

Package ‘treekoR’

May 8, 2025

Type Package

Title Cytometry Cluster Hierarchy and Cellular-to-phenotype
Associations

Version 1.16.0

biocViews Clustering, DifferentialExpression, FlowCytometry,
ImmunoOncology, MassSpectrometry, SingleCell, Software,
StatisticalMethod, Visualization

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

Encoding UTF-8

LazyData false

RoxygenNote 7.2.3

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

VignetteBuilder knitr

Config/testthat/edition 3

git_url <https://git.bioconductor.org/packages/treekoR>

git_branch RELEASE_3_21

git_last_commit d8a669a
git_last_commit_date 2025-04-15
Repository Bioconductor 3.21
Date/Publication 2025-05-07
Author Adam Chan [aut, cre],
Ellis Patrick [ctb]
Maintainer Adam Chan <adam.s.chan@sydney.edu.au>

Contents

addFreqBars	2
addHeatMap	3
addTree	4
colourTree	5
DeBiasi_COVID_CD8_samp	6
findChildren	7
geometricMean	7
getCellGMeans	8
getCellProp	9
getClusterTree	10
getParentProp	11
getTotalProp	11
getTreeResults	12
hopachToPhylo	13
plotInteractiveHeatmap	14
plotSigScatter	16
runEdgeRTests	16
runGLMMTests	17
runHOPACH	17
testTree	18
Index	20

addFreqBars	<i>Title</i>
-------------	--------------

Description

a function to add the frequency bars for each cluster

Usage

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

Arguments

<code>p</code>	a phylogenetic tree plot created from the <code>ggtree()</code> function
<code>clusters</code>	a vector representing the cell type or cluster of each cell (can be character or numeric)
<code>offset</code>	distance between the heatmap and frequency bars
<code>bar_length</code>	length of bar with max frequency
<code>bar_width</code>	width of each frequency bar
<code>freq_labels</code>	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

addHeatMap

Title

Description

a function to add a heatmap of cluster medians alongisde 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 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
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	<i>Title</i>
---------	--------------

Description

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

Usage

```
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


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

DeBiasi_COVID_CD8_samp

COVID-19 Sample data

Description

Data from a 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 subsetting

Usage

```
data(COVIDSampleData)
```

Format

An object of class "SingeCellExperiment"

Source

[FlowRepository](#)

References

De Biasi et al. (2020) Nat Commun 11, 3434 ([Nature](#))

Examples

```
data(COVIDSampleData)
```

findChildren	<i>findChildren</i>
--------------	---------------------

Description

findChildren

Usage

```
findChildren(tree)
```

Arguments

tree	a ggtree object
------	-----------------

Value

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

geometricMean	<i>geometricMean</i>
---------------	----------------------

Description

getCellGMeans helper function

Usage

```
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

getCellGMeans

Description

getCellGMeans

Usage

```
getCellGMeans(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

getCellProp

Description

getCellProp

Usage

```
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

```
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	<i>getClusterTree</i> 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
```

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

getParentProp	<i>getParentProp</i>
---------------	----------------------

Description

getCellProp helper function

Usage

```
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	<i>getTotalProp</i>
--------------	---------------------

Description

getCellProp helper function

Usage

```
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	<i>getTreeResults</i>
----------------	-----------------------

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

```
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)
```

hopachToPhylo	<i>hopachToPhylo</i>
---------------	----------------------

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
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)))
phylo <- hopachToPhylo(hopach_res)
```

plotInteractiveHeatmap

Title

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

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	<i>plotSigScatter</i>
----------------	-----------------------

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	<i>runEdgeRTests</i>
---------------	----------------------

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

runGLMMTests

runGLMMTests

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

runHOPACH

runHOPACH

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

```
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)))
```

testTree

testTree

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

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

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

```
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)
```

Index

* **datasets**

DeBiasi_COVID_CD8_samp, [6](#)

addFreqBars, [2](#)

addHeatMap, [3](#)

addTree, [4](#)

colourTree, [5](#)

DeBiasi_COVID_CD8_samp, [6](#)

findChildren, [7](#)

geometricMean, [7](#)

getCellMeans, [8](#)

getCellProp, [9](#)

getClusterTree, [10](#)

getParentProp, [11](#)

getTotalProp, [11](#)

getTreeResults, [12](#)

hopachToPhylo, [13](#)

plotInteractiveHeatmap, [14](#)

plotSigScatter, [16](#)

runEdgeRTests, [16](#)

runGLMMTests, [17](#)

runHOPACH, [17](#)

testTree, [18](#)