Package ‘treekoR’

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Type Package

Title Cytometry Cluster Hierarchy and Cellular-to-phenotype Associations

Version 1.10.0


Description treekoR is a novel framework that aims to utilise the hierarchical nature of single cell cytometry data to find robust and interpretable associations between cell subsets and patient clinical end points. These associations are aimed to recapitulate the nested proportions prevalent in workflows involving manual gating, which are often overlooked in workflows using automatic clustering to identify cell populations. We developed treekoR to:

- Derive a hierarchical tree structure of cell clusters; quantify a cell types as a proportion relative to all cells in a sample (%total), and, as the proportion relative to a parent population (%parent);
- Perform significance testing using the calculated proportions; and provide an interactive html visualisation to help highlight key results.

Depends R (>= 4.1)

Imports stats, utils, tidyR, dplyr, data.table, ggiraph, ggplot2, hopach, ape, ggtree, patchwork, SingleCellExperiment, diffcyt, edgeR, lme4, multcomp

License GPL-3

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RoxygenNote 7.2.3

Suggests knitr, rmarkdown, BiocStyle, CATALYST, testthat (>= 3.0.0)

VignetteBuilder knitr

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**R topics documented:**

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**Description**

a function to add the frequency bars for each cluster
addHeatMap

Usage

addFreqBars(  
p,  
clusters,  
offset = 0.75,  
bar_length = 3,  
bar_width = 0.4,  
freq_labels = FALSE
)

Arguments

p a phylogenetic tree plot created from the ggtree() function
clusters a vector representing the cell type or cluster of each cell (can be character or numeric)
offset distance between the heatmap and frequency bars
bar_length length of bar with max frequency
bar_width width of each frequency bar
freq_labels boolean indicated whether or not to show frequency bar labels

Value

an interactive ggplot graph object with frequency bars of clusters alongside heatmap of cluster median expression

Description

a function to add a heatmap of cluster medians alongside the phylogenetic tree

Usage

addHeatMap(  
p,  
cluster_medians,  
offset = 0.5,  
width = 1,  
expand_y_lim = 20,  
low = "#313695",  
mid = "ivory",  
high = "#A50026",  
colnames_angle = 90,  
metric_name = "Column z-score"
)
Arguments

**p**
- a phylogenetic tree plot created from the ggtree() function

**cluster_medians**
- a dataframe with the cluster medians. The row numbers of the clusters median data frame should correspond to the nodes in the phylo tree. The column names should also correspond to the labels you want to use

**offset**
- the distance between the tree plot and heatmap

**width**
- width of each tile in the heatmap

**expand_y_lim**
- white space below heatmap

**low**
- colour used for low values on heatmap

**mid**
- colour used for medium values on heatmap

**high**
- colour used for large values on heatmap

**colnames_angle**
- angle for x-axis label

**metric_name**
- legend title

Value

an interactive ggplot graph object with heatmap of median cluster expressions plotted alongside hierarchical tree

---

**addTree**

**Title**

Description

a function to create a skeleton tree diagram to display significance testing results on each node

Usage

```r
addTree(p, offset = 0.3, font_size = 2.5, hjust = 0)
```

Arguments

**p**
- a phylogenetic tree plot created from the ggtree() function

**offset**
- distance between leaf nodes on the tree and their labels

**font_size**
- font size of leaf labels

**hjust**
- horizontal justification as defined in ggplot2

Value

a ggtree graph object with the hierarchical tree of clusters and corresponding labels
colourTree

Description

Adding statistical test results onto the tree by using colourful nodes and branches. Takes a ggtree object with test results for each node and returns a ggtree graph object.

Usage

colourTree(
  tree,
  point_size = 1.5,
  high = "#00c434",
  low = "purple",
  mid = "ivory2"
)

Arguments

tree a tree plot created from the ggtree() function with p$data containing test statistic and p-
point_size size of nodes in the tree
high colour for large values
low colour for low values
mid colour for middle values

Value

an interactive ggplot graph object, plotting the hierarchical tree of clusters with nodes and branches coloured by the significance testing results.

Examples

library(SingleCellExperiment)
data(COVIDSampleData)

sce <- DeBiassi_COVID_CD8_samp
exprs <- t(assay(sce, "exprs"))
clusters <- colData(sce)$cluster_id
classes <- colData(sce)$condition
samples <- colData(sce)$sample_id

clust_tree <- getClusterTree(exprs,
  clusters,
  hierarchy_method="hopach")
tested_tree <- testTree(clust_tree$clus_tree, 
clusts=clusters, 
samples=samples, 
classes=classes)

colourTree(tested_tree)

DeBiasi_COVID_CD8_samp

COVID-19 Sample data

**Description**

Data from an experiment investigating T cell compositions between COVID-19 patients and healthy control. This data has been transformed using a arcsinh transform using a co-factor of 5 and randomly subsetted.

**Usage**

data(COVIDSampleData)

**Format**

An object of class "SingeCellExperiment"

**Source**

FlowRepository

**References**


**Examples**

data(COVIDSampleData)
**findChildren**

**Description**

findChildren

**Usage**

```r
callChildren(tree)
```

**Arguments**

- `tree` a ggtree object

**Value**

a ggtree object with the data containing a column with the clusters contained in each node

---

**geometricMean**

**Description**

getCellGMeans helper function

**Usage**

```r
geometricMean(x, na.rm = TRUE)
```

**Arguments**

- `x` vector containing numeric values
- `na.rm` whether or not to ignore NA values

**Value**

geomtric mean of vector x
getCellGMeans

Description

getCellGMeans

Usage

ggetCellGMeans(phylo, exprs, clusters, samples, classes)

Arguments

phylo a phylogram with tip.labels corresponding to cell types/cluster contained in 'clusters' vector
exprs a dataframe containing single cell expression data
clusters a vector representing the cell type or cluster of each cell (can be character or numeric). If numeric, cluster names need to be consecutive starting from 1.
samples a vector identifying the patient each cell belongs to
classes a vector containing the patient outcome/class each cell belongs to

Value

a dataframe containing proportions calculated for each sample

Examples

library(SingleCellExperiment)
data(COVIDSampleData)
sce <- DeBiasi_COVID_CD8_samp
exprs <- t(assay(sce, "exprs"))
clusters <- colData(sce)$cluster_id
classes <- colData(sce)$condition
samples <- colData(sce)$sample_id

clust_tree <- getClusterTree(exprs,
    clusters,
    hierarchy_method="hopach")

means_df <- getCellGMeans(clust_tree$clust_tree,
    exprs=exprs,
    clusters=clusters,
    samples=samples,
    classes=classes)
**getCellProp**

---

**Description**

getCellProp

**Usage**

```r
getCellProp(phylo, clusters, samples, classes, excl_top_node_parent = TRUE)
```

**Arguments**

- **phylo**: a phylogram with tip.labels corresponding to cell types/cluster contained in 'clusters' vector
- **clusters**: a vector representing the cell type or cluster of each cell (can be character or numeric). If numeric, cluster names need to be consecutive starting from 1.
- **samples**: a vector identifying the patient each cell belongs to
- **classes**: a vector containing the patient outcome/class each cell belongs to
- **excl_top_node_parent**: a boolean indicating whether the for cell types with the highest node as their parent

**Value**

a dataframe containing proportions calculated for each sample

**Examples**

```r
library(SingleCellExperiment)
data(COVIDSampleData)
sce <- DeBiasi_COVID_CD8_samp
exprs <- t(assay(sce, "exprs"))
clusters <- colData(sce)$cluster_id
classes <- colData(sce)$condition
samples <- colData(sce)$sample_id

clust_tree <- getClusterTree(exprs,
                           clusters,
                           hierarchy_method="hopach")

prop_df <- getCellProp(clust_tree$clust_tree,
                       clusters=clusters,
                       samples=samples,
                       classes=classes)
```
getClusterTree

getClusterTree This function takes a CATALYST sce with clusters and creates a hierarchical tree

Description
getClusterTree This function takes a CATALYST sce with clusters and creates a hierarchical tree

Usage
getClusterTree(
  exprs,
  clusters,
  hierarchy_method = "hopach",
  hopach_kmax = 5,
  hopach_K = 10,
  scale_exprs = TRUE
)

Arguments
exprs a dataframe containing single cell expression data
clusters a vector representing the cell type or cluster of each cell (can be character or numeric). If numeric, cluster names need to be consecutive starting from 1.
hierarchy_method a string indicating the hierarchical tree construction method to be used
hopach_kmax integer between 1 and 9 specifying the maximum number of children at each node in the tree
hopach_K positive integer specifying the maximum number of levels in the tree. Must be 15 or less, due to computational limitations (overflow)
scale_exprs boolean indicating whether to scale median cluster expression data before constructing hierarchical tree

Value
a list containing the cluster median frequencies and a phylogram of the hierarchical tree

Examples
library(SingleCellExperiment)
data(COVIDSampleData)
sce <- DeBiasi_COVID_CD8_samp
exprs <- t(assay(sce, "exprs"))
clusters <- colData(sce)$cluster_id
classes <- colData(sce)$condition
samples <- colData(sce)$sample_id
**getParentProp**

```r
clust_tree <- getClusterTree(exprs,
                           clusters,
                           hierarchy_method="hopach")
```

---

**getParentProp**

**Description**

getCellProp helper function

**Usage**

```r
getParentProp(vars1, vars2, n_cells)
```

**Arguments**

- **vars1**  
  name of cell type, matching to column in `n_cells`
- **vars2**  
  name of parent cell type, matching to column in `n_cells`
- **n_cells**  
  matrix of counts of each cell type per sample

**Value**

a vector containing the proportions of cell type `vars1` as a percent of parent `vars2` per sample

---

**getTotalProp**

**Description**

getCellProp helper function

**Usage**

```r
getTotalProp(vars1, n_cells, n_cells_pat)
```

**Arguments**

- **vars1**  
  name of cell type, matching to column in `n_cells`
- **n_cells**  
  matrix of counts of each cell type per sample
- **n_cells_pat**  
  vector containing number of cells per sample

**Value**

a vector containing the proportions of cell type `vars1` as a percent of total per sample
**getTreeResults**

**Description**

getTreeResults

**Usage**

getTreeResults(testedTree, sort_by = "parent")

**Arguments**

testedTree  a ggtree object outputed from testTree()

sort_by  whether to sort by p-values testing via proportions to parent or p-values testing via absolute proportions. Values can be c(NA, "parent", "all")

**Value**

a dataframe with hierarchical tree nodes, corresponding clusters and corresponding significance testing results

**Examples**

```r
library(SingleCellExperiment)
data(COVIDSampleData)
sce <- DeBiasi_COVID_CD8_samp
exprs <- t(assay(sce, "exprs"))
clusters <- colData(sce)$cluster_id
classes <- colData(sce)$condition
samples <- colData(sce)$sample_id

clust_tree <- getClusterTree(exprs,
                           clusters,
                           hierarchy_method="hopach")

tested_tree <- testTree(clust_tree$clust_tree,
                        clusters=clusters,
                        samples=samples,
                        classes=classes,
                        pos_class_name=NULL)

res_df <- getTreeResults(tested_tree)

head(res_df, 10)
```
Description

hopachToPhylo

Usage

hopachToPhylo(res)

Arguments

res an object returned from the runHOPACH() function

Value

a phylogram converted from the outputted list from the runHOPACH function

Examples

library(SingleCellExperiment)
library(data.table)
data(COVIDSampleData)

sce <- DeBiasi_COVID_CD8_samp
eprs <- t(assay(sce, "exprs"))
custers <- colData(sce)$cluster_id
classes <- colData(sce)$condition
samples <- colData(sce)$sample_id

clust_med_dt <- as.data.table(eprs)
clust_med_dt[, cluster_id := clusters]
res <- clust_med_dt[, lapply(.SD, median, na.rm=TRUE), by=cluster_id]
res2 <- res[, .SD, .SDcols = !c('cluster_id')]

hopach_res <- runHOPACH(as.data.frame(scale(res2)))
phylo <- hopachToPhylo(hopach_res)
plotInteractiveHeatmap

Description

This function takes a hierarchical tree which has been tested for proportion to all and proportion to parent cluster.

Usage

plotInteractiveHeatmap(
  testedTree,
  clust_med_df,
  clusters,
  svg_width = 13,
  svg_height = 9,
  tr_offset = 0.3,
  tr_font_size = 2,
  tr_point_size = 1.5,
  tr_col_high = "#00c434",
  tr_col_low = "purple",
  tr_col_mid = "ivory2",
  hm_offset = 1,
  hm_tile_width = 1,
  hm_expand_y_lim = 20,
  hm_col_high = "#cc2010",
  hm_col_mid = "#fff8de",
  hm_col_low = "#66a6cc",
  fb_offset = 0.75,
  fb_bar_length = 3,
  fb_bar_width = 0.4,
  fb_freq_labels = FALSE
)

Arguments

- **testedTree**: a ggtree object that has been run through the testTree.
- **clust_med_df**: a dataframe with the cluster medians. The rownumbers of the clusters median data frame should correspond to the nodes in the phylo tree. The column names should also correspond to the labels you want to use.
- **clusters**: a vector representing the cell type or cluster of each cell (can be character or numeric).
- **svg_width**: width of svg canvas.
- **svg_height**: height of svf canvas.
- **tr_offset**: distance between leaf nodes on the tree and their labels.
plotInteractiveHeatmap

tr_font_size  font size of leaf labels
tr_point_size  size of each node in the tree
tr_col_high  colour used for high test statistics, coloured on the nodes and branches of the tree
tr_col_low  colour used for low test statistics, coloured on the nodes and branches of the tree
tr_col_mid  colour used for medium test statistics, coloured on the nodes and branches of the tree
hm_offset  distance between the tree and the heatmap
hm_tile_width  width of each tile in the heatmap
hm_expand_y_lim  white space below heatmap
hm_col_high  colour used for large values on heatmap
hm_col_mid  colour used for medium values on heatmap
hm_col_low  colour used for low values on heatmap
fb_offset  distance between the heatmap and frequency bars
fb_bar_length  length of bar with max frequency
fb_bar_width  width of each frequency bar
fb_freq_labels  boolean indicated whether or not to show frequency bar labels

Value

an interactive ggplot object containing the hierarchical tree of clusters coloured by significance testing results, with corresponding heatmap and a scatterplot comparing significance when testing using proportions to parent vs. absolute proportions

Examples

library(SingleCellExperiment)
data(COVIDSampleData)

sce <- DeBiasi_COVID_CD8_samp
exprs <- t(assay(sce, "exprs"))
clusters <- colData(sce)$cluster_id
classes <- colData(sce)$condition
samples <- colData(sce)$sample_id

clust_tree <- getClusterTree(exprs,
  clusters,
  hierarchy_method="hopach")

tested_tree <- testTree(clust_tree$clust_tree,
  clusters=clusters,
  samples=samples,
  classes=classes)

plotInteractiveHeatmap(tested_tree,
  clust_med_df = clust_tree$median_freq,
  clusters=clusters)
plotSigScatter

Description
plotSigScatter

Usage
plotSigScatter(testedTree, scatter_tooltip, max_val)

Arguments
- **testedTree**: an output from the function testTree()
- **scatter_tooltip**: vector containing tooltips for interactive plot
- **max_val**: maximum value to set as x/y axis limits

Value
a ggplot object, containing test statistics from testing proportions relative to parent against the test statistics from testing absolute proportions.

runEdgeRTests

Description
This function runs edgeR using the treekoR inputs across all nodes of the hierarchical tree of clusters, adapted from the diffcyt workflow

Usage
runEdgeRTests(td, clusters, samples, classes, pos_class_name)

Arguments
- **td**: a dataframe of data from ggtree object
- **clusters**: a vector representing the cell type or cluster of each cell (can be character or numeric). If numeric, cluster names need to be consecutive starting from 1.
- **samples**: a vector identifying the patient each cell belongs to
- **classes**: a vector containing the patient outcome/class each cell belongs to
- **pos_class_name**: a character indicating which class should be treated as positive

Value
a dataframe with pvalues, test statistic (signed -log10(p)), and FDR
Description

This function runs GLMM using the treekoR inputs across all nodes of the hierarchical tree of clusters, adapted from the diffcyt workflow. (Unable to get direction of test statistic currently)

Usage

runGLMMTests(td, clusters, samples, classes, pos_class_name, neg_class_name)

Arguments

td a dataframe of data from ggtree object
clusters a vector representing the cell type or cluster of each cell (can be character or numeric). If numeric, cluster names need to be consecutive starting from 1.
samples a vector identifying the patient each cell belongs to
classes a vector containing the patient outcome/class each cell belongs to
pos_class_name a character indicating which class should be treated as positive
neg_class_name a character indicating which class should be treated as negative

Value

a dataframe with pvalues and test statistics

Description

runHOPACH

Usage

runHOPACH(data, K = 10, kmax = 5, dissimilarity_metric = "cor")

Arguments

data dataframe containing the median expression of the clusters/cell types
K positive integer specifying the maximum number of levels in the tree. Must be 15 or less, due to computational limitations (overflow)
kmax integer between 1 and 9 specifying the maximum number of children at each node in the tree
dissimilarity_metric metric used to calculate dissimilarities between clusters/cell types
Value

a list containing the groups each cluster belongs to at each level of the hopach tree

Examples

```r
library(SingleCellExperiment)
library(data.table)
data(COVIDSampleData)

sce <- DeBiasi_COVID_CD8_samp
exprs <- t(assay(sce, "exprs"))
clusters <- colData(sce)$cluster_id
classes <- colData(sce)$condition
samples <- colData(sce)$sample_id

clust_med_dt <- as.data.table(exprs)
clust_med_dt[, cluster_id := clusters]
res <- clust_med_dt[, lapply(.SD, median, na.rm=TRUE), by=cluster_id]
res2 <- res[, .SD, .SDcols = !c('cluster_id')]
hopach_res <- runHOPACH(as.data.frame(scale(res2)))
```

Description

This function takes a hierarchical tree of the cluster medians of a cytometry dataset, and then uses this structure to perform t-tests between conditions of patients testing for difference using the proportion of cluster relative to sample’s n and proportion of cluster relative to sample’s n of hierarchical parent cluster. Takes a ggtree object and returns a ggtree object with testing results appended in the data

Usage

```r
testTree(
  phylo,
  clusters,
  samples,
  classes,
  sig_test = "ttest",
  p_adjust = NULL,
  pos_class_name = NULL
)
```
testTree

**Arguments**

- `phylo` a `ggtree` object
- `clusters` a vector representing the cell type or cluster of each cell (can be character or numeric). If numeric, cluster names need to be consecutive starting from 1.
- `samples` a vector identifying the patient each cell belongs to
- `classes` a vector containing the patient outcome/class each cell belongs to
- `sig_test` a character, either "ttest" or "wilcox" indicating the significance test to be used
- `p_adjust` a character, indicating whether p-value adjustment should be performed. Valid values are in stats::p.adjust.methods
- `pos_class_name` a character indicating which class is positive

**Value**
a `ggtree` object with significance testing results in embedded data

**Examples**

```r
library(SingleCellExperiment)
data(COVIDSampleData)
sce <- DeBiasi_COVID_CD8_samp
exprs <- t(assay(sce, "exprs"))
clusters <- colData(sce)$cluster_id
classes <- colData(sce)$condition
samples <- colData(sce)$sample_id

clust_tree <- getClusterTree(exprs,
                           clusters,
                           hierarchy_method="hopach")

tested_tree <- testTree(clust_tree$clust_tree,
                        clusters=clusters,
                        samples=samples,
                        classes=classes,
                        sig_test="ttest",
                        pos_class_name=NULL)
```
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