

Package ‘phemd’

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Type Package

Title Phenotypic EMD for comparison of single-cell samples

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Description Package for comparing and generating a low-dimensional embedding of multiple single-cell samples.

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aggregateSamples	<i>Aggregate expression data from all samples</i>
------------------	---

Description

Takes initial Phemd object and returns object with additional data frame in slot @data_aggregate containing cells aggregated from all samples (to be used for further analyses e.g. Monocle 2 trajectory building / pseudotime mapping / cell clustering)

Usage

```
aggregateSamples(obj, max_cells = 12000)
```

Arguments

obj	'Phemd' object containing raw expression data and associated metadata
max_cells	Maximum number of cells across all samples to be included in final matrix on which Monocle 2 will be run

Details

Subsamples cells as necessary based on max_cells. If subsampling is performed, an equal number of cells are subsampled from each sample

Value

Same as input 'Phemd' object with additional slot 'data_aggregate' containing aggregated expression data (num_markers x num_cells)

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
```

all_expn_data	<i>Single-cell RNA-seq expression data for melanoma samples</i>
---------------	---

Description

This dataset contains normalized single-cell RNA-seq expression data for 19 melanoma samples (immune cells).

Usage

```
data(all_expn_data)
```

Format

A list of length 19 with each element representing a distinct sample. Each list element (sample) is a matrix with dimension num_genes x num_cells.

Source

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72056>

References

Tirosh, I. et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* 352, 189–196 (2016)

all_genes

All genes included in (subsampling) melanoma single-cell RNA-seq expression data

Description

This object contains 100 genes measured in melanoma single-cell RNA-seq expression data.

Usage

```
data(all_genes)
```

Format

Vector of length 100 representing row names of each matrix in melanoma expression dataset

Source

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72056>

References

Tirosh, I. et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* 352, 189–196 (2016)

`assignCellClusterNearestNode`*Assign cells to a reference cell subtype*

Description

Assigns each cell in `cur_cells` to a cluster based on nearest cell in Monocle 2 tree

Usage

```
assignCellClusterNearestNode(cur_cells, ref_cells, ref_cell_labels,  
  cell_model = c("monocle2", "seurat"))
```

Arguments

<code>cur_cells</code>	Matrix of cells to be assigned to clusters (Dim: <i>num_cells</i> x <i>num_markers</i>)
<code>ref_cells</code>	Matrix of cells used to build reference Monocle 2 tree (Dim: <i>num_monocle_cells</i> x <i>num_markers</i>)
<code>ref_cell_labels</code>	Vector of length <i>num_monocle_cells</i> containing Monocle 2 cell branch assignments
<code>cell_model</code>	Either "monocle2" or "seurat" depending on method used to model cell state space

Details

Private method (not exported in namespace). Uses RANN package for fast knn search

Value

Vector of length *num_cells* representing cluster assignments for each cell in *cur_cells*

Examples

```
## Not run:  
cur_cells_cluster_labels <- assignCellClusterNearestNode(cur_cells_expn_data,  
  clustered_cells_expn_data, clustered_cells_cluster_labels, cell_model='monocle2')  
  
## End(Not run)
```

batchIDs	<i>Accessor function for batch ID for each sample</i>
----------	---

Description

Accessor function for batch ID for each sample

Usage

```
batchIDs(obj)
```

Arguments

obj Phemd object

Value

Vector of length num_samples representing the experiment (batch) in which the sample was profiled

Examples

```
phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
batch_metadata <- batchIDs(phemdObj)
```

bindSeuratObj	<i>Attach 'seurat' object to 'Phemd' object</i>
---------------	---

Description

Allows user to attach batch-normalized reference cell data from Seurat into 'Phemd' object containing raw expression data and metadata

Usage

```
bindSeuratObj(phemd_obj, seurat_obj, batch.colname = "plt")
```

Arguments

phemd_obj Phemd object initialized using createDataObj
seurat_obj S4 'seurat' object containing batch-normalized reference cell data
batch.colname Name of column in Seurat object that denotes batch ID

Value

'Phemd' object containing with attached Seurat object

Examples

```

my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_seuratObj <- Seurat::CreateSeuratObject(counts = t(all_expn_data[[1]]), project = "A")
my_seuratObj <- Seurat::FindVariableFeatures(object = my_seuratObj)
my_seuratObj <- Seurat::ScaleData(object = my_seuratObj, do.scale=FALSE, do.center=FALSE)
my_seuratObj <- Seurat::RunPCA(object = my_seuratObj, pc.genes = colnames(all_expn_data[[1]]), do.print = FALSE)
my_seuratObj <- Seurat::FindNeighbors(my_seuratObj, reduction = "pca", dims.use = 1:10)
my_seuratObj <- Seurat::FindClusters(my_seuratObj, resolution = 0.6, print.output = 0, save.SNN = TRUE)
my_phemdObj <- bindSeuratObj(my_phemdObj, my_seuratObj)

```

celltypeFreqs

Accessor function for cell subtype distribution for each sample

Description

Accessor function for cell subtype distribution for each sample

Usage

```
celltypeFreqs(obj)
```

Arguments

obj Phemd object

Value

Matrix representing cell subtype relative frequencies for each sample (num_samples x num_genes)

Examples

```

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
celltype_weights <- celltypeFreqs(phemdObj)

```

clusterIndividualSamples

Computes cell subtype abundances for each sample

Description

Takes as input a Phemd object with all single-cell expression data of all single-cell samples in @data slot and Monocle2 object (already embedded and ordered) in @monocle_obj slot. Returns updated object with cell subtype frequencies of each sample in @data_cluster_weights slot

Usage

```

clusterIndividualSamples(obj, verbose = FALSE,
  cell_model = c("monocle2", "seurat"))

```

Arguments

obj	'Phemd' object containing single-cell expression data of all samples in @data slot and Monocle2 object (already embedded and ordered) in @monocle_obj slot
verbose	Boolean that determines whether progress (sequential processing of samples) should be printed. FALSE by default
cell_model	Either "monocle2" or "seurat" depending on method used to model cell state space

Details

embedCells and orderCellsMonocle need to be called before calling this function.

Value

'Phemd' object with cell subtype frequencies of each sample in @data_cluster_weights slot

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model = 'gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
my_phemdObj_final <- clusterIndividualSamples(my_phemdObj_monocle)
```

compareSamples	<i>Computes EMD distance matrix representing pairwise dissimilarity between samples</i>
----------------	---

Description

Takes as input a Phemd object with cell subtype relative frequencies for each sample in @data_cluster_weights slot and ground distance matrix (representing cell subtype pairwise dissimilarity) in @emd_dist_mat slot. Returns distance matrix representing pairwise dissimilarity between samples

Usage

```
compareSamples(obj)
```

Arguments

obj	'Phemd' object containing cell subtype relative frequencies for each sample in @data_cluster_weights slot and ground distance matrix (representing cell subtype dissimilarity) in @emd_dist_mat slot
-----	--

Details

Requires 'transport' and 'pracma' packages

Value

Distance matrix of dimension `num_samples` x `num_samples` representing pairwise dissimilarity between samples

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model = 'gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
my_phemdObj_final <- clusterIndividualSamples(my_phemdObj_monocle)
my_phemdObj_final <- generateGDM(my_phemdObj_final)
my_EMD_mat <- compareSamples(my_phemdObj_final)
```

createDataObj	<i>Create 'Phemd' object</i>
---------------	------------------------------

Description

Wrapper function to create 'Phemd' object containing raw expression data and metadata

Usage

```
createDataObj(data, markers, snames, datatype = "list",
              valtype = "counts")
```

Arguments

<code>data</code>	List of length <code>num_samples</code> containing expression data; each element is of size <code>num_cells</code> x <code>num_markers</code> . Alternately a <code>SingleCellExperiment</code> object.
<code>markers</code>	Vector containing marker names (i.e. column names of <code>all_data</code>)
<code>snames</code>	Vector containing sample names (i.e. names of samples contained in <code>all_data</code>)
<code>datatype</code>	Either "list" or "sce" (<code>SingleCellExperiment</code> with genes x cells)
<code>valtype</code>	Type of assay data (i.e. "counts", "normcounts", "logcounts", "tpm", "cpm") if <code>datatype</code> is "sce"

Details

Note that each element in list can have different number of rows (i.e. number of cells in each sample can vary).

Value

'Phemd' object containing raw multi-sample expression data and associated metadata

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
```

drawColnames45	<i>Rotates heatmap marker labels 45 degrees</i>
----------------	---

Description

Overwrites default draw_colnames in the pheatmap package

Usage

```
drawColnames45(coln, gaps, ...)
```

Arguments

coln	Column names
gaps	Spacing of labels
...	Additional parameters to be passed to gpar

Details

To be used with pheatmap plotting function; not to be called directly. Thanks to Josh O'Brien at <http://stackoverflow.com/questions/15505607>

Value

Formatted marker labels in heatmap

Examples

```
#Not to be called directly
```

embedCells	<i>Generate Monocle2 embedding</i>
------------	------------------------------------

Description

Takes as input a Phemd object with aggregated data and returns updated object with Monocle2 object in @monocle_obj slot

Usage

```
embedCells(obj, data_model = "negbinomial_sz", ...)
```

Arguments

obj	'Phemd' object containing aggregated data
data_model	One of the following: 'negbinomial_sz', 'negbinomial', 'tobit', 'uninormal', 'gaussianff'. See "Family Function" table at the following link for more details on selecting the proper one. http://cole-trapnell-lab.github.io/monocle-release/docs/#getting-started-with-monocle
...	Additional parameters to be passed to reduceDimension function

Details

Wrapper function for reduceDimension in Monocle 2 package. aggregateSamples needs to be called before running this function.

Value

Same as input 'Phemd' object with additional Monocle2 object in @monocle_obj slot

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_lg <- embedCells(my_phemdObj_lg, data_model = 'gaussianff', sigma=0.02, maxIter=2)
```

gaussianffLocal	<i>Models expression data using generalized linear model with Gaussian error</i>
-----------------	--

Description

Useful for modeling pre-normalized single-cell expression data.

Usage

```
gaussianffLocal(dispersion = 0, parallel = FALSE, zero = NULL)
```

Arguments

dispersion	Dispersion parameter. If 0, then estimate as described in VGAM 1.0-5 documentation.
parallel	A logical or formula. If a formula, the response of the formula should be a logical and the terms of the formula indicates whether or not those terms are parallel.
zero	An integer-valued vector specifying which linear/additive predictors are modelled as intercepts only. The values must be from the set 1...M where M is the number of columns of the matrix response.

Details

Private method (not to be called by user directly). Requires VGAM package. Obtained from VGAM v1.0-5 (<https://www.rdocumentation.org/packages/VGAM/versions/1.0-5/topics/gaussianff>)

Value

Generalized linear model with Gaussian error

GDM

*Accessor function for EMD ground distance matrix***Description**

Accessor function for EMD ground distance matrix

Usage

```
GDM(obj)
```

Arguments

obj A Phemd object

Value

Sqaure matrix representing pairwise distances between cell subtypes

Examples

```
phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
gdm <- GDM(phemdObj)
```

generateGDM

*Computes ground distance matrix based on cell embedding***Description**

Takes as input a Phemd object with Monocle2 object (already embedded and ordered) in @monocle_obj slot. Returns updated object with ground distance matrix representing pairwise distance between 2 cell subtypes based on cell state embedding.

Usage

```
generateGDM(obj, cell_model = c("monocle2", "seurat"),
  expn_type = "aligned")
```

Arguments

obj 'Phemd' object containing Monocle2 object (already embedded and ordered) in @monocle_obj slot

cell_model Method by which cell state was modeled (either "monocle2" or "seurat")

expn_type Data type to use to determine cell-type dissimilarities

Details

embedCells and orderCellsMonocle need to be called before calling this function. Requires 'igraph' package

Value

Phemd object with ground distance matrix (to be used in EMD computation) in @data_cluster_weights slot

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model = 'gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
my_phemdObj_final <- clusterIndividualSamples(my_phemdObj_monocle)
my_phemdObj_final <- generateGDM(my_phemdObj_final)
```

getArithmeticCentroids

Get arithmetic centroids (coordinates)

Description

Takes initial list and returns a matrix with row i representing the arithmetic centroid of cluster i

Usage

```
getArithmeticCentroids(ref_clusters)
```

Arguments

ref_clusters list containing each cluster of interest (each list element is a matrix of dimension num_cells x num_markers)

Details

Private method (not exported in namespace)

Value

Matrix of dimension num_cluster x num_markers; row i representing the arithmetic centroid of cluster i

Examples

```
## Not run:
cluster_centroids <- getArithmeticCentroids(ref_clusters)

## End(Not run)
```

getCellYield	<i>Gets cell yield of each sample as a table</i>
--------------	--

Description

Gets cell yield (number of viable cells) of each single-cell sample in decreasing order

Usage

```
getCellYield(myobj, cluster_assignments = NULL)
```

Arguments

myobj	phemdObj object containing expression data for each sample in 'data' slot
cluster_assignments	Vector of cluster assignments to be included as additional column in output table (optional)

Value

Data frame representing cell yield of each sample

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model = 'gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
my_phemdObj_final <- clusterIndividualSamples(my_phemdObj_monocle)
my_phemdObj_final <- generateGDM(my_phemdObj_final)
my_EMD_mat <- compareSamples(my_phemdObj_final)
cluster_assignments <- groupSamples(my_EMD_mat, distfun = 'hclust', ncluster=4)
getCellYield(my_phemdObj_final, cluster_assignments)
```

getSampleCelltypeFreqs	<i>Returns cell subtype distribution for each sample as a table</i>
------------------------	---

Description

Returns cell subtype distribution for each single-cell sample along with (optional) final inhibitor cluster assignment

Usage

```
getSampleCelltypeFreqs(myobj, cluster_assignments = NULL)
```

Arguments

myobj phemdObj object containing expression data for each sample in 'data' slot
 cluster_assignments Vector of cluster assignments to be included as additional column in output table
 (optional)

Value

Data frame representing relative frequencies of each cell subtype along with (optional) final inhibitor cluster assignment for each single-cell sample

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model = 'gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
my_phemdObj_final <- clusterIndividualSamples(my_phemdObj_monocle)
my_phemdObj_final <- generateGDM(my_phemdObj_final)
my_EMD_mat <- compareSamples(my_phemdObj_final)
cluster_assignments <- groupSamples(my_EMD_mat, distfun = 'hclust', ncluster=4)
getSampleCelltypeFreqs(my_phemdObj_final, cluster_assignments)
```

getSampleHistsByCluster

Gets cell subtype frequency histograms for each sample by cluster ID

Description

Gets relative frequency ("weights") of cell subtypes ("bins" or "signatures") in each single-cell sample

Usage

```
getSampleHistsByCluster(myobj, cluster_assignments,
  cell_model = c("monocle2", "seurat"))
```

Arguments

myobj phemdObj object containing cell subtype relative frequency in @data_cluster_weights slot
 cluster_assignments Vector containing group assignments for each sample in myobj
 cell_model Method by which cell state was modeled (either "monocle2" or "seurat")

Details

groupSamples must be called before calling this function. Saves plots in directory called "individual_inhibs"

Value

List of lists, with outer list representing sample cluster ID and inner list representing cell subtype frequencies of given sample

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model = 'gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
my_phemdObj_final <- clusterIndividualSamples(my_phemdObj_monocle)
my_phemdObj_final <- generateGDM(my_phemdObj_final)
my_EMD_mat <- compareSamples(my_phemdObj_final)
cluster_assignments <- groupSamples(my_EMD_mat, distfun = 'hclust', ncluster=4)
weights_by_cluster <- getSampleHistsByCluster(my_phemdObj_final, cluster_assignments)
```

getSampleSizes

Retrieve single-cell sample sizes

Description

Takes initial list of single-cell samples and returns vector containing number of cells in each sample.

Usage

```
getSampleSizes(data_list)
```

Arguments

`data_list` List of length `num_samples` (each element has dimension `num_cells` x `num_markers`)

Details

Private method (not exported in namespace)

Value

Vector of length `num_samples` representing number of cells in each sample

Examples

```
## Not run:
sample_sizes <- getSampleSizes(all_expn_data)

## End(Not run)
```

groupSamples	<i>Performs community detection on sample-sample distance matrix</i>
--------------	--

Description

Takes sample-sample distance matrix as input and returns group assignments for each sample

Usage

```
groupSamples(distmat, distfun = "hclust", ncluster = NULL,
             method = "complete")
```

Arguments

distmat	Distance matrix of dimension num_samples x num_samples representing pair-wise dissimilarity between samples
distfun	Method of partitioning network of samples (currently either 'hclust' or 'pam')
ncluster	Optional parameter specifying total number of sample groups
method	Optional parameter for hierarchical clustering (see "hclust" documentation)

Details

By default, uses 'kgs' (Kelley-Gardner-Sutcliffe) method for determining optimal number of groups. Alternatively, can take user-specified number of groups). Requires 'cluster' and 'maptree' packages.

Value

Vector containing group assignments for each sample (same order as row-order of distmat) based on user-specified partitioning method (e.g. hierarchical clustering)

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model = 'gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
my_phemdObj_final <- clusterIndividualSamples(my_phemdObj_monocle)
my_phemdObj_final <- generateGDM(my_phemdObj_final)
my_EMD_mat <- compareSamples(my_phemdObj_final)
cluster_assignments <- groupSamples(my_EMD_mat, distfun = 'hclust', ncluster=4)
```

heatmap_genes	<i>Genes to be used when plotting heatmap for melanoma single-cell RNA-seq expression data</i>
---------------	--

Description

This object contains genes to be used when plotting heatmap for melanoma single-cell RNA-seq expression data.

Usage

```
data(heatmap_genes)
```

Format

Vector of length 42 representing selected genes for plotting heatmap.

Source

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72056>

References

Tirosh, I. et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* 352, 189–196 (2016)

identifyCentroids	<i>Identify cluster centroids (cell names)</i>
-------------------	--

Description

Takes initial list and returns list of cell names representing centroid of cluster

Usage

```
identifyCentroids(ref_clusters)
```

Arguments

ref_clusters list containing each cluster of interest (each list element is a matrix of dimension num_cells x num_markers)

Details

Private method (not exported in namespace)

Value

List of names; element i represents the name of the cell in cluster i that is closest to the centroid (arithmetic mean) of cluster i

Examples

```
## Not run:
centroid_names <- identifyCentroids(ref_clusters)

## End(Not run)
```

monocleInfo	<i>Accessor function for stored Monocle object</i>
-------------	--

Description

Accessor function for stored Monocle object

Usage

```
monocleInfo(obj)
```

Arguments

obj A Phemd object.

Value

An object of class 'CellDataSet' (from Monocle)

Examples

```
phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
monocle_obj <- monocleInfo(phemdObj)
```

orderCellsMonocle	<i>Compute Monocle2 cell state and pseudotime assignments</i>
-------------------	---

Description

Takes as input a Phemd object with Monocle2 object and returns updated object with Monocle2 object containing cell state and pseudotime assignments

Usage

```
orderCellsMonocle(obj, ...)
```

Arguments

obj 'Phemd' object containing initial Monocle 2 object
 ... Additional parameters to be passed into orderCells function

Details

Wrapper function for `orderCells` in Monocle 2 package. `embedCells` needs to be called before calling this function.

Value

Same as input 'Phemd' object with updated Monocle2 object in `@monocle_obj` slot containing cell state and pseudotime assignments

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model='gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
```

Phemd

*Phemd class***Description**

The main PhEMD class to store single-cell expression data.

Fields

`data` List of matrices, each of which represents a single-cell sample (num_cells x num_genes)
`markers` Column names (e.g. genes) for each element (i.e. data matrix) in "data"
`snames` Sample ID for each element in "data"
`data_aggregate` Numeric matrix representing expression data for cells from all experimental conditions (rows = markers, cols = cells)
`data_subsample_idx` List of vectors each representing the indices of elements in "data" that were subsampled and combined to form "data_aggregate"
`subsampled_bool` Boolean represent whether or not subsampling was performed in the data aggregation process
`monocle_obj` Data object of type "CellDataSet" that is the core Monocle data structure
`data_cluster_weights` Matrix representing cell subtype relative frequencies for each sample (num_samples x num_genes)
`emd_dist_mat` Matrix representing pairwise distances between each pair of cell subtypes
`seurat_obj` Object of type "Seurat" that is the core Seurat data structure
`experiment_ids` Vector of length num_samples representing the experiment (batch) in which the sample was profiled

Phemd-methods

Setter function for protein / gene markers

Description

Setter function for protein / gene markers

Setter function for stored expression data

Setter function for single-cell expression data aggregated from multiple samples

Setter function for indices of cells subsampled from each sample during aggregation

Setter function for boolean denoting whether cells were subsampled from each sample during aggregation

Setter function for Monocle2 CellDataSet object for experiment

Setter function for Seurat object for experiment

Setter function for cell subtype frequencies of each single-cell sample

Setter function for batch IDs of each single-cell sample

Setter function for EMD ground distance matrix

Usage

```
selectMarkers(obj) <- value
```

```
## S4 replacement method for signature 'Phemd'  
selectMarkers(obj) <- value
```

```
rawExpn(obj) <- value
```

```
## S4 replacement method for signature 'Phemd'  
rawExpn(obj) <- value
```

```
pooledCells(obj) <- value
```

```
## S4 replacement method for signature 'Phemd'  
pooledCells(obj) <- value
```

```
subsampledIdx(obj) <- value
```

```
## S4 replacement method for signature 'Phemd'  
subsampledIdx(obj) <- value
```

```
subsampledBool(obj) <- value
```

```
## S4 replacement method for signature 'Phemd'  
subsampledBool(obj) <- value
```

```
monocleInfo(obj) <- value
```

```
## S4 replacement method for signature 'Phemd'  
monocleInfo(obj) <- value
```

```

seuratInfo(obj) <- value

## S4 replacement method for signature 'Phemd'
seuratInfo(obj) <- value

celltypeFreqs(obj) <- value

## S4 replacement method for signature 'Phemd'
celltypeFreqs(obj) <- value

batchIDs(obj) <- value

## S4 replacement method for signature 'Phemd'
batchIDs(obj) <- value

GDM(obj) <- value

## S4 replacement method for signature 'Phemd'
GDM(obj) <- value

```

Arguments

obj	A Phemd object
value	Assignment object

Value

Updated Phemd object
 Updated Phemd object
 Updated Phemd object
 Updated Phemd object
 Updated Phemd object
 Updated Phemd object containing Seurat object
 Updated Phemd object
 Updated Phemd object
 Updated Phemd object

Examples

```

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
new_genes <- all_genes
new_genes[1] <- 'IL2R'
selectMarkers(phemdObj) <- new_genes

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
new_expn_data <- all_expn_data
new_expn_data <- lapply(new_expn_data, function(x) {log2(x+1)})
rawExpn(phemdObj) <- new_expn_data

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))

```

```

aggregated_data <- t(do.call(rbind,all_expn_data))
pooledCells(phemdObj) <- aggregated_data

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
subsampldIdxList<- rep(list(1:10), length(all_expn_data)) #subsampld cells 1-10 from each sample
subsampldIdx(phemdObj) <- subsampldIdxList

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
subsampldBool(phemdObj) <- TRUE

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
mydata <- pooledCells(phemdObj)
myCellDataSet <- newCellDataSet(mydata,phenoData=NULL, expressionFamily=VGAM::negbinomial.size())
monocleInfo(phemdObj) <- myCellDataSet

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_seuratObj <- Seurat::CreateSeuratObject(counts = t(all_expn_data[[1]]), project = "A")
seuratInfo(phemdObj) <- my_seuratObj

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
myCellTypeFreqs <- matrix(rexp(length(all_expn_data)*10, rate=.1), ncol=10)
myCellTypeFreqs <- apply(myCellTypeFreqs, 1, function(x) {x / sum(x)})
celltypeFreqs(phemdObj) <- myCellTypeFreqs

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_seuratObj <- Seurat::CreateSeuratObject(counts = t(all_expn_data[[1]]), project = "A")
seuratInfo(phemdObj) <- my_seuratObj
batchIDs(phemdObj) <- rep('A', length(all_expn_data))

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
cluster_locs <- 1:10
myGDM <- as.matrix(dist(cluster_locs))
GDM(phemdObj) <- myGDM

```

plotCellYield

Plot cell yield of each sample as bar plot

Description

Plots cell yield (number of viable cells) of each single-cell sample in decreasing order as horizontal bar plot

Usage

```

plotCellYield(myobj, labels = NULL, cmap = NULL, font_sz = 0.6,
              w = 8, h = 9.5)

```

Arguments

myobj	Phmed object containing expression data for each sample in 'data' slot
labels	Vector containing group labels for samples (optional). If not provided, bars will be of uniform color (blue)

cmap	Vector containing colors by which histogram bars should be colored (optional)
font_sz	Scaling factor for font size of sample names in barplot
w	Width of plot in inches
h	Height of plot in inches

Value

None

Examples

```

my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model = 'gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
my_phemdObj_final <- clusterIndividualSamples(my_phemdObj_monocle)
my_phemdObj_final <- generateGDM(my_phemdObj_final)
my_EMD_mat <- compareSamples(my_phemdObj_final)
cluster_assignments <- groupSamples(my_EMD_mat, distfun = 'hclust', ncluster=4)
plotCellYield(my_phemdObj_final, labels=cluster_assignments, font_sz = 0.8)

```

plotEmbeddings

Plots Monocle2 cell embedding plots

Description

Takes as input a Phemd object containing either a Monocle2 object or Seurat object (already embedded and ordered) and plots cell embedding plots side by side. Optionally saves to specified folder.

Usage

```

plotEmbeddings(obj, cell_model = c("monocle2", "seurat"), cmap = NULL,
  w = 4, h = 5, pt_sz = 1, ndims = NULL)

```

Arguments

obj	'Phemd' object containing Monocle 2 object
cell_model	Method by which cell state was modeled (either "monocle2" or "seurat")
cmap	User-specified colormap to use to color cell state embedding (optional)
w	Width of plot in inches
h	Height of plot in inches
pt_sz	Scalar factor for point size
ndims	Number of dimensions to use for dimensionality reduction in case it hasn't been performed yet (only relevant when using Seurat data as input)

Details

embedCells and orderCellsMonocle need to be called before calling this function. Required additional packages: 'RColorBrewer', 'cowplot'

Value

Colormap (vector of colors) used to color Monocle2 cell state embedding

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model='gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
cmap <- plotEmbeddings(my_phemdObj_monocle)
```

plotGroupedSamplesDmap

Plot diffusion map embedding of samples based on distance matrix

Description

Visualizes diffusion map for network of samples based on square distance matrix (sample-sample pairwise dissimilarity)

Usage

```
plotGroupedSamplesDmap(my_distmat, cluster_assignments, pt_sz = 1,
  n_dim = 3, pt_label = NULL, cmap = NULL, w = 8, h = 5,
  scale.y = 1, angle = 40, autosave = FALSE, ...)
```

Arguments

my_distmat	phemdObj object containing sample names in @snames slot
cluster_assignments	Vector containing group assignments for each sample
pt_sz	Size of points representing samples in plot (scaling factor)
n_dim	Number of dimensions for embedding (either 2 or 3)
pt_label	Vector of sample names corresponding to each point (same order as samples in my_distmat and cluster_assignments)
cmap	Vector containing colors by which points should be colored (corresponding to cluster_assignments)
w	Width of plot in inches
h	Height of plot in inches
scale.y	Scaling factor for diffusion map y-axis
angle	Rotation factor for diffusion map plot
autosave	Boolean denoting whether or not to save output diffusion map
...	Additional parameters to be passed to DiffusionMap function

Details

Requires 'destiny' package

Value

DiffusionMap object containing biological sample embedding and associated metadata

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model = 'gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
my_phemdObj_final <- clusterIndividualSamples(my_phemdObj_monocle)
my_phemdObj_final <- generateGDM(my_phemdObj_final)
my_EMD_mat <- compareSamples(my_phemdObj_final)
cluster_assignments <- groupSamples(my_EMD_mat, distfun = 'hclust', ncluster=4)
printClusterAssignments(cluster_assignments, my_phemdObj_final, '.', overwrite=TRUE)
dm <- plotGroupedSamplesDmap(my_EMD_mat, cluster_assignments, pt_sz=2)
```

plotHeatmaps

Plot heatmap of cell subtypes

Description

Takes as input a Phemd object containing either a Monocle2 or Seurat object (already embedded and ordered) and saves heatmap describing cell subtypes to specified folder

Usage

```
plotHeatmaps(obj, cell_model = c("monocle2", "seurat"),
  selected_genes = NULL, w = 8, h = 5, ...)
```

Arguments

obj	'Phemd' object containing Monocle 2 object
cell_model	Method by which cell state was modeled (either "monocle2" or "seurat")
selected_genes	Vector containing gene names to include in heatmap (optional)
w	Width of plot in inches
h	Height of plot in inches
...	Additional parameters to be passed on to pheatmap function

Details

embedCells and orderCellsMonocle need to be called before calling this function. Required additional package: 'pheatmap'

Value

Heatmap containing expression values for each cell subtype. If `cell_model` is 'seurat', then returns a list of heatmaps (1 for each batch) that may be subsequently plotted individually

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_lg <- selectFeatures(my_phemdObj_lg, selected_genes)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model = 'gaussianff',
pseude_expr=0, sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
myheatmap <- plotHeatmaps(my_phemdObj_monocle, cell_model='monocle2')
```

`plotSummaryHistograms` *Plots cell subtype frequency histograms summarizing each group of samples*

Description

Visualizes plots of relative frequency ("weights") of cell subtypes ("bins" or "signatures") summarizing each group of single-cell samples. Each summary histogram is computed by taking the bin-wise mean of all samples in the group

Usage

```
plotSummaryHistograms(myobj, cluster_assignments,
  cell_model = c("monocle2", "seurat"), cmap = NULL, ncol.plot = 4,
  ax.lab.sz = 2.5, title.sz = 3)
```

Arguments

<code>myobj</code>	Phemd object containing cell subtype relative frequency in <code>@data_cluster_weights</code> slot
<code>cluster_assignments</code>	Vector containing group assignments for each sample in <code>myobj</code>
<code>cell_model</code>	Method by which cell state was modeled (either "monocle2" or "seurat")
<code>cmap</code>	Vector containing colors by which histogram bars should be colored (optional)
<code>ncol.plot</code>	Number of columns to use to plot multi-panel histogram plot
<code>ax.lab.sz</code>	Scaling factor for axis labels (default 2.5)
<code>title.sz</code>	Scaling factor for plot title (default 3)

Details

`groupSamples` must be called before calling this function. Saves plots in directory called "summary_inhibs"

Value

None

pooledCells	<i>Accessor function for aggregated cells used for cell subtype definition</i>
-------------	--

Description

Accessor function for aggregated cells used for cell subtype definition

Usage

```
pooledCells(obj)
```

Arguments

obj	Phemd object
-----	--------------

Value

Numeric matrix representing expression data for cells from all experimental conditions (rows = markers, cols = cells)

Examples

```
phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
cells_aggregated <- pooledCells(phemdObj)
```

printClusterAssignments	<i>Writes samples to file based on community detection group assignments</i>
-------------------------	--

Description

Takes vector of cluster assignments and phemdObj containing sample names and writes sample groups to file

Usage

```
printClusterAssignments(cluster_assignments, obj, dest,
  overwrite = FALSE)
```

Arguments

cluster_assignments	Vector containing group assignments for each sample
obj	phemdObj object containing sample names in @snames slot
dest	Path to existing directory where output should be saved
overwrite	Boolean representing whether or not to overwrite contents of "dest" with output of printClusterAssignments

Details

Order of samples in `obj@snames` is assumed to be the same as the order of group assignments in `cluster_assignments`

Value

None

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_monocle <- embedCells(my_phemdObj_lg, data_model = 'gaussianff', sigma=0.02, maxIter=2)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
my_phemdObj_final <- clusterIndividualSamples(my_phemdObj_monocle)
my_phemdObj_final <- generateGDM(my_phemdObj_final)
my_EMD_mat <- compareSamples(my_phemdObj_final)
cluster_assignments <- groupSamples(my_EMD_mat, distfun = 'hclust', ncluster=4)
printClusterAssignments(cluster_assignments, my_phemdObj_final, '.', overwrite=TRUE)
```

rawExpn

Accessor function for stored multi-sample raw expression data

Description

Accessor function for stored multi-sample raw expression data

Usage

```
rawExpn(obj)
```

Arguments

`obj` A Phemd object.

Value

List of matrices, each of which represents a single-cell sample

Examples

```
phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
raw_expn_data <- rawExpn(phemdObj)
```

removeTinySamples	<i>Remove samples with too few cells</i>
-------------------	--

Description

Removes samples from Phemd that have fewer cells than min_sz

Usage

```
removeTinySamples(obj, min_sz = 20)
```

Arguments

obj	'Phemd' object containing raw expression data and associated metadata
min_sz	Minimum number of cells in each sample to be retained

Details

Note: If used, this function must be called before (and not after) the aggregateSamples function is called

Value

'Phemd' object containing raw multi-sample expression data and associated metadata (same as input minus removed samples)

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10) #removes samples with fewer than 10 cells
```

retrieveRefClusters	<i>Retrieve reference cell clusters</i>
---------------------	---

Description

Takes initial Phemd struct and returns cell clusters as assigned by clustering algorithm (i.e. Monocle 2)

Usage

```
retrieveRefClusters(obj, cell_model = c("monocle2", "seurat"),
  expn_type = "aligned", ndim = 10)
```

Arguments

obj	Phemd struct containing Monocle2 object and underlying expression data
cell_model	String representing data model for cell state space (Seurat or Monocle 2)
expn_type	String representing whether to return raw expression values or coordinates in dimensionality-reduced, aligned feature space (only relevant for Seurat data models)
ndim	Number of dimensions (e.g. CCA) to use (only relevant for Seurat data models)

Details

Private method (not exported in namespace)

Value

List of data matrices; each list element is of size num_cells_in_cluster x num_markers and represents a distinct cell cluster

Examples

```
## Not run:
cluster_expression_data <- retrieveRefClusters(my_phemdObj)

## End(Not run)
```

selected_genes	<i>Genes to be used when performing clustering and trajectory analyses on melanoma single-cell RNA-seq expression data</i>
----------------	--

Description

This object contains genes to be used when performing clustering and trajectory analyses on melanoma single-cell RNA-seq expression data.

Usage

```
data(selected_genes)
```

Format

Vector of length 44 representing selected genes for performing computational analyses such as generating cell embeddings and clustering cell subtypes.

Source

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72056>

References

Tirosh, I. et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* 352, 189–196 (2016)

selectFeatures	<i>Perform feature selection on aggregated data</i>
----------------	---

Description

Takes as input a Phemd object with aggregated data and returns updated object after performing feature selection on aggregated data

Usage

```
selectFeatures(obj, selected_genes)
```

Arguments

obj 'Phemd' object containing aggregated data
selected_genes Vector containing names of genes to use for downstream analyses

Details

aggregateSamples needs to be called before running this function

Value

Same as input 'Phemd' object after performing feature-selection based dimensionality reduction on aggregated expression data

Examples

```
my_phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
my_phemdObj_lg <- removeTinySamples(my_phemdObj, 10)
my_phemdObj_lg <- aggregateSamples(my_phemdObj_lg, max_cells=1000)
my_phemdObj_lg <- selectFeatures(my_phemdObj_lg, selected_genes=c('TP53',
'EGFR', 'KRAS', 'FOXP3', 'LAG3'))
```

selectMarkers	<i>Accessor function for gene/protein markers measured in experiment</i>
---------------	--

Description

Accessor function for gene/protein markers measured in experiment

Usage

```
selectMarkers(obj)
```

Arguments

obj Phemd object

Value

Vector representing gene/protein markers corresponding to expression matrices

Examples

```
phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
genes <- selectMarkers(phemdObj)
```

seuratInfo

Accessor function for stored Seurat object within Phemd object

Description

Accessor function for stored Seurat object within Phemd object

Usage

```
seuratInfo(obj)
```

Arguments

obj A Phemd object.

Value

An object of class 'Seurat'

Examples

```
phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
seurat_obj <- seuratInfo(phemdObj)
```

sNames

Accessor function for identifiers of all single-cell samples in experiment

Description

Accessor function for identifiers of all single-cell samples in experiment

Usage

```
sNames(obj)
```

Arguments

obj Phemd object

Value

Vector representing sample names corresponding to expression matrices

Examples

```
phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
sampleIDs <- sNames(phemdObj)
```

snames_data	<i>Sample names for melanoma single-cell RNA-seq expression data</i>
-------------	--

Description

This object contains sample names corresponding to samples contained in melanoma expression data.

Usage

```
data("snames_data")
```

Format

Vector of length 19 representing sample names corresponding to order of samples in all_expn_data in melanomaData dataset.

Source

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72056>

References

Tirosh, I. et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* 352, 189–196 (2016)

subsampledBool	<i>Accessor function for whether or not cells were subsampled when aggregated for cell subtype analysis</i>
----------------	---

Description

Accessor function for whether or not cells were subsampled when aggregated for cell subtype analysis

Usage

```
subsampledBool(obj)
```

Arguments

obj Phemd object

Value

Boolean represent whether or not subsampling was performed in the data aggregation process

Examples

```
phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
subsampled <- subsampledBool(phemdObj)
```

subsampledIdx *Accessor function for aggregated cells used for cell subtype definition*

Description

Accessor function for aggregated cells used for cell subtype definition

Usage

```
subsampledIdx(obj)
```

Arguments

obj Phemd object

Value

List of vectors each representing the indices of elements in `rawExpn(obj)` that were subsampled and combined to form "data_aggregate"

Examples

```
phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
subsampled_idx_list <- subsampledIdx(phemdObj)
```

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