

Package ‘GeneNMF’

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Type Package

Title Non-Negative Matrix Factorization for Single-Cell Omics

Version 0.9.2

Description A collection of methods to extract gene programs from single-cell gene expression data using non-negative matrix factorization (NMF). 'GeneNMF' contains functions to directly interact with the 'Seurat' toolkit and derive interpretable gene program signatures.

Depends R (>= 4.3.0)

Imports RcppML, Matrix, stats, methods, utils, Seurat (>= 4.3.0), cluster, lsa, irlba, pheatmap, dendextend, viridis, colorspace

Suggests knitr, rmarkdown, fgsea, msigdb

VignetteBuilder knitr

URL <https://github.com/carmonalab/GeneNMF>

BugReports <https://github.com/carmonalab/GeneNMF/issues>

License GPL-3

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dropMetaPrograms	<i>Drop meta-programs</i>
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Description

Remove meta-programs from the GeneNMF results. This can be desirable if one or more MPs are redundant or have poor metrics (e.g. low sample coverage or low silhouette score).

Usage

```
dropMetaPrograms(mp.res, dropMP = NULL)
```

Arguments

mp.res	The meta-programs object generated by getMetaPrograms
dropMP	A vector with the names of the MPs to be dropped

Value

The input meta-program object, minus the dropped MPs

Examples

```
library(Seurat)
data(sampleObj)
geneNMF_programs <- multiNMF(list(sampleObj), k=5)
geneNMF_metaprograms <- getMetaPrograms(geneNMF_programs, nMP=3)
geneNMF_metaprograms_filtered <- dropMetaPrograms(geneNMF_metaprograms, dropMP = c("MP2"))
```

`findVariableFeatures_wfilters`*Find variable features*

Description

Select highly variable genes (HVG) from an expression matrix. Genes from a blocklist (e.g. cell cycling genes, mitochondrial genes) can be excluded from the list of variable genes, as well as genes with very low or very high average expression

Usage

```
findVariableFeatures_wfilters(  
  obj,  
  nfeatures = 2000,  
  genesBlockList = NULL,  
  min.exp = 0.01,  
  max.exp = 3  
)
```

Arguments

<code>obj</code>	A Seurat object containing an expression matrix
<code>nfeatures</code>	Number of top HVG to be returned
<code>genesBlockList</code>	Optionally takes a vector or list of vectors of gene names. These genes will be ignored for HVG detection. This is useful to mitigate effect of genes associated with technical artifacts or batch effects (e.g. mitochondrial, heat-shock response). If set to 'NULL' no genes will be excluded
<code>min.exp</code>	Minimum average normalized expression for HVG. If lower, the gene will be excluded
<code>max.exp</code>	Maximum average normalized expression for HVG. If higher, the gene will be excluded

Value

Returns the input Seurat object `obj` with the calculated highly variable features accessible through `VariableFeatures(obj)`

Examples

```
data(sampleObj)  
sampleObj <- findVariableFeatures_wfilters(sampleObj, nfeatures=100)
```

`getDataMatrix`*Extract data matrix from Seurat object*

Description

Get the gene expression matrix from a Seurat object, optionally centered and/or subset on highly variable genes

Usage

```
getDataMatrix(  
  obj,  
  assay = "RNA",  
  slot = "data",  
  hvg = NULL,  
  center = FALSE,  
  scale = FALSE,  
  non_negative = TRUE  
)
```

Arguments

<code>obj</code>	Seurat object
<code>assay</code>	Get data matrix from this assay
<code>slot</code>	Get data matrix from this slot (=layer)
<code>hvg</code>	List of variable genes to subset the matrix. If NULL, uses all genes
<code>center</code>	Whether to center the data matrix
<code>scale</code>	Whether to scale the data matrix
<code>non_negative</code>	Enforce non-negative values for NMF

Value

Returns a sparse data matrix (cells per genes), subset according to the given parameters

Examples

```
data(sampleObj)  
matrix <- getDataMatrix(sampleObj)
```

getMetaPrograms *Extract consensus gene programs (meta-programs)*

Description

Run it over a list of NMF models obtained using [multiNMF](#); it will determine gene programs that are consistently observed across samples and values of k.

Usage

```
getMetaPrograms(
  nmf.res,
  nMP = 10,
  specificity.weight = 5,
  weight.explained = 0.5,
  max.genes = 200,
  metric = c("cosine", "jaccard"),
  hclust.method = "ward.D2",
  min.confidence = 0.5,
  remove.empty = TRUE
)
```

Arguments

nmf.res	A list of NMF models obtained from multiNMF
nMP	Total number of meta-programs
specificity.weight	A parameter controlling how specific gene should be for each program. ‘specificity.weight=0’ no constraint on specificity, and positive values impose increasing specificity.
weight.explained	Fraction of NMF weights explained by selected genes. For example if weight.explained=0.5, all genes that together account for 50% of NMF weights are used to return program signatures.
max.genes	Max number of genes for each programs
metric	Metric to calculate pairwise similarity between programs
hclust.method	Method to build similarity tree between individual programs
min.confidence	Percentage of programs in which a gene is seen (out of programs in the corresponding program tree branch/cluster), to be retained in the consensus metaprograms
remove.empty	Whether to remove meta-programs with no genes above confidence threshold

Value

Returns a list with i) 'metaprograms.genes' top genes for each meta-program; ii) 'metaprograms.metrics' dataframe with meta-programs statistics: a) freq. of samples where the MP is present, b) average silhouette width, c) mean similarity (cosine or Jaccard), d) number of genes in MP, e) number of gene programs in MP; iii) 'metaprogram.composition' dataframe containing the number of individual for each sample that contributed to the consensus MPs; iv) 'programs.similarity': matrix of similarities (Jaccard or cosine) between meta-programs; v) 'programs.tree': hierarchical clustering of meta-programs (hclust tree); vi) 'programs.clusters': meta-program identity for each program

Examples

```
library(Seurat)
data(sampleObj)
geneNMF_programs <- multiNMF(list(sampleObj), k=5)
geneNMF_metaprograms <- getMetaPrograms(geneNMF_programs, nMP=3)
```

getNMFgenes

Get list of genes for each NMF program

Description

Run it over a list of NMF models obtained using `multiNMF()`

Usage

```
getNMFgenes(
  nmf.res,
  specificity.weight = 5,
  weight.explained = 0.5,
  max.genes = 200
)
```

Arguments

<code>nmf.res</code>	A list of NMF models obtained using <code>multiNMF()</code>
<code>specificity.weight</code>	A parameter controlling how specific gene should be for each program. 'specificity.weight=0' no constraint on specificity, and positive values impose increasing specificity.
<code>weight.explained</code>	Fraction of NMF weights explained by selected genes. For example if <code>weight.explained=0.5</code> , all genes that together account for 50% of NMF weights are used to return program signatures.
<code>max.genes</code>	Max number of genes for each program

Value

Returns a list of top genes for each gene program found by multiNMF()

Examples

```
library(Seurat)
data(sampleObj)
geneNMF_programs <- multiNMF(list(sampleObj), k=5)
geneNMF_genes <- getNMFgenes(geneNMF_programs)
```

multiNMF

Run NMF on a list of Seurat objects

Description

Given a list of Seurat objects, run non-negative matrix factorization on each sample individually, over a range of target NMF components (k).

Usage

```
multiNMF(
  obj.list,
  assay = "RNA",
  slot = "data",
  k = 5:6,
  hvg = NULL,
  nfeatures = 2000,
  L1 = c(0, 0),
  min.exp = 0.01,
  max.exp = 3,
  center = FALSE,
  scale = FALSE,
  min.cells.per.sample = 10,
  hvg.blocklist = NULL,
  seed = 123
)
```

Arguments

obj.list	A list of Seurat objects
assay	Get data matrix from this assay
slot	Get data matrix from this slot (=layer)
k	Number of target components for NMF (can be a vector)
hvg	List of pre-calculated variable genes to subset the matrix. If hvg=NULL it calculates them automatically

nfeatures	Number of HVG, if calculate_hvg=TRUE
L1	L1 regularization term for NMF
min.exp	Minimum average log-expression value for retaining genes
max.exp	Maximum average log-expression value for retaining genes
center	Whether to center the data matrix
scale	Whether to scale the data matrix
min.cells.per.sample	Minimum number of cells per sample (smaller samples will be ignored)
hvg.blocklist	Optionally takes a vector or list of vectors of gene names. These genes will be ignored for HVG detection. This is useful to mitigate effect of genes associated with technical artifacts and batch effects (e.g. mitochondrial), and to exclude TCR and BCR adaptive immune(clone-specific) receptors. If set to 'NULL' no genes will be excluded
seed	Random seed

Value

Returns a list of NMF programs, one for each sample and for each value of 'k'. The format of each program in the list follows the structure of [nmf](#) factorization models.

Examples

```
library(Seurat)
data(sampleObj)
geneNMF_programs <- multiNMF(list(sampleObj), k=5)
```

multiPCA

Run PCA on a list of Seurat objects

Description

Given a list of Seurat objects, run non-negative PCA factorization on each sample individually.

Usage

```
multiPCA(
  obj.list,
  assay = "RNA",
  slot = "data",
  k = 4:5,
  hvg = NULL,
  nfeatures = 500,
  min.exp = 0.01,
  max.exp = 3,
```

```

    min.cells.per.sample = 10,
    center = FALSE,
    scale = FALSE,
    hvg.blocklist = NULL,
    seed = 123
  )

```

Arguments

<code>obj.list</code>	A list of Seurat objects
<code>assay</code>	Get data matrix from this assay
<code>slot</code>	Get data matrix from this slot (=layer)
<code>k</code>	Number of target components for PCA
<code>hvg</code>	List of pre-calculated variable genes to subset the matrix. If <code>hvg=NULL</code> it calculates them automatically
<code>nfeatures</code>	Number of HVG, if <code>calculate_hvg=TRUE</code>
<code>min.exp</code>	Minimum average log-expression value for retaining genes
<code>max.exp</code>	Maximum average log-expression value for retaining genes
<code>min.cells.per.sample</code>	Minimum number of cells per sample (smaller samples will be ignored)
<code>center</code>	Whether to center the data matrix
<code>scale</code>	Whether to scale the data matrix
<code>hvg.blocklist</code>	Optionally takes a vector or list of vectors of gene names. These genes will be ignored for HVG detection. This is useful to mitigate effect of genes associated with technical artifacts and batch effects (e.g. mitochondrial), and to exclude TCR and BCR adaptive immune(clone-specific) receptors. If set to 'NULL' no genes will be excluded
<code>seed</code>	Random seed

Value

Returns a list of non-negative PCA programs, one for each sample. The format of each program in the list follows the structure of [nmf](#) factorization models.

Examples

```

library(Seurat)
data(sampleObj)
geneNMF_programs <- multiPCA(list(sampleObj), k=5)

```

plotMetaPrograms *Visualizations for meta-programs*

Description

Generates a clustered heatmap for meta-program similarities (by Jaccard index or Cosine similarity). This function is intended to be run on the object generated by [getMetaPrograms](#), which contains a pre-calculated tree of pairwise similarities between clusters (as a 'hclust' object).

Usage

```
plotMetaPrograms(
  mp.res,
  similarity.cutoff = c(0, 1),
  scale = "none",
  downsample = 1000,
  showtree = TRUE,
  palette = viridis(100, option = "A", direction = -1),
  annotation_colors = NULL,
  main = "Clustered Heatmap",
  show_rownames = FALSE,
  show_colnames = FALSE,
  ...
)
```

Arguments

<code>mp.res</code>	The meta-programs object generated by getMetaPrograms
<code>similarity.cutoff</code>	Min and max values for similarity metric
<code>scale</code>	Heatmap rescaling (passed to pheatmap as 'scale')
<code>downsample</code>	Limit max number of samples in heatmap, to avoid overloading the graphics
<code>showtree</code>	Whether to plot the hierarchical clustering tree
<code>palette</code>	Heatmap color palette (passed to pheatmap as 'color')
<code>annotation_colors</code>	Color palette for MP annotations
<code>main</code>	Heatmap title
<code>show_rownames</code>	Whether to display individual program names as rows
<code>show_colnames</code>	Whether to display individual program names as cols
<code>...</code>	Additional parameters for pheatmap

Value

Returns a clustered heatmap of MP similarities, in ggplot2 format

Examples

```
library(Seurat)
data(sampleObj)
geneNMF_programs <- multiNMF(list(sampleObj), k=5)
geneNMF_metaprograms <- getMetaPrograms(geneNMF_programs, nMP=3)
plotMetaPrograms(geneNMF_metaprograms)
```

runGSEA

Run Gene set enrichment analysis

Description

Utility function to run Gene Set Enrichment Analysis (GSEA) against gene sets from MSigDB. Note: this is an optional function, which is conditional to the installation of suggested packages fgsea and msigdb.

Usage

```
runGSEA(
  genes,
  universe = NULL,
  category = "H",
  subcategory = NULL,
  species = "Homo sapiens",
  custom.db = NULL,
  pval.thr = 0.05
)
```

Arguments

genes	A vector of genes
universe	Background universe of gene symbols (passed on to fgsea::fora)
category	GSEA main category/collection (e.g. "H" or "C5")
subcategory	GSEA subcategory/subcollection
species	Species for GSEA analysis. For a list of the available species, type msigdb::msigdb_species()
custom.db	A custom database of signatures, to be used instead of MSigDB. Provide custom.db as a named list, where the list names are the signature names, and the list elements are the signatures (as vectors).
pval.thr	Min p-value to include results

Value

Returns a table of enriched gene programs from GSEA

Examples

```

data(sampleObj)
geneset <- c("BANK1", "CD22", "CD79A", "CD19", "IGHD", "IGHG3", "IGHM")
#test is conditional on availability of suggested packages
if (requireNamespace("fgsea", quietly=TRUE) &
    requireNamespace("msigbr", quietly=TRUE)) {
  gsea_res <- runGSEA(geneset,
    universe=rownames(sampleObj),
    category = "C8")
}

```

runNMF

*Compute NMF as a low-dim embedding for Seurat***Description**

Compute NMF embeddings for single-cell dataset, and store them in the Seurat data structure. They can be used as an alternative to PCA for downstream analyses.

Usage

```

runNMF(
  obj,
  assay = "RNA",
  slot = "data",
  k = 10,
  new.reduction = "NMF",
  seed = 123,
  L1 = c(0, 0),
  hvg = NULL,
  center = FALSE,
  scale = FALSE
)

```

Arguments

obj	A seurat object
assay	Get data matrix from this assay
slot	Get data matrix from this slot (=layer)
k	Number of components for low-dim representation
new.reduction	Name of new dimensionality reduction
seed	Random seed
L1	L1 regularization term for NMF
hvg	Which genes to use for the reduction
center	Whether to center the data matrix
scale	Whether to scale the data matrix

Value

Returns a Seurat object with a new dimensionality reduction (NMF)

Examples

```
data(sampleObj)
sampleObj <- runNMF(sampleObj, k=8)
```

sampleObj

Sample dataset to test GeneNMF installation

Description

A Seurat object containing single-cell transcriptomes (scRNA-seq) for 50 cells and 20729 genes. Single-cell UMI counts were normalized using a standard log-normalization: counts for each cell were divided by the total counts for that cell and multiplied by 10,000, then natural-log transformed using 'log1p'.

This is a subsample of 25 predicted B cells and 25 predicted NK cells from the large scRNA-seq PBMC dataset published by Hao et al. (doi:10.1016/j.cell.2021.04.048) and available as UMI counts at https://atlas.fredhutch.org/data/nygc/multimodal/pbmc_multimodal.h5seurat

Usage

```
sampleObj
```

Format

A sparse matrix of 50 cells and 20729 genes.

Source

[doi:10.1016/j.cell.2021.04.048](https://doi.org/10.1016/j.cell.2021.04.048)

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