Package ‘OMICsPCA’

April 4, 2024

Type Package

Title An R package for quantitative integration and analysis of multiple omics assays from heterogeneous samples

Version 1.20.0

Date 21/08/2018

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Depends R (>= 3.5.0), OMICsPCAdata

Suggests knitr, RUnit, BiocGenerics

Description OMICsPCA is an analysis pipeline designed to integrate multi OMICs experiments done on various subjects (e.g. Cell lines, individuals), treatments (e.g. disease/control) or time points and to analyse such integrated data from various various angles and perspectives. In it's core OMICsPCA uses Principal Component Analysis (PCA) to integrate multiomics experiments from various sources and thus has ability to over data insufficiency issues by using the integrated data as representatives. OMICsPCA can be used in various application including analysis of overall distribution of OMICs assays across various samples /individuals /time points; grouping assays by user-defined conditions; identification of source of variation, similarity/dissimilarity between assays, variables or individuals.

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biocViews ImmunoOncology, MultipleComparison, PrincipalComponent, DataRepresentation, Workflow, Visualization, DimensionReduction, Clustering, BiologicalQuestion, EpigeneticsWorkflow, Transcription, GeneticVariability, GUI, BiomedicalInformatics, Epigenetics, FunctionalGenomics, SingleCell

Imports HelloRanges, fpc, stats, MultiAssayExperiment, pdftools, methods, grDevices, utils, clValid, NbClust, cowplot, rmarkdown, kableExtra, rtracklayer, IRanges, GenomeInfoDb, reshape2, ggplot2, factoextra, rgl, corrplot, MASS, graphics, FactoMineR, PerformanceAnalytics, tidyr, data.table, cluster, magick
analyse_individuals

Quick analysis and visualization of the individuals/annotations/rows (e.g. Tss, gene) from integrated Assay.

Description

This function displays scatterplot of the individuals from integrated assays. This allows the display of scatterplots both in 2D and 3D.
analyse_integrated_individuals

Usage

analyze_individuals(name, Assay, choice, PC, group,
    groupinfo = NULL, ...)

Arguments

name               Name of the "PCA" object containing the assay data
Assay              Name of an Assay
choice             1 = 2D scatterplot on selected PCs through PC argument.
                    2 = 3D scatterplot on selected PCs through PC argument

                    for details of the choices see the vignette
PC                  A vector of numbers corresponding to principal components
group              A vector of group names. Required only if choice = 2
groupinfo          output of create_group, or similar object containing group information.
...                additional arguments allowed to base function "plot3d" of package "rgl"

Value

Displays various plots and tables as per the combination of input arguments

Author(s)

Subhadeep Das <subhadeep1024@gmail.com>

Examples

PCAlist <- integrate_variables(assays = c(“H2a”, “H3k4me1”,
                    “H3k9ac”), name = multi_assay,
                    groups = c(“WE”, “RE”), groupinfo = groupinfo_ext,
                    scale.unit = FALSE, graph = FALSE)

analyze_individuals(name = PCAlist,
                    Assay = “H3k9ac”, groupinfo = groupinfo_ext,
                    choice = 1, PC = c(1,2))

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analyse_integrated_individuals

Quick analysis and visualization of the individuals/annotations/rows
(e.g. Tss, gene)

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Description

This function works similarly as "analyze_individuals()". The only extra argument needs to be
supplied here is "start_end", (if choice = 1.) returned by "integrate_pca()"
analyse_integrated_individuals

Usage

analyse_integrated_individuals(
  name, choice = 1, geom = "point",
  PC = c(1,2), groupinfo = NULL, ...)

Arguments

name      Name of the "PCA" object containing the integrated PCA.
choice    1 = 2D scatterplot on selected PCs through PC argument.
          2 = 3D scatterplot on selected PCs through PC argument
          for details of the choices see the vignette
geom      Used when choice = 1. Available options are: "point", "text", c("point","text").
          default = "point"
PC        A vector of numbers corresponding to principal components
groupinfo same as integrate_variables()
...       additional arguments allowed to base function "plot3d" of package "rgl"

Value

Displays various plots and tables as per the combination of input arguments

Author(s)

Subhadeep Das <subhadeep1024@gmail.com>

Examples

eclude <- list(0,c(1,9))

int_PCA <- integrate_pca(assays = c("H2az", "H3k9ac"), name = multi_assay, mergetype = 2,
                          exclude = exclude,
                          groupinfo = groupinfo_ext, graph = FALSE)

name = int_PCA$int_PCA

analyse_integrated_individuals(
  name = name,
  choice = 2, PC = c(1,2,3),
  col = c("RED", "BLACK", "GREEN"),
  groupinfo = groupinfo_ext)
analyse_integrated_variables

Quick analysis and visualization of the integrated Assays by integrate() function.

Description

This function is designed for a quick analysis and visualization of the data integrated by "integrate-Assays()" function. It works similarly as analyse_variables. In addition to that it takes an additinal argument "start_end" returned by "integrateAssays()".

Usage

analyse_integrated_variables(start_end = start_end, Assay = "all", name, choice, title = NULL, PC = 1, var_type = "contrib", ...)

Arguments

- start_end: a list as returned by "integrate_pca()"
- Assay: Name of an Assay. default = "all"
- name: Name of the "PCA" object returned by "integrate_pca()"
- choice: 1 = variance barplot 2 = Loadings of cell lines on selected PCs 3 = hCorrelation matrix 4 = Squared loadings of Cell lines on a PC 5 = Contribution of Cell lines on a PC for details of the choices see the vignette
- title: title of plots
- PC: The PC on which the plots will be drawn. used in choice 4 and 5. default = 1
- var_type: Used when choice = 2. available options are: "coord", "cos2" and "contrib"
- ... additional arguments allowed to base functions "used in various choices. See vignettes for details.

Value

Displays various plots and tables as per the combination of input arguments

Author(s)

Subhadeep Das <subhadeep1024@gmail.com>
**Examples**

```r
exclude <- list(0,c(1,9))

int_PCA <- integrate_pca(assays = c("H2az", "H3k9ac"), name = multi_assay, mergetype = 2, exclude = exclude, graph = FALSE)

start_end = int_PCA$start_end

name = int_PCA$int_PCA

analyse_integrated_variables(start_end = start_end, name = name, choice = 1, title = "variance barplot", assay = 1, addlabels = TRUE)
```

**Description**

This function is designed for a quick analysis and visualization of the data integrated by integrate() function. It takes 3 compulsory arguments name, Assay and choice. The type of analysis should be selected through the "choice" argument. This function acts as a wrapper around a collection of functions of package "factoextra" and "corrplot" and thus can take additional arguments specific and non-conflicting to such functions.

**Usage**

```r
analyse_variables(name, assay, choice, title = NULL, PC = 1, var_type = "contrib", ...)
```

**Arguments**

- **name**: Name of the "PCA" object
- **Assay**: Name of an Assay.
- **choice**: 1 = variance barplot
  2 = Loadings of cell lines on selected PCs
  3 = hCorrelation matrix
  4 = Squared loadings of Cell lines on a PC
  5 = Contribution of Cell lines on a PC
  for details of the choices see the vignette
- **title**: Title of the plot. default = NULL
- **PC**: used in choice = 4 and 5. Indicates the PC on which choice 4 and 5 will depend. default = 1
**chart_correlation**

var_type used in choice = 2. available options are: "coord", "cos2" and "contrib". default = "contrib"

extra arguments passed through the functions imported from various packages. See vignettes for details.

**Value**

Displays various plots and tables as per the combination of input arguments

**Author(s)**

Subhadeep Das <subhadeep1024@gmail.com>

**Examples**

```r
PCAlist <- integrate_variables(assays = c("H2az","H3k4me1", "H3k9ac"), name = multi_assay, groups = c("WE","RE"), groupinfo = groupinfo_ext, scale.unit = FALSE, graph = FALSE)

analyse_variables(name = PCAlist, assay = "H2az", choice = 1, title = "variance barplot", addlabels = TRUE)
```

**Description**

This function creates pairwise correlation plots and tables; scatter plots and histograms on selected groups. The type of results should be passed through the argument 'choice'. This is a wrapper on various functions and thus can take additional and non-conflicting arguments specific to them.

**Usage**

```r
chart_correlation(name, assay, groups, choice, groupinfo = NULL, ...)
```

**Arguments**

- **name**: Name of the "MultiAssayExperiment" object containing the assay data
- **Assay**: Name of an assay
- **groups**: Name of all or subset of groups
choice

"table" = correlation table

"scatter" = scatterplot of each pair of columns/variables of selected groups

"hist" = histogram of each column/variables

"all" = all of 1,2,3 together

groupinfo

output of create_group or similar object.

... additional arguments for base functions "chart.Correlation" and "pairs"

Value
Displays various plots and tables as per the combination of input arguments

Author(s)
Subhadeep Das <subhadeep1024@gmail.com>

Examples

chart_correlation(name = multi_assay,
Assay = "H2az", groupinfo = groupinfo_ext,
   groups = "WE", choice = "table")

cluster

Cluster data points

Description
Clustering of data points using various algorithms

Usage
cluster(name, n = NULL, graph = NULL, choice, title = NULL, ...)

Arguments
name
name of the dataframe or matrix object containing the coordinates of data points.
The output of "extract()" may be directly put here.
n
Number of clusters

graph
logical. Plots the clusterplot on first 2 dimensions if set TRUE

choice
Clustering algorithm to use. Available choices are: "density", "kmeans", "pam"
title
Title of the plot

... additional non-conflicting arguments to cluster functions
Value

returns a list containing the cluster and plot information

Author(s)

Subhadeep Das

References


Examples

```r
exclude <- list(0,c(1,9))

int_PCA <- integrate_pca(Assays = c("H2az", "H3k9ac"),
                          groupinfo = groupinfo,
                          name = multi_assay, mergetype = 2,
                          exclude = exclude, graph = FALSE)

name = int_PCA$int_PCA

data <- extract(name = name, PC = c(1:4),
                groups = c("WE","RE"), integrated = TRUE,
                rand = 300, groupinfo = groupinfo_ext)

clusters <- cluster(name = data, n = 2,
                     choice = "kmeans",
                     title = "kmeans on 2 clusters")
```
cluster_boxplot  

Comparison of clusters by boxplot

Description

boxplot of the assay values in each cluster

Usage

cluster_boxplot(name, Assay, clusterobject, clustercolumn, choice = NULL)

Arguments

name

Assay  
name of the assay which is to be compared

clusterobject  
a cluster object returned by the cluster() function

clustercolumn  
the column in the clusterobject containing the group membership information

choice  
type of graph

Value

a ggplot object

Author(s)

Subhadeep Das

Examples

bp <- cluster_boxplot(name = multi_assay,  
Assay = "H2az", clusterobject = clustered_data,  
clustercolumn = 5)

bp
cluster_parameters

Detection of algorithm and number of clusters

Description

Detection of appropriate clustering algorithm and cluster number for given data using "clvalid" and "NbClust" in background for cluster validation.

Usage

cluster_parameters(name, comparisonAlgorithm = "clValid", optimal = FALSE, n = 2:6, clusteringMethods = c("kmeans", "pam"), validationMethods = c("internal", "stability"), distance = "euclidean", ...)

Arguments

name
dataframe returned by "extract()".

optimal
logical. If TRUE, returns a dataframe of optimal results

n
a vector of numbers corresponding to the number of clusters to be tested or validated

comparisonAlgorithm
2 choices available: "clValid" or "NbClust"

clusteringMethods
a vector of single or multiple names of clustering algorithms. available choices are:
1) if comparisonAlgorithm = "clValid" : "hierarchical", "kmeans", "diana", "fanny", "som", "model", "sota", "pam", "clara" and "agnes"
2) if comparisonAlgorithm = "NbClust" : "ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median", "centroid", "kmeans"

validationMethods
name of the method to validate clusters. Available options (one or more):
1) if comparisonAlgorithm = "clValid" then one of:
2) if comparisonAlgorithm = "NbClust" then one or more of:
"internal", "stability", and "biological"

distance
metric used to calculate distance matrix. options:
1) for "clValid" :
cluster_parameters

"euclidean", "correlation", and "manhattan".
2) for "NbClust":
This must be one of: "euclidean", "maximum", "manhattan", "canberra", "binary", "minkowski"
... additional non-conflicting arguments to "clValid" or "NbClust"

Value

1) for "clValid"
an object of class "clValid" (optimal = FALSE) or a dataframe of optimal values (optimal = TRUE)
2) for "NbClust"
a list of:
- All.index, All.CriticalValues, Best.nc and Best.partition.
See the help pages of "clValid" (?clValid) and "NbClust" (?NbClust) for more details.

Author(s)

Subhadeep Das

References

Journal of Statistical Software 25(4) http://www.jstatsoft.org/v25/i04

the Relevant Number of Clusters in a Data Set.", "Journal of Statistical Software, 61(6), 1-36.",
"URL http://www.jstatsoft.org/v61/i06/".

Examples

exclude <- list(0, c(1, 9))

int_PCA <- integrate_pca(assays = c("H2az", "H3k9ac"),
groupinfo = groupinfo,
name = multi_assay, mergetype = 2,
exclude = exclude, graph = FALSE)

name = int_PCA$int_PCA

data <- extract(name = name, PC = c(1:4),
groups = c("WE", "RE"), integrated = TRUE, rand = 600, groupinfo = groupinfo_ext)

#### Using "clValid" ####

clusterstats <- cluster_parameters(name = data,
optimal = FALSE, n = 2:4, comparisonAlgorithm = "clValid",
distance = "euclidean", clusteringMethods = c("kmeans"),
create_group

validationMethods = c("internal")

create_group

Subsets an Assay dataframe into smaller groups

Description

This function subsests an user defined Assay into smaller groups according to user supplied instructions.

Usage

create_group(name, group_names = NULL,
 group_name = NULL,
 grouping_factor = NULL,
 comparison = NULL,
 condition = NULL
)

Arguments

name Name of the "MultiAssayExperiment" object containing the assay data
 group_names A vector containing the user defined names of the groups to be created
 grouping_factor name of the dataframe on which grouping will be done
 comparison A vector of comparison symbols such as >, <, ==, >=, <=,%in%, etc
 condition A vector of conditions corresponding to 'comparison'. "condition" should be a vector or range of digits (e.g. c(1,3,7,9) or 1:5) if%in% is chosen as comparison. Otherwise, a single digit should be chosen.

Value

returns the group membership of individuals as a dataframe

Author(s)

Subhadeep Das <subhadeep1024@gmail.com>

Examples

groupinfo <- create_group(name = multi_assay,
 group_names = c("WE","RE","NE","IntE"),
 grouping_factor = "CAGE",
 comparison = c(">","%in%","==","%in%"),
 condition = c("25","1:5","0","6:24"))

head(groupinfo)
**Description**

descriptor() produces panels of boxplots displaying the distribution of various factors (e.g. ChIP-seq of H3k9ac histone modification) in percentage of cell lines.

choice = 1 output is collection of boxplots explaining the distribution of percentage of cell lines having or overlapping with the input factors (e.g. H3k9ac).

choice = 2 displays the distribution of percentage of cell lines having or overlapping with increasing number of factors for the selected group (e.g. WE or RE etc). If this option is selected, then a group name should be provided through choice2group.

**Usage**

descriptor(name, factors, groups, choice = 1, choice2group = NULL, title = NULL, groupinfo = NULL)

**Arguments**

name
Name of the "MultiAssayExperiment" object containing the assay data

factors
A vector containing the name of the Assays

groups
Name of all or subset of groups

choice
Numerical value between 1 and 2. See Description and vignettes for details of plots generated by this 2 choices

choice2group
Used when choice is 2. This argument allows a name of group.

title
Title of the plot

groupinfo
output of create_group() or similar object

**Value**

Displays various plots as per the combination of various input arguments.

**Author(s)**

Subhadeep Das <subhadeep1024@gmail.com>

**Examples**

descriptor(name = multi_assay, factors = c("H2az","H3k9ac","H3k4me1"), groups = c("WE","RE","IntE","NE"), choice = 1, title = "Distribution of percentage of cell types overlapping..."
extract

with various factors",
groupinfo = groupinfo_ext)

extract extraction of projected cooordinates from "PCA" object.

Description

tion to extract projected coordinates of individuals on principal components.

Usage

extract(name, groups, integrated = TRUE, Assay = NULL,
rand = NULL, PC = c(1,2,3,4), groupinfo = NULL)

Arguments

name name of the OMICs object.
groups one or more groups created by or supplied through "create_group()"
integrated whether to extract coordinates from integrated or individual assay.
Assay name of the assay if integrated = FALSE
rand number of rows to be extracted randomly.
PC principal component numbers. The projected informaton on this PCs will be
extracted.
groupinfo same as integrate_variables()

Value

a data frame of the extracted value.

Author(s)

Subhadeep Das

Examples

exclude <- list(0,c(1,9))

int_PCA <- integrate_pca( Assays = c("H2az",
"H3k9ac"),
groupinfo = groupinfo,
name = multi_assay, mergetype = 2,
exclude = exclude, graph = FALSE)

name = int_PCA$int_PCA
data <- extract(name = name, PC = c(1:4),
groups = c("WE","RE"), integrated = TRUE, rand = 600,
groupinfo = groupinfo_ext)

### extract_assay

**Data extraction from "MultiAssayExperiment" object**

#### Description
Extraction of individuals according to user supplied group and Assay

#### Usage

```r
extract_assay(name, Assay, groups, groupinfo = NULL,
             addgroupnames = TRUE)
```

#### Arguments

- `name`: name of the "OMICs" object from which data is to be retrieved.
- `Assay`: name of the "Assay" from which data is to be retrieved.
- `groups`: name of the "groups" from which data is to be retrieved.
- `groupinfo`: output of `create_group()` or similar object
- `addgroupnames`: logical argument. If "TRUE", adds the group membership info of returned individuals

#### Value

A data frame containing the intended information

#### Author(s)
Subhadeep Das

#### Examples

```r
x <- extract_assay(name = multi_assay, Assay = "H2az",
                   groups = c("WE","RE"), groupinfo = groupinfo_ext)

x
```
**integrate_pca**

Integration of multiple Assays into linear combinations by PCA.

**Description**

This function integrates multiple assays read by InputOMICs() into linear combinations using PCA. Following earlier analyses, user may want to exclude some variables/columns/cell lines which should be supplied through the "exclude" argument (see vignettes for more explanation).

PCA is done by the function PCA() from package "FactoMineR". Additional arguments may be supplied to PCA() through "...".

**Usage**

```r
integrate_pca(Assays, name, exclude, mergetype = 1, groupinfo = NULL, ...)
```

**Arguments**

- **Assays**: A vector of names of an Assays read by "InputOMICs()"
- **name**: Name of the "MultiAssayExperiment" object containing the assay data
- **exclude**: A list that indicates which columns to exclude. See vignettes for details
- **mergetype**: 2 options:
  - 1 = subset of groups should be entered by user and PCA is run on this subset to integrate Assays into PCs
  - 2 = PCA is run on all groups
- **groupinfo**: same as plot_density()
- **...**: additional arguments supplied to function "PCA()" from package "FactoMineR"

**Details**

The "mergetype" argument lets user decide whether to combine all groups or a subset of groups created by "create_group". If mergetype is set to 1, "integrateAssays" asks for the name of groups which should be a character or character vector (see vignettes for more details).

**Value**

"integrateAssays()" returns a list containing the start and end column of each Assay as ordered in the "Assays" argument.

**Author(s)**

Subhadeep Das <subhadeep1024@gmail.com>
Examples

```r
int_PCA <- integrate_pca(Assays = c("H2az", "H3k9ac"), name = multi_assay, mergetype = 2, exclude = list(0,c(1,9)), graph = FALSE, groupinfo = groupinfo_ext)
```

**Description**

If an assay (e.g. ChIP-seq for the histone modification H3k9ac) is done on multiple cells/conditions/treatment/time, it might be, sometimes, necessary to integrate or combine them. Such combination may be done by several techniques like Principal Component Analysis (PCA) or Factor Analysis (FA). This function integrates an assay experimented on multiple cells or conditions into many linear combinations using PCA.

**Usage**

```r
integrate_variables(Assays, name, groups, groupinfo = NULL, ...)
```

**Arguments**

- **Assays**: A vector of names of an assays
- **name**: Name of the "MultiAssayExperiment" object containing the assay data
- **groups**: Name of all or subset of groups
- **groupinfo**: output of `create_group` or similar object containing group information
- **...**: Additional arguments supplied to "PCA()" imported from "FactoMineR"

**Value**

an object of class "PCA"

**Author(s)**

Subhadeep Das <subhadeep1024@gmail.com>

**Examples**

```r
PCAlist <- integrate_variables(Assays = c("H2az","H3k4me1","H3k9ac"), name = multi_assay, groups = c("WE","RE"), groupinfo = groupinfo_ext, scale.unit = FALSE, graph = FALSE)
```
**Description**

Intersects a factor file and an annotation file.

**Usage**

```
intersect(fact, anno, ...)
```

**Arguments**

- `fact`: Full path of the directory containing only the bed (.bed) files corresponding to several cell lines of the specified factor.
- `anno`: Full path of the annotation file (e.g. gene, TSS, exon etc.).
- `...`: Additional arguments received from "prepareDataset()" and supplied to "R_bedtools_intersect()".

**Value**

A list containing the intersections of the files (paths of which are supplied through the arguments).

**Author(s)**

Subhadeep Das

**Examples**

```r
anno <- system.file("extdata/annotation2/TSS_groups.bed", package = "OMICsPCAdataset")
fact <- system.file("extdata/factors2/demofactor", package = "OMICsPCAdataset")
Cells <- intersect(fact = fact, anno = anno)
Cells
```
merge_cells

Storage of intersected factors from multiple cell types as columns into a dataframe

Description
This function takes the output list of the function "intersect" and stores the intersected value of the factor and annotation files into a dataframe. The values corresponding to the entries in annotation file represents the corresponding value of in each cell line. If an entry is not found in a factor file, its corresponding value will be 0.

Usage
merge_cells(list, Cells)

Arguments
list Full path of the file containing name of the Annotations (e.g. gene) in a single column
Cells output list of function "integrate()"

Value
A dataframe which rows corresponding to each entry in annotation file and columns corresponding to the intersected value with the corresponding annotation in each cell line.

Author(s)
Subhadeep Das

Examples
anno <- system.file("extdata/annotation2/TSS_groups.bed", package = "OMICsPCAdata")
fact <- system.file("extdata/factors2/demofactor", package = "OMICsPCAdata")
Cells <- intersect(fact = fact, anno = anno)
list <- system.file("extdata/annotation2/TSS_list", package = "OMICsPCAdata")
merged_Cells <- merge_cells(list = list, Cells = Cells)
head(merged_Cells)
plot_density

Visualization of Density of various groups on Principal components

Description
This function displays density of individuals

Usage
plot_density(name, Assay, PC = 1, groups,
groupinfo = NULL, ...)

Arguments
name Name of the "PCA" object containing the assay data
Assay Name of an Assay
PC A number corresponding to the principal component on which density is to be calculated
groups A vector of names or subset of names of groups
groupinfo same as integrate_variables()
... additional arguments of base function "geom_density" from package "ggplot2"

Value
a "gg" "ggplot" object

Author(s)
Subhadeep Das <subhadeep1024@gmail.com>

Examples

PCAlst <- integrate_variables(Assays = c("H2az","H3k4me1",
"H3k9ac"), name = multi_assay,
groups = c("WE","RE"), groupinfo = groupinfo_ext,
scale.unit = FALSE, graph = FALSE)
densityplot <- plot_density(name = PCAlst,
Assay = "H2az", groupinfo = groupinfo_ext,
PC = 1, groups = c("WE","RE"),
adjust = 1)
plot_density_3D  

Visualization of 3D Density of various groups on Principal components

Description

This function displays density of individuals from various groups created by create_group(). This wraps "persp" from package "graphics" and thus takes additional graphical input shown in example.

Usage

plot_density_3D(name, Assay, group, PC1 = 1, PC2 = 2, static = FALSE, gridsize = 100, groupinfo = NULL, ...)

Arguments

name  
Name of the "PCA" object containing the assay data
Assay  
Name of an Assay
group  
Names of a group
PC1, PC2  
Numbers corresponding to the principal components on which density is to be calculated
static  
Logical if TRUE a static plot is generated. default = FALSE
gridsize  
A number used in kernel smoothing. default is 100
groupinfo  
Same as integrate_variables()
...  
additional arguments passed to base function "persp" from package "graphics"

Details

2D density is calculated using the "kde2d" function from package "MASS" which use kernel density estimation (KDE) to calculate density of 2D data. If the variance on either or both of the PCs are 0, the KDE can’t be calculated.

Value

Displays 3D density plots of PCs

Author(s)

Subhadeep Das <subhadeep1024@gmail.com>
Examples

```r
PCAlist <- integrate_variables(Assays = c("H2az"),
                              name = multi_assay,
                              groups = c("WE","RE"), groupinfo = groupinfo_ext,
                              scale.unit = FALSE, graph = FALSE)

plot_density_3D(name = PCAlist, Assay = "H2az",
                group = "WE", PC1 = 1, PC2 = 2, grid_size = 100,
                static = FALSE, groupinfo = groupinfo_ext)
```

---

**plot_integrated_density**

*Visualization of Density of various groups on Principal components of integrated assays*

---

**Description**

This function works similarly as "plot_density()". See the man page of "plot_density()" for details.

**Usage**

```r
plot_integrated_density(name, PC = 1, groups,
                        groupinfo = NULL, ...)
```

**Arguments**

- `name`: Name of the integrated "PCA" object
- `PC`: A number corresponding to the principal component on which density is to be calculated. default = 1
- `groups`: A vector of names or subset of names of groups
- `groupinfo`: same as plot_density()
- `...`: additional arguments allowed to base function "geom_density" of package "ggplot2"

**Value**

a "gg" ggplot object

**Author(s)**

Subhadeep Das <subhadeep1024@gmail.com>
Examples

exclude <- list(0, c(1, 9))

int_PCA <- integrate_pca(assays = c("H2az", "H3k9ac"), name = multi_assay, mergetype = 2, exclude = exclude, groupinfo = groupinfo_ext, graph = FALSE)

name = int_PCA$int_PCA

densityplot <- plot_integrated_density(name = name, PC = 1, groups = c("WE", "RE", "IntE", "NE"), groupinfo = groupinfo_ext)

# additional graphical functions (e.g. xlim, ylim, theme) may be
# added with densityplot (see section VIII. Density analysis)

densityplot

plot_integrated_density_3D

Visualization of 3D Density of various groups on Principal components of multiple Assays

Description

works similarly as "plot_density_3D". See vignettes and man page of "plot_density_3D" for more details.

Usage

plot_integrated_density_3D(name, PC1 = 1, PC2 = 2, group, gridsize = 100, static = FALSE, groupinfo = NULL, ...)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>name</td>
<td>Name of the integrated &quot;PCA&quot; object</td>
</tr>
<tr>
<td>PC1, PC2</td>
<td>Numbers corresponding to the principal components on which density is to be calculated</td>
</tr>
<tr>
<td>group</td>
<td>Names of a group</td>
</tr>
<tr>
<td>gridsize</td>
<td>A number used in kernel smoothing. default is 100</td>
</tr>
<tr>
<td>static</td>
<td>Logical if TRUE a static plot is generated. default = FALSE</td>
</tr>
<tr>
<td>groupinfo</td>
<td>same as integrate_variables()</td>
</tr>
<tr>
<td>...</td>
<td>additional arguments allowed to base function &quot;persp&quot; of package &quot;graphics&quot;</td>
</tr>
</tbody>
</table>
Details

2D density is calculated using the "kde2d" function from package "MASS" which uses kernel density estimation (KDE) to calculate density of 2D data. If the variance on either or both of the PCs are 0, the KDE can’t be calculated.

Value

Displays 3D density plots.

Author(s)

Subhadeep Das <subhadeep1024@gmail.com>

Examples

```r
exclude <- list(0, c(1, 9))

int_PCA <- integrate_pca(assays = c("H2az", "H3k9ac"), name = multi_assay, mergetype = 2, exclude = exclude, groupinfo = groupinfo_ext, graph = FALSE)

name = int_PCA$int_PCA

plot_integrated_density_3D(name = name, PC1 = 1, PC2 = 2, group = c("WE", "RE"), gridsize = 100, static = FALSE, groupinfo = groupinfo_ext)
```

prepare_dataset

**Intersects and merge data from multiple experiments into a dataframe**

Description

This function intersects feature files (e.g. ChIP-seq, RNA seq) with annotation files (e.g. exons, genes) and represents the intersected values of each annotation from all cell lines in a data frame.

Usage

```r
prepare_dataset(factdir, annofile, annolist, ...)
```

Arguments

- **factdir**: Full path of directory containing (only) feature (e.g. ChIP-seq, CAGE) files in bed format
- **annofile**: Full path of the annotation file (e.g. TSS, exon, gene) in bed format
- **annolist**: Full path of the file containing name of the annotations (e.g. name of exons)
- **...**: Additional arguments to pass to "R_bedtools_intersect()" through "intersect()"
Details

If an annotation (e.g. gene) does not present in an assay file (e.g ChIP-seq of H3k9ac in the Cell Gm12878), this function puts a 0 as its value.

Value

a dataframe of intersected values.

Author(s)

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Examples

```r
anno <- system.file("extdata/annotation2/TSS_groups.bed", package = "OMICsPCAdata")
list <- system.file("extdata/annotation2/TSS_list", package = "OMICsPCAdata")
fact <- system.file("extdata/factors2/demofactor", package = "OMICsPCAdata")
prepare_dataset(factdir = fact, annofile = anno, annolist = list)
```
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