Package 'SpatialDecon'

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Title Deconvolution of mixed cells from spatial and/or bulk gene expression data

Version 1.22.0

Description Using spatial or bulk gene expression data, estimates abundance of mixed cell types within each observation. Based on ``Advances in mixed cell deconvolution enable quantification of cell types in spatial transcriptomic data", Danaher (2022). Designed for use with the NanoString GeoMx platform, but applicable to any gene expression data.

Depends R (>= 4.0.0)

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`SpatialDecon-package` *SpatialDecon: A package for estimating mixed cell type abundance in the regions of spatially-resolved gene expression studies*

Description

The SpatialDecon package estimates mixed cell type abundance in the regions of spatially-resolved gene expression studies, using the method of Danaher & Kim (2020), "Advances in mixed cell deconvolution enable quantification of cell types in spatially-resolved gene expression data." It is also appropriate to apply to bulk gene expression data.

functions

Functions to help set up deconvolution:

- derive_GeoMx_background Estimates the background levels from GeoMx experiments
- collapseCellTypes reformats deconvolution results to merge closely-related cell types
- download_profile_matrix Downloads a cell profile matrix.
- safeTME: a data object, a matrix of immune cell profiles for use in tumor-immune deconvolution.

Deconvolution functions:

- spatialdecon runs the core deconvolution function
- reverseDecon runs a transposed/reverse deconvolution problem, fitting the data as a function of cell abundance estimates. Used to measure genes' dependency on cell mixing and to calculate gene residuals from cell mixing.

Plotting functions:

- florets Plot cell abundance on a specified x-y space, with each point a cockscomb plot showing the cell abundances of that region/sample.
- TIL_barplot Plot abundances of tumor infiltrating lymphocytes (TILs) estimated from the safeTME cell profile matrix

Examples

```
data(mini_geomx_dataset)
data(safeTME)
data(safeTME.matches)
# estimate background:
mini_geomx_dataset$bg <- derive_GeoMx_background(
  norm = mini_geomx_dataset$normalized,
  probepool = rep(1, nrow(mini_geomx_dataset$normalized)),
  negnames = "NegProbe"
)
# run basic decon:
res0 <- spatialdecon(
  norm = mini_geomx_dataset$normalized,
  bg = mini_geomx_dataset$bg,
  X = safeTME
)
# run decon with bells and whistles:
res <- spatialdecon(
  norm = mini_geomx_dataset$normalized,
  bg = mini_geomx_dataset$bg,
  X = safeTME,
  cellmerges = safeTME.matches,
  cell_counts = mini_geomx_dataset$annot$nuclei,
  is_pure_tumor = mini_geomx_dataset$annot$AOI.name == "Tumor"
)
```

---

cellcols *Default colors for the cell types in the safeTME matrix*

Description

A named vector of colors, giving colors for the cell types of the safeTME matrix.

Usage

```
cellcols
```

Format

A named vector

`collapseCellTypes` *Collapse related cell types within a deconvolution result*

Description

Given the input of an SpatialDecon result output and a list of which cell types to combine, returns a reshaped deconvolution result object with the specified cell types merged.

Usage

```
collapseCellTypes(fit, matching)
```

Arguments

`fit`	The object (a list) returned by the SpatialDecon algorithm
`matching`	A list object holding the mapping from beta's cell names to official cell names. See str(safeTME.matches) for an example.

Value

A reshaped deconvolution result object

Examples

```
data(mini_geomx_dataset)
data(safeTME)
data(safeTME.matches)
# estimate background:
mini_geomx_dataset$bg <- derive_GeoMx_background(
  norm = mini_geomx_dataset$normalized,
  probepool = rep(1, nrow(mini_geomx_dataset$normalized)),
  negnames = "NegProbe"
)
# run basic decon:
res0 <- spatialdecon(
  norm = mini_geomx_dataset$normalized,
  bg = mini_geomx_dataset$bg,
  X = safeTME
)
res1 <- collapseCellTypes(
    fit = res0,
    matching = safeTME.matches
)
```

`create_profile_matrix` *Create Custom Cell Profile Matrix*

Description

Create custom cell profile matrix using single cell data. The average gene expression for each cell type is returned.

Usage

```
create_profile_matrix(
  mtx,
  cellAnnots,
  cellTypeCol,
  cellNameCol,
  matrixName = "Custom",
  outDir = "./",
  geneList = NULL,
  normalize = FALSE,
  scalingFactor = 5,
  minCellNum = 15,
  minGenes = 100,
  discardCellTypes = FALSE
)
```

Arguments

`mtx`	gene x cell count matrix
`cellAnnots`	cell annotations with cell type and cell name as columns
`cellTypeCol`	column containing cell type
`cellNameCol`	column containing cell ID/name
`matrixName`	name of final profile matrix
`outDir`	path to desired output directory, set to NULL if matrix should not be written
`geneList`	gene list to filter profile matrix to
`normalize`	Should data be normalized? (TRUE/FALSE) if TRUE data will be normalize using total gene count
`scalingFactor`	what should all values be multiplied by for final matrix, set to 1 if no scaling is wanted
`minCellNum`	minimum number of cells of one type needed to create profile, exclusive
`minGenes`	minimum number of genes expressed in a cell, exclusive
`discardCellTypes`	should cell types be filtered for types like mitotic, doublet, low quality, unknown, etc.

Value

A custom cell profile matrix genes (rows) by cell types (columns), matrix gets written to disk and outDir

Examples

```
cellNames <- paste0("Cell", seq_len(1500))
geneNames <- paste0("Gene", seq_len(1500))
mtx <- matrix(data=sample(size = length(cellNames)*length(geneNames),
                          replace = TRUE,
                          x = c(0,seq_len(100)),
                          prob = c(0.6784, rep(0.0075, 15), rep(0.005, 25),
                                   rep(0.002, 25), rep(0.001, 35))),
                          ncol = length(cellNames), nrow = length(geneNames),
                          dimnames = list(geneNames, cellNames))
cellAnnots <- as.data.frame(cbind(CellID=cellNames,
                                  cellType=sample(size = length(cellNames),
                                                  replace = TRUE,
                                                  x = c("A", "B", "C", "D"),
                                                  prob = c(0.1, 0.4, 0.3, 0.2))))
table(cellAnnots$cellType)
profile_matrix <- create_profile_matrix(mtx = mtx,
                                        cellAnnots = cellAnnots,
                                        cellTypeCol = "cellType",
                                        cellNameCol = "CellID",
                                        minGenes = 10,
                                        scalingFactor = 1)
head(profile_matrix)
```

`derive_GeoMx_background` *Derive background at the scale of the normalized data for GeoMx data*

Description

Estimates per-datapoint background levels from a GeoMx experiment. In studies with two or more probe pools, different probes will have different background levels. This function provides a convenient way to account for this phenomenon.

Usage

```
derive_GeoMx_background(norm, probepool, negnames)
```

Arguments

`norm`	Matrix of normalized data, genes in rows and segments in columns. Must include negprobes, and must have rownames.
`probepool`	Vector of probe pool names for each gene, aligned to the rows of "norm".
`negnames`	Names of all negProbes in the dataset. Must be at least one neg.name within each probe pool.

Value

A matrix of expected background values, in the same scale and dimensions as the "norm" argument.

Examples

```
data(mini_geomx_dataset)
# estimate background:
mini_geomx_dataset$bg <- derive_GeoMx_background(
  norm = mini_geomx_dataset$normalized,
  probepool = rep(1, nrow(mini_geomx_dataset$normalized)),
  negnames = "NegProbe"
)
```

`download_profile_matrix` *Download a cell profile matrix*

Description

Download a cell profile matrix from the online library

Usage

```
download_profile_matrix(species, age_group, matrixname)
```

Arguments

`species`	species of profile matrix
`age_group`	age_group of profile matrix, if fetal mouse please add the developmental stage separated with /, i.e. Fetal/E14.5
`matrixname`	name of profile matrix

Details

Valid matrices can be found on the github site https://github.com/Nanostring-Biostats/CellProfileLibrary/tree/master

Value

A cell profile matrix, suggested cell groups, and paper metadata

Examples

```
download_profile_matrix(species = "Human", age_group = "Adult", matrixname = "Colon_HCA")
head(profile_matrix)
print(cellGroups)
print(metadata)
```

florets *Draw coxcomb plots as points in a graphics window*

Description

Draws a scatterplot where each point is a circular barplot, intended to show decon results

Usage

```
florets(
  x,
  y,
  b,
  col = NULL,
  legendwindow = FALSE,
  rescale.by.sqrt = TRUE,
  border = NA,
  add = FALSE,
  cex = 1,
  bty = "n",
  xaxt = "n",
  yaxt = "n",
  xlab = "",
  ylab = "",
  ...
)
```

Arguments

x	Vector of x coordinates
y	Vector of y coordinates
b	matrix or cell abundances, with columns aligned with the elements of x and y
col	vector of colors, aligned to the rows of b.
legendwindow	Logical. If TRUE, the function draws a color legend in a new window
rescale.by.sqrt	Logical, for whether to rescale b by its square root to make value proportional to shape area, not shape length.
border	Color of pie segment border, defauls to NA/none
add	Logical. If TRUE, the function draws florets atop an existing graphics device (TRUE) or call a new device (FALSE).
cex	Floret size. Florets are scaled relative to the range of x and y; this further scales up or down.
bty	bty argument passed to plot()
xaxt	xaxt argument passed to plot()

yaxt	yaxt argument passed to plot()
xlab	xlab, defaults to ""
ylab	ylab, defaults to ""
...	additional arguments passed to plot()

Value

Draws a coxcomb plot, returns no data.

Examples

```
data(mini_geomx_dataset)
data(safeTME)
# estimate background:
mini_geomx_dataset$bg <- derive_GeoMx_background(
  norm = mini_geomx_dataset$normalized,
  probepool = rep(1, nrow(mini_geomx_dataset$normalized)),
  negnames = "NegProbe"
)
# run basic decon:
res0 <- spatialdecon(
  norm = mini_geomx_dataset$normalized,
  bg = mini_geomx_dataset$bg,
  X = safeTME
)
# draw florets:
florets(
  x = mini_geomx_dataset$annot$x,
  y = mini_geomx_dataset$annot$y,
  b = res0$beta, cex = 2
)
```

---

mean.resid.sd *Genes' biological variability in immune deconvolution from TCGA.*

Description

Genes' biological SDs, as estimated from immune deconvolution from TCGA. Used to weight genes in spatialdecon.

Usage

```
mean.resid.sd
```

Format

A named vector giving SDs of 1179 genes.

`mergeTumorIntoX` *Estimate a tumor-specific profile and merge it with the pre-specified cell profile matrix (X)*

Description

Given the input of "tumor-only" AOI's, estimates an collection of tumor-specific expression profiles and merges them with the immune cell expression training matrix. The process:

1. log2/normalized data from tumor-only AOIs is clustered with hclust, and cutree() is used to define clusters.
2. 2. Each cluster's geomean profile is merged into the immune cell profile matrix.

Usage

```
mergeTumorIntoX(norm, bg, pure_tumor_ids, X, K = 10)
```

Arguments

`norm`	matrix of normalized data
`bg`	matrix of expected background, on the scale of norm.
`pure_tumor_ids`	Vector identifying columns of norm that are pure tumor. Can be indices, logicals or column names.
`X`	The training matrix
`K`	the number of clusters to fit

Value

an updated X matrix with new columns, "tumor.1", "tumor.2", ...

Examples

```
data(mini_geomx_dataset)
data(safeTME)
mini_geomx_dataset$bg <- derive_GeoMx_background(
  norm = mini_geomx_dataset$normalized,
  probepool = rep(1, nrow(mini_geomx_dataset$normalized)),
  negnames = "NegProbe"
)
safeTME.with.tumor <- mergeTumorIntoX(
  norm = mini_geomx_dataset$norm,
  bg = mini_geomx_dataset$bg,
  pure_tumor_ids = mini_geomx_dataset$annot$AOI.name == "Tumor",
  X = safeTME,
  K = 3
)
```

mini_geomx_dataset *Small example GeoMx data*

Description

A miniature GeoMx dataset used by the spatialdecon examples.

Usage

```
mini_geomx_dataset
```

Format

A list with the following elements:

- normalized: normalized data matrix
- raw: raw data matrix
- annot: AOI annotation data frame

mini_singleCell_dataset *Mini human colon single cell dataset*

Description

Random 250 cells and most informative genes (CV > 10) between cell types from Kinchen, J. et al. Structural Remodeling of the Human Colonic Mesenchyme in Inflammatory Bowel Disease. Cell 175, 372-386.e17 (2018).

Usage

```
mini_singleCell_dataset
```

Format

A list with the following elements:

- mtx: sparse count matrix
- annots: cell type annotation data frame

nsclc *Large example GeoMx data*

Description

A GeoMx dataset with dense AOIs gridded over a NSCLC tumor. Each AOI is split into tumor and microenvironment segments.

Usage

```
nsclc
```

Format

GeoMxSet Object

reverseDecon *Reverse deconvolution*

Description

Performs "reverse deconvolution", modelling each gene expression's ~ cell scores. Returns a matrix of "fitted" expression values, a matrix of residuals, a matrix of reverse decon coefficients for genes * cells.

Usage

```
reverseDecon(norm, beta, epsilon = NULL)
```

Arguments

norm	Matrix of normalized data, with genes in rows and observations in columns
beta	Matrix of cell abundance estimates, with cells in rows and observations in columns. Columns are aligned to "norm".
epsilon	All y and yhat values are thresholded up to this point when performing decon. Essentially says, "ignore variability in counts below this threshold."

Value

A list:

- coefs, a matrix of coefficients for genes * cells, where element i,j is interpreted as "every unit increase in cell score j is expected to increase expression of gene i by _".
- yhat, a matrix of fitted values, in the same dimension as norm

- resids, a matrix of log2-scale residuals from the reverse decon fit, in the same dimension as norm
- cors, a vector giving each gene's correlation between fitted and observed expression
- resid.sd, a vector of each gene's residual SD, a metric of how much variability genes have independend of cell mixing.

Examples

```
data(mini_geomx_dataset)
data(safeTME)
# estimate background:
mini_geomx_dataset$bg <- derive_GeoMx_background(
  norm = mini_geomx_dataset$normalized,
  probepool = rep(1, nrow(mini_geomx_dataset$normalized)),
  negnames = "NegProbe"
)
# run basic decon:
res0 <- spatialdecon(
  norm = mini_geomx_dataset$normalized,
  bg = mini_geomx_dataset$bg,
  X = safeTME
)
# run reverse decon:
rdecon <- reverseDecon(
  norm = mini_geomx_dataset$norm,
  beta = res0$beta
)
```

---

runCollapseCellTypes *Run collapseCellTypes*

Description

Runs collapseCellTypes from an S4 object

Given the input of an SpatialDecon result output and a list of which cell types to combine, returns a reshaped deconvolution result object with the specified cell types merged.

Usage

```
runCollapseCellTypes(object, ...)

## S4 method for signature 'NanoStringGeoMxSet'
runCollapseCellTypes(object, matching = NULL)
```

Arguments

object	An S4 object such as a GeoMxSet object
...	Arguments passed to collapseCellTypes
matching	A list object holding the mapping from beta's cell names to official cell names. See str(safeTME.matches) for an example.

Value

A reshaped deconvolution result object

Examples

```
library(GeomxTools)
datadir <- system.file("extdata", "DSP_NGS_Example_Data", package = "GeomxTools")
demoData <- readRDS(file.path(datadir, "/demoData.rds"))

demoData <- shiftCountsOne(demoData)
target_demoData <- aggregateCounts(demoData)

target_demoData <- normalize(target_demoData, "quant")

# run basic decon:
res0 <- runspatialdecon(object = target_demoData,
                        norm_elt = "exprs_norm",
                        raw_elt = "exprs")

# run reverse decon:
target_demoData <- runReverseDecon(object = target_demoData,
                                   norm_elt = "exprs_norm",
                                   beta = pData(res0)$beta)
```

---

runErrorModel *Apply error model to estimate technical SD from raw counts*

Description

Based on raw counts, uses past data to estimate each raw count's log-scale SD from technical noise. Specifies different error models for different platforms.

Usage

```
runErrorModel(counts, platform = "general")
```

Arguments

counts	vector or matrix of raw counts
platform	String specifying which platform was used to create "rawCounts". Default to "dsp", for digital spatial profiler/ aka GeoMx. Other options include "ncounter", "rsem", "quantile", and "st" for spatial transcriptomics/visium.

Value

a matrix of log2-scale SDs

Examples

```
library(GeomxTools)
datadir <- system.file("extdata", "DSP_NGS_Example_Data", package = "GeomxTools")
demoData <- readRDS(file.path(datadir, "/demoData.rds"))

demoData <- shiftCountsOne(demoData)
target_demoData <- aggregateCounts(demoData)

sd_from_noise <- runErrorModel(counts = exprs(target_demoData), platform = "dsp")
wts <- 1 / sd_from_noise
```

---

runMergeTumorIntoX *Run MergeTumorIntoX*

Description

Runs mergeTumorIntoX from an S4 object

A wrapper for applying mergeTumorIntoX to a NanostringGeomxSet object.

Usage

```
runMergeTumorIntoX(object, ...)

## S4 method for signature 'NanoStringGeoMxSet'
runMergeTumorIntoX(object, X, K = 10, pure_tumor_ids = NULL, norm_elt = NULL)
```

Arguments

object	An S4 object such as a GeoMxSet object
...	Arguments passed to mergeTumorIntoX
X	The training matrix
K	the number of clusters to fit
pure_tumor_ids	Vector identifying columns of norm that are pure tumor. Can be indices, logicals or column names.
norm_elt	norm data element in assayData

Value

updated X matrix with new columns, "tumor.1", "tumor.2", ...

Examples

```
library(GeomxTools)
datadir <- system.file("extdata", "DSP_NGS_Example_Data", package = "GeomxTools")
demoData <- readRDS(file.path(datadir, "/demoData.rds"))

demoData <- shiftCountsOne(demoData)
target_demoData <- aggregateCounts(demoData)

target_demoData <- normalize(target_demoData, "quant")

data(safeTME)
tumor.ids <- as.logical(sample(x = c("TRUE","FALSE"), size = 88, replace = TRUE))
safeTME.with.tumor <- runMergeTumorIntoX(object = target_demoData,
                                         X = safeTME,
                                         K = 3,
                                         pure_tumor_ids = tumor.ids,
                                         norm_elt = "exprs_norm")
```

---

runReverseDecon *Run Reversedecon*

Description

Runs reversedecon from an S4 object

A wrapper for applying reversedecon to a NanostringGeomxSet object.

Usage

```
runReverseDecon(object, ...)

## S4 method for signature 'NanoStringGeoMxSet'
runReverseDecon(object, norm_elt = NULL, beta, epsilon = NULL)
```

Arguments

object	An S4 object such as a GeoMxSet object
...	Arguments passed to reversedecon
norm_elt	normalized data element in assayData.
beta	Matrix of cell abundance estimates, with cells in columns and observations in rows. Columns are aligned to "norm".
epsilon	All y and yhat values are thresholded up to this point when performing decon. Essentially says, "ignore variability in counts below this threshold."

Value

a valid GeoMx S4 object including the following items:

- in fData
  - coefs, a matrix of coefficients for genes * cells, where element i,j is interpreted as "every unit increase in cell score j is expected to increase expression of gene i by _".
  - cors, a vector giving each gene's correlation between fitted and observed expression
  - resid.sd, a vector of each gene's residual SD, a metric of how much variability genes have independend of cell mixing.
- in assayData
  - yhat, a matrix of fitted values, in the same dimension as norm
  - resids, a matrix of log2-scale residuals from the reverse decon fit, in the same dimension as norm

Examples

```
library(GeomxTools)
datadir <- system.file("extdata", "DSP_NGS_Example_Data", package = "GeomxTools")
demoData <- readRDS(file.path(datadir, "/demoData.rds"))

demoData <- shiftCountsOne(demoData)
target_demoData <- aggregateCounts(demoData)

target_demoData <- normalize(target_demoData, "quant")

# run basic decon:
res0 <- runspatialdecon(object = target_demoData,
                        norm_elt = "exprs_norm",
                        raw_elt = "exprs")

# run reverse decon:
target_demoData <- runReverseDecon(object = target_demoData,
                                   norm_elt = "exprs_norm",
                                   beta = pData(res0)$beta)
```

`runspatialdecon` *Run spatialdecon*

Description

Runs spatialdecon from an S4 object

A wrapper for applying spatialdecon to a NanostringGeomxSet object.

A wrapper for applying spatialdecon to the Spatial data element in a Seurat object. Unlike spatialdecon, which expects a normalized data matrix, this function operates on raw counts. Scaling for total cells

Usage

```
runspatialdecon(object, ...)

## S4 method for signature 'NanoStringGeoMxSet'
runspatialdecon(
  object,
  X = NULL,
  norm_elt = NULL,
  raw_elt = NULL,
  wts = NULL,
  resid_thresh = 3,
  lower_thresh = 0.5,
  align_genes = TRUE,
  is_pure_tumor = NULL,
  n_tumor_clusters = 10,
  cell_counts = NULL,
  cellmerges = NULL,
  maxit = 1000
)

## S4 method for signature 'Seurat'
runspatialdecon(
  object,
  X = NULL,
  bg = 0.1,
  wts = NULL,
  resid_thresh = 3,
  lower_thresh = 0.5,
  align_genes = TRUE,
  is_pure_tumor = NULL,
  n_tumor_clusters = 10,
  cell_counts = NULL,
  cellmerges = NULL,
  maxit = 1000
)
```

Arguments

object	An S4 object such as a Seurat object that includse a "Spatial" element in the "assays" slot or a GeoMxSet object
...	Arguments passed to spatialdecon
X	Cell profile matrix. If NULL, the safeTME matrix is used.
norm_elt	normalized data element in assayData in NanostringGeomxSet object
raw_elt	raw data element in assayData in NanostringGeomxSet object
wts	Optional, a matrix of weights.
resid_thresh	A scalar, sets a threshold on how extreme individual data points' values can be (in log2 units) before getting flagged as outliers and set to NA.

`lower_thresh` A scalar. Before log2-scale residuals are calculated, both observed and fitted values get thresholded up to this value. Prevents log2-scale residuals from becoming extreme in points near zero.

`align_genes` Logical. If TRUE, then Y, X, bg, and wts are row-aligned by shared genes.

`is_pure_tumor` A logical vector denoting whether each AOI consists of pure tumor. If specified, then the algorithm will derive a tumor expression profile and merge it with the immune profiles matrix.

`n_tumor_clusters`
Number of tumor-specific columns to merge into the cell profile matrix. Has an impact only when is_pure_tumor argument is used to indicate pure tumor AOIs. Takes this many clusters from the pure-tumor AOI data and gets the average expression profile in each cluster. Default 10.

`cell_counts` Number of cells estimated to be within each sample. If provided alongside norm_factors, then the algorithm will additionally output cell abundance esimtates on the scale of cell counts.

`cellmerges` A list object holding the mapping from beta's cell names to combined cell names. If left NULL, then defaults to a mapping of granular immune cell definitions to broader categories.

`maxit` Maximum number of iterations. Default 1000.

`bg` Expected background counts. Either a scalar applied equally to all points in the count matrix, or a matrix with the same dimensions as the count matrix in GetAssayData(object, assay = "Spatial"). Recommended to use a small non-zero value, default of 0.1.

Value

decon results in list or in GeoMxSet object

For GeoMxSet object, if not given cellmerges and cell_counts, a valid GeoMx S4 object including the following items

- In pData
  - beta: matrix of cell abundance estimates, cells in rows and observations in columns
  - p: matrix of p-values for H0: beta == 0
  - t: matrix of t-statistics for H0: beta == 0
  - se: matrix of standard errors of beta values
  - prop_of_all: rescaling of beta to sum to 1 in each observation
  - prop_of_nontumor: rescaling of beta to sum to 1 in each observation, excluding tumor abundance estimates
  - sigmas: covariance matrices of each observation's beta estimates
- In assayData
  - yhat: a matrix of fitted values
  - resids: a matrix of residuals from the model fit. (log2(pmax(y, lower_thresh)) - log2(pmax(xb, lower_thresh))).
- In experimentData

  - SpatialDeconMatrix: the cell profile matrix used in the decon fit.

if given cellmerges, the valid GeoMx S4 object will additionally include the following items

- In pData
  - beta.granular: cell abundances prior to combining closely-related cell types
  - sigma.granular: sigmas prior to combining closely-related cell types

if given cell_counts, the valid GeoMx S4 object will additionally include the following items

- In pData
  - cell.counts: beta rescaled to estimate cell numbers, based on prop_of_all and nuclei count

if given both cellmerges and cell_counts, the valid GeoMx S4 object will additionally include the following items

- In pData
  - cell.counts.granular: cell.counts prior to combining closely-related cell types

For Seurat Object, if not given cellmerges and cell_counts, a list including the following items:

- beta: matrix of cell abundance estimates, cells in rows and observations in columns
- p: matrix of p-values for H0: beta == 0
- t: matrix of t-statistics for H0: beta == 0
- se: matrix of standard errors of beta values
- prop_of_all: rescaling of beta to sum to 1 in each observation
- prop_of_nontumor: rescaling of beta to sum to 1 in each observation, excluding tumor abundance estimates
- yhat: a matrix of fitted values
- resids: a matrix of residuals from the model fit. (log2(pmax(y, lower_thresh)) - log2(pmax(xb, lower_thresh))).
- X: the cell profile matrix used in the decon fit.
- sigmas: covariance matrices of each observation's beta estimates

if given cellmerges, the list will additionally include the following items

- beta.granular: cell abundances prior to combining closely-related cell types
- sigma.granular: sigmas prior to combining closely-related cell types

if given cell_counts, the list will additionally include the following items

- cell.counts: beta rescaled to estimate cell numbers, based on prop_of_all and nuclei count

if given both cellmerges and cell_counts, the list will additionally include the following items

- cell.counts.granular: cell.counts prior to combining closely-related cell types

Examples

```
## GeoMxSet Object ##
library(GeomxTools)
datadir <- system.file("extdata", "DSP_NGS_Example_Data", package = "GeomxTools")
demoData <- readRDS(file.path(datadir, "/demoData.rds"))

demoData <- shiftCountsOne(demoData)
target_demoData <- aggregateCounts(demoData)

target_demoData <- normalize(target_demoData, "quant")

demoData <- runspatialdecon(object = target_demoData,
                            norm_elt = "exprs_norm",
                            raw_elt = "exprs")

## Seurat Object ##
# get dataset
con <- gzcon(url("https://github.com/almaan/her2st/raw/master/data/ST-cnts/G1.tsv.gz"))
txt <- readLines(con)
temp <- read.table(textConnection(txt), sep = "\t", header = TRUE, row.names = 1)
# parse data
raw = t(as.matrix(temp))
norm = sweep(raw, 2, colSums(raw), "/") * mean(colSums(raw))
x = as.numeric(substr(rownames(temp), 1, unlist(gregexpr("x", rownames(temp))) - 1))
y = -as.numeric(substr(rownames(temp),
                 unlist(gregexpr("x", rownames(temp))) + 1, nchar(rownames(temp))))
# put into a seurat object:
andersson_g1 = SeuratObject::CreateSeuratObject(counts = raw, assay="Spatial")
andersson_g1@meta.data$x = x
andersson_g1@meta.data$y = y

res <- runspatialdecon(andersson_g1)
str(res)
```

`safeTME` *SafeTME matrix*

Description

A matrix of expression profiles of 906 genes over 18 cell types.

Usage

```
safeTME
```

Format

A matrix with 906 genes (rows) and 18 cell types (columns)

safeTME.matches — *Mapping from granularly-defined cell populations to broaded cell populations*

Description

Mapping from granularly-defined cell populations to broaded cell populations, for use by the convertCellTypes function.

Usage

```
safeTME.matches
```

Format

A list. Each element of the list contains the granular cell types that roll up to a single coarse cell type.

spatialdecon — *Mixed cell deconvolution of spatiall-resolved gene expression data*

Description

Runs the spatialdecon algorithm with added optional functionalities. Workflow is:

1. compute weights from raw data
2. Estimate a tumor profile and merge it into the cell profiles matrix
3. run deconvolution once
4. remove poorly-fit genes from first round of decon
5. re-run decon with cleaned-up gene set
6. combine closely-related cell types
7. compute p-values
8. rescale abundance estimates, to proportions of total, proportions of immune, cell counts

Usage

```
spatialdecon(
  norm,
  bg,
  X = NULL,
  raw = NULL,
  wts = NULL,
  resid_thresh = 3,
  lower_thresh = 0.5,
```

```
  align_genes = TRUE,
  is_pure_tumor = NULL,
  n_tumor_clusters = 10,
  cell_counts = NULL,
  cellmerges = NULL,
  maxit = 1000
)
```

Arguments

norm	p-length expression vector or p * N expression matrix - the actual (linear-scale) data
bg	Same dimension as norm: the background expected at each data point.
X	Cell profile matrix. If NULL, the safeTME matrix is used.
raw	Optional for using an error model to weight the data points. p-length expression vector or p * N expression matrix - the raw (linear-scale) data
wts	Optional, a matrix of weights.
resid_thresh	A scalar, sets a threshold on how extreme individual data points' values can be (in log2 units) before getting flagged as outliers and set to NA.
lower_thresh	A scalar. Before log2-scale residuals are calculated, both observed and fitted values get thresholded up to this value. Prevents log2-scale residuals from becoming extreme in points near zero.
align_genes	Logical. If TRUE, then Y, X, bg, and wts are row-aligned by shared genes.
is_pure_tumor	A logical vector denoting whether each AOI consists of pure tumor. If specified, then the algorithm will derive a tumor expression profile and merge it with the immune profiles matrix.
n_tumor_clusters	Number of tumor-specific columns to merge into the cell profile matrix. Has an impact only when is_pure_tumor argument is used to indicate pure tumor AOIs. Takes this many clusters from the pure-tumor AOI data and gets the average expression profile in each cluster. Default 10.
cell_counts	Number of cells estimated to be within each sample. If provided alongside norm_factors, then the algorithm will additionally output cell abundance esimtates on the scale of cell counts.
cellmerges	A list object holding the mapping from beta's cell names to combined cell names. If left NULL, then defaults to a mapping of granular immune cell definitions to broader categories.
maxit	Maximum number of iterations. Default 1000.

Value

a list:

- beta: matrix of cell abundance estimates, cells in rows and observations in columns
- sigmas: covariance matrices of each observation's beta estimates

- p: matrix of p-values for H0: beta == 0
- t: matrix of t-statistics for H0: beta == 0
- se: matrix of standard errors of beta values
- prop_of_all: rescaling of beta to sum to 1 in each observation
- prop_of_nontumor: rescaling of beta to sum to 1 in each observation, excluding tumor abundance estimates
- cell.counts: beta rescaled to estimate cell numbers, based on prop_of_all and nuclei count
- beta.granular: cell abundances prior to combining closely-related cell types
- sigma.granular: sigmas prior to combining closely-related cell types
- cell.counts.granular: cell.counts prior to combining closely-related cell types
- resids: a matrix of residuals from the model fit. (log2(pmax(y, lower_thresh)) - log2(pmax(xb, lower_thresh))).
- X: the cell profile matrix used in the decon fit.

Examples

```
data(mini_geomx_dataset)
data(safeTME)
data(safeTME.matches)
# estimate background:
mini_geomx_dataset$bg <- derive_GeoMx_background(
  norm = mini_geomx_dataset$normalized,
  probepool = rep(1, nrow(mini_geomx_dataset$normalized)),
  negnames = "NegProbe"
)
# run basic decon:
res0 <- spatialdecon(
  norm = mini_geomx_dataset$normalized,
  bg = mini_geomx_dataset$bg,
  X = safeTME
)
# run decon with bells and whistles:
res <- spatialdecon(
  norm = mini_geomx_dataset$normalized,
  bg = mini_geomx_dataset$bg,
  X = safeTME,
  cellmerges = safeTME.matches,
  cell_counts = mini_geomx_dataset$annot$nuclei,
  is_pure_tumor = mini_geomx_dataset$annot$AOI.name == "Tumor"
)
```

TIL_barplot *Barplot of abundance estimates*

Description

Draw barplot of the "betas" from a decon fit

Usage

```
TIL_barplot(mat, draw_legend = FALSE, main = "", col = NULL, ...)
```

Arguments

mat	Matrix of cell proportions or abundances, in the same dimensions output by spatialdecon (cells in rows, observations in columns). User is free to re-order columns/observations in whatever order is best for display.
draw_legend	Logical. If TRUE, the function draws a legend in a new plot frame.
main	Title for barplot
col	Vector of colors for cell types. Defaults to pre-set colors for the safeTME cell types.
...	Arguments passed to barplot()

Value

Draws a barplot.

Examples

```
data(mini_geomx_dataset)
data(safeTME)
# estimate background:
mini_geomx_dataset$bg <- derive_GeoMx_background(
  norm = mini_geomx_dataset$normalized,
  probepool = rep(1, nrow(mini_geomx_dataset$normalized)),
  negnames = "NegProbe"
)
# run basic decon:
res0 <- spatialdecon(
  norm = mini_geomx_dataset$normalized,
  bg = mini_geomx_dataset$bg,
  X = safeTME
)
# run barplot:
TIL_barplot(mat = res0$beta)
# run barplot and draw a color legend
TIL_barplot(mat = res0$beta, draw_legend = TRUE)
```

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