Package ‘pagoda2’

March 2, 2022

**Title**  Single Cell Analysis and Differential Expression

**Version**  1.0.9

**Description**  Analyzing and interactively exploring large-scale single-cell RNA-seq datasets. ‘pagoda2’ primarily performs normalization and differential gene expression analysis, with an interactive application for exploring single-cell RNA-seq datasets. It performs basic tasks such as cell size normalization, gene variance normalization, and can be used to identify subpopulations and run differential expression within individual samples. ‘pagoda2’ was written to rapidly process modern large-scale scRNAseq datasets of approximately 1e6 cells. The companion web application allows users to explore which gene expression patterns form the different subpopulations within your data. The package also serves as the primary method for preprocessing data for conos, <https://github.com/kharchenkolab/conos>. This package interacts with data available through the 'p2data' package, which is available in a 'drat' repository. To access this data package, see the instructions at <https://github.com/kharchenkolab/pagoda2>. The size of the 'p2data' package is approximately 6 MB.

**License**  GPL-3

**Copyright**  See the file COPYRIGHTS for various pagoda2 copyright details

**Encoding**  UTF-8

**Depends**  R (>= 3.5.0), Matrix, igraph

**biocViews**

**Imports**  dendsort, drat, fastcluster, graphics, grDevices, irlba, magrittr, MASS, mgcv, methods, N2R, parallel, plyr, R.utils, Rcpp, rjson, rlang, R6, RMTstat, Rook, Rtsne, score (> = 0.1.1), stats, urltools, utils

**RoxygenNote**  7.1.1

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BugReports: https://github.com/kharchenkolab/pagoda2/issues

NeedsCompilation: yes

LinkingTo: Rcpp, RcppArmadillo, RcppProgress, RcppEigen

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**armaCor**

armaCor - matrix column correlations. Allows faster matrix correlations with armadillo. Similar to cor() call, will calculate correlation between matrix columns.

### Description

armaCor - matrix column correlations. Allows faster matrix correlations with armadillo. Similar to cor() call, will calculate correlation between matrix columns.

### Usage

armaCor(mat)

### Arguments

- **mat**: matrix

### Value

matrix with columns as correlations

**basicP2proc**

Perform basic 'pagoda2' processing, i.e. adjust variance, calculate pca reduction, make knn graph, identify clusters with multilevel, and generate largeVis and tSNE embeddings.

### Description

Perform basic 'pagoda2' processing, i.e. adjust variance, calculate pca reduction, make knn graph, identify clusters with multilevel, and generate largeVis and tSNE embeddings.

### Usage

basicP2proc(
  cd,
  n.cores = 1,
  n.odgenes = 3000,
  nPcs = 100,
  k = 30,
  perplexity = 50,
  log.scale = TRUE,
  trim = 10,
  keep.genes = NULL,
  min.cells.per.gene = 0,
  min.transcripts.per.cell = 100,
get.largevis = TRUE,
get.tsne = TRUE,
make.geneknn = TRUE
)

Arguments

- **cd**: count matrix whereby rows are genes, columns are cells.
- **n.cores**: numeric Number of cores to use (default=1)
- **n.odgenes**: numeric Number of top overdispersed genes to use (default=3e3)
- **nPcs**: numeric Number of PCs to use (default=100)
- **k**: numeric Default number of neighbors to use in kNN graph (default=30)
- **perplexity**: numeric Perplexity to use in generating tSNE and largeVis embeddings (default=50)
- **log.scale**: boolean Whether to use log scale normalization (default=TRUE)
- **trim**: numeric Number of cells to trim in winsorization (default=10)
- **keep.genes**: optional set of genes to keep from being filtered out (even at low counts) (default=NULL)
- **min.cells.per.gene**: numeric Minimal number of cells required for gene to be kept (unless listed in keep.genes) (default=0)
- **min.transcripts.per.cell**: numeric Minimumal number of molecules/reads for a cell to be admitted (default=100)
- **get.largevis**: boolean Whether to calculate largeVis embedding (default=TRUE)
- **get.tsne**: boolean Whether to calculate tSNE embedding (default=TRUE)
- **make.geneknn**: boolean Whether pre-calculate gene kNN (for gene search) (default=TRUE)

Value

- a new 'Pagoda2' object

---

**basicP2web**  
Generate a 'pagoda2' web application from a 'Pagoda2' object

Description

Generate a 'pagoda2' web application from a 'Pagoda2' object

Usage

basicP2web(p2, app.title = "Pagoda2", extraWebMetadata = NULL, n.cores = 4)
Arguments

- **p2**  
  a 'Pagoda2' object
- **app.title**  
  name of application as displayed in the browser title (default='Pagoda2')
- **extraWebMetadata**  
  additional metadata generated by p2.metadata.from.factor (default=NULL)
- **n.cores**  
  numeric Number of cores to use for differential expression calculation (default=4)

Value

A 'pagoda2' web object

---

**buildWijMatrix**  
Rescale the weights in an edge matrix to match a given perplexity. From 'largeVis', <https://github.com/elbamos/largeVis>

Description

Rescale the weights in an edge matrix to match a given perplexity. From 'largeVis', <https://github.com/elbamos/largeVis>

Usage

```r
buildWijMatrix(x, threads = NULL, perplexity = 50)
```

Arguments

- **x**  
  An edgematrix, either an 'edgematrix' object or a sparse matrix.
- **threads**  
  numeric The maximum number of threads to spawn (default=NULL). Determined automatically if NULL (default=NULL)
- **perplexity**  
  numeric Given perplexity (default=50)

Value

A list with the following components:

- **'dist'** An [N,K] matrix of the distances to the nearest neighbors.
- **'id'** An [N,K] matrix of the node indexes of the nearest neighbors. Note that this matrix is 1-indexed, unlike most other matrices in this package.
- **'k'** The number of nearest neighbors.
calcMulticlassified

Description

Returns a list vector with the number of cells that are present in more than one selections in the provided p2 selection object

Usage

calcMulticlassified(sel)

Arguments

sel a pagoda2 selection as generated by readPagoda2SelectionFile

Value

list vector with the number of cells that are present in more than one selections in the provided p2 selection object

cellsPerSelectionGroup

Get the number of cells in each selection group

Description

Get the number of cells in each selection group

Usage

cellsPerSelectionGroup(selection)

Arguments

selection a pagoda2 selection list

Value

a named vector of cell numbers in each groups
collapse.aspect.clusters

*Collapse aspect patterns into clusters*

**Description**

Collapse aspect patterns into clusters

**Usage**

```
collapse.aspect.clusters(d, dw, ct, scale = TRUE, pick.top = FALSE)
```

**Arguments**

- `d`: matrix of normalized aspect patterns (rows: significant aspects, columns: cells), normally the output $xv$ in `tamr`, the combined pathways that show similar expression patterns
- `dw`: corresponding weight matrix to parameter `d`
- `ct`: clusters, the output of fastcluster::hclust()
- `scale`: boolean Whether to scale aspects (default=TRUE)
- `pick.top`: boolean Whether to pick top aspects (default=FALSE)

**Value**

list of clusters from matrix of normalized aspect patterns and clusters from the corresponding weight matrix

---

compareClusterings

*Compare two different clusterings provided as factors by plotting a normalised heatmap*

**Description**

Compare two different clusterings provided as factors by plotting a normalised heatmap

**Usage**

```
compareClusterings(cl1, cl2, filename = NA)
```

**Arguments**

- `cl1`: clustering 1, a named factor
- `cl2`: clustering 2, a named factor
- `filename`: an optional filename to save the plot instead of displaying it, will be passed to pheatmap (default=NA)
extendedP2proc

Value
invisible summary table that gets plotted

extendedP2proc  Perform extended 'Pagoda2' processing. Generate organism specific GO environment and calculate pathway overdispersion.

Description
Perform extended 'Pagoda2' processing. Generate organism specific GO environment and calculate pathway overdispersion.

Usage
extendedP2proc(p2, organism = "hs")

Arguments

p2 the 'Pagoda2' object
organism character Organisms hs (Homo Sapiens), mm (M. Musculus, mouse) or dr (D. Rerio, zebrafish) (default='hs')

Value
list of a 'Pagoda2' object and go.env

factorFromP2Selection

Returns a factor of cell membership from a p2 selection object the factor only includes cells present in the selection. If the selection contains multiclassified cells an error is raised

Description
Returns a factor of cell membership from a p2 selection object the factor only includes cells present in the selection. If the selection contains multiclassified cells an error is raised

Usage
factorFromP2Selection(sel, use.internal.name = FALSE, flatten = FALSE)

Arguments

sel a pagoda2 selection as generated by readPagoda2SelectionFile
use.internal.name boolean Whether to use field 'internal.name' as factor names (default=FALSE)
flatten boolean Whether to ignore multiclassified cells, overwriting randomly (default=FALSE)
factorToP2selection

Value
factor of cell membership from a p2 selection object. The factor only includes cells present in the selection.

factorListToMetadata

Description
Converts a list of factors into 'pagoda2' metadata optionally filtering down to the cells present in the provided 'pagoda2' app.

Usage
factorListToMetadata(factor.list, p2 = NULL)

Arguments
factor.list list of factors named by the cell identifier
p2 'pagoda2' app to filter the factors by, optional (default=NULL)

Value
'pagoda2' web metadata object

factorToP2selection

Description
Converts a names factor to a p2 selection object if colors are provided it assigns those, otherwise uses a rainbow palette.

Usage
factorToP2selection(cl, col = NULL)

Arguments
cl factor
col names vector of colors (default=NULL)

Value
a p2 selection object (list)
gene.vs.molecule.cell.filter

Filter cells based on gene/molecule dependency

Description

Filter cells based on gene/molecule dependency

Usage

gene.vs.molecule.cell.filter(
  countMatrix,
  min.cell.size = 500,
  max.cell.size = 50000,
  p.level = min(0.001, 1/ncol(countMatrix)),
  alpha = 0.1,
  plot = TRUE,
  do.par = TRUE
)

Arguments

countMatrix    input count matrix to be filtered
min.cell.size  numeric Min allowed cell size (default=500)
max.cell.size  numeric Max allowed cell size (default=5e4)
p.level        numeric Statistical confidence level for deviation from the main trend, used for cell filtering (default=min(1e-3, 1/ncol(countMatrix)))
alpha          numeric Shading of the confidence band (default=0.1)
plot           boolean Plot the molecule distribution and the gene/molecule dependency fit (default=TRUE)
do.par         boolean Reset graphical parameters prior to plotting (default=TRUE)

Value

a filtered matrix
generateClassificationAnnotation

*Given a cell clustering (partitioning) and a set of user provided selections generate a cleaned up annotation of cluster groups that can be used for classification*

**Description**

Given a cell clustering (partitioning) and a set of user provided selections generate a cleaned up annotation of cluster groups that can be used for classification

**Usage**

```r
generateClassificationAnnotation(clustering, selections)
```

**Arguments**

- `clustering` a factor that provides the clustering
- `selections` a p2 selection object that provided by the web interfact user

**Value**

a named factor that can be used for classification

---

get.control.geneset

*Get a control geneset for cell scoring using the method described in Puram, Bernstein (Cell, 2018)*

**Description**

Get a control geneset for cell scoring using the method described in Puram, Bernstein (Cell, 2018)

**Usage**

```r
get.control.geneset(data, signature, n.bins = 25, n.genes.per.bin = 100)
```

**Arguments**

- `data` matrix of expression, rows are cell, columns are genes
- `signature` character vector The signature to evaluate, a character vector of genes
- `n.bins` numeric Number of bins to put the genes in (default=25)
- `n.genes.per.bin` numeric Number of genes to get from each bin (default=100)

**Value**

a character vector that can be used as a background signature
get.de.geneset  Generate differential expression genesets for the web app given a cell grouping by calculating DE sets between each cell set and everything else

Description
Generate differential expression genesets for the web app given a cell grouping by calculating DE sets between each cell set and everything else

Usage
get.de.geneset(pagObj, groups, prefix = "de_")

Arguments
- pagObj: pagoda object
- groups: named factor to do the de by
- prefix: character Prefix to assign to genesets generated (default="de_")

Value
a `pagoda2` web object

getCellsInSelections  Returns all the cells that are in the designated selections. Given a pagoda2 selections object and the names of some selections in it returns the names of the cells that are in these selections removed any duplicates

Description
Returns all the cells that are in the designated selections. Given a pagoda2 selections object and the names of some selections in it returns the names of the cells that are in these selections removed any duplicates

Usage
getCellsInSelections(p2selections, selectionNames)

Arguments
- p2selections: a p2 selections object
- selectionNames: the names of some selections in th p2 object
getClusterLabelsFromSelection

Assign names to the clusters, given a clustering vector and a set of selections. This function will use a set of pagoda2 cell selection to identify the clusters in a named factor. It is meant to be used to import user defined annotations that are defined as selections into a more formal categorization of cells that are defined by cluster. To help with this the function allows a percent of cells to have been classified in the selections into multiple groups, something which may be the result of the users making wrong selections. The percent of cells allows to be multiselected in any given group is defined by multiClassCutoff. Furthermore the method will assign each cluster to a selection only if the most popular cluster to the next most popular exceed the ambiguous.ratio in terms of cell numbers. If a cluster does not satisfy this condition it is not assigned.

Usage

getClusterLabelsFromSelection(
    clustering,
    selections,
    multiClassCutoff = 0.3,
    ambiguous.ratio = 0.5
)

Arguments

clustering a named factor of clusters, where every entry is a cell
selections a pagoda2 selection object
multiClassCutoff numeric Percent of cells in any one cluster that can be multiassigned (default=0.3)
getColorsFromP2Selection

ambiguous.ratio
numeric Ratio of first and second cell numbers for any cluster to produce a valid clustering (default=0.5)

Value
a data.frame with two columns, one for cluster and one for selections, each cluster appears only once

getColorsFromP2Selection
Retrieves the colors of each selection from a p2 selection object as a names vector of strings

Description
Retrieves the colors of each selection from a p2 selection object as a names vector of strings

Usage
getColorsFromP2Selection(sel)

Arguments
sel pagoda2 selection object

Value
a named vector of hex colours

getIntExtNamesP2Selection
Get a mapping form internal to external names for the specified selection object

Description
Get a mapping form internal to external names for the specified selection object

Usage
gGetIntExtNamesP2Selection(x)

Arguments
x p2 selection object
Value

list of names from the specified selection object

---

hierDiffToGenesets

Converts the output of hierarchical differential expression aspects into genesets that can be loaded into a 'pagoda2' web app to retrieve the genes that make the geneset interactively

Description

Converts the output of hierarchical differential expression aspects into genesets that can be loaded into a 'pagoda2' web app to retrieve the genes that make the geneset interactively

Usage

hierDiffToGenesets(output)

Arguments

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</thead>
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Value

a geneset that can be loaded into p2 web genesets

---

make.p2.app

Generate a Rook Server app from a 'Pagoda2' object. This generates a 'pagoda2' web object from a 'Pagoda2' object by automating steps that most users will want to run. This function is a wrapper about the 'pagoda2' web constructor. (Advanced users may wish to use that constructor directly.)

Description

Generate a Rook Server app from a 'Pagoda2' object. This generates a 'pagoda2' web object from a 'Pagoda2' object by automating steps that most users will want to run. This function is a wrapper about the 'pagoda2' web constructor. (Advanced users may wish to use that constructor directly.)
Usage

```r
make.p2.app(
  r, dendrogramCellGroups, additionalMetadata = list(),
  geneSets, show.depth = TRUE, show.batch = TRUE,
  show.clusters = TRUE, appname = "Pagoda2 Application",
  innerOrder = NULL, orderDend = FALSE,
  appmetadata = NULL
)
```

Arguments

- **r**: a `Pagoda2` object
- **dendrogramCellGroups**: a named factor of cell groups, used to generate the main dendrogram, limits zoom in
- **additionalMetadata**: a list of metadata other than depth, batch and cluster that are automatically added (default=list())
- **geneSets**: a list of genesets to show
- **show.depth**: boolean Include depth as a metadata row (default=TRUE)
- **show.batch**: boolean Include batch as a metadata row (default=TRUE)
- **show.clusters**: boolean Include clusters as a metadata row (default=TRUE)
- **appname**: character Application name (default="Pagoda2 Application")
- **innerOrder**: Ordering of cells inside the clusters provided in dendrogramCellGroups (default=NULL). This should be one of "odPCA", "reductdist", "graphbased", "knn". Defaults to NULL
- **orderDend**: boolean Whether to order dendrogram (default=FALSE)
- **appmetadata**: a `pagoda2` web application metadata (default=NULL)

Value

a `pagoda2` web object that presents a Rook compatible interface
**minMaxScale**

*Scale the designated values between the range of 0 and 1*

**Description**

Scale the designated values between the range of 0 and 1

**Usage**

```
minMaxScale(x)
```

**Arguments**

- **x** values to scale

**Value**

the scaled values

**Examples**

```r
e.example_matrix = matrix(rep(1:5), 3)
minMaxScale(example_matrix)
```

**namedNames**

*Get a vector of the names of an object named by the names themselves. This is useful with lapply when passing names of objects as it ensures that the output list is also named.*

**Description**

Get a vector of the names of an object named by the names themselves. This is useful with lapply when passing names of objects as it ensures that the output list is also named.

**Usage**

```
namedNames(g)
```

**Arguments**

- **g** an objects on which we can call names()

**Value**

vector with names of object
p2.generate.dr.go

Generate a GO environment for human for overdispersion analysis for the back end

Description
Generate a GO environment for human for overdispersion analysis for the back end

Usage
p2.generate.dr.go(r)

Arguments
- r: a 'Pagoda2' object

Value
- a GO environment object

p2.generate.go

Generate a GO environment for the organism specified

Description
Generate a GO environment for the organism specified

Usage
p2.generate.go(
  r,
  organism = NULL,
  go2all.egs = NULL,
  eg.alias2eg = NULL,
  min.env.length = 5
)

Arguments
- r: a 'Pagoda2' object
- organism: the organism (default=NULL). Currently 'hs' (human), 'mm' (mouse) and 'dr' (zebrafish) are supported.
- go2all.egs: mappings between a given GO identifier and all of the Entrez Gene identifiers annotated at that GO term or to one of its child nodes in the GO ontology (default=NULL)
p2.generate.mouse.go

eg.alias2eg  mappings between common gene symbol identifiers and entrez gene identifiers (default=NULL)
min.env.length  numeric Minimum environment length (default=5)

Description
Generate a GO environment for mouse for overdispersion analysis for the back end

Usage
p2.generate.mouse.go(r)

Arguments
r  a 'Pagoda2' object

Value
a GO environment object

p2.generate.human.go  Generate a GO environment for human for overdispersion analysis for the back end

Description
Generate a GO environment for human for overdispersion analysis for the back end

Usage
p2.generate.human.go(r)

Arguments
r  a 'Pagoda2' object

Value
a GO environment object
Create 'PAGODA1' web application from a 'Pagoda2' object 'PAGODA1' found here, with 'SCDE':<https://www.bioconductor.org/packages/release/bioc/html/scde.html>

**Usage**

```r
p2.make.pagoda1.app(
  p2,
  col.cols = NULL,
  row.clustering = NULL,
  title = "pathway clustering",
  zlim = NULL,
  embedding = NULL,
  inner.clustering = TRUE,
  groups = NULL,
  clusterType = NULL,
  embeddingType = NULL,
  veloinfo = NULL,
  type = "PCA",
  min.group.size = 1,
  batch.colors = NULL,
  n.cores = 10
)
```

**Arguments**

- **p2** : 'Pagoda2' object
- **col.cols** : Matrix of column colors (default=NULL). Useful for visualizing cell annotations such as batch labels.
- **row.clustering** : Row dendrogram (default=NULL)
- **title** : character Title to use (default="pathway clustering")
- **zlim** : Range of the normalized gene expression levels (default=NULL). Input as a list: c(lower_bound, upper_bound). Values outside this range will be Winsorized. Useful for increasing the contrast of the heatmap visualizations. If NULL, set to the 5th and 95th percentiles.
- **embedding** : A 2-D embedding of the cells (PCA, tSNE, etc.), passed as a data frame with two columns (two dimensions) and rows corresponding to cells (row names have to match cell names) (default=NULL).
- **inner.clustering** : boolean Whether to get overall cell clustering (default=TRUE).
p2.metadata.from.factor

Generate a list metadata structure that can be passed to a 'pagoda2' web object constructor as additional metadata given a named factor

Description

Generate a list metadata structure that can be passed to a 'pagoda2' web object constructor as additional metadata given a named factor

Usage

p2.metadata.from.factor(
  metadata,
  displayname = NULL,
  s = 1,
  v = 1,
  start = 0,
  end = NULL,
  pal = NULL
)
**Arguments**

- **metadata**: named factor with metadata for individual cells, names must correspond to cells
- **displayname**: character Name to display for the metadata (default=NULL)
- **s**: numeric Value for rainbow palette (default=1)
- **v**: numeric Value for rainbow palette (default=1)
- **start**: numeric Starting value (default=0)
- **end**: numeric Ending value (default=NULL)
- **pal**: optional vector of colours to use, if provided overrides s,v,start and end parameters (default=NULL)

**Value**

list of data, levels, palette to be passed to 'pagoda2' web object constructor

---

**Description**

Generate a 'pagoda2' web object from a 'Pagoda2' object using hierarchical differential expression

**Usage**

```r
p2.toweb.hdea(p2, title = "")
```

**Arguments**

- **p2**: p2 object
- **title**: character Name of the pagoda object (default="")

**Value**

a 'pagoda2' web object
Description

Modified 'PAGODA1' app (from 'SCDE') for browsing 'pagoda2' results. Refer to 'ViewPagodaAppOld' and 'make.pagoda.app()' in 'SCDE'

Public fields

- **results**  Result object returned by scde.expression.difference() (default=NULL). Note to browse group posterior levels, use return.posteriors = TRUE in the scde.expression.difference() call.
- **type** Either 'counts' or a name of a 'reduction' in the 'Pagoda2' object
- **genes** List of genes to display in the Detailed clustering panel (default=list())
- **batch** Any batch or other known confounders to be included in the visualization as a column color track (default=NULL)
- **pathways** character vector Pathway or gene names (default=NULL)
- **name** App name (needs to be altered only if adding more than one app to the server using the 'server' parameter) (default=NULL)
- **trim** Trim quantity used for Winsorization for visualization
- **embedding** Embedding information (default=NULL)
- **veloinfo** Velocity information (default=NULL)
- **goenv** environment mapping pathways to genes (default=NULL)
- **renv** Global environment (default=NULL)

Methods

**Public methods:**
- p2ViewPagodaApp$new()
- p2ViewPagodaApp$getgenecldata()
- p2ViewPagodaApp$call()
- p2ViewPagodaApp$clone()

**Method new():** Initialize p2ViewPagodaApp class

*Usage:*

```r
p2ViewPagodaApp$new(  
  results,  
  pathways,  
  genes,  
  goenv,  
  batch = NULL,  
  name = "pathway overdispersion",  
)```
trim = 1.1/nrow(p2$counts),
  embedding = NULL,
  type,
  veloinfo = NULL
)

Arguments:
results  Result object returned by scde.expression.difference(). Note to browse group
  posterior levels, use return.posteriors = TRUE in the scde.expression.difference()
call.
pathways  character vector Pathway or gene names (default=NULL)
genes  list Genes to display in the Detailed clustering panel (default=list())
goenv  Environment mapping pathways to genes (default=NULL)
batch  Any batch or other known confounders to be included in the visualization as a column
color track (default=NULL)
name  string App name (needs to be altered only if adding more than one app to the server using
  the 'server' parameter) (default="pathway overdispersion")
trim  numeric Trim quantity used for Winsorization for visualization (default=1.1/nrow(p2$counts)
  whereby the 'counts' from the 'Pagoda2' object is the gene count matrix, normalized on to-
tal counts (default=NULL)
embedding  Embedding information (default=NULL)
type  Either 'counts' or a name of a 'reduction' in the 'pagoda2' object
veloinfo  Velocity information (default=NULL)

Returns:  new 'p2ViewPagodaApp' object

Method getgenecldata(): Helper function to get the heatmap data for a given set of genes

Usage:
p2ViewPagodaApp$getgenecldata(genes = NULL, gcl = NULL, ltrim = 0)

Arguments:
genes  character vector Gene names (default=NULL)
gcl  pathway or gene-weighted PCA (default=NULL). If NULL, uses tp2c.view.pathways(self$genes,
  self$results$p2, goenv=goenv, vhc=self$results$hvc, plot=FALSE, trim=ltrim, n.genes=Inf).
ltrim  numeric Winsorization trim that should be applied (default=0)

Returns:  heatmap data for a given set of genes

Method call(): Call Rook application. Using client-side ExtJS framework and Inchlib HTML5
canvas libraries to create the graphical user interface for PAGODA

Usage:
p2ViewPagodaApp$call(env)

Arguments:
env  The environment argument is a true R environment object which the application is free to
  modify. Please see the Rook documentation for more details.

Returns:  modified 'PAGODA1' app

Method clone(): The objects of this class are cloneable with this method.
Usage:

pagoda.reduce.loading.redundancy

Arguments:

depth Whether to make a deep clone.

description

Collapse aspects driven by the same combinations of genes. (Aspects are some pattern across cells e.g. sequencing depth, or PC corresponding to an undesired process such as ribosomal pathway variation.) Examines PC loading vectors underlying the identified aspects and clusters of aspects based on a product of loading and score correlation (raised to corr.power). Clusters of aspects driven by the same genes are determined based on the parameter "distance.threshold".

Usage

pagoda.reduce.loading.redundancy(
  tam,
  pwpca,
  clpca = NULL,
  plot = FALSE,
  cluster.method = "complete",
  distance.threshold = 0.01,
  corr.power = 4,
  abs = TRUE,
  n.cores = 1,
  ...
)

Arguments

tam output of pagoda.top.aspects(), i.e. a list structure containing the following items: xv: a matrix of normalized aspect patterns (rows: significant aspects, columns: cells) xvw: corresponding weight matrix gw: set of genes driving the significant aspects df: text table with the significance testing results

pwpca output of pagoda.pathway.wPCA(), i.e. a list of weighted PCA info for each valid gene set
pagoda.reduce.redundancy

collapse aspects driven by similar patterns (i.e. separate the same sets of cells) Examines PC loading vectors underlying the identified aspects and clusters aspects based on score correlation. Clusters of aspects driven by the same patterns are determined based on the distance.threshold.

Description

Collapse aspects driven by similar patterns (i.e. separate the same sets of cells) Examines PC loading vectors underlying the identified aspects and clusters aspects based on score correlation. Clusters of aspects driven by the same patterns are determined based on the distance.threshold.

Usage

pagoda.reduce.redundancy(
  tamr,
  distance.threshold = 0.2,
  cluster.method = "complete",
  distance = NULL,
  ...)

Value

a list structure analogous to that returned by pagoda.top.aspects(), but with addition of a $cnam element containing a list of aspects summarized by each row of the new (reduced) $xv and $xvw
Arguments

tamr
combined pathways that show similar expression patterns, output of pagoda.reduce.loading.redundancy()
distance.threshold
numeric Similarity threshold for grouping interdependent aspects (default=0.2)
cluster.method
character One of the standard clustering methods to be used (default="complete")
distance
distance matrix (default=NULL)
weighted.correlation
boolean Whether to use a weighted correlation in determining the similarity of patterns (default=TRUE)
plot
boolean Whether to show plot (default=FALSE)
top
boolean Restrict output to the top N aspects of heterogeneity (default=Inf, i.e. no restriction)
trim
numeric Winsorization trim to use prior to determining the top aspects (default=0)
abs
boolean Whether to use absolute correlation (default=FALSE)
...  
additional arguments are passed to the pagoda.view.aspects() method during plotting

Value

List structure analogous to that returned by pagoda.top.aspects(), but with addition of a $cnam element containing a list of aspects summarized by each row of the new (reduced) $xv and $xvw

pagoda2WebApp-class

pagoda2WebApp class to create 'pagoda2' web applications via a Rook server

Description

pagoda2WebApp class to create 'pagoda2' web applications via a Rook server
Fields

originalP2object Input 'Pagoda2' object
name string Display name for the application
mat Embedding
cellmetadata Metadata associated with 'Pagoda2' object
mainDendrogram Dendrogram from hclust() of all cells in the 'Pagoda2' object
geneSets Gene sets in the 'Pagoda2' object
rookRoot Rook server root directory
appmetadata pagoda2 web application metadata

pagoda2WebApp_arrayToJSON

Description

Serialise an R array to a JSON object

Arguments

arr An array (default=NULL)

Value

Serialised version of the array in JSON, which includes dimension information as separate fields

pagoda2WebApp_availableAspectsJSON

Description

Parse pathways from originalP2object$misc$pathwayOD$xv into JSON

Value

JSON with parsed cell order from mainDendrogram$cellorder
Description

Handle httpd server calls

Arguments

env  The environment argument is a true R environment object which the application is free to modify. Please see the Rook documentation for more details.

Description

Parse cellmetadata into JSON

Value

JSON with parsed cellmetadata

Description

Parse mainDendrogram$cellorder into JSON

Value

JSON with parsed cell order from mainDendrogram$cellorder
pagoda2WebApp_geneInformationJSON

Description
Parse originalP2Object$misc$varinfo[,c("m","qv")]] into JSON

Value
JSON with parsed information from genename, dispersion, mean gene expression

pagoda2WebApp_generateDendrogramOfGroups

Generate a dendrogram of groups

Description
Generate a dendrogram of groups

Arguments
dendrogramCellGroups
Cell groups to input into hclust()

Value
List of hcGroups, cellorder, and cluster.sizes

pagoda2WebApp_generateEmbeddingStructure

Generate information about the embeddings we are exporting

Value
List with embeddings
Description

Generate a JSON list representation of the gene kNN network

Arguments

graph Input graph

Value

JSON with gene kNN network

Description

Compress the embedding

Arguments

reduc reduction
embed embedding

Value

compressed embedding as JSON
pagoda2WebApp_packCompressFloat64Array

Description
Compress float64 array

Arguments
v float64 array

Value
compressed array

pagoda2WebApp_packCompressInt32Array

Description
Compress int32 array

Arguments
v int32 array

Value
compressed array

pagoda2WebApp_readStaticFile

Description
Read a static file from the filesystem, and put in the response

Arguments
filename path to filename

Value
Content to display or error page
pagoda2WebApp_reducedDendrogramJSON

**Description**
Parse dendrogram into JSON

**Value**
JSON with parsed dendrogram

pagoda2WebApp_serializeToStaticFast

**Description**
Convert serialized file to static file

**Arguments**
- **binary.filename**
  path to binary file (default=NULL)
- **verbose**
  boolean Whether to give verbose output (default=FALSE)

**Value**
static file written by WriteListToBinary(expL=exportList, outfile=binary.filename, verbose=verbose)

pagoda2WebApp_serverLog

**Description**
Logging function for console

**Arguments**
- **message**
  Message to output for the console

**Value**
printed message
pagoda2WebApp_sparseMatList

pagoda2WebApp_sparseMatList

Description

Create simple List from sparse Matrix with Dimnames as JSON

Arguments

matsparse Sparse matrix

Value

List with slots i, p, x

pathway.pc.correlation.distance

Calculate correlation distance between PC magnitudes given a number of target dimensions

Description

Calculate correlation distance between PC magnitudes given a number of target dimensions

Usage

pathway.pc.correlation.distance(pcc, xv, n.cores = 1, target.ndf = NULL)

Arguments

pcc weighted PC magnitudes e.g. scde::pagoda.pathway.wPCA() gives the weighted PC magnitudes for each gene provided; e.g. scde::pagoda.gene.clusters() gives the weighted PC magnitudes for de novo gene sets identified by clustering on expression

xv a matrix of normalized aspect patterns (rows: significant aspects, columns: cells)

n.cores numeric Number of cores to use (default=1)

target.ndf numeric Target dimensions (default=NULL)

Value

correlation distance matrix, akin to stats dist
plotMulticlassified  
*Plot multiclassified cells per selection as a percent barplot*

**Description**
Plot multiclassified cells per selection as a percent barplot

**Usage**

```r
plotMulticlassified(sel)
```

**Arguments**

- `sel` pagoda2 selection object

**Value**

ggplot2 object

---

plotOneWithValues  
*Plot the embedding of a 'Pagoda2' object with the given values*

**Description**
Plot the embedding of a 'Pagoda2' object with the given values

**Usage**

```r
plotOneWithValues(
  p2obj, 
  values, 
  title = "", 
  type = "PCA", 
  embeddingType = "tSNE"
)
```

**Arguments**

- `p2obj` the 'Pagoda2' object
- `values` the values to plot, fed into p2obj$plotEmbedding(colors=values)
- `title` character Title for the plot (default="")
- `type` character Type reduction on which the embedding is based on (default="PCA")
- `embeddingType` character Type of embedding to plot (default="tSNE")

**Value**

NULL, simply updates p2obj$plotEmbedding()
plotSelectionOverlaps

Get a dataframe and plot summarising overlaps between selection of a pagoda2 selection object ignore self overlaps

Description

Get a dataframe and plot summarising overlaps between selection of a pagoda2 selection object ignore self overlaps

Usage

plotSelectionOverlaps(sel)

Arguments

sel a pagoda2 selection object

Value

a list that contains a ggplot2 object and a datatable with the overlaps data

projectKNNs

Project a distance matrix into a lower-dimensional space. (from elbamos/largeVis)

Description

Takes as input a sparse matrix of the edge weights connecting each node to its nearest neighbors, and outputs a matrix of coordinates embedding the inputs in a lower-dimensional space.

Usage

projectKNNs(
  wij,
  dim = 2,
  sgd_batches = NULL,
  M = 5,
  gamma = 7,
  alpha = 1,
  rho = 1,
  coords = NULL,
  useDegree = FALSE,
  momentum = NULL,
  seed = NULL,
  threads = NULL,
  verbose = getOption("verbose", TRUE)
)

}
Arguments

wij  A symmetric sparse matrix of edge weights, in C-compressed format, as created with the Matrix package.
dim numeric The number of dimensions for the projection space (default=2)
sgd_batches numeric The number of edges to process during SGD (default=NULL). Defaults to a value set based on the size of the dataset. If the parameter given is between 0 and 1, the default value will be multiplied by the parameter.
M numeric (largeVis) The number of negative edges to sample for each positive edge (default=5).
gamma numeric (largeVis) The strength of the force pushing non-neighbor nodes apart (default=7).
alpha numeric (largeVis) The hyperparameter in the distance function (default=1). The default distance function, \(1/(1 + \alpha ||y_i - y_j||^2)\). The function relates the distance between points in the low-dimensional projection to the likelihood that the two points are nearest neighbors. Increasing \(\alpha\) tends to push nodes and their neighbors closer together; decreasing \(\alpha\) produces a broader distribution. Setting \(\alpha\) to zero enables the alternative distance function. \(\alpha\) below zero is meaningless.
rho numeric (largeVis) Initial learning rate (default=1)
coords An initialized coordinate matrix (default=NULL)
useDegree boolean Whether to use vertex degree to determine weights in negative sampling (if TRUE) or the sum of the vertex’s edges (if FALSE) (default=FALSE)
momentum numeric If not NULL, SGD with momentum is used, with this multiplier, which must be between 0 and 1 (default=NULL). Note that momentum can drastically speed-up training time, at the cost of additional memory consumed.
seed numeric Random seed to be passed to the C++ functions (default=NULL). Sampled from hardware entropy pool if NULL (the default). Note that if the seed is not NULL (the default), the maximum number of threads will be set to 1 in phases of the algorithm that would otherwise be non-deterministic.
threads numeric The maximum number of threads to spawn (default=NULL). Determined automatically if NULL (the default).
verbose boolean Verbosity (default=getOption("verbose", TRUE))

Details

The algorithm attempts to estimate a \(\text{dim}\)-dimensional embedding using stochastic gradient descent and negative sampling.

The objective function is:

\[
O = \sum_{(i,j) \in E} w_{ij} (\log f(||p(e_{ij} = 1||)) + \sum_{k=1}^M E_{jk} p_{s(j)} \gamma \log(1 - f(||p(e_{ijk} = 1||))))
\]

where \(f()\) is a probabilistic function relating the distance between two points in the low-dimensional projection space, and the probability that they are nearest neighbors.
The default probabilistic function is $1/(1 + \alpha |x|^2)$. If $\alpha$ is set to zero, an alternative probabilistic function, $1/(1 + \exp(x^2))$ will be used instead.

Note that the input matrix should be symmetric. If any columns in the matrix are empty, the function will fail.

**Value**

A dense $[N,D]$ matrix of the coordinates projecting the $w_{ij}$ matrix into the lower-dimensional space.

**Note**

If specified, seed is passed to the C++ and used to initialize the random number generator. This will not, however, be sufficient to ensure reproducible results, because the initial coordinate matrix is generated using the R random number generator. To ensure reproducibility, call `set.seed` before calling this function, or pass it a pre-allocated coordinate matrix.

The original paper called for weights in negative sampling to be calculated according to the degree of each vertex, the number of edges connecting to the vertex. The reference implementation, however, uses the sum of the weights of the edges to each vertex. In experiments, the difference was imperceptible with small (MNIST-size) datasets, but the results seems aesthetically preferable using degree. The default is to use the edge weights, consistent with the reference implementation.

---

**read.10x.matrices**  
*Quick loading of 10X CellRanger count matrices*

**Description**

Quick loading of 10X CellRanger count matrices

**Usage**

```r
read.10x.matrices(matrixPaths, version = "V3", n.cores = 1, verbose = TRUE)
```

**Arguments**

- `matrixPaths`  
  a single path to the folder containing matrix.mtx, genes.tsv and barcodes.tsv files, OR a named list of such paths

- `version`  
  string Version of 10x output to read (default='V3'). Must be one of 'V2' or 'V3'.

- `n.cores`  
  numeric Cores to utilize in parallel (default=1)

- `verbose`  
  boolean Whether to output verbose output (default=TRUE)

**Value**

A sparse matrix representation of the data (or a list of sparse matrices if a list of paths was passed)
**read10xMatrix**

*This function reads a matrix generated by the 10x processing pipeline from the specified directory and returns it. It aborts if one of the required files in the specified directory do not exist.*

**Description**

This function reads a matrix generated by the 10x processing pipeline from the specified directory and returns it. It aborts if one of the required files in the specified directory do not exist.

**Usage**

```r
read10xMatrix(path, version = "V3", transcript.id = "SYMBOL", verbose = TRUE)
```

**Arguments**

- `path` string Location of 10x output
- `version` string Version of 10x output to read (default='V3'). Must be one of 'V2' or 'V3'.
- `transcript.id` string Transcript identifier to use (default='SYMBOL'). Must be either 'SYMBOL' (e.g. "Sox17") or 'ENSEMBL' (e.g. "ENSMUSG00000025902"). This value is case-sensitive.
- `verbose` boolean Whether to return verbose output

**Value**

parsed 10x outputs into a matrix

---

**readPagoda2SelectionAsFactor**

*Read a pagoda2 cell selection file and return it as a factor while removing any multiclassified cells*

**Description**

Read a pagoda2 cell selection file and return it as a factor while removing any multiclassified cells

**Usage**

```r
readPagoda2SelectionAsFactor(filepath, use.internal.name = FALSE)
```
**readPagoda2SelectionFile**

**Arguments**
- **filepath** name of the selection file
- **use.internal.name** boolean Use field 'internal.name' as factor names (default=FALSE). Passed to factorFromP2Selection

**Value**
a name factor with the membership of all the cells that are not multiclassified

---

**readPagoda2SelectionFile**

*Reads a 'pagoda2' web app exported cell selection file exported as a list of list objects that contain the name of the selection, the color (as a hex string) and the identifiers of the individual cells*

**Description**

Reads a 'pagoda2' web app exported cell selection file exported as a list of list objects that contain the name of the selection, the color (as a hex string) and the identifiers of the individual cells

**Usage**

```r
readPagoda2SelectionFile(filepath)
```

**Arguments**
- **filepath** the path of the file load

---

**removeSelectionOverlaps**

*Remove cells that are present in more than one selection from all the selections they are in*

**Description**

Remove cells that are present in more than one selection from all the selections they are in

**Usage**

```r
removeSelectionOverlaps(selections)
```

**Arguments**
- **selections** a pagoda2 selections list
score.cells.nb0  

Score cells by getting mean expression of genes in signatures

Description
Score cells by getting mean expression of genes in signatures

Usage
score.cells.nb0(data, signature)

Arguments

- **data**: matrix
- **signature**: the genes in the signature

Value
cell scores

score.cells.puram  

Puram, Bernstein (Cell, 2018) Score cells as described in Puram, Bernstein (Cell, 2018)

Description
Puram, Bernstein (Cell, 2018) Score cells as described in Puram, Bernstein (Cell, 2018)

Usage
score.cells.puram(data, signature, correct = TRUE, show.plot = FALSE, ...)

Arguments

- **data**: matrix of expression, rows are cell, columns are genes
- **signature**: character vector The signature to evaluate, a character vector of genes
- **correct**: boolean Perform background correction by getting a semi-random geneset (default=TRUE)
- **show.plot**: boolean If corrected values are calculated show plot of corrected vs original scores (default=FALSE)
- **...**: options for get.control.geneset()
sgdBatches

Value

a score for each cell

---

sgdBatches Calculate the default number of batches for a given number of vertices and edges. The formula used is the one used by the 'largeVis' reference implementation. This is substantially less than the recommendation $E \times 10000$ in the original paper.

Description

Calculate the default number of batches for a given number of vertices and edges. The formula used is the one used by the 'largeVis' reference implementation. This is substantially less than the recommendation $E \times 10000$ in the original paper.

Usage

sgdBatches(N, E = 150 * N/2)

Arguments

N Number of vertices
E Number of edges (default = 150*N/2)

Value

The recommended number of sgd batches.

Examples

# Observe that increasing K has no effect on processing time
N <- 70000 # MNIST
K <- 10:250
plot(K, sgdBatches(rep(N, length(K)), N * K / 2))

# Observe that processing time scales linearly with N
N <- c(seq(from = 1, to = 10000, by = 100), seq(from = 10000, to = 10000000, by = 100))
plot(N, sgdBatches(N))
**show.app**

*Directly open the 'pagoda2' web application and view the 'p2web' application object from our R session*

**Description**

Directly open the 'pagoda2' web application and view the 'p2web' application object from our R session

**Usage**

```r
show.app(app, name, port, ip, browse = TRUE, server = NULL)
```

**Arguments**

- **app**: 'pagoda2' application object
- **name**: character Name of the application to view
- **port**: numeric Port number
- **ip**: numeric IP address
- **browse**: boolean Whether to load the app into an HTML browser (default=TRUE)
- **server**: server If NULL, will grab server with get.scde.server(port=port, ip=ip) (default=NULL)

**Value**

application within browser

**subsetSignatureToData**

*Subset a gene signature to the genes in the given matrix with optional warning if genes are missing*

**Description**

Subset a gene signature to the genes in the given matrix with optional warning if genes are missing

**Usage**

```r
subsetSignatureToData(data, signature, raise.warning = TRUE)
```

**Arguments**

- **data**: matrix
- **signature**: character vector The gene signature from which to subset a character vector of genes
- **raise.warning**: boolean Warn if genes are missing (default=TRUE)
Value

The filtered subset of gene signatures

Description

View pathway or gene-weighted PCA ‘Pagoda2’ version of the function pagoda.show.pathways() Takes in a list of pathways (or a list of genes), runs weighted PCA, optionally showing the result.

Usage

tp2c.view.pathways(pathways,
p2,
goenv = NULL,
batch = NULL,
n.genes = 20,
two.sided = TRUE,
n.pc = rep(1, length(pathways)),
colcols = NULL,
zlim = NULL,
labRow = NA,
vhc = NULL,
cexCol = 1,
cexRow = 1,
nstarts = 50,
row.order = NULL,
show.Colv = TRUE,
plot = TRUE,
trim = 1.1/nrow(p2$counts),
showPC = TRUE,
...)

Arguments

pathways character vector of pathway or gene names
p2 'Pagoda2' object
goenv environment mapping pathways to genes (default=NULL)
batch factor (corresponding to rows of the model matrix) specifying batch assignment of each cell, to perform batch correction (default=NULL).
validateSelectionsObject

Validates a pagoda2 selection object

validateSelectionsObject(selections)

Arguments

selections the pagoda2 selection object to be validated

Value

a logical value indicating if the object is valid
webP2proc

Generate a 'pagoda2' web object

Description

Generate a 'pagoda2' web object

Usage

webP2proc(
  p2,
  additionalMetadata = NULL,
  title = "Pagoda2",
  make.go.sets = TRUE,
  make.de.sets = TRUE,
  go.env = NULL,
  make.gene.graph = TRUE,
  appmetadata = NULL
)

Arguments

p2 a 'Pagoda2' object
additionalMetadata 'pagoda2' web metadata object (default=NULL)
title character string Title for the web app (default='Pagoda2')
make.go.sets boolean Whether GO sets should be made (default=TRUE)
make.de.sets boolean Whether differential expression sets should be made (default=TRUE)
go.env the GO environment used for the overdispersion analysis (default=NULL)
make.gene.graph logical specifying if the gene graph should be make, if FALSE the find similar
genes functionality will be disabled on the web app
appmetadata 'pagoda2' web application metadata (default=NULL)

Value

a 'pagoda2' web application
writeGenesAsPagoda2Selection

writes a list of genes as a gene selection that can be loaded in the web interface

Description

Writes a list of genes as a gene selection that can be loaded in the web interface

Usage

writeGenesAsPagoda2Selection(name, genes, filename)

writeGenesAsPagoda2Selection

set.seed(0)
mat <- matrix(c(rnorm(5*10, mean=0, sd=1), rnorm(5*10, mean=5, sd=1)), 10, 10) # random matrix
mat[1,1] <- 1000 # make outlier
range(mat) # look at range of values
win.mat <- winsorize.matrix(mat, 0.1)
range(win.mat) # note outliers removed

winsorize.matrix

Sets the ncol(mat)*trim top outliers in each row to the next lowest value same for the lowest outliers

Description

Sets the ncol(mat)*trim top outliers in each row to the next lowest value same for the lowest outliers

Usage

winsorize.matrix(mat, trim)

Arguments

mat Numeric matrix
trim numeric Fraction of outliers (on each side) that should be Winsorized, or (if the value is >= 1) the number of outliers to be trimmed on each side

Value

Winsorized matrix

Examples

set.seed(0)
mat <- matrix(c(rnorm(5*10, mean=0, sd=1), rnorm(5*10, mean=5, sd=1)), 10, 10) # random matrix
mat[1,1] <- 1000 # make outlier
range(mat) # look at range of values
win.mat <- winsorize.matrix(mat, 0.1)
range(win.mat) # note outliers removed
writePagoda2SelectionFile

Arguments

- **name**: the name of the selection
- **genes**: a string vector of the gene names
- **filename**: the filename to save to

Value

NULL, writes to filepath the list of genes as a gene selection that can be loaded in the web interface

```
writePagoda2SelectionFile
   Writes a pagoda2 selection object as a p2 selection file that be be
   loaded to the web interface
```

Description

Writes a pagoda2 selection object as a p2 selection file that be be loaded to the web interface

Usage

```
writePagoda2SelectionFile(sel, filepath)
```

Arguments

- **sel**: pagoda2 selection object
- **filepath**: name of file to which to write

Value

NULL, writes to filepath the pagoda2 selection object as a p2 selection file that be be loaded to the web interface
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