL,
  minoverlap = 500,
  verbose = TRUE,
  ref = "hg38",
  nthreads = 1,
  rebin_function = rebin_helper
)

Arguments

mat A matrix of peaks x cells
bin_width Width of bins to produce in base pairs (minimum 500) (50000)
custom_annotation A GenomicRanges object specifying the new features to count the matrix on instead of recounting on genomic bins. If not NULL, takes precedence over bin_width.
minoverlap Minimum overlap between the original bins and the new features to consider the peak as overlapping the bin. We recommend to put this number at exactly half of the original bin size (e.g. 500bp for original bin size of 1000bp) so that no original bins are counted twice. (500)
verbose Verbose
ref Reference genome to use (hg38)
nthreads Number of threads to use for parallel processing
reduce_dims_scExp

Value
A sparse matrix of larger bins or peaks.

Examples
```r
mat = create_scDataset_raw()$mat
binned_mat = rebin_matrix(mat, bin_width = 10e6)
dim(binned_mat)
```

reduce_dims_scExp | Reduce dimensions (PCA, TSNE, UMAP)

Description
Reduce dimensions (PCA, TSNE, UMAP)

Usage
```r
reduce_dims_scExp(
scExp,
dimension_reductions = c("PCA", "UMAP"),
n = 10,
batch_correction = FALSE,
batch_list = NULL,
remove_PC = NULL,
verbose = TRUE
)
```

Arguments
- `scExp` A SingleCellExperiment object.
- `dimension_reductions` A character vector of methods to apply. (c("PCA", "TSNE", "UMAP"))
- `n` Numbers of dimensions to keep for PCA. (50)
- `batch_correction` Do batch correction ? (FALSE)
- `batch_list` List of characters. Names are batch names, characters are sample names.
- `remove_PC` A vector of string indicating which principal components to remove before downstream analysis as probably correlated to library size. Should be under the form : 'Component_1', 'Component_2', ... Recommended when using 'TFIDF' normalization method. (NULL)
- `verbose` Print messages ? (TRUE)

Value
A SingleCellExperiment object containing feature spaces. See `reduceDims()`.
Examples

```r
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp = normalize_scExp(scExp, "CPM")
scExp = reduce_dims_scExp(scExp, dimension_reductions=c("PCA", "UMAP"))
```

reduce_dim_batch_correction

*Reduce dimension with batch corrections*

**Description**

Reduce dimension with batch corrections

**Usage**

```r
reduce_dim_batch_correction(scExp, mat, batch_list, n)
```

**Arguments**

- `scExp` SingleCellExperiment
- `mat` The normalized count matrix
- `batch_list` List of batches
- `n` Number of PCs to keep

**Value**

A list containing the SingleCellExperiment with batch info and the corrected pca

---

remove_chr_M_fun

*Remove chromosome M from scExprownames*

**Description**

Remove chromosome M from scExprownames

**Usage**

```r
remove_chr_M_fun(scExp, verbose)
```

**Arguments**

- `scExp` A SingleCellExperiment
- `verbose` Print ?
Value

A SingleCellExperiment without chromosome M (mitochondrial chr)

Description

Remove non canonical chromosomes from scExp

Usage

remove_non_canonical_fun(scExp, verbose)

Arguments

scExp A SingleCellExperiment
verbose Print ?

Value

A SingleCellExperiment without non canonical chromosomes (random,unknown, contigs etc...)

Description

Results of hypergeometric gene set enrichment test

Usage

results_enrichmentTest(  differentialGenes,  enrichment_qval,  GeneSets,  GeneSetsDf,  GenePool )
Arguments

differentialGenes
   Genes significantly over / under expressed

enrichment_qval
   Adjusted p-value threshold above which a pathway is considered significative

GeneSets
   List of pathways

GeneSetsDf
   Data.frame of pathways

GenePool
   Pool of possible genes for testing

Value

A data.frame with pathways passing q.value threshold
run_pairwise_tests

Run pairwise tests

Description

Run pairwise tests

Usage

run_pairwise_tests(affectation,
    by,
    counts,
    feature,
    method,
    progress = NULL,
    BPPARAM = BiocParallel::bpparam())

Arguments

affectation       An annotation data.frame with cell_cluster and cell_id columns
by               = A character specifying the column of the object containing the groups of cells
to compare.
counts              Count matrix
feature             Feature data.frame
method             DA method, Wilcoxon or edgeR
progress       A shiny Progress instance to display progress bar.
BPPARAM      BPPARAM object for multiprocessing. See bpparam for more informations.
               Will take the default BPPARAM set in your R session.

Value

A list containing objects for DA function

run_tsne_scExp

Run tsne on single cell experiment

Description

Run tsne on single cell experiment
Usage

```r
run_tsne_scExp(scExp, verbose = FALSE)
```

Arguments

- `scExp` A SingleCellExperiment Object
- `verbose` Print ?

Value

A colored kable with the number of cells per sample for display

---

Description

Data from a single-cell ChIP-seq experiment against H3K4me3 active mark from two cell lines, Jurkat B cells and Ramos T cells from Grosselin et al., 2019. The count matrices, on 5kb bins, were given to ChromSCape and the filtering parameter was set to 3% of cells active in regions and subsampled down to 150 cells per sample. After correlation filtering, the experiment is composed of respectively 51 and 55 cells from Jurkat & Ramos and 5499 5kbp-genomic bins where signal is located.

Usage

```r
data("scExp")
```

Format

`scExp` - a SingleCellExperiment with 106 cells and 5499 features (genomic bins) in hg38:

- `chr` A SingleCellExperiment

Details

The scExp is composed of:

- counts and normcounts assays, PCA, UMAP, and Correlation matrix in reducedDims(scExp)
- Assignation of genes to genomic bins in rowRanges(scExp)
- Cluster information in colData(scExp) correlation
- Hierarchical clustering dendogram in metadata$hc_cor
- Consensus clustering raw data in metadata$consclust
- Consensus clustering cluster-consensus and item consensus dataframes in metadata$sicl
- Differential analysis in metadata$diff
- Gene Set Analysis in metadata$enr
separate_BAM_into_clusters

Separate BAM files into cell cluster BAM files

Description

Separate BAM files into cell cluster BAM files

Usage

separate_BAM_into_clusters(affectation, odir, merged_bam)

Arguments

- **affectation**: An annotation data.frame containing cell_id and cell_cluster columns
- **odir**: A valid output directory path
- **merged_bam**: A list of merged bam file paths

@importFrom Rsamtools filterBam ScanBamParam

Value

Create one BAM per cluster from one BAM per condition

separator_count_mat

Determine Count matrix separator ("tab" or "," )

Description

Determine Count matrix separator ("tab" or "," )

Usage

separator_count_mat(path_to_matrix)

Arguments

- **path_to_matrix**: A path towards the count matrix to check
**Value**

A character separator

---

**smoothBin**  
*Smooth a vector of values with nb_bins left and right values*

---

**Description**

Smooth a vector of values with nb_bins left and right values

**Usage**

```r
smoothBin(bin_score, nb_bins = 10)
```

**Arguments**

- **bin_score**: A numeric vector of values to be smoothed
- **nb_bins**: Number of values to take left and right

---

**Value**

A smooth vector of the same size

---

**subsample_scExp**  
*Subsample scExp*

---

**Description**

Randomly sample x cells from each sample in a SingleCellExperiment to return a subsampled SingleCellExperiment with all samples having maximum n cells. If n is higher than the number of cell in a sample, this sample will not be subsampled.

**Usage**

```r
subsample_scExp(scExp, n_cell_per_sample = 500, n_cell_total = NULL)
```

**Arguments**

- **scExp**: A SingleCellExperiment
- **n_cell_per_sample**: An integer number of cells to subsample for each sample. Exclusive with n_cells_total. (500)
- **n_cell_total**: An integer number of cells to subsample in total. Exclusive with n_cell_per_sample (NULL).
Value

A subsampled SingleCellExperiment

Examples

```r
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp_sub = subsample_scExp(scExp,50)
## Not run: num_cell_scExp(scExp_sub)
```

subset_bam_call_peaks  Peak calling on cell clusters

Description

This function does peak calling on each cell population in order to refine gene annotation for large bins. For instance, a 50000bp bins might contain the TSS of several genes, while in reality only one or two of these genes are overlapping the signal (peak). To do so, first in-silico cell sorting is applied based on previously defined clusters contained in the SingleCellExperiment. Taking BAM files of each sample as input, samtools pools then splits reads from each cell barcode into 1 BAM file per cell cluster (pseudo-bulk). Then MACS2 calls peaks on each cluster. The peaks are aggregated and merged if closer to a certain distance defined by user (10000bp). Then,

This function takes as input a SingleCellExperiment, that must contain a 'cell_cluster' column in it's colData, an output directory where to store temporary files, the list of BAM files corresponding to each sample and containing the cell barcode information as a tag (for instance tag CB:Z:xxx, XB:Z:xxx or else...) or single-cell BED files containing the raw reads and corresponding to the 'barcode' column metadata, the p.value used by MACS2 to distinguish significant peaks, the reference genome (either hg38 or mm10), the maximal merging distance in bp and a data.frame containing gene TSS genomic coordinates of corresponding genome (if set to NULL, will automatically load geneTSS). The output is a SingleCellExperiment with GRanges object containing ranges of each merged peaks that falls within genomic bins of the SingleCellExperiment, saving the bin range as additional column (window_chr, window_start, window_end), as well as the closest genes and their distance relative to the peak. The peaks may be present in several rows if multiple genes are close / overlap to the peaks.

Note that the user must have MACS2 installed and available in the PATH. Users can open command terminal and type `which macs2` to verify the availability of these programs. Will only work on unix operating system. Check operating system with `print(.Platform)`.

Usage

```r
subset_bam_call_peaks(
  scExp,
  odir,
  input,
  format = "BAM",
```

```r```
subset_bam_call_peaks

```r
p.value = 0.05,
ref = "hg38",
peak_distance_to_merge = 10000,
geneTSS_annotation = NULL,
run_coverage = FALSE,
progress = NULL
)
```

**Arguments**

- `scExp` A SingleCellExperiment object
- `odir` Output directory where to write temporary files and each cluster’s BAM file
- `input` A character vector of file paths to each sample’s BAM file, containing cell barcode information as tags. BAM files can be paired-end or single-end.
- `format` Format of the input data, either "BAM" or "scBED".
- `p.value` a p-value to use for MACS2 to determine significant peaks. (0.05)
- `ref` A reference genome, either hg38 or mm10. ('hg38')
- `peak_distance_to_merge` Maximal distance to merge peaks together after peak calling, in bp. (10000)
- `geneTSS_annotation` A data.frame annotation of genes TSS. If NULL will automatically load Gen- code list of genes fro specified reference genome.
- `run_coverage` Create coverage tracks (.bw) for each cluster?
- `progress` A shiny Progress instance to display progress bar.

**Details**

The BED files of the peaks called for each clusters, as well as the merged peaks are written in the output directory.

**Value**

A SingleCellExperiment with refined annotation

**Examples**

```r
## Not run:
data("scExp")
subset_bam_call_peaks(scExp, "path/to/out/", list("sample1" = "path/to/BAM/sample1.bam", "sample2" = "path/to/BAM/sample2.bam"),
p.value = 0.05, ref = "hg38", peak_distance_to_merge = 10000,
geneTSS_annotation = NULL)

## End(Not run)
```
**summary_DA**  
*Summary of the differential analysis*

**Description**
Summary of the differential analysis

**Usage**

```r
summary_DA(scExp, qval.th = 0.01, logFC.th = 1, min.percent = 0.01)
```

**Arguments**

- `scExp`: A SingleCellExperiment object containing consclust with selected number of cluster.
- `qval.th`: Adjusted p-value threshold. (0.01)
- `logFC.th`: Fold change threshold. (1)
- `min.percent`: Minimum fraction of cells having the feature active to consider it as significantly differential. (0.01)

**Value**
A table summary of the differential analysis

**Examples**

```r
data('scExp')
summary_DA(scExp)
```

---

**swapAltExp_sameColData**  
*Swap main & alternative Experiments, with fixed colData*

**Description**
Swap main & alternative Experiments, with fixed colData

**Usage**

```r
swapAltExp_sameColData(scExp, alt)
```

**Arguments**

- `scExp`: A SingleCellExperiment
- `alt`: Name of the alternative experiment
A swapped SingleCellExperiment with the exact same colData.

Examples

```r
data(scExp)
swapAltExp_sameColData(scExp, "peaks")
```

---

**table_enriched_genes_scExp**

*C Mahmood, K Jones, A Marques, J Jombart

This function creates a data table of enriched gene sets based on a SingleCellExperiment object.

### Description

Creates table of enriched genes sets

### Usage

```r
table_enriched_genes_scExp(
  scExp,
  set = "Both",
  group = "C1",
  enr_class_sel = c("c1_positional", "c2_curated", "c3_motif", "c4_computational", "c5_GO", "c6_oncogenic", "c7_immunologic", "hallmark")
)
```

### Arguments

- `scExp` A SingleCellExperiment object containing list of enriched gene sets.
- `set` A character vector, either 'Both', 'Overexpressed' or 'Underexpressed'. (‘Both’)
- `group` The "group" name from differential analysis. Can be the cluster name or the custom name in case of a custom differential analysis.
- `enr_class_sel` Which classes of gene sets to show. (c('c1_positional', 'c2_curated', ...))

### Value

A DT::data.table of enriched gene sets.

### Examples

```r
data("scExp")
## Not run: table_enriched_genes_scExp(scExp)
```
Description

Warning for differential_analysis_scExp

Usage

warning_DA(scExp, by, de_type, method, block, group, ref)

Arguments

scExp A SingleCellExperiment object containing consclust with selected number of cluster.
by = A character specifying the column of the object containing the groups of cells to compare. Exclusive with de_type == custom
de_type Type of comparisons. Either 'one_vs_rest', to compare each cluster against all others, or 'pairwise' to make 1 to 1 comparisons. ('one_vs_rest')
method Wilcoxon or edgerGLM
block Use batches as blocking factors ?
group If de_type is custom, the group to compare (data.frame), must be a one-column data.frame with cell_clusters or sample_id as character in rows
ref If de_type is custom, the reference to compare (data.frame), must be a one-column data.frame with cell_clusters or sample_id as character in rows

Value

Warnings or Errors if the input are not correct

Description

warning_filter_correlated_cell_scExp

warning_filter_correlated_cell_scExp

warning_filter_correlated_cell_scExp
Usage

warning_filter_correlated_cell_scExp(
    scExp,
    random_iter,
    corr_threshold,
    percent_correlation,
    run_tsne,
    downsample,
    verbose
)

Arguments

scExp A SingleCellExperiment object containing 'Cor', a correlation matrix, in reducedDims.
random_iter Number of random matrices to create to calculate random correlation scores. (50)
corr_threshold Quantile of random correlation score above which a cell is considered to be 'correlated' with another cell. (99)
percent_correlation Percentage of the cells that any cell must be 'correlated' to in order to not be filtered. (1)
run_tsne Re-run tsne ? (FALSE)
downsample Number of cells to calculate correlation filtering threshold ? (2500)
verbose (TRUE)

Value

Warnings or Errors if the input are not correct

Description

A warning helper for plot_reduced_dim_scExp

Usage

warning_plot_reduced_dim_scExp(
    scExp,
    color_by,
    reduced_dim,
    downsample,
transparency,
size,
max_distanceToTSS,
annotate_clusters,
min_quantile,
max_quantile
)

Arguments

scExp A SingleCellExperiment Object
color_by Feature used for coloration
reduced_dim Reduced Dimension used for plotting
downsample Number of cells to downsample
transparency Alpha parameter, between 0 and 1
size Size of the points.
max_distanceToTSS Numeric. Maximum distance to a gene’s TSS to consider a region linked to a gene.
annotate_clusters A logical indicating if clusters should be labelled. The 'cell_cluster' column should be present in metadata.
min_quantile The lower threshold to remove outlier cells, as quantile of cell embeddings (between 0 and 0.5).
max_quantile The upper threshold to remove outlier cells, as quantile of cell embeddings (between 0.5 and 1).

Value

Warning or errors if the inputs are not correct

Description

Warning for raw_counts_to_sparse_matrix
Usage

```r
warning_raw_counts_to_sparse_matrix(
  files_dir_list,
  file_type = c("scBAM", "scBED", "SparseMatrix"),
  peak_file = NULL,
  n_bins = NULL,
  bin_width = NULL,
  genebody = NULL,
  extendPromoter = 2500,
  verbose = TRUE,
  ref = "hg38"
)
```

Arguments

- `files_dir_list`: A named character vector of directories containing the raw files.
- `file_type`: Input file(s) type(s) ("scBED","scBAM","SparseMatrix").
- `peak_file`: A file containing genomic location of peaks (NULL).
- `n_bins`: The number of bins to tile the genome (NULL).
- `bin_width`: The size of bins to tile the genome (NULL).
- `genebody`: Count on genes (body + promoter) ? (NULL).
- `extendPromoter`: If counting on genes, number of base pairs to extend up or downstream of TSS (2500).
- `verbose`: Verbose (TRUE).
- `ref`: Reference genome to use (hg38).

Value

Error or warnings if the input are not correct

---

Wrapper around `FeatureMatrix` function from Signac Package

Description

Wrapper around `FeatureMatrix` function from Signac Package.
wrapper_Signac_FeatureMatrix

Usage

```r
wrapper_Signac_FeatureMatrix(
  files_dir_list,
  which,
  ref = "hg38",
  process_n = 2000,
  set_future_plan = TRUE,
  verbose = TRUE,
  progress = NULL
)
```

Arguments

- `files_dir_list`: A named character vector of directories containing the files. The names correspond to sample names.
- `which`: A GenomicRanges containing the features to count on.
- `ref`: Reference genome to use (hg38). Chromosomes that are not present in the canonical chromosomes of the given reference genome will be excluded from the matrix.
- `process_n`: Number of regions to load into memory at a time, per thread. Processing more regions at once can be faster but uses more memory. (2000)
- `set_future_plan`: Set ‘multisession’ plan within the function (TRUE). If TRUE, the previous plan (e.g. future::plan()) will be set back on exit.
- `verbose`: Verbose (TRUE).
- `progress`: Progress object for Shiny.

Details

Signac & future are not required packages for ChromSCape as they are required only for the fragment matrix calculations. To use this function, install Signac package first (future will be installed as a dependency). For the simplicity of the application & optimization, the function by default sets future::plan("multisession") with workers = future::availableCores(omit = 1) in order to allow parallel processing with Signac. On exit the plan is re-set to the previously set future plan. Note that future multisession may have trouble running when VPN is on. To run in parallel, first deactivate your VPN if you encounter long runtimes.

Value

A sparse matrix of features x cells

References

Stuart el al., Multimodal single-cell chromatin analysis with Signac bioRxiv https://doi.org/10.1101/2020.11.09.373613
Examples

```r
## Not run:
gr_bins = define_feature("hg38", bin_width = 50000)
wrapper_Signac_FeatureMatrix("/path/to/dir_containing_fragment_files",
gr_bins, ref = "hg38")

## End(Not run)
```
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