

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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LazyData true

Depends R (>= 3.5), monocle

Imports SingleCellExperiment, RColorBrewer, igraph, transport, pracma, cluster, Rtsne, destiny, Seurat, RANN, ggplot2, maptree, pheatmap, scatterplot3d, VGAM, methods, grDevices, graphics, stats, utils, cowplot, S4Vectors, BiocGenerics, SummarizedExperiment, Biobase, phateR, reticulate

Config/reticulate list(packages = list(list(package = `phate`)))

Suggests knitr

VignetteBuilder knitr

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R topics documented:

aggregateSamples	3
all_expn_data	3

all_genes	4
assignCellClusterNearestNode	5
batchIDs	6
bindSeuratObj	6
celltypeFreqs	7
clusterIndividualSamples	7
compareSamples	8
createDataObj	9
drawColnames45	10
embedCells	11
gaussianffLocal	12
GDM	12
generateGDM	13
getArithmeticCentroids	14
getCellYield	15
getSampleCelltypeFreqs	15
getSampleHistsByCluster	16
getSampleSizes	17
groupSamples	18
heatmap_genes	19
identifyCentroids	19
monocleInfo	20
orderCellsMonocle	20
phateInfo	21
Phemd	22
Phemd-methods	22
plotCellYield	25
plotEmbeddings	26
plotGroupedSamplesDmap	27
plotHeatmaps	28
plotSummaryHistograms	29
pooledCells	30
printClusterAssignments	31
rawExpn	32
removeTinySamples	32
retrieveRefClusters	33
selected_genes	34
selectFeatures	34
selectMarkers	35
seuratInfo	35
sNames	36
snames_data	37
subsampedBool	37
subsampedIdx	38

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", "phate")  
)
```

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", "seurat", or "phate" 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 cell-state embedding generated by embedCells. Returns updated object with cell subtype frequencies of each sample that may be retrieved by the 'celltypeFreqs' accessor function.

Usage

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

Arguments

obj	'Phemd' object containing single-cell expression data of all samples in @data slot and cell-state embedding object generated and stored using the embedCells function.
verbose	Boolean that determines whether progress (sequential processing of samples) should be printed. FALSE by default
cell_model	Either "monocle2", "seurat", or "phate" depending on method used to model cell state space

Details

embedCells (and orderCellsMonocle if using the Monocle2 embedding technique) needs to be called before calling this function.

Value

'Phemd' object with cell subtype frequencies of each sample that can be retrieved using the 'cell-typeFreqs' accessor function

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

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

Description

Takes as input a Phemd object with aggregated data and returns updated object containing cell-state embedding

Usage

```
embedCells(
  obj,
  cell_model = c("monocle2", "seurat", "phate"),
  data_model = "negbinomial_sz",
  phate_ncluster = 8,
  phate_cluster_seed = NULL,
  ...
)
```

Arguments

obj	'Phemd' object containing aggregated data
cell_model	Method to use to generate cell-state embedding. Currently supports "phate" and "monocle2". If using the Seurat to model the cell-state space, please identify cell subtypes as outlined in the Seurat software package and then use the bindSeuratObj function.
data_model	Only relevant if cell_model = "monocle2". 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-m
phate_ncluster	Only relevant if cell_model = "phate". Number of cell state clusters to return when using PHATE
phate_cluster_seed	Only relevant if cell_model = "phate". Seed to use when performing cell state clustering (optional)
...	Additional parameters to be passed to reduceDimension function for Monocle or phate function for PHATE

Details

aggregateSamples needs to be called before running this function.

Value

Same as input 'Phemd' object containing additional cell-state embedding object

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, cell_model='monocle2', data_model = 'gaussianff', sigma=0.02, ma
```

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	<i>Accessor function for EMD ground distance matrix</i>
-----	---

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 containing cell-state embedding object. Returns updated object with ground distance matrix representing pairwise distances between distinct cell subtypes based on cell state embedding.

Usage

```
generateGDM(
  obj,
  cell_model = c("monocle2", "seurat", "phate"),
  expn_type = "reduced",
  ndim = 8
)
```

Arguments

obj 'Phemd' object containing cell-state embedding object

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

expn_type Data type to use to determine cell-type dissimilarities

ndim Number of embedding dimensions to be used for computing cell-type dissimilarity (optional)

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 to identify groups of similar samples</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	A 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)
...	Optional additional parameters to be passed to diffusionKmeans method

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, cell_model = 'monocle2', data_model = 'gaussianff', sigma=0)
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 Monocle2 object initialized using embedCells
 ... 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 cell-state embedding object containing cell state 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, cell_model='monocle2', data_model='gaussianff', sigma=0.02)
my_phemdObj_monocle <- orderCellsMonocle(my_phemdObj_monocle)
```

phateInfo

Accessor function for stored phate object

Description

Accessor function for stored phate object

Usage

```
phateInfo(obj)
```

Arguments

obj A Phemd object.

Value

An object of class 'phate' (from phateR)

Examples

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

Phemd	<i>Phemd class</i>
-------	--------------------

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 class "Seurat" that is the core Seurat data structure
phate_obj Object of class "phate" that is the core PHATE data structure
experiment_ids Vector of length num_samples representing the experiment (batch) in which the sample was profiled

Phemd-methods	<i>Setter function for protein / gene markers</i>
---------------	---

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 phate 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

phateInfo(obj) <- value

## S4 replacement method for signature 'Phemd'
phateInfo(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 containing phate 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))
subsamplingIdxList<- rep(list(1:10), length(all_expn_data)) #subsampling cells 1-10 from each sample
subsamplingIdx(phemdObj) <- subsamplingIdxList

phemdObj <- createDataObj(all_expn_data, all_genes, as.character(snames_data))
subsamplingBool(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))
#my_phateObj <- phateR::phate(all_expn_data[[1]])
phateInfo(phemdObj) <- list()

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", "phate"),
  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", "seurat", or "phate")
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 = NULL,
  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, Seurat, or PHATE object (already embedded and clustered) and plots heatmap characterizing cell subtypes

Usage

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

Arguments

obj	'Phemd' object containing cell-state embedding object
cell_model	Method by which cell state was modeled ("monocle2", "seurat", or "phate")
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 if using Monocle2) 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", "phate"),
  cmap = NULL,
  ncol.plot = 4,
  ax.lab.sz = 2.5,
  title.sz = 3
)
```

Arguments

myobj	Phemd 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", "seurat", or "phate")
cmap	Vector containing colors by which histogram bars should be colored (optional)
ncol.plot	Number of columns to use to plot multi-panel histogram plot
ax.lab.sz	Scaling factor for axis labels (default 2.5)
title.sz	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

Writes samples to file based on community detection group assignments

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	<i>Accessor function for stored multi-sample raw expression data</i>
---------	--

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     Retrieve reference cell clusters
```

Description

Takes initial Phemd struct and returns cell clusters as assigned by clustering algorithm (e.g. PHATE or Monocle2)

Usage

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

Arguments

obj	Phemd struct containing cell-state embedding object and underlying expression data
cell_model	String representing data model for cell-state space ("seurat", "monocle2", or "phate")
expn_type	String representing whether to return raw expression values or coordinates in dimensionality-reduced feature space
ndim	Number of dimensions in reduced dimensionality space (e.g. PHATE / CCA) to use (only relevant in reduced dimensionality space)

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

Accessor function for gene/protein markers measured in experiment

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	<i>Accessor function for identifiers of all single-cell samples in experiment</i>
--------	---

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

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)
```

Index

* datasets

- all_expn_data, 3
- all_genes, 4
- heatmap_genes, 19
- selected_genes, 34
- snames_data, 37

aggregateSamples, 3

all_expn_data, 3

all_genes, 4

assignCellClusterNearestNode, 5

batchIDs, 6

batchIDs<- (Phemd-methods), 22

batchIDs<- , Phemd-method
(Phemd-methods), 22

bindSeuratObj, 6

celltypeFreqs, 7

celltypeFreqs<- (Phemd-methods), 22

celltypeFreqs<- , Phemd-method
(Phemd-methods), 22

clusterIndividualSamples, 7

compareSamples, 8

createDataObj, 9

drawColnames45, 10

embedCells, 11

gaussianffLocal, 12

GDM, 12

GDM<- (Phemd-methods), 22

GDM<- , Phemd-method (Phemd-methods), 22

generateGDM, 13

getArithmeticCentroids, 14

getCellYield, 15

getSampleCelltypeFreqs, 15

getSampleHistsByCluster, 16

getSampleSizes, 17

groupSamples, 18

heatmap_genes, 19

identifyCentroids, 19

monocleInfo, 20

monocleInfo<- (Phemd-methods), 22

monocleInfo<- , Phemd-method
(Phemd-methods), 22

orderCellsMonocle, 20

phateInfo, 21

phateInfo<- (Phemd-methods), 22

phateInfo<- , Phemd-method
(Phemd-methods), 22

Phemd, 22

Phemd, ANY, ANY-method (Phemd-methods), 22

Phemd, character, ANY-method
(Phemd-methods), 22

Phemd-class (Phemd), 22

Phemd-methods, 22

plotCellYield, 25

plotEmbeddings, 26

plotGroupedSamplesDmap, 27

plotHeatmaps, 28

plotSummaryHistograms, 29

pooledCells, 30

pooledCells<- (Phemd-methods), 22

pooledCells<- , Phemd-method
(Phemd-methods), 22

printClusterAssignments, 31

rawExpn, 32

rawExpn<- (Phemd-methods), 22

rawExpn<- , Phemd-method (Phemd-methods),
22

removeTinySamples, 32

retrieveRefClusters, 33

selected_genes, 34

selectFeatures, 34

selectMarkers, 35

selectMarkers<- (Phemd-methods), 22

selectMarkers<- , Phemd-method
(Phemd-methods), 22

seuratInfo, 35

seuratInfo<- (Phemd-methods), 22

seuratInfo<- , Phemd-method
(Phemd-methods), 22

sNames, [36](#)
snames_data, [37](#)
subsamp1edBool, [37](#)
subsamp1edBool<- (Phemd-methods), [22](#)
subsamp1edBool<- ,Phemd-method
 (Phemd-methods), [22](#)
subsamp1edIdx, [38](#)
subsamp1edIdx<- (Phemd-methods), [22](#)
subsamp1edIdx<- ,Phemd-method
 (Phemd-methods), [22](#)