Package 'ChromSCape'

October 21, 2025

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

Version 1.19.1

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, vizualisation, clustering, differential analysis and gene set analysis.

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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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BugReports https://github.com/vallotlab/ChromSCape/issues

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2 Contents

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Author Pacome Prompsy [aut, cre] (ORCID: https://orcid.org/0000-0003-4375-7583), Celine Vallot [aut] (ORCID: https://orcid.org/0000-0003-1601-2359)
Maintainer Pacome Prompsy <pacome.pr@gmail.com></pacome.pr@gmail.com>

Contents

annotation_from_merged_peaks
annotToCol2
anocol_binary
anocol_categorical
bams_to_matrix_indexes
beds_to_matrix_indexes
calculate_CNA
calculate_cyto_mat
calculate_gain_or_loss
calculate_logRatio_CNA
call_macs2_merge_peaks
ce11.chromosomes
ce11.GeneTSS
changeRange
CheA3_TF_nTargets
check_correct_datamatrix
choose_cluster_scExp
choose_perplexity
col2hex
colors_scExp
combine_datamatrix
combine_enrichmentTests
comparable_variables
CompareedgeRGLM
CompareWilcox
concatenate_scBed_into_clusters
consensus_clustering_scExp
correlation_and_hierarchical_clust_scExp
count_coverage
create_project_folder
create_sample_name_mat
create_scDataset_raw
create_scExp
DA_custom
DA one vs rest

Contents 3

DA_pairwise	30
define_feature	31
detect_samples	32
differential_activation	32
differential_analysis_scExp	33
distPearson	35
enrichmentTest	35
enrich_TF_ChEA3_genes	36
enrich_TF_ChEA3_scExp	36
exclude_features_scExp	37
feature_annotation_scExp	38
filter_correlated_cell_scExp	39
filter_genes_with_refined_peak_annotation	40
filter_scExp	41
find_clusters_louvain_scExp	42
find_top_features	43
generate_analysis	44
generate_count_matrix	46
generate_coverage_tracks	
generate_feature_names	
generate_report	
gene_set_enrichment_analysis_scExp	
getExperimentNames	
getMainExperiment	
get_color_dataframe_from_input	
get_cyto_features	
get_genomic_coordinates	
get_most_variable_cyto	
get_pathway_mat_scExp	
gg_fill_hue	
groupMat	
H1proportion	
has_genomic_coordinates	57
hclustAnnotHeatmapPlot	57
hg38.chromosomes	58
hg38.cytoBand	59
hg38.GeneTSS	59
imageCol	60
import_count_input_files	60
import_scExp	61
index_peaks_barcodes_to_matrix_indexes	62
inter_correlation_scExp	63
intra_correlation_scExp	63
launchApp	64
load_MSIGdb	65
merge_MACS2_peaks	65
mm10.chromosomes	66
mm10.cytoBand	66
mm10.GeneTSS	67
normalize_scExp	67
num_cell_after_cor_filt_scExp	
num_cell_after_OC_filt_scExp	69
HILLI CARL ZUNA VIV. HIL MALAD	(19

4 Contents

num_cell_before_cor_filt_scExp	69
num_cell_in_cluster_scExp	70
num_cell_scExp	70
oca_irlba_for_sparseMatrix	71
olot_cluster_consensus_scExp	71
olot_correlation_PCA_scExp	72
olot_coverage_BigWig	73
olot_differential_summary_scExp	74
olot_differential_volcano_scExp	74
olot_distribution_scExp	75
blot_gain_or_loss_barplots	76
olot_heatmap_scExp	77
olot_inter_correlation_scExp	77
olot_intra_correlation_scExp	78
plot_most_contributing_features	79
plot_percent_active_feature_scExp	80
olot_pie_most_contributing_chr	81
plot_reduced_dim_scExp	81
plot_reduced_dim_scExp_CNA	83
olot_top_TF_scExp	83
olot_violin_feature_scExp	84
preprocessing_filtering_and_reduction	85
preprocess_CPM	
preprocess_feature_size_only	
preprocess_RPKM	
preprocess_TFIDF	
preprocess_TPM	
rawfile_ToBigWig	
aw_counts_to_sparse_matrix	
read_count_mat_with_separated_chr_start_end	
read_sparse_matrix	
rebin_helper	93
ebin matrix	93
educe_dims_scExp	
reduce dim batch correction	95
remove_chr_M_fun	96
remove_non_canonical_fun	96
results_enrichmentTest	97
etrieve_top_bot_features_pca	97
un_pairwise_tests	98
run_tsne_scExp	
ccExp	
separate_BAM_into_clusters	
-	
separator_count_mat	
smoothBin	
subsample_scExp	
subset_bam_call_peaks	
summary_DA	
swapAltExp_sameColData	
able_enriched_genes_scExp	
warning_DA	
varning inter correlated cell schan	107

```
      warning_plot_reduced_dim_scExp
      107

      warning_raw_counts_to_sparse_matrix
      108

      wrapper_Signac_FeatureMatrix
      109

      Index
      111
```

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

annotToCol2

annot To Col 2

Description

annot To Col 2

Usage

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

6 anocol_binary

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?

 ${\tt plotLegendFile} \ \ Which \ file \ to \ plot \ legend \ ?$

Value

A matrix of continuous or discrete colors

Examples

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

anocol_binary

Helper binary column for anocol function

Description

Helper binary column for anocol function

Usage

```
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 binrary columns colored

anocol_categorical 7

anocol_categorical

Helper binary column for anocol function

Description

Helper binary column for anocol function

Usage

```
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 binrary 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 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

8 calculate_CNA

```
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 cyto band. See calculate_gain_or_loss

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

Usage

```
calculate_CNA(
   scExp,
   control_samples = unique(scExp$sample_id)[1],
   ref_genome = c("hg38", "mm10", "ce11")[1],
   quantiles_to_define_gol = c(0.05, 0.95)
)
```

calculate_cyto_mat 9

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", "ce11")[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".

calculate_gain_or_loss

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

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

Arguments

quantiles

scExp A SingleCellExperiment with "logRatio_cytoBand" reducedDim slot filled. See ${\tt calculate_logRatio_CNA}$

controls Sample IDs or Cell IDs of the normal sample to take as reference.

Quantiles of normal log2-ratio distribution below/above which cytoband is con-

sidered 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

```
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

```
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

```
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

12 ce11.chromosomes

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

ce11.chromosomes

Data.frame of chromosome length - cel1

Description

This data frame provides the length of each "canonical" chromosomes of C Elegans genome build cell.

Usage

```
data("ce11.chromosomes")
```

Format

ce11.chromosomes - a data frame with 7 chromosomes and 3 variables:

```
chr Chromosome - characterstart Start of the chromosome (bp) - integerend End of the chromosome (bp) - integer
```

Value

ce11.chromosomes - a data frame with 7 chromosomes and 3 variables

ce11.GeneTSS

ce11.GeneTSS

Data.frame of gene TSS - cell

Description

This dataframe was extracted from Gencode v25 and report the Transcription Start Site of each gene in the C Elegans genome build ce11

Usage

```
data("ce11.GeneTSS")
```

Format

```
ce11.GeneTSS - a data frame with 20,051 genes and 5 variables:
```

```
chr Chromosome - character
```

start Start of the gene (TSS) - integer

end End of the gene - integer

Gene Gene symbol - character

strand Srand - character

Value

ce11.GeneTSS - a data frame with 20,051 genes and 5 variables

changeRange

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

14 CheA3_TF_nTargets

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

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

Format

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

Value

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

References

Keenan AB, Torre D, Lachmann A, Leong AK, Wojciechowicz M, Utti V, Jagodnik K, Kropiwnicki E, Wang Z, Ma'ayan A (2019) ChEA3: transcription factor enrichment analysis by orthogonal omics integration. Nucleic Acids Research. doi: 10.1093/nar/gkz446

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

```
data("CheA3_TF_nTargets")
head(CheA3_TF_nTargets)
```

```
check_correct_datamatrix
```

Check if matrix rownames are well formated and correct if needed

Description

Throws warnings / error if matrix is in the wrong format

Usage

```
check_correct_datamatrix(datamatrix_single, sample_name = "")
```

Arguments

Value

A sparseMatrix in the right rownames format

Description

This functions takes as input a SingleCellExperiment object and a number of cluster to select. It outputs a SingleCellExperiment object with each cell assigned to a correlation cluster in colData. Also calculates a hierarchical clustering of the consensus associations calculated by Consensus-ClusterPlus.

Usage

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

Arguments

scExp A SingleCellExperiment object containing consclust in metadata.

nclust Number of cluster to pick (3)

consensus Use consensus clustering results instead of simple hierarchical clustering? (FALSE)

hc_linkage A linkage method for hierarchical clustering. See cor. ('ward.D')

16 col2hex

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)
```

Arguments

cname

Color name

Value

The HEX color code of a particular color

colors_scExp 17

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

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

Arguments

Value

A combined sparse matrix

```
combine_enrichmentTests
```

Run enrichment tests and combine into list

Description

Run enrichment tests and combine into list

Usage

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

Arguments

diff Differential list enrichment_qval

Adusted p-value threshold above which a pathway is considered significative list

qval.th Differential analysis adjusted p.value threshold logFC.th Differential analysis log-fold change threshold

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

differential. (0.01)

comparable_variables 19

annotFeat_long Long annotation

peak_distance Maximum gene to peak distance

refined_annotation

Refined annotation data.frame if peak calling is done

GeneSets List of pathways

GeneSetsDf Data.frame of pathways

GenePool Pool of possible genes for testing

progress A shiny Progress instance to display progress bar.

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

scExp A SingleCellExperiment

allExp A logical indicating wether alternative experiments comparable variables should

also be fetch.

Value

A character vector with the comparable variable names

CompareedgeRGLM Creates a summary table with the number of genes under- or overex-

pressed 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

```
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

```
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$ID
ref_group = list("C1"=scExp_cf$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)
```

CompareWilcox 21

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 conteract 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

Examples

```
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$ID
ref_group = list("C1"=scExp_cf$cell_id[which(scExp_cf$cell_cluster=="C1")])
groups = list("C2"=scExp_cf$cell_id[which(scExp_cf$cell_cluster=="C2")])
myres = CompareWilcox(as.matrix(SingleCellExperiment::normcounts(scExp_cf)),
annot=as.data.frame(SingleCellExperiment::colData(scExp_cf)),
    ref_group=ref_group,groups=groups, featureTab=featureTab)
```

```
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

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

Runs consensus hierarchical clustering on PCA feature space of scExp object. Plot consensus scores for each number of clusters. See ConsensusClusterPlus - Wilkerson, M.D., Hayes, D.N. (2010). ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. Bioinformatics, 2010 Jun 15;26(12):1572-3.

Usage

```
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 abosulte 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' heirarchical (hclust), 'pam' for paritioning 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')
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 functions 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

ConsensusClusterPlus package by Wilkerson, M.D., Hayes, D.N. (2010). ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. Bioinformatics, 2010 Jun 15;26(12):1572-3.

Examples

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

24 count_coverage

```
correlation_and_hierarchical_clust_scExp

Correlation and hierarchical clustering
```

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')

Details

This functions 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 & hiearchical clustering.

Examples

```
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.

create_project_folder 25

Usage

```
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

canonical_chr GenomicRanges of the chromosomes to read the BAM file.

norm_factor Then number of cells or total number of reads in the given sample, for normal-

ization.

n_smoothBin Number of bins left and right to smooth the signal.

ref Genomic reference read_size Length of the reads

original_bins Original bins GenomicRanges in case the format is raw

matrix.

Value

A binned GenomicRanges that can be readily exported into bigwig file.

```
create_project_folder Create ChromSCape project folder
```

Description

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

Usage

```
create_project_folder(
  output_directory,
  analysis_name = "Analysis_1",
  ref_genome = c("hg38", "mm10", "ce11")[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.

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

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

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

Number of samples nb_samples

Character vector of sample names samples_names

Value

A matrix

create_scDataset_raw 27

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))
)
```

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

```
# Creating a basic sparse 600 genomic bins x 300 cells matrix and annotation
1 = create_scDataset_raw()
head(1$mat)
head(1$annot)
head(1$batches)

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

# Specifying species
mouse_1 = create_scDataset_raw(ref="mm10")

# Specifying batches
batch_1 = create_scDataset_raw(nsamp=4, batch_id = factor(c(1,1,2,2)))
```

28 create_scExp

```
# Peaks of different size as features
peak_1 = create_scDataset_raw(featureType="peak")
head(peak_1$mat)

# Genes as features
gene_1 = create_scDataset_raw(featureType="gene")
head(gene_1$mat)
```

create_scExp

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

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 canconical 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).
                 A data frame containing informations on cells. Should have the same number of
annot
                 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)
                 Name of the mainExpName e.g. 'bins', 'peaks'... ("default")
mainExpName
verbose
                 (TRUE)
```

Value

Returns a SingleCellExperiment object.

DA_custom 29

Examples

```
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp</pre>
```

DA_custom

Differential Analysis Custom in 'One vs One' mode

Description

Differential Analysis Custom in 'One vs One' mode

Usage

```
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 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

DA_one_vs_rest

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 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

define_feature 31

Usage

```
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

Description

Define the features on which reads will be counted

Usage

```
define_feature(ref = c("hg38","mm10", "ce11")[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 divinding genome into fixed width bins

genebody A logical indicating if feature should be counted in genebodies and promoter.

extendPromoter Extension length before TSS (2500).

32 differential_activation

Value

A GRanges object

Examples

```
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

```
detect_samples(barcodes, nb_samples = 1)
```

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

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

```
differential_activation
```

Find Differentialy Activated Features (One vs All)

Description

Based on the statement that single-cell epigenomic dataset are very sparse, specifically when analysis 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

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

Arguments

scExp A SingleCellExperiment object containing consclust with selected number of

cluster.

by Which grouping to run the marker enrichment?

verbose Print?

progress A shiny Progress instance to display progress bar.

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 SingleCellExperiment 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()
)
```

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		

re

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 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.

distPearson 35

Value

Returns a SingleCellExperiment object containing a differential list.

Examples

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

distPearson

distPearson

Description

distPearson

Usage

distPearson(m)

Arguments

m

A matrix

Value

A dist object

enrichmentTest

enrichment Test

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

Keenan AB, Torre D, Lachmann A, Leong AK, Wojciechowicz M, Utti V, Jagodnik K, Kropiwnicki E, Wang Z, Ma'ayan A (2019) ChEA3: transcription factor enrichment analysis by orthogonal omics integration. Nucleic Acids Research. doi: 10.1093/nar/gkz446 +

Examples

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

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

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

exclude_features_scExp

Arguments

scExp	A SingleCellExperiment object containing list of differential features.
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

```
data("scExp")
scExp = enrich_TF_ChEA3_scExp(
    scExp,
    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)

```
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</pre>
```

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', 'mm10' or 'ce11'. ('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))</pre>
```

```
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.

Usage

```
filter_correlated_cell_scExp(scExp, random_iter = 5,
corr_threshold = 99, percent_correlation = 1,
downsample = 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 functions 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

```
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 41

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.

Usage

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

Arguments

Value

Returns a filtered SingleCellExperiment object.

```
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)</pre>
```

```
find_clusters_louvain_scExp
```

Build SNN graph and find cluster using Louvain Algorithm

Description

Build SNN graph and find cluster using Louvain Algorithm

Usage

```
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)

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

find_top_features 43

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 th

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).

(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

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

44 generate_analysis

generate_analysis

Generate a complete ChromSCape analysis

Description

Generate a complete ChromSCape analysis

Usage

```
generate_analysis(input_data_folder,
analysis_name = "Analysis_1",
output_directory = "./",
input_data_type = c("scBED", "DenseMatrix", "SparseMatrix", "scBAM")[1],
feature_count_on = c("bins", "genebody", "peaks")[1],
feature_count_parameter = 50000,
rebin_sparse_matrix = FALSE,
ref_genome = c("hg38","mm10", "ce11")[1],
run = c("filter", "CNA","cluster", "consensus", "coverage",
"DA", "GSA", "report")[c(1,3,5,6,7,8)],
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.
```

generate_analysis 45

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.

rebin_sparse_matrix

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

The genome of reference. ref_genome

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

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.

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

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

Number of clusters to force choice of clusters. n_clust

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.

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

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.

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

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.

```
## 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.

```
generate_coverage_tracks(
   scExp_cf,
   input,
   odir,
   format = "scBED",
   ref_genome = c("hg38", "mm10", "ce11")[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.

```
## 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)
```

48 generate_report

```
generate_feature_names
```

Generate feature names

Description

Generate feature names

Usage

```
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.

Usage

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
```

Runs Gene Set Enrichment Analysis on genes associated with differential features

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.

```
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
)
```

50 getExperimentNames

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', 'mm10', 'ce11'. ('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 auto-

matically 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)

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 recommanding 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)
```

getExperimentNames

Get experiment names from a SingleCellExperiment

Description

Get experiment names from a SingleCellExperiment

```
getExperimentNames(scExp)
```

getMainExperiment 51

Arguments

scExp

A SingleCellExperiment with named mainExp and altExps.

Value

Character vector of unique experiment names

Examples

```
data(scExp)
getExperimentNames(scExp)
```

getMainExperiment

Get Main experiment of a SingleCellExperiment

Description

Get Main experiment of a SingleCellExperiment

Usage

```
getMainExperiment(scExp)
```

Arguments

scExp

 $A\ Single Cell Experiment\ with\ named\ main Exp\ and\ alt Exps.$

Value

The swapped SingleCellExperiment towards "main" experiment

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

52 get_cyto_features

Description

Get color dataframe from shiny::colorInput

Usage

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

Arguments

```
input Shiny input object

levels_selected

Names of the features

color_by Which feature color to retrieve
input_id_prefix

Prefix in front of the feature names
```

Value

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

Description

Map the 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 canconical chromosomes), and are removed from the returned object.

Usage

```
get_cyto_features(scExp, ref_genome = c("hg38", "mm10", "ce11")[1])
```

Arguments

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

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

```
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.

```
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
feature_GRanges = get_genomic_coordinates(scExp)</pre>
```

```
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 interseting 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).

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

```
get_pathway_mat_scExp(
    scExp,
    pathways,
    max_distanceToTSS = 1000,
    ref = "hg38",
    GeneSetClasses = c("c1_positional", "c2_curated", "c3_motif", "c4_computational",
        "c5_G0", "c6_oncogenic", "c7_immunologic", "hallmark"),
    progress = NULL
)
```

gg_fill_hue 55

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)#' @param ref

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")
```

gg_fill_hue gg_fill_hue

Description

gg_fill_hue

Usage

gg_fill_hue(n)

Arguments

n num hues

Value

A color in HEX format

56 H1proportion

groupMat

groupMat

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

H1 proportion

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

```
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)</pre>
```

hclustAnnotHeatmapPlot

hclust Annot Heatmap Plot

Description

hclust Annot Heat map Plot

```
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
)
```

58 hg38.chromosomes

Arguments

x A correlation matrix
 hc An helust 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

Value

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

hg38.cytoBand 59

hg38.cytoBand

Data.frame of cytoBandlocation - 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 cytobands 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

Value

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

hg38.GeneTSS

Data.frame of gene TSS - hg38

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

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

Format

hg38.GeneTSS - a data frame with 32,937 genes and 5 variables:

chr Chromosome - character

start Start of the gene (TSS) - integer

end End of the gene - integer

Gene Gene symbol - character

Strand - character

Value

hg38.GeneTSS - a data frame with 32,937 genes and 5 variables

imageCol

imageCol

Description

imageCol

Usage

```
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
```

Value

A rectangular image

```
import_count_input_files
```

Import and count input files depending on their format

Description

Import and count input files depending on their format

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

import_scExp 61

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

 $import_scExp$

Read single-cell matrix(ces) into scExp

Description

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

Usage

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

Arguments

file_paths A character vector of file names towards single cell epigenomic matrices (fea-

tures x cells) (must be .txt / .tsv)

remove_pattern A string pattern to remove from the sample names. Can be a regexp.

wards temporary files. (NULL)

Value

A list containing:

• datamatrix: a sparseMatrix of features x cells

• annot_raw: an annotation of cells as data.frame

Examples

```
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

Usage

```
inter_correlation_scExp(
    scExp_cf,
    by = c("sample_id", "cell_cluster")[1],
    reference_group = unique(scExp_cf[[by]])[1],
    other_groups = unique(scExp_cf[[by]]),
    fullCor = 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".

A logical specifying if the correlation matrix was calculated on the entire set of

cells (TRUE).

Value

fullCor

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

Examples

```
data(scExp)
inter_correlation_scExp(scExp)
```

```
intra_correlation_scExp
```

Calculate intra correlation between cluster or samples

Description

Calculate intra correlation between cluster or samples

64 launchApp

Usage

```
intra_correlation_scExp(
   scExp_cf,
   by = c("sample_id", "cell_cluster")[1],
   fullCor = 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

```
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

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

Arguments

```
launch.browser Wether to launch browser or not
... Additional parameters passed to runApp
```

Value

Launches the shiny application

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

load_MSIGdb 65

load_MSIGdb

Load and format MSIGdb pathways using msigdbr package

Description

Load and format MSIGdb pathways using msigdbr package

Usage

```
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

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

Arguments

Value

Peaks as GRanges

66 mm10.cytoBand

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 chromosomes and 3 variables:

```
chr Chromosome - character
```

start Start of the chromosome (bp) - integer

end End of the chromosome (bp) - integer

Value

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

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 403 cytobands 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

Value

mm10.cytoBand - a data frame with $403\ cytobands$ and $4\ variables.$

mm10.GeneTSS 67

mm10.GeneTSS

Data.frame of gene TSS - mm10

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")
```

Strand - character

Format

```
mm10.GeneTSS - a data frame with 27,916 genes and 5 variables:

chr Chromosome name - character

start Start of the gene (TSS) - integer

end End of the gene - integer

Gene Gene symbol - character
```

Value

mm10.GeneTSS - a data frame with 27,916 genes and 5 variables

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.

type

Which normalization to apply. Either 'CPM', 'TFIDF', 'RPKM', 'TPM' or 'feature_size_only'. Note that for all normalization by size (RPKM, TPM, feature_size_only), the features must have defined genomic coordinates.

Value

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

Examples

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

Description

Number of cells before & after correlation filtering

Usage

```
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

```
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)
```

Description

Table of cells before / after QC

Usage

```
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

```
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,as.data.frame(SingleCellExperiment::colData(scExp)), counts(scExp))
## End(Not run)</pre>
```

```
num_cell_before_cor_filt_scExp
```

Table of number of cells before correlation filtering

Description

Table of number of cells before correlation filtering

Usage

```
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

70 num_cell_scExp

Examples

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

```
num_cell_in_cluster_scExp
```

Number of cells in each cluster

Description

Number of cells in each cluster

Usage

```
num_cell_in_cluster_scExp(scExp)
```

Arguments

scExp

A SingleCellExperiment object containing chromatin groups.

Value

A formatted kable of cell assignation to each cluster.

Examples

```
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))</pre>
```

```
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 increamental 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)
```

Arguments

x A sparse normalized matrix (features x cells)n_compThe 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.

```
{\tt plot\_cluster\_consensus\_scExp} \\ {\tt Plot\ cluster\ consensus}
```

Description

Plot cluster consensus score for each k as a bargraph.

```
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

```
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 cal-

culate 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

```
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

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

Arguments

Value

A coverage plot annotated with genes

```
data(scExp)
```

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

plot_distribution_scExp 75

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")
```

```
plot_distribution_scExp
```

Plotting distribution of signal

Description

Plotting distribution of signal

Usage

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

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

```
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 Plot Gain or Loss of cytobands of the most variables cytobands

Usage

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

Arguments

scExp	$A \ Single Cell Experiment \ with \ "logRatio_cytoBand" \ reduced Dim \ slot \ filled. \ See \ calculate_logRatio_CNA$
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

```
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])])

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 77

plot_heatmap_scExp	Plot cell correlation heatmap with annotations
proc_neatmap_occxp	1 tot cett corretation neather with annotations

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,
    downsample = 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
downsample	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

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

```
plot_inter_correlation_scExp
```

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

Description

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

Usage

```
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 Downsample for plotting

Value

A violin plot of inter-correlation

Examples

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

```
plot_intra_correlation_scExp
```

Violin plot of intra-correlation distribution

Description

Violin plot of intra-correlation distribution

```
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

Usage

```
plot_most_contributing_features(
   scExp,
   component = "Component_1",
   n_top_bot = 10
)
```

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

Details

If a gene TSS is within 10,000bp of the region, the name of the gene(s) will be displayed instead of the region

Value

A barplot of top and bottom features with the largest absolute value in the component of interest

```
data(scExp)
plot_most_contributing_features(scExp, component = "Component_1")
```

Description

Barplot of the % of active cells for a given features

Usage

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

Arguments

scExp A SingleCellExperiment

gene A character specifying the gene to plot

by Color violin by cell_cluster or sample_id ("cell_cluster")

highlight A specific group to highlight in a one vs all fashion

downsample Downsample for plotting (5000)

max_distanceToTSS

Numeric. Maximum distance to a gene's TSS to consider a region linked to a

gene. (1000)

Value

A violin plot of intra-correlation

```
data(scExp)
plot_percent_active_feature_scExp(scExp, "UBXN10")
```

```
plot_pie_most_contributing_chr
```

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

Description

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 eature 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.	
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 reprensentation

```
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")
```

```
{\tt 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.

Examples

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

```
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

```
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

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

Arguments

scExp A SingleCellExperiment

gene A character specifying the gene to plot

by Color violin by cell_cluster or sample_id ("cell_cluster")

downsample Downsample for plotting (5000)

max_distanceToTSS

Numeric. Maximum distance to a gene's TSS to consider a region linked to a

gene. (1000)

Value

A violin plot of intra-correlation

Examples

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

```
preprocessing_filtering_and_reduction
```

Preprocess and filter matrix annotation data project folder to SCE

Description

Preprocess and filter matrix annotation data project folder to SCE

```
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
)
```

86 preprocess_CPM

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

are sample names.

Value

A SingleCellExperiment object containing feature spaces.

Examples

```
raw <- create_scDataset_raw()
scExp = preprocessing_filtering_and_reduction(raw$mat, raw$annot)</pre>
```

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

```
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp = preprocess_CPM(scExp)
head(SingleCellExperiment::normcounts(scExp))</pre>
```

```
\begin{tabular}{ll} preprocess\_feature\_size\_only \\ Preprocess\ scExp\ -\ size\ only \\ \end{tabular}
```

Description

Preprocess scExp - size only

Usage

```
preprocess_feature_size_only(scExp)
```

Arguments

scExp

A SingleCellExperiment Object

Value

A SingleCellExperiment object.

Examples

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

preprocess_RPKM

Preprocess scExp - Read per Kilobase Per Million (RPKM)

Description

Preprocess scExp - Read per Kilobase Per Million (RPKM)

Usage

```
preprocess_RPKM(scExp)
```

Arguments

scExp

A SingleCellExperiment Object

88 preprocess_TFIDF

Value

A SingleCellExperiment object.

Examples

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

preprocess_TFIDF

 $Preprocess\ scExp\ -\ TF\text{-}IDF$

Description

Preprocess scExp - TF-IDF

Usage

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

Arguments

scExp A SingleCellExperiment Object

scale A numeric to multiply the matrix in order to have human readeable 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.

```
raw <- create_scDataset_raw()
scExp = create_scExp(raw$mat, raw$annot)
scExp = preprocess_TFIDF(scExp)
head(SingleCellExperiment::normcounts(scExp))</pre>
```

preprocess_TPM 89

preprocess_TPM

Preprocess scExp - Transcripts per Million (TPM)

Description

Preprocess scExp - Transcripts per Million (TPM)

Usage

```
preprocess_TPM(scExp)
```

Arguments

scExp

A SingleCellExperiment Object

Value

A SingleCellExperiment object.

Examples

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

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

```
rawfile_ToBigWig(
  input,
  BigWig_filename,
  format = "BAM",
  bin_width = 150,
  norm_factor,
  n_smoothBin = 5,
  ref = "hg38",
  read_size = 101,
  original_bins = NULL,
  quantile_for_peak_calling = 0.85
)
```

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.

BigWig_filename

Path to write the output BigWig file

format File format, either "BAM" or "BED"

bin_width Bin size for coverage

norm_factor Then number of cells or total number of reads in the given sample, for normal-

ization.

n_smoothBin Number of bins for smoothing values

ref Reference genome. read_size Length of the reads.

original_bins Original bins GenomicRanges in case the format is raw matrix.

quantile_for_peak_calling

The quantile to define the threshold above which signal is considered as a peak.

Value

Writes in the output directory a bigwig file displaying the cumulative coverage of cells and a basic set of peaks called by taking all peaks above a given threshold

Writes a BigWig file as output

```
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(
   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", "ce11")[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	<pre>Input file(s) type(s) ('scBED','scBAM','FragmentFile')</pre>
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

References

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

```
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)

92 read_sparse_matrix

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"

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", "ce11")[1],
  verbose = TRUE
)
```

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

rebin_helper 93

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)</pre>
```

rebin_helper

Rebin Helper for rebin_matrix function

Description

Rebin Helper for rebin_matrix function

Usage

```
rebin_helper(mat_df)
```

Arguments

mat_df

A data frame corresponding to sparse matrix indexes & values.

Value

a data.frame grouped mean-summarised by col and new_row

rebin_matrix

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

Description

This functions 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 canconical 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 disminish the number of peaks overlapping multiple region. Any peak smaller than the minimum overlapp threshold will be dismissed. Therefore, library size might be slightly different from peaks to bins if signal was duplicated into multiple bins or ommitted due to peaks smaller than minimum overlap.

94 reduce_dims_scExp

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 predecency over

bin_width.

minoverlap Minimum overlap between the original bins and the new features to consider the

peak as overlapping the bin . We recommand 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 paralell processing

rebin_function A function to use to rebin the matrix.

Value

A sparse matrix of larger bins or peaks.

Examples

```
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

```
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')) 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

```
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"))</pre>
```

```
reduce_dim_batch_correction
```

Reduce dimension with batch corrections

Description

Reduce dimension with batch corrections

```
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

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

Arguments

scExp A SingleCellExperiment

verbose Print?

Value

A SingleCellExperiment without chromosome M (mitochondrial chr)

```
remove_non_canonical_fun
```

 $Remove\ non\ canonical\ chromosomes\ from\ scExp$

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...)

results_enrichmentTest 97

```
results_enrichmentTest
```

Resutls of hypergeometric gene set enrichment test

Description

Run hypergeometric enrichment test and combine significant pathways into a data.frame

Usage

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

Arguments

differentialGenes

Genes significantly over / under expressed

enrichment_qval

Adusted 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

```
retrieve_top_bot_features_pca
```

Retrieve Top and Bot most contributing features of PCA

Description

Retrieve Top and Bot most contributing features of PCA

```
retrieve_top_bot_features_pca(
  pca,
  counts,
  component,
  n_top_bot,
  absolute = FALSE
)
```

98 run_pairwise_tests

Arguments

A matrix/data.frame of rotated data рса the normalized counts used for PCA counts

component the componenent of interest

n_top_bot the number of top & bot features to take

If TRUE, return the top features in absolute values instead. absolute

Value

a data.frame of top bot contributing features in PCA

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

An annotation data.frame with cell_cluster and cell_id columns affectation

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

A shiny Progress instance to display progress bar. progress

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_scExp

Run tsne on single cell experiment

Description

Run tsne on single cell experiment

Usage

```
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

scExp

A SingleCellExperiment outputed by ChromSCape

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 5kbp 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

```
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 dengogram in metadata\$hc_cor
- Consensus clustering raw data in metadata\$consclust
- Consensus clustering cluster-consensus and item consensus dataframes in metadata\$icl
- Differential analysis in metadata\$diff
- Gene Set Analysis in metadata\$enr

Value

A scExp - a SingleCellExperiment with 106 cells and 5499 features

Examples

```
data("scExp")
plot_reduced_dim_scExp(scExp)
plot_reduced_dim_scExp(scExp,color_by = "cell_cluster")
plot_heatmap_scExp(scExp)
plot_differential_volcano_scExp(scExp, "C1")
plot_differential_summary_scExp(scExp)
```

```
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 101

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

 ${\sf smoothBin}$

Smooth a vector of values with nb_bins left and righ values

Description

Smooth a vector of values with nb_bins left and righ values

Usage

```
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

@importFrom BiocParallel bpvec

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

```
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

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

```
subset_bam_call_peaks Peak calling on cell clusters
```

Description

This functions 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 cooridnates 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 closests 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

```
subset_bam_call_peaks(
    scExp,
    odir,
    input,
    format = "BAM",
    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, mm10 or ce11. ('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 Gencode 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.

104 summary_DA

Value

A SingleCellExperiment with refinded annotation

Examples

```
## 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

```
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)

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

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

```
swapAltExp_sameColData
```

Swap main & alternative Experiments, with fixed colData

Description

Swap main & alternative Experiments, with fixed colData

Usage

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

Arguments

scExp A SingleCellExperiment

alt Name of the alternative experiment

Value

A swapped SingleCellExperiment with the exact same colData.

Examples

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

```
table_enriched_genes_scExp
```

Creates table of enriched genes sets

Description

Creates table of enriched genes sets

```
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")
)
```

106 warning_DA

Arguments

scE	¢р	A SingleCellExperiment object containing list of enriched gene sets.
set		A character vector, either 'Both', 'Overexpressed' or 'Underexpressed'. ('Both')
grou	qp	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

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

warning_DA V	Varning for differential_analysis_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
```

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 re-

ducedDims.

(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)

 ${\tt run_tsne} \qquad \qquad {\tt Re-run \ tsne} \ ? \ ({\tt FALSE})$

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 (be-

tween 0 and 0.5).

max_quantile The upper threshold to remove outlier cells, as quantile of cell embeddings (be-

tween 0.5 and 1).

Value

Warning or errors if the inputs are not correct

Description

Warning for raw_counts_to_sparse_matrix

Usage

```
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 directory containing the raw files
                  Input file(s) type(s) ('scBED','scBAM','SparseMatrix')
file_type
peak_file
                  A file containing genomic location of peaks (NULL)
                  The number of bins to tile the genome (NULL)
n_bins
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_Signac_FeatureMatrix
```

Wrapper around 'FeatureMatrix' function from Signac Package

Description

Wrapper around 'FeatureMatrix' function from Signac Package

```
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 corre-

spond 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 canon-

ical chromosomes of the given reference genome will be excluded from the ma-

trix.

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 defaults 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

```
## 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)
```

Index

* datasets	cor, 15, 24, 77
cell.chromosomes, 12	<pre>correlation_and_hierarchical_clust_scExp,</pre>
ce11.GeneTSS, 13	24
CheA3_TF_nTargets, 14	count_coverage, 24
hg38.chromosomes, 58	create_project_folder, 25
hg38.cytoBand, 59	create_sample_name_mat, 26
hg38.GeneTSS, 59	create_scDataset_raw, 27
mm10.chromosomes, 66	create_scExp, 28
mm10.cytoBand, 66	
mm10.GeneTSS, 67	DA_custom, 29
scExp, 99	DA_one_vs_rest, 30
	DA_pairwise, 30
<pre>annotation_from_merged_peaks, 5</pre>	define_feature, 31
annotToCol2, 5	detect_samples, 32
anocol_binary, 6	differential_activation, 32
anocol_categorical, 7	differential_analysis_scExp, 33, 33
	distPearson, 35
<pre>bams_to_matrix_indexes, 7</pre>	
beds_to_matrix_indexes, 8	enrich_TF_ChEA3_genes, 36
bpparam, 7, 8, 21, 29–31, 34, 39, 42, 61, 91, 98	enrich_TF_ChEA3_scExp, 36
1 1	enrichmentTest, 35
calculate_CNA, 8	exclude_features_scExp, 37
calculate_cyto_mat, 8, 9, 11	
calculate_gain_or_loss, 8, 9, 10, 83	feature_annotation_scExp, 38
calculate_logRatio_CNA, 8-10, 11, 76	filter_correlated_cell_scExp, 39
call_macs2_merge_peaks, 11	filter_genes_with_refined_peak_annotation,
ce11.chromosomes, 12	40
ce11. GeneTSS, 13	filter_scExp, 41
changeRange, 13	find_clusters_louvain_scExp, 42
CheA3_TF_nTargets, 14	find_top_features, 43
check_correct_datamatrix, 15	cons not anniahment analysis as Eyn 40
chisq.test, 33	gene_set_enrichment_analysis_scExp, 49
choose_cluster_scExp, 15, 47	generate_analysis, 44
choose_perplexity, 16	generate_count_matrix, 46
col2hex, 16 colors_scExp, 17	generate_coverage_tracks, 46
• •	generate_feature_names, 48
combine_datamatrix, 17	generate_report, 48
combine_enrichmentTests, 18	get_color_dataframe_from_input, 52
comparable_variables, 19 CompareedgeRGLM, 19	get_cyto_features, 52
	get_genomic_coordinates, 53
CompareWilcox, 21	get_most_variable_cyto, 54
concatenate_scBed_into_clusters, 22 consensus_clustering_scExp, 22	get_pathway_mat_scExp, 54
ConsensusClusterIng_SCEXp, 22 ConsensusClusterPlus, 22	getExperimentNames, 50
Consensuscius lei Fius, 22	getMainExperiment, 51

INDEX

gg_fill_hue, 55 groupMat, 56	preprocess_TFIDF, 88 preprocess_TPM, 89
Illamonantian 56	preprocessing_filtering_and_reduction,
H1proportion, 56	85
has_genomic_coordinates, 57	
hclustAnnotHeatmapPlot, 57	raw_counts_to_sparse_matrix, 90
hg38.chromosomes, 58	rawfile_ToBigWig, 89
hg38.cytoBand, 59	<pre>read_count_mat_with_separated_chr_start_end,</pre>
hg38.GeneTSS, 59	91
:C-1 60	read_sparse_matrix, 92
imageCol, 60	rebin_helper, 93
<pre>import_count_input_files, 60</pre>	rebin_matrix, 93
import_scExp, 61	reduce_dim_batch_correction, 95
<pre>index_peaks_barcodes_to_matrix_indexes,</pre>	reduce_dims_scExp, 94
62	remove_chr_M_fun,96
inter_correlation_scExp, 63	remove_non_canonical_fun, 96
intra_correlation_scExp, 63	results_enrichmentTest,97
7	retrieve_top_bot_features_pca, 97
launchApp, 64	run_pairwise_tests, 98
load_MSIGdb, 65	run_tsne_scExp, 99
	runApp, <i>64</i>
merge_MACS2_peaks, 65	117
mm10.chromosomes, 66	scExp, 99
mm10.cytoBand, 66	separate_BAM_into_clusters, 100
mm10.GeneTSS, 67	separator_count_mat, 101
	smoothBin, 101
normalize_scExp, 67	subsample_scExp, 102
num_cell_after_cor_filt_scExp, 68	subset_bam_call_peaks, 102
num_cell_after_QC_filt_scExp, 69	summary_DA, 104
num_cell_before_cor_filt_scExp, 69	swapAltExp_sameColData, 105
num_cell_in_cluster_scExp, 70	
num_cell_scExp, 70	table_enriched_genes_scExp, 105
pca_irlba_for_sparseMatrix,71	
	warning_DA, 106
plot_cluster_consensus_scExp, 71	<pre>warning_filter_correlated_cell_scExp,</pre>
plot_correlation_PCA_scExp, 72	107
plot_coverage_BigWig, 73	<pre>warning_plot_reduced_dim_scExp, 107</pre>
plot_differential_summary_scExp, 74	<pre>warning_raw_counts_to_sparse_matrix,</pre>
plot_differential_volcano_scExp, 74	108
plot_distribution_scExp, 75	wrapper_Signac_FeatureMatrix, 109
plot_gain_or_loss_barplots, 76	,
plot_heatmap_scExp, 77	
plot_inter_correlation_scExp, 77	
plot_intra_correlation_scExp, 78	
plot_most_contributing_features, 79	
plot_percent_active_feature_scExp, 80	
plot_pie_most_contributing_chr, 81	
plot_reduced_dim_scExp, 81	
plot_reduced_dim_scExp_CNA, 83	
plot_top_TF_scExp, 83	
plot_violin_feature_scExp, 84	
preprocess_CPM, 86	
preprocess_feature_size_only, 87	
preprocess_RPKM, 87	