Package 'UCell'

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Type Package

Title Rank-based signature enrichment analysis for single-cell data

Version 2.8.0

Description UCell is a package for evaluating gene signatures in single-cell datasets.
UCell signature scores, based on the Mann-
Whitney U statistic, are robust to dataset size and heterogeneity, and their calculation
demands less computing time and memory than other available methods, enabling the processing of large datasets in a few minutes even
on machines with limited computing power. UCell can be applied to any single-
cell data matrix, and includes functions to directly
interact with SingleCellExperiment and Seurat objects.

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`AddModuleScore_UCell` *Calculate module enrichment scores from single-cell data (Seurat interface)*

Description

Given a Seurat object, calculates module/signature enrichment scores at single-cell level using the Mann-Whitney U statistic. UCell scores are normalized U statistics (between 0 and 1), and they are mathematically related to the Area under the ROC curve (see Mason and Graham)

Usage

```
AddModuleScore_UCell(
  obj,
  features,
  maxRank = 1500,
  chunk.size = 100,
  BPPARAM = NULL,
  ncores = 1,
  storeRanks = FALSE,
```

```
  w_neg = 1,
  assay = NULL,
  slot = "counts",
  ties.method = "average",
  force.gc = FALSE,
  name = "_UCell"
)
```

Arguments

obj	Seurat object
features	A list of signatures, for example: list(Tcell_signature = c("CD2","CD3E","CD3D"), Myeloid_signature = c("SPI1","FCER1G","CSF1R")) You can also specify positive and negative gene sets by adding a + or - sign to genes in the signature; see an example below
maxRank	Maximum number of genes to rank per cell; above this rank, a given gene is considered as not expressed.
chunk.size	Number of cells to be processed simultaneously (lower size requires slightly more computation but reduces memory demands)
BPPARAM	A BiocParallel::bpparam() object that tells UCell how to parallelize. If provided, it overrides the ncores parameter.
ncores	Number of processors to parallelize computation. If BPPARAM = NULL, the function uses BiocParallel::MulticoreParam(workers=ncores)
storeRanks	Store ranks matrix in Seurat object ('UCellRanks' assay) for fast subsequent computations. This option may demand large amounts of RAM.
w_neg	Weight on negative genes in signature. e.g. w_neg=1 weighs equally up- and down-regulated genes, w_neg=0.5 gives 50% less importance to negative genes
assay	Pull out data from this assay of the Seurat object (if NULL, use DefaultAssay(obj))
slot	Pull out data from this slot (layer in v5) of the Seurat object
ties.method	How ranking ties should be resolved - passed on to data.table::frank
force.gc	Explicitly call garbage collector to reduce memory footprint
name	Name tag that will be appended at the end of each signature name, "_UCell" by default (e.g. signature score in meta data will be named: Myeloid_signature_UCell)

Details

In contrast to Seurat's AddModuleScore, which is normalized by binning genes of similar expression at the population level, UCell scores depend only on the gene expression ranks of individual cell, and therefore they are robust across datasets regardless of dataset composition.

Value

Returns a Seurat object with module/signature enrichment scores added to object meta data; each score is stored as the corresponding signature name provided in features followed by the tag given in name (or "_UCell" by default)

Examples

```
library(UCell)
gene.sets <- list(Tcell = c("CD2","CD3E","CD3D"),
                  Myeloid = c("SPI1","FCER1G","CSF1R"))
data(sample.matrix)
obj <- Seurat::CreateSeuratObject(sample.matrix)

obj <- AddModuleScore_UCell(obj,features = gene.sets)
head(obj[[]])

## Using positive and negative gene sets
gene.sets <- list()
gene.sets$Tcell_gd <- c("TRDC+","TRGC1+","TRGC2+","TRDV1+",
    "TRAC-","TRBC1-","TRBC2-")
gene.sets$NKcell <- c("FGFBP2+", "SPON2+", "KLRF1+",
    "FCGR3A+", "CD3E-","CD3G-")
obj <- AddModuleScore_UCell(obj, features = gene.sets, name=NULL)
head(obj$NKcell)
```

---

calculate_Uscore *Calculate rankings and scores for query data and given signature set*

Description

Calculate rankings and scores for query data and given signature set

Usage

```
calculate_Uscore(
  matrix,
  features,
  maxRank = 1500,
  chunk.size = 100,
  BPPARAM = NULL,
  ncores = 1,
  w_neg = 1,
  ties.method = "average",
  storeRanks = FALSE,
  force.gc = FALSE,
  name = "_UCell"
)
```

Arguments

matrix	Input data matrix
features	List of signatures
maxRank	Rank cutoff (1500)

chunk.size	Cells per sub-matrix (100)
BPPARAM	A BioParallel object to instruct UCell how to parallelize
ncores	Number of cores to use for parallelization
w_neg	Weight on negative signatures
ties.method	How to break ties, for data.table::frankv method ("average")
storeRanks	Store ranks? (FALSE)
force.gc	Force garbage collection? (FALSE)
name	Suffix for metadata columns ("_UCell")

Value

A list of signature scores

`check_genes` *Check genes*

Description

Check if all genes in signatures are found in data matrix - otherwise add zero counts in data-matrix to complete it

Usage

```
check_genes(matrix, features)
```

Arguments

matrix	Input data matrix
features	List of genes that must be present (otherwise they are added)

Value

Same input matrix, extended to comprise any missing genes

check_signature_names *Check signature names and add standard names is missing*

Description

Check signature names and add standard names is missing

Usage

```
check_signature_names(features)
```

Arguments

features	List of signatures for scoring

Value

The input list of signatures, with standard names if provided un-named

data_to_ranks_data_table *Calculate per-cell feature rankings*

Description

Calculate per-cell feature rankings

Usage

```
data_to_ranks_data_table(data, ties.method = "average")
```

Arguments

data	Expression data matrix
ties.method	How to break ties (passed on to data.table::frankv)

Value

A data.table of ranks

knn_smooth_scores *Smoothing scores by KNN*

Description

Smoothing scores by KNN

Usage

```
knn_smooth_scores(matrix = NULL, nn = NULL, decay = 0.1, up.only = FALSE)
```

Arguments

matrix	Input data matrix
nn	A nearest neighbor object returned by BiocNeighbors::findKNN
decay	Exponential decay for nearest neighbor weight: (1-decay)^n
up.only	If set to TRUE, smoothed scores will only be allowed to increase by smoothing

Value

A dataframe of knn-smoothed scores

rankings2Uscore *Get signature scores from pre-computed rank matrix*

Description

Get signature scores from pre-computed rank matrix

Usage

```
rankings2Uscore(
  ranks_matrix,
  features,
  chunk.size = 100,
  w_neg = 1,
  BPPARAM = NULL,
  ncores = 1,
  force.gc = FALSE,
  name = "_UCell"
)
```

Arguments

ranks_matrix	A rank matrix
features	List of signatures
chunk.size	How many cells per matrix chunk
w_neg	Weight on negative signatures
BPPARAM	A BioParallel object to instruct UCell how to parallelize
ncores	How many cores to use for parallelization?
force.gc	Force garbage collection to recover RAM? (FALSE)
name	Name suffix for metadata columns ("_UCell")

Value

A list of signature scores

---

sample.matrix *Sample dataset to test UCell installation*

Description

A sparse matrix (class "dgCMatrix") of single-cell transcriptomes (scRNA-seq) for 30 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 a subsample of T cells from the large scRNA-seq PBMC dataset published by Hao et al. and available as UMI counts at https://atlas.fredhutch.org/data/nygc/multimodal/pbmc_multimodal.h5seurat

Usage

```
sample.matrix
```

Format

A sparse matrix of 30 cells and 20729 genes.

Source

https://doi.org/10.1016/j.cell.2021.04.048

ScoreSignatures_UCell *Calculate module enrichment scores from single-cell data*

Description

Given a gene vs. cell matrix, calculates module/signature enrichment scores on single-cell level using Mann-Whitney U statistic. UCell scores are normalized U statistics (between 0 and 1), and they are mathematically related to the Area under the ROC curve (see Mason and Graham) These scores only depend on the gene expression ranks of individual cell, and therefore they are robust across datasets regardless of dataset composition.

Usage

```
ScoreSignatures_UCell(
  matrix = NULL,
  features,
  precalc.ranks = NULL,
  maxRank = 1500,
  w_neg = 1,
  name = "_UCell",
  assay = "counts",
  chunk.size = 100,
  BPPARAM = NULL,
  ncores = 1,
  ties.method = "average",
  force.gc = FALSE
)
```

Arguments

matrix	Input matrix, either stored in a SingleCellExperiment object or as a raw matrix. dgCMatrix format supported.
features	A list of signatures, for example: list(Tcell_signature = c("CD2","CD3E","CD3D"), Myeloid_signature = c("SPI1","FCER1G","CSF1R")) You can also specify positive and negative gene sets by adding a + or - sign to genes in the signature; see an example below
precalc.ranks	If you have pre-calculated ranks using StoreRankings_UCell, you can specify the pre-calculated ranks instead of the gene vs. cell matrix.
maxRank	Maximum number of genes to rank per cell; above this rank, a given gene is considered as not expressed. Note: this parameter is ignored if precalc.ranks are specified
w_neg	Weight on negative genes in signature. e.g. w_neg=1 weighs equally up- and down-regulated genes, w_neg=0.5 gives 50% less importance to negative genes
name	Name suffix appended to signature names
assay	The sce object assay where the data is to be found

chunk.size	Number of cells to be processed simultaneously (lower size requires slightly more computation but reduces memory demands)
BPPARAM	A BiocParallel::bpparam() object that tells UCell how to parallelize. If provided, it overrides the ncores parameter.
ncores	Number of processors to parallelize computation. If BPPARAM = NULL, the function uses BiocParallel::MulticoreParam(workers=ncores)
ties.method	How ranking ties should be resolved - passed on to data.table::frank
force.gc	Explicitly call garbage collector to reduce memory footprint

Value

Returns input SingleCellExperiment object with UCell scores added to altExp

Examples

```
library(UCell)
# Using sparse matrix
data(sample.matrix)
gene.sets <- list( Tcell_signature = c("CD2","CD3E","CD3D"),
                Myeloid_signature = c("SPI1","FCER1G","CSF1R"))
scores <- ScoreSignatures_UCell(sample.matrix, features=gene.sets)
head(scores)

# Using sce object
library(SingleCellExperiment)
data(sample.matrix)
my.sce <- SingleCellExperiment(list(counts=sample.matrix))
gene.sets <- list( Tcell_signature = c("CD2","CD3E","CD3D"),
                Myeloid_signature = c("SPI1","FCER1G","CSF1R"))
my.sce <- ScoreSignatures_UCell(my.sce, features=gene.sets)
altExp(my.sce, 'UCell')
```

SmoothKNN.Seurat *Smooth signature scores by kNN*

Description

This function performs smoothing of single-cell scores by weighted average of the k-nearest neighbors. It can be useful to 'impute' scores by neighboring cells and partially correct data sparsity. While this function has been designed to smooth UCell scores, it can be applied to any numerical metadata contained in SingleCellExperiment or Seurat objects

Usage

```
## S3 method for class 'Seurat'
SmoothKNN(
  obj = NULL,
  signature.names = NULL,
  reduction = "pca",
  k = 10,
  decay = 0.1,
  up.only = FALSE,
  BNPARAM = AnnoyParam(),
  BPPARAM = SerialParam(),
  suffix = "_kNN",
  assay = NULL,
  slot = "data",
  sce.expname = NULL,
  sce.assay = NULL
)

## S3 method for class 'SingleCellExperiment'
SmoothKNN(
  obj = NULL,
  signature.names = NULL,
  reduction = "PCA",
  k = 10,
  decay = 0.1,
  up.only = FALSE,
  BNPARAM = AnnoyParam(),
  BPPARAM = SerialParam(),
  suffix = "_kNN",
  assay = NULL,
  slot = "data",
  sce.expname = c("UCell", "main"),
  sce.assay = NULL
)

SmoothKNN(
  obj = NULL,
  signature.names = NULL,
  reduction = "pca",
  k = 10,
  decay = 0.1,
  up.only = FALSE,
  BNPARAM = AnnoyParam(),
  BPPARAM = SerialParam(),
  suffix = "_kNN",
  assay = NULL,
  slot = "data",
  sce.expname = c("UCell", "main"),
```

```
  sce.assay = NULL
)
```

Arguments

obj	Input object - either a SingleCellExperiment object or a Seurat object.
signature.names	The names of the signatures (or any numeric metadata column) for which to calculate kNN-smoothed scores
reduction	Which dimensionality reduction to use for kNN smoothing. It must be already present in the input object.
k	Number of neighbors for kNN smoothing
decay	Exponential decay for nearest neighbor weight: (1-decay)^n
up.only	If set to TRUE, smoothed scores will only be allowed to increase by smoothing
BNPARAM	A BiocNeighborParam object specifying the algorithm to use for kNN calculation.
BPPARAM	A `BiocParallel::bpparam()` object for parallel computing, e.g. MulticoreParam or SnowParam
suffix	Suffix to append to metadata columns for the new knn-smoothed scores
assay	For Seurat objects only - do smoothing on expression data from this assay. When NULL, only looks in metadata
slot	For Seurat objects only - do smoothing on expression data from this slot
sce.expname	For sce objects only - which experiment stores the signatures to be smoothed. Set to 'main' for smoothing gene expression stored in the main sce experiment.
sce.assay	For sce objects only - pull data from this assay

Value

An augmented `obj` with the smoothed signatures. If `obj` is a Seurat object, smoothed signatures are added to metadata; if `obj` is a SingleCellExperiment object, smoothed signatures are returned in a new altExp. See the examples below.

Examples

```
#### Using Seurat ####
library(Seurat)
gene.sets <- list(Tcell = c("CD2","CD3E","CD3D"),
                  Myeloid = c("SPI1","FCER1G","CSF1R"))
data(sample.matrix)
obj <- Seurat::CreateSeuratObject(sample.matrix)
# Calculate UCell scores
obj <- AddModuleScore_UCell(obj,features = gene.sets, name=NULL)
# Run PCA
obj <- FindVariableFeatures(obj) |> NormalizeData() |> ScaleData() |> RunPCA(npcs=5)
# Smooth signatures
obj <- SmoothKNN(obj, k=3, signature.names=names(gene.sets))
head(obj[[]])
```

```
#### Using SingleCellExperiment ####
library(SingleCellExperiment)
library(scater)
data(sample.matrix)
sce <- SingleCellExperiment(list(counts=sample.matrix))
gene.sets <- list( Tcell = c("CD2","CD3E","CD3D"),
                   Myeloid = c("SPI1","FCER1G","CSF1R"))
# Calculate UCell scores
sce <- ScoreSignatures_UCell(sce, features=gene.sets, name=NULL)
# Run PCA
sce <- logNormCounts(sce)
sce <- runPCA(sce, scale=TRUE, ncomponents=5)
# Smooth signatures
sce <- SmoothKNN(sce, k=3, signature.names=names(gene.sets))
# See results
altExp(sce, 'UCell')
assays(altExp(sce, 'UCell'))
# Plot on UMAP
sce <- runUMAP(sce, dimred="PCA")
plotUMAP(sce, colour_by = "Tcell_kNN", by_exprs_values = "UCell_kNN")
```

`split_data.matrix` *Split data matrix into smaller sub-matrices ('chunks')*

Description

Split data matrix into smaller sub-matrices ('chunks')

Usage

```
split_data.matrix(matrix, chunk.size = 100)
```

Arguments

`matrix`	Input data matrix
`chunk.size`	How many cells to include in each sub-matrix

Value

A list of sub-matrices, each with size n_features x chunk_size

StoreRankings_UCell *Calculate and store gene rankings for a single-cell dataset*

Description

Given a gene vs. cell matrix, calculates the rankings of expression for all genes in each cell.

Usage

```
StoreRankings_UCell(
  matrix,
  maxRank = 1500,
  chunk.size = 100,
  BPPARAM = NULL,
  ncores = 1,
  assay = "counts",
  ties.method = "average",
  force.gc = FALSE
)
```

Arguments

matrix	Input matrix, either stored in a SingleCellExperiment object or as a raw matrix. dgCMatrix format supported.
maxRank	Maximum number of genes to rank per cell; above this rank, a given gene is considered as not expressed
chunk.size	Number of cells to be processed simultaneously (lower size requires slightly more computation but reduces memory demands)
BPPARAM	A BiocParallel::bpparam() object that tells UCell how to parallelize. If provided, it overrides the ncores parameter.
ncores	Number of processors to parallelize computation. If BPPARAM = NULL, the function uses BiocParallel::MulticoreParam(workers=ncores)
assay	Assay where the data is to be found (for input in 'sce' format)
ties.method	How ranking ties should be resolved - passed on to data.table::frank
force.gc	Explicitly call garbage collector to reduce memory footprint

Details

While ScoreSignatures_UCell can be used 'on the fly' to evaluate signatures in a query dataset, it requires recalculating gene ranks at every execution. If you have a large dataset and plan to experiment with multiple signatures, evaluating the same dataset multiple times, this function allows you to store pre-calculated ranks so they do not have to be recomputed every time. Pre-calculated ranks can then be applied to the function ScoreSignatures_UCell to evaluate gene signatures in a significantly faster way on successive iterations.

Value

Returns a sparse matrix of pre-calculated ranks that can be used multiple times to evaluate different signatures

Examples

```
library(UCell)
data(sample.matrix)
ranks <- StoreRankings_UCell(sample.matrix)
ranks[1:5,1:5]
gene.sets <- list( Tcell_signature = c("CD2","CD3E","CD3D"),
                   Myeloid_signature = c("SPI1","FCER1G","CSF1R"))
scores <- ScoreSignatures_UCell(features=gene.sets, precalc.ranks=ranks)
head(scores)
```

`UCell` *UCell: Robust and scalable single-cell gene signature scoring*

Description

UCell is an R package for scoring gene signatures in single-cell datasets. UCell scores, based on the Mann-Whitney U statistic, are robust to dataset size and heterogeneity, and their calculation demands relatively less computing time and memory than most other methods, enabling the processing of large datasets (> 10^5 cells). UCell can be applied to any cell vs. gene data matrix, and includes functions to directly interact with Seurat and SingleCellExperiment objects.

UCell functions

- `ScoreSignatures_UCell` Calculate module enrichment scores from single-cell data. Given a gene vs. cell matrix (either as sparse matrix or stored in a SingleCellExperiment object), it calculates module/signature enrichment scores. This score depends only on the gene activity ranks of individual cell, and therefore is robust across datasets.
- `AddModuleScore_UCell` A wrapper for UCell to interact directly with Seurat objects. Given a Seurat object and a set of signatures, it calculates enrichment scores on single-cell level and returns them into the meta.data of the input Seurat object.
- `StoreRankings_UCell` Calculates and stores gene rankings for a single-cell dataset. Given a gene vs. cell matrix and a set of signatures, it calculates the rankings of expression for all genes in each cell. It can then be applied to the function ScoreSignatures_UCell to evaluate gene signatures on the gene expression ranks of individual cells.
- `SmoothKNN` Perform signature score smoothing using a weighted average of the scores of the first k nearest neighbors (kNN). It can be useful to 'impute' scores by neighboring cells and partially correct data sparsity. While this function has been designed to smooth UCell scores, it can be applied to any numerical metadata contained in SingleCellExperiment or Seurat objects

Gene signatures

UCell evaluates the strength of gene signatures (or gene sets) in individual cells of your dataset. You may specify positive and negative (up- or down-regulated) genes in signatures. See the examples below:

```
markers <- list()
markers$Tcell_CD4 <- c("CD4","CD40LG")
markers$Tcell_CD8 <- c("CD8A","CD8B")
markers$Tcell_Treg <- c("FOXP3","IL2RA")
markers$Tcell_gd <- c("TRDC+", "TRGC1+", "TRGC2+",
                      "TRDV1+","TRAC-","TRBC1-","TRBC2-")
markers$Tcell_NK <- c("FGFBP2+", "SPON2+", "KLRF1+",
                      "FCGR3A+", "CD3E-","CD3G-")
```

If you don't specify +/- for genes, they are assumed to be all as a positive set. The UCell score is calculated as:

$$U = max(0, U^+ - w_{neg} * U^-)$$

where U^+ and U^- are respectively the UCell scores for the positive and negative set, and w_neg is a weight on the negative set. When no negative set of genes is present, $U = U^+$

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References

UCell: robust and scalable single-cell gene signature scoring. Massimo Andreatta & Santiago J Carmona (2021) CSBJ https://doi.org/10.1016/j.csbj.2021.06.043

See Also

Useful links:

- https://github.com/carmonalab/UCell
- Report bugs at https://github.com/carmonalab/UCell/issues

u_stat *Calculate Mann Whitney U from a vector of ranks*

Description

Maximum sum of ranks, rank_sum_max: len_sig*max_Rank Minimum sum of ranks, rank_sum_min: len_sig*(len_sig + 1)/2 Maximum U statistic, Umax: Maximum sum of ranks - Minimum sum of ranks Minimum U statistic, Umin: 0 Normalized U statistic (0 to 1), Unorm: (U - Umin)/(Umax-Umin) = U/Umax UCell score (0 to 1): 1 - Unorm

Usage

```
u_stat(rank_value, maxRank = 1000, sparse = FALSE)
```

Arguments

rank_value	A vector of ranks
maxRank	Max number of features to include in ranking
sparse	Whether the vector of ranks is in sparse format

Details

Any rank > maxRank is set to maxRank

Value

Normalized U statistic for the vector of ranks

u_stat_signature_list *Calculate U scores for a list of signatures, given a rank matrix*

Description

Calculate U scores for a list of signatures, given a rank matrix

Usage

```
u_stat_signature_list(
  sig_list,
  ranks_matrix,
  maxRank = 1000,
  sparse = FALSE,
  w_neg = 1
)
```

Arguments

sig_list	A list of signatures
ranks_matrix	Matrix of pre-computed ranks
maxRank	Max number of features to include in ranking, for u_stat function
sparse	Whether the vector of ranks is in sparse format
w_neg	Weight on negative signatures

Value

A matrix of U scores

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