Package 'scDataviz'

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Type Package

Title scDataviz: single cell dataviz and downstream analyses

Version 1.22.0

Description In the single cell World, which includes flow cytometry, mass cytometry, single-cell RNA-seq (scRNA-seq), and others, there is a need to improve data visualisation and to bring analysis capabilities to researchers even from non-technical backgrounds. scDataviz attempts to fit into this space, while also catering for advanced users. Additonally, due to the way that scDataviz is designed, which is based on SingleCellExperiment, it has a 'plug and play' feel, and immediately lends itself as flexibile and compatibile with studies that go beyond scDataviz. Finally, the graphics in scDataviz are generated via the ggplot engine, which means that users can 'add on' features to these with ease.

BugReports https://github.com/kevinblighe/scDataviz/issues

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Depends R (>= 4.0), S4Vectors, SingleCellExperiment,

Imports ggplot2, ggrepel, flowCore, umap, Seurat, reshape2, scales, RColorBrewer, corrplot, stats, grDevices, graphics, utils, MASS, matrixStats, methods

Suggests PCAtools, cowplot, BiocGenerics, RUnit, knitr, kableExtra, rmarkdown

URL https://github.com/kevinblighe/scDataviz

biocViews SingleCell, ImmunoOncology, RNASeq, GeneExpression, Transcription, FlowCytometry, MassSpectrometry, DataImport

VignetteBuilder knitr

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`basetheme` *Package-wide, non-user function used to set a base* `ggplot2` *theme.*

Description

Package-wide, non-user function used to set a base `ggplot2` theme.

Usage

```
basetheme(
  titleLabSize,
  subtitleLabSize,
  captionLabSize,
  axisLabSize,
  xlabAngle,
  xlabhjust,
  xlabvjust,
  ylabAngle,
  ylabhjust,
  ylabvjust,
  legendPosition,
  legendLabSize
)
```

Arguments

titleLabSize	Size of plot title.
subtitleLabSize	Size of plot subtitle.
captionLabSize	Size of plot caption.
axisLabSize	Size of x- and y-axis labels.
xlabAngle	Rotation angle of x-axis labels.
xlabhjust	Horizontal adjustment of x-axis labels.
xlabvjust	Vertical adjustment of x-axis labels.
ylabAngle	Rotation angle of y-axis labels.
ylabhjust	Horizontal adjustment of y-axis labels.
ylabvjust	Vertical adjustment of y-axis labels.
legendPosition	Position of legend ('top', 'bottom','left', 'right', 'none').
legendLabSize	Size of plot legend text.

Details

Package-wide, non-user function used to set a base ggplot2 theme.

Value

A list object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create a theme
th <- basetheme(
  titleLabSize = 16,
  subtitleLabSize = 12,
  captionLabSize = 12,
  axisLabSize = 16,
  xlabAngle = 0,
  xlabhjust = 0.5,
  xlabvjust = 0.5,
  ylabAngle = 0,
  ylabhjust = 0.5,
  ylabvjust = 0.5,
  legendPosition = 'none',
  legendLabSize = 12)
```

clusKNN *A wrapper function for* Seurat*'s* FindNeighbors *and* FindClusters.

Description

A wrapper function for Seurat's FindNeighbors and FindClusters.

Usage

```
clusKNN(
  indata,
  reducedDim = "UMAP",
  dimColnames = c("UMAP1", "UMAP2"),
  clusterAssignName = "Cluster",
  distance.matrix = FALSE,
  k.param = 20,
  compute.SNN = TRUE,
  prune.SNN = 1/15,
  nn.method = "rann",
  annoy.metric = "euclidean",
  nn.eps = 0,
  verbose = TRUE,
  force.recalc = FALSE,
  modularity.fxn = 1,
  initial.membership = NULL,
  weights = NULL,
  node.sizes = NULL,
  resolution = 0.8,
  method = "matrix",
  algorithm = 1,
  n.start = 10,
  n.iter = 10,
  random.seed = 0,
  group.singletons = TRUE,
  temp.file.location = NULL,
  edge.file.name = NULL,
  overwrite = FALSE
)
```

Arguments

indata	A data-frame or matrix, or SingleCellExperiment object. If a SingleCellExperiment object, the cell-to-cluster assignments will be added as a new column, specified by clusterAssignName, to the input object's metadata; if a data-frame or matrix, only the cluster assignment vector is returned.
reducedDim	A reduced dimensional component stored within indata. e.g., PCA or UMAP.

`dimColnames` The column names of the dimensions to use.

`clusterAssignName`
The new column name in the metadata that will contain the determined cell-to-cluster assignments.

`distance.matrix`
Refer to `?Seurat::FindNeighbors`.

`k.param` Refer to `?Seurat::FindNeighbors`.

`compute.SNN` Refer to `?Seurat::FindNeighbors`.

`prune.SNN` Refer to `?Seurat::FindNeighbors`.

`nn.method` Refer to `?Seurat::FindNeighbors`.

`annoy.metric` Refer to `?Seurat::FindNeighbors`.

`nn.eps` Refer to `?Seurat::FindNeighbors`.

`verbose` Refer to `?Seurat::FindNeighbors`.

`force.recalc` Refer to `?Seurat::FindNeighbors`.

`modularity.fxn` Refer to `?Seurat::FindClusters`.

`initial.membership`
Refer to `?Seurat::FindClusters`.

`weights` Refer to `?Seurat::FindClusters`.

`node.sizes` Refer to `?Seurat::FindClusters`.

`resolution` Refer to `?Seurat::FindClusters`.

`method` Refer to `?Seurat::FindClusters`.

`algorithm` Refer to `?Seurat::FindClusters`.

`n.start` Refer to `?Seurat::FindClusters`.

`n.iter` Refer to `?Seurat::FindClusters`.

`random.seed` Refer to `?Seurat::FindClusters`.

`group.singletons`
Refer to `?Seurat::FindClusters`.

`temp.file.location`
Refer to `?Seurat::FindClusters`.

`edge.file.name` Refer to `?Seurat::FindClusters`.

`overwrite` When the input object is a SingleCellExperiment, enabling this will result in the overwriting, with the new cluster assignments, of any column in your metadata that has the same name as `clusterAssignName`.

Details

A wrapper function for Seurat's FindNeighbors and FindClusters.

Value

A `SingleCellExperiment` or `numeric` object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat <- jitter(matrix(
  MASS::rnegbin(rexp(1000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat) <- paste0('CD', 1:ncol(mat))
rownames(mat) <- paste0('cell', 1:nrow(mat))

clusKNN(mat)
```

`contourPlot` *Draw a contour plot, typically relating to co-ordinates of a 2-dimensional reduction / embedding, typically contained within a* `SingleCellExperiment` *object.*

Description

Draw a contour plot, typically relating to co-ordinates of a 2-dimensional reduction / embedding, typically contained within a `SingleCellExperiment` object.

Usage

```
contourPlot(
  indata,
  reducedDim = "UMAP",
  dimColnames = c("UMAP1", "UMAP2"),
  lowcol = "darkblue",
  highcol = "darkred",
  alpha = c(0, 0.5),
  contour = "black",
  bins = 300,
  legendPosition = "right",
  legendLabSize = 12,
  legendIconSize = 5,
  legendKeyHeight = 2.5,
  xlim = NULL,
  ylim = NULL,
  celllab = NULL,
  labSize = 3,
  drawConnectors = TRUE,
  widthConnectors = 0.5,
  colConnectors = "black",
```

```
  xlab = dimColnames[1],
  xlabAngle = 0,
  xlabhjust = 0.5,
  xlabvjust = 0.5,
  ylab = dimColnames[2],
  ylabAngle = 0,
  ylabhjust = 0.5,
  ylabvjust = 0.5,
  axisLabSize = 16,
  title = "Cellular density and contours",
  subtitle = "",
  caption = ifelse(is(indata, "SingleCellExperiment"), paste0("Total cells, ",
    nrow(as.data.frame(reducedDim(indata, reducedDim))), "; Bins, ", bins),
    paste0("Total cells, ", nrow(indata), "; Bins, ", bins)),
  titleLabSize = 16,
  subtitleLabSize = 12,
  captionLabSize = 12,
  hline = NULL,
  hlineType = "longdash",
  hlineCol = "black",
  hlineWidth = 0.4,
  vline = NULL,
  vlineType = "longdash",
  vlineCol = "black",
  vlineWidth = 0.4,
  gridlines.major = TRUE,
  gridlines.minor = TRUE,
  borderWidth = 0.8,
  borderColour = "black",
  verbose = TRUE
)
```

Arguments

indata	A data-frame or matrix, or SingleCellExperiment object. If a data-frame or matrix, columns named in dimColnames will be extracted from the data and used to generate the contour plot. If a SingleCellExperiment object, a reduction named by reducedDim will be taken from your object and used to generate the contour plot, again using columns whose names are specified in dimColnames.
reducedDim	A reduced dimensional embedding stored within indata, e.g., PCA or UMAP.
dimColnames	The column names of the dimensions to use.
lowcol	Shade for low-density contours.
highcol	Shade for high-density contours.
alpha	Control the gradient of colour transparency, with 1 being opaque.
contour	The colour of the contour lines.
bins	The number of bins that determine the overall density values.

legendPosition	Position of legend ('top', 'bottom', 'left', 'right','none').
legendLabSize	Size of plot legend text.
legendIconSize	Size of plot legend icons / symbols.
legendKeyHeight	Height of the legend key.
xlim	Limits of the x-axis.
ylim	Limits of the y-axis.
celllab	A vector containing any cells that the user wishes to label in the plot.
labSize	Size of labels.
drawConnectors	Logical, indicating whether or not to connect plot labels to their corresponding points by line connectors.
widthConnectors	Line width of connectors.
colConnectors	Line colour of connectors.
xlab	Label for x-axis.
xlabAngle	Rotation angle of x-axis labels.
xlabhjust	Horizontal adjustment of x-axis labels.
xlabvjust	Vertical adjustment of x-axis labels.
ylab	Label for y-axis.
ylabAngle	Rotation angle of y-axis labels.
ylabhjust	Horizontal adjustment of y-axis labels.
ylabvjust	Vertical adjustment of y-axis labels.
axisLabSize	Size of x- and y-axis labels.
title	Plot title.
subtitle	Plot subtitle.
caption	Plot caption.
titleLabSize	Size of plot title.
subtitleLabSize	Size of plot subtitle.
captionLabSize	Size of plot caption.
hline	Draw one or more horizontal lines passing through this/these values on y-axis. For single values, only a single numerical value is necessary. For multiple lines, pass these as a vector, e.g., c(60,90).
hlineType	Line type for hline ('blank', 'solid', 'dashed', 'dotted','dotdash', 'longdash', 'twodash').
hlineCol	Colour of hline.
hlineWidth	Width of hline.
vline	Draw one or more vertical lines passing through this/these values on x-axis. For single values, only a single numerical value is necessary. For multiple lines, pass these as a vector, e.g., c(60,90).

vlineType	Line type for vline ('blank', 'solid', 'dashed', 'dotted','dotdash', 'longdash', 'twodash').
vlineCol	Colour of vline.
vlineWidth	Width of vline.
gridlines.major	Logical, indicating whether or not to draw major gridlines.
gridlines.minor	Logical, indicating whether or not to draw minor gridlines.
borderWidth	Width of the border on the x and y axes.
borderColour	Colour of the border on the x and y axes.
verbose	Boolean (TRUE / FALSE) to print messages to console or not.

Details

Draw a contour plot, typically relating to co-ordinates of a 2-dimensional reduction / embedding, typically contained within a SingleCellExperiment object.

Value

A ggplot2 object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat <- jitter(matrix(
  MASS::rnegbin(rexp(1000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat) <- paste0('CD', 1:ncol(mat))

u <- umap::umap(mat)$layout
colnames(u) <- c('UMAP1','UMAP2')

contourPlot(u)
```

---

downsampleByVar *Downsample an input data-frame or matrix based on variance.*

Description

Downsample an input data-frame or matrix based on variance.

Usage

```
downsampleByVar(x, varianceFactor = 0.1, verbose = TRUE)
```

Arguments

x	Input data-matrix.
varianceFactor	Removes this proportion of variables based on lesser variance.
verbose	Boolean (TRUE / FALSE) to print messages to console or not.

Details

Downsample an input data-frame or matrix based on variance.

Value

A `matrix` object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat <- jitter(matrix(
  MASS::rnegbin(rexp(1000, rate=.1), theta = 4.5),
  ncol = 20))

downsampleByVar(mat, varianceFactor = 0.1)
```

---

importData *Import a data-frame or matrix, and associated metadata, to a* `SingleCellExperiment` *object.*

Description

Import a data-frame or matrix, and associated metadata, to a `SingleCellExperiment` object.

Usage

```
importData(
  mat,
  assayname,
  metadata = NULL,
  downsampleVar = NULL,
  verbose = TRUE
)
```

Arguments

mat	A data-frame or matrix of expression values. Data-frames will be coerced to matrices.
assayname	Name of the SingleCellExperiment assay slot in which data will be stored.
metadata	Metadata associated with the data contained in 'mat'. A strict rule is enforced requiring that rownames(metadata) == rownames(mat).
downsampleVar	Downsample based on variance. Removes this proportion of variables (rows) based on lesser variance. This is applied on a per sample basis. If user wishes to apply this globally on the final merged dataset, then set this to 0 and remove based on variance manually after object creation.
verbose	Boolean (TRUE / FALSE) to print messages to console or not.

Details

Import a data-frame or matrix, and associated metadata, to a SingleCellExperiment object.

Value

A SingleCellExperiment object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat <- jitter(matrix(
  MASS::rnegbin(rexp(50000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat) <- paste0('CD', 1:ncol(mat))
rownames(mat) <- paste0('cell', 1:nrow(mat))

metadata <- data.frame(
  group = rep('A', nrow(mat)),
  row.names = rownames(mat),
  stringsAsFactors = FALSE)

sce <- importData(mat,
  assayname = 'normcounts',
  metadata = metadata)
```

markerEnrichment *Find enriched markers per identified cluster and calculate cluster abundances across these for samples and metadata variables.*

Description

Find enriched markers per identified cluster and calculate cluster abundances across these for samples and metadata variables.

Usage

```
markerEnrichment(
  indata,
  meta = NULL,
  assay = "scaled",
  sampleAbundances = TRUE,
  sampleID = "sample",
  studyvarID = NULL,
  clusterAssign = metadata(indata)[["Cluster"]],
  funcSummarise = function(x) mean(x, na.rm = TRUE),
  method = "Z",
  prob = 0.1,
  limits = c(-1.96, 1.96),
  verbose = TRUE
)
```

Arguments

indata	A data-frame or matrix, or SingleCellExperiment object. If a data-frame or matrix, this should relate to expression data (cells as columns; genes as rows). If a SingleCellExperiment object, data will be extracted from an assay component named by assay.
meta	If 'indata' is a non-SingleCellExperiment object, meta must be activated and relate to a data-frame of metadata that aligns with the columns of indata, and that also contains a column name specified by studyvarID.
assay	Name of the assay slot in indata from which data will be taken, assuming indata is a SingleCellExperiment object.
sampleAbundances	Logical, indicating whether or not to calculate cluster abundances across study samples.
sampleID	If sampleAbundances == TRUE, a column name from the provided metadata representing over which sample cluster abundances will be calculated.
studyvarID	A column name from the provided metadata representing a condition or trait over which cluster abundances will be calculated.

clusterAssign	A vector of cell-to-cluster assignments. This can be from any source but must align with your cells / variables. There is no check to ensure this when 'indata' is not a SingleCellExperiment object.
funcSummarise	A mathematical function used to summarise expression per marker per cluster.
method	Type of summarisation to apply to the data for final marker selection. Possible values include Z or quantile. If Z, limits relate to lower and upper Z-score cut-offs for low|high markers. The defaults of -1.96 and +1.96 are equivalents of p<0.05 on a two-tailed distribution. If quantile, prob will be used to define the nth lower and 1 - nth upper quantiles, which will be used for selecting low|high markers.
prob	See details for method.
limits	See details for method.
verbose	Boolean (TRUE / FALSE) to print messages to console or not.

Details

Find enriched markers per identified cluster and calculate cluster abundances across these for samples and metadata variables. markerEnrichment first collapses your input data's expression profiles from the level of cells to the level of clusters based on a mathematical function specified by funcSummarise. It then either selects, per cluster, low|high markers via quantiles, or transforms this collapsed data to global Z-scores and selects low|high markers based on Z-score cut-offs.

Value

A data.frame object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat <- jitter(matrix(
  MASS::rnegbin(rexp(1000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat) <- paste0('CD', 1:ncol(mat))
rownames(mat) <- paste0('cell', 1:nrow(mat))

u <- umap::umap(mat)$layout
colnames(u) <- c('UMAP1','UMAP2')
rownames(u) <- rownames(mat)
clus <- clusKNN(u)

metadata <- data.frame(
  group = c(rep('PB1', 25), rep('PB2', 25)),
  row.names = rownames(u))

markerEnrichment(t(mat), meta = metadata,
```

```
  sampleAbundances = FALSE,
  studyvarID = 'group', clusterAssign = clus)
```

`markerExpression` *Highlight the individual marker expression profile across a 2-dimensional reduction / embedding, typically contained within a* `SingleCellExperiment` *object. By default, this function plots the expression profile of 6 randomly-selected markers from your data.*

Description

Highlight the individual marker expression profile across a 2-dimensional reduction / embedding, typically contained within a `SingleCellExperiment` object. By default, this function plots the expression profile of 6 randomly-selected markers from your data.

Usage

```
markerExpression(
  indata,
  layout = NULL,
  assay = "scaled",
  reducedDim = "UMAP",
  dimColnames = c("UMAP1", "UMAP2"),
  markers = sample(rownames(indata), 6),
  ncol = 3,
  nrow = 2,
  col = c("darkblue", "yellow"),
  colMidpoint = 0,
  alpha = c(0, 1),
  pointSize = 0.5,
  legendPosition = "right",
  legendLabSize = 12,
  legendKeyHeight = 2.5,
  xlim = NULL,
  ylim = NULL,
  celllab = NULL,
  labSize = 3,
  drawConnectors = TRUE,
  widthConnectors = 0.5,
  colConnectors = "black",
  xlab = dimColnames[1],
  xlabAngle = 0,
  xlabhjust = 0.5,
  xlabvjust = 0.5,
  ylab = dimColnames[2],
  ylabAngle = 0,
```

```
  ylabhjust = 0.5,
  ylabvjust = 0.5,
  axisLabSize = 16,
  stripLabSize = 16,
  title = "Individual marker expression",
  subtitle = "",
  caption = ifelse(is(indata, "SingleCellExperiment"), paste0("Total cells, ",
   nrow(as.data.frame(reducedDim(indata, reducedDim)))), paste0("Total cells, ",
    nrow(layout))),
  titleLabSize = 16,
  subtitleLabSize = 12,
  captionLabSize = 12,
  hline = NULL,
  hlineType = "longdash",
  hlineCol = "black",
  hlineWidth = 0.4,
  vline = NULL,
  vlineType = "longdash",
  vlineCol = "black",
  vlineWidth = 0.4,
  gridlines.major = TRUE,
  gridlines.minor = TRUE,
  borderWidth = 0.8,
  borderColour = "black"
)
```

Arguments

indata	A data-frame or matrix, or SingleCellExperiment object. If a data-frame or matrix, this should relate to expression data (cells as columns; genes as rows). If a SingleCellExperiment object, data will be extracted from an assay component named by assay.
layout	If 'indata' is a non-SingleCellExperiment object, layout must be activated and relate to a 2-dimensional reduction / embedding, although, technically, any data-frame or matrix of numbers will be accepted, provided that it aligns with the dimensions of indata, and provided that it contains columns as specified in dimColnames.
assay	Name of the assay slot in 'indata' from which data will be taken, assuming indata is a SingleCellExperiment object.
reducedDim	A reduced dimensional component stored within indata, e.g., PCA or UMAP.
dimColnames	The column names of the dimensions to use.
markers	Vector containing marker names to plot.
ncol	Number of columns for faceting.
nrow	Number of rows for faceting.
col	Colours used for generation of fill gradient according to expression values. Can be 2 or 3 colours.

colMidpoint	Mid-point (expression value) for the colour range. Only used when 3 colours are specified by col.
alpha	Control the gradient of colour transparency, with 1 being opaque.
pointSize	Size of plotted points.
legendPosition	Position of legend ('top', 'bottom', 'left', 'right','none').
legendLabSize	Size of plot legend text.
legendKeyHeight	Height of the legend key.
xlim	Limits of the x-axis.
ylim	Limits of the y-axis.
celllab	A vector containing any cells that the user wishes to label in the plot.
labSize	Size of labels.
drawConnectors	Logical, indicating whether or not to connect plot labels to their corresponding points by line connectors.
widthConnectors	Line width of connectors.
colConnectors	Line colour of connectors.
xlab	Label for x-axis.
xlabAngle	Rotation angle of x-axis labels.
xlabhjust	Horizontal adjustment of x-axis labels.
xlabvjust	Vertical adjustment of x-axis labels.
ylab	Label for y-axis.
ylabAngle	Rotation angle of y-axis labels.
ylabhjust	Horizontal adjustment of y-axis labels.
ylabvjust	Vertical adjustment of y-axis labels.
axisLabSize	Size of x- and y-axis labels.
stripLabSize	Size of the strip (marker) labels.
title	Plot title.
subtitle	Plot subtitle.
caption	Plot caption.
titleLabSize	Size of plot title.
subtitleLabSize	Size of plot subtitle.
captionLabSize	Size of plot caption.
hline	Draw one or more horizontal lines passing through this/these values on y-axis. For single values, only a single numerical value is necessary. For multiple lines, pass these as a vector, e.g., c(60,90).
hlineType	Line type for hline ('blank', 'solid', 'dashed', 'dotted','dotdash', 'longdash', 'twodash').

hlineCol	Colour of hline.
hlineWidth	Width of hline.
vline	Draw one or more vertical lines passing through this/these values on x-axis. For single values, only a single numerical value is necessary. For multiple lines, pass these as a vector, e.g., c(60,90).
vlineType	Line type for vline (`'blank'`, `'solid'`, `'dashed'`, `'dotted'`,`'dotdash'`, `'longdash'`, `'twodash'`).
vlineCol	Colour of vline.
vlineWidth	Width of vline.
gridlines.major	Logical, indicating whether or not to draw major gridlines.
gridlines.minor	Logical, indicating whether or not to draw minor gridlines.
borderWidth	Width of the border on the x and y axes.
borderColour	Colour of the border on the x and y axes.

Details

Highlight the individual marker expression profile across a 2-dimensional reduction / embedding, typically contained within a `SingleCellExperiment` object. By default, this function plots the expression profile of 6 randomly-selected markers from your data.

Value

A `ggplot2` object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat <- jitter(matrix(
MASS::rnegbin(rexp(1000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat) <- paste0('CD', 1:ncol(mat))
rownames(mat) <- paste0('cell', 1:nrow(mat))

u <- umap::umap(mat)$layout
colnames(u) <- c('UMAP1','UMAP2')
rownames(u) <- rownames(mat)

markerExpression(t(mat), layout = u)
```

markerExpressionPerCluster

Generate box-and-whisker plots illustrating marker expression per k-NN identified cluster. By default, 5 randomly-selected clusters are selected, and the expression profiles of 10 randomly-selected markers are plot across these.

Description

Generate box-and-whisker plots illustrating marker expression per k-NN identified cluster. By default, 5 randomly-selected clusters are selected, and the expression profiles of 10 randomly-selected markers are plot across these.

Usage

```
markerExpressionPerCluster(
  indata,
  assay = "scaled",
  clusters = sample(unique(metadata(indata)[["Cluster"]]), 5),
  clusterAssign = metadata(indata)[["Cluster"]],
  markers = sample(rownames(indata), 10),
  ncol = 5,
  nrow = 2,
  legendPosition = "none",
  legendLabSize = 12,
  legendKeyHeight = 2.5,
  xlim = NULL,
  ylim = NULL,
  yfixed = FALSE,
  xlab = "Marker",
  xlabAngle = 90,
  xlabhjust = 0.5,
  xlabvjust = 0.5,
  ylab = "Expression",
  ylabAngle = 0,
  ylabhjust = 0.5,
  ylabvjust = 0.5,
  axisLabSize = 16,
  stripLabSize = 16,
  title = "Marker expression per cluster",
  subtitle = "",
  caption = "",
  titleLabSize = 16,
  subtitleLabSize = 12,
  captionLabSize = 12,
  borderWidth = 0.8,
  borderColour = "black",
```

```
  verbose = TRUE
)
```

Arguments

indata	A data-frame or matrix, or SingleCellExperiment object. If a data-frame or matrix, this should relate to expression data (cells as columns; genes as rows). If a SingleCellExperiment object, data will be extracted from an assay component named by assay.
assay	Name of the assay slot in indata from which data will be taken, assuming indata is a SingleCellExperiment object.
clusters	Vector containing clusters to plot.
clusterAssign	A vector of cell-to-cluster assignments. This can be from any source but must align with your cells / variables. There is no check to ensure this when indata is not a SingleCellExperiment object.
markers	Vector containing marker names to plot.
ncol	Number of columns for faceting.
nrow	Number of rows for faceting.
legendPosition	Position of legend ('top', 'bottom', 'left', 'right','none').
legendLabSize	Size of plot legend text.
legendKeyHeight	Height of the legend key.
xlim	Limits of the x-axis.
ylim	Limits of the y-axis.
yfixed	Logical, specifying whether or not to fix the y-axis scales across all clusters when faceting.
xlab	Label for x-axis.
xlabAngle	Rotation angle of x-axis labels.
xlabhjust	Horizontal adjustment of x-axis labels.
xlabvjust	Vertical adjustment of x-axis labels.
ylab	Label for y-axis.
ylabAngle	Rotation angle of y-axis labels.
ylabhjust	Horizontal adjustment of y-axis labels.
ylabvjust	Vertical adjustment of y-axis labels.
axisLabSize	Size of x- and y-axis labels.
stripLabSize	Size of the strip labels.
title	Plot title.
subtitle	Plot subtitle.
caption	Plot caption.
titleLabSize	Size of plot title.

subtitleLabSize	Size of plot subtitle.
captionLabSize	Size of plot caption.
borderWidth	Width of the border on the x and y axes.
borderColour	Colour of the border on the x and y axes.
verbose	Boolean (TRUE / FALSE) to print messages to console or not.

Details

Generate box-and-whisker plots illustrating marker expression per k-NN identified cluster. By default, 5 randomly-selected clusters are selected, and the expression profiles of 10 randomly-selected markers are plot across these.

Value

A ggplot2 object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat <- jitter(matrix(
  MASS::rnegbin(rexp(5000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat) <- paste0('CD', 1:ncol(mat))
rownames(mat) <- paste0('cell', 1:nrow(mat))

clus <- clusKNN(mat)
markerExpressionPerCluster(t(mat), clusters = c(0, 1),
  clusterAssign = clus)
```

---

metadataPlot *Colour shade a 2-dimensional reduction / embedding based on metadata, typically contained within a* SingleCellExperiment *object.*

Description

Colour shade a 2-dimensional reduction / embedding based on metadata, typically contained within a SingleCellExperiment object.

Usage

```
metadataPlot(
  indata,
  meta = NULL,
  reducedDim = "UMAP",
  dimColnames = c("UMAP1", "UMAP2"),
  colby = NULL,
  colkey = NULL,
  pointSize = 0.5,
  legendPosition = "right",
  legendLabSize = 12,
  legendIconSize = 5,
  xlim = NULL,
  ylim = NULL,
  celllab = NULL,
  labSize = 3,
  drawConnectors = TRUE,
  widthConnectors = 0.5,
  colConnectors = "black",
  xlab = dimColnames[1],
  xlabAngle = 0,
  xlabhjust = 0.5,
  xlabvjust = 0.5,
  ylab = dimColnames[2],
  ylabAngle = 0,
  ylabhjust = 0.5,
  ylabvjust = 0.5,
  axisLabSize = 16,
  title = "Metadata plot",
  subtitle = "",
  caption = ifelse(is(indata, "SingleCellExperiment"), paste0("Total cells, ",
   nrow(as.data.frame(reducedDim(indata, reducedDim)))), paste0("Total cells, ",
    nrow(meta))),
  titleLabSize = 16,
  subtitleLabSize = 12,
  captionLabSize = 12,
  hline = NULL,
  hlineType = "longdash",
  hlineCol = "black",
  hlineWidth = 0.4,
  vline = NULL,
  vlineType = "longdash",
  vlineCol = "black",
  vlineWidth = 0.4,
  gridlines.major = TRUE,
  gridlines.minor = TRUE,
  borderWidth = 0.8,
  borderColour = "black"
```

```
)
```

Arguments

indata	A data-frame or matrix, or SingleCellExperiment object. If a data-frame or matrix, columns named in dimColnames will be extracted from the data and used to generate the plot. If a SingleCellExperiment object, a reduction named by reducedDim will be taken from your object and used to generate the plot, again using columns whose names are specified in dimColnames.
meta	If 'indata' is a non-SingleCellExperiment object, 'meta' must be activated and relate to a data-frame of metadata that aligns with the rows of indata, and that also contains a column name specified by colby.
reducedDim	A reduced dimensional embedding stored within indata, e.g., PCA or UMAP.
dimColnames	The column names of the dimensions to use.
colby	If NULL, all points will be coloured differently. If not NULL, the value is assumed to be a column name in metadata(indata) relating to some grouping / categorical variable.
colkey	Vector of name-value pairs relating to value passed to 'col', e.g., c(A='forestgreen', B='gold').
pointSize	Size of plotted points.
legendPosition	Position of legend ('top', 'bottom', 'left', 'right','none').
legendLabSize	Size of plot legend text.
legendIconSize	Size of plot legend icons / symbols.
xlim	Limits of the x-axis.
ylim	Limits of the y-axis.
celllab	A vector containing any cells that the user wishes to label in the plot.
labSize	Size of labels.
drawConnectors	Logical, indicating whether or not to connect plot labels to their corresponding points by line connectors.
widthConnectors	Line width of connectors.
colConnectors	Line colour of connectors.
xlab	Label for x-axis.
xlabAngle	Rotation angle of x-axis labels.
xlabhjust	Horizontal adjustment of x-axis labels.
xlabvjust	Vertical adjustment of x-axis labels.
ylab	Label for y-axis.
ylabAngle	Rotation angle of y-axis labels.
ylabhjust	Horizontal adjustment of y-axis labels.
ylabvjust	Vertical adjustment of y-axis labels.
axisLabSize	Size of x- and y-axis labels.

title	Plot title.
subtitle	Plot subtitle.
caption	Plot caption.
titleLabSize	Size of plot title.
subtitleLabSize	Size of plot subtitle.
captionLabSize	Size of plot caption.
hline	Draw one or more horizontal lines passing through this/these values on y-axis. For single values, only a single numerical value is necessary. For multiple lines, pass these as a vector, e.g., c(60,90).
hlineType	Line type for hline ('blank', 'solid', 'dashed', 'dotted','dotdash', 'longdash', 'twodash').
hlineCol	Colour of hline.
hlineWidth	Width of hline.
vline	Draw one or more vertical lines passing through this/these values on x-axis. For single values, only a single numerical value is necessary. For multiple lines, pass these as a vector, e.g., c(60,90).
vlineType	Line type for vline ('blank', 'solid', 'dashed', 'dotted','dotdash', 'longdash', 'twodash').
vlineCol	Colour of vline.
vlineWidth	Width of vline.
gridlines.major	Logical, indicating whether or not to draw major gridlines.
gridlines.minor	Logical, indicating whether or not to draw minor gridlines.
borderWidth	Width of the border on the x and y axes.
borderColour	Colour of the border on the x and y axes.

Details

Colour shade a 2-dimensional reduction / embedding based on metadata, typically contained within a SingleCellExperiment object.

Value

A ggplot2 object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat <- jitter(matrix(
  MASS::rnegbin(rexp(1000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat) <- paste0('CD', 1:ncol(mat))
rownames(mat) <- paste0('cell', 1:nrow(mat))

u <- umap::umap(mat)$layout
colnames(u) <- c('UMAP1','UMAP2')
rownames(u) <- rownames(mat)

metadata <- data.frame(
  group = c(rep('PB1', 25), rep('PB2', 25)),
  row.names = rownames(u))

metadataPlot(u, meta = metadata, colby = 'group')
```

`performUMAP` *Perform UMAP on an input data-frame or matrix, or* `SingleCellExperiment` *object, using the basic R implementation of UMAP.*

Description

Perform UMAP on an input data-frame or matrix, or `SingleCellExperiment` object, using the basic R implementation of UMAP.

Usage

```
performUMAP(
  indata,
  config = NULL,
  assay = "scaled",
  reducedDim = NULL,
  dims = seq_len(20),
  newDimName = NULL,
  useMarkers = NULL,
  verbose = TRUE
)
```

Arguments

`indata`	A data-frame or matrix, or `SingleCellExperiment` object. If a data-frame or matrix, only the derived co-ordinates for the first two dimensions are returned. If a `SingleCellExperiment` object, UMAP is performed on the assay named by `assay`, and the co-ordinates for the first two dimensions are stored as a reduced dimension named by `reducedDim`.

config	UMAP configuration settings
assay	Name of the assay slot in indata from which data will be taken, assuming indata is a SingleCellExperiment object.
reducedDim	A dimensional reduction / embedding stored within indata, e.g., PCA. If activated, UMAP will be performed on this object in place of the assay component specified by assay.
dims	If 'reducedDim' is activated, the number of dimensions to use.
newDimName	Name for the new dimensional embedding that will be produced. If nothing is selected for neither this nor reducedDim, then the new name will be UMAP. If nothing is selected for this, but, e.g., PCA is selected for reducedDim, then the new name will be UMAP_PCA.
useMarkers	Before performing UMAP, subset the data for these markers.
verbose	Boolean (TRUE / FALSE) to print messages to console or not.

Details

Perform UMAP on an input data-frame or matrix, or SingleCellExperiment object, using the basic R implementation of UMAP.

Value

A SingleCellExperiment object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat <- jitter(matrix(
  MASS::rnegbin(rexp(1000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat) <- paste0('CD', 1:ncol(mat))

performUMAP(mat)
```

---

plotClusters *Highlight cell-to-cluster assignments across a 2-dimensional reduction / embedding.*

Description

Highlight cell-to-cluster assignments across a 2-dimensional reduction / embedding.

Usage

```
plotClusters(
  indata,
  clusterVector = NULL,
  reducedDim = "UMAP",
  dimColnames = c("UMAP1", "UMAP2"),
  clusterColname = "Cluster",
  pointSize = 0.5,
  legendPosition = "none",
  legendLabSize = 12,
  xlim = NULL,
  ylim = NULL,
  label = TRUE,
  labSize = 5,
  drawConnectors = TRUE,
  widthConnectors = 0.5,
  colConnectors = "black",
  xlab = dimColnames[1],
  xlabAngle = 0,
  xlabhjust = 0.5,
  xlabvjust = 0.5,
  ylab = dimColnames[2],
  ylabAngle = 0,
  ylabhjust = 0.5,
  ylabvjust = 0.5,
  axisLabSize = 16,
  title = "k-nearest neighbour (k-NN) clusters",
  subtitle = "",
  caption = ifelse(is(indata, "SingleCellExperiment"), paste0("Total cells, ",
   nrow(as.data.frame(reducedDim(indata, reducedDim)))), paste0("Total cells, ",
    length(clusterVector))),
  titleLabSize = 16,
  subtitleLabSize = 12,
  captionLabSize = 12,
  hline = NULL,
  hlineType = "longdash",
  hlineCol = "black",
  hlineWidth = 0.4,
  vline = NULL,
  vlineType = "longdash",
  vlineCol = "black",
  vlineWidth = 0.4,
  gridlines.major = TRUE,
  gridlines.minor = TRUE,
  borderWidth = 0.8,
  borderColour = "black",
  verbose = TRUE
)
```

Arguments

indata	A data-frame or matrix, or SingleCellExperiment object. If a data-frame or matrix, columns named in dimColnames will be extracted from the data and used to generate the plot. If a SingleCellExperiment object, a reduction named by reducedDim will be taken from your object and used to generate the plot, again using columns whose names are specified in dimColnames.
clusterVector	If indata is a non-SingleCellExperiment object, clusterVector must be non-NULL and relate to a cell-to-cluster assignment whose length matches nrow(indata).
reducedDim	A reduced dimensional embedding stored within 'indata', e.g., PCA or UMAP.
dimColnames	The column names of the dimensions to use.
clusterColname	The column name in the metadata of indata that contains the cell-to-cluster assignment, assuming indata is a SingleCellExperiment object.
pointSize	Size of plotted points.
legendPosition	Position of legend ('top', 'bottom', 'left', 'right','none').
legendLabSize	Size of plot legend text.
xlim	Limits of the x-axis.
ylim	Limits of the y-axis.
label	Logical, indicating whether or not to label the clusters.
labSize	Size of labels.
drawConnectors	Logical, indicating whether or not to connect plot labels to their corresponding cluster islands by line connectors.
widthConnectors	Line width of connectors.
colConnectors	Line colour of connectors.
xlab	Label for x-axis.
xlabAngle	Rotation angle of x-axis labels.
xlabhjust	Horizontal adjustment of x-axis labels.
xlabvjust	Vertical adjustment of x-axis labels.
ylab	Label for y-axis.
ylabAngle	Rotation angle of y-axis labels.
ylabhjust	Horizontal adjustment of y-axis labels.
ylabvjust	Vertical adjustment of y-axis labels.
axisLabSize	Size of x- and y-axis labels.
title	Plot title.
subtitle	Plot subtitle.
caption	Plot caption.
titleLabSize	Size of plot title.
subtitleLabSize	Size of plot subtitle.

captionLabSize	Size of plot caption.
hline	Draw one or more horizontal lines passing through this/these values on y-axis. For single values, only a single numerical value is necessary. For multiple lines, pass these as a vector, e.g., c(60,90).
hlineType	Line type for hline ('blank', 'solid', 'dashed', 'dotted','dotdash', 'longdash', 'twodash').
hlineCol	Colour of hline.
hlineWidth	Width of hline.
vline	Draw one or more vertical lines passing through this/these values on x-axis. For single values, only a single numerical value is necessary. For multiple lines, pass these as a vector, e.g., c(60,90).
vlineType	Line type for vline ('blank', 'solid', 'dashed', 'dotted','dotdash', 'longdash', 'twodash').
vlineCol	Colour of vline.
vlineWidth	Width of vline.
gridlines.major	Logical, indicating whether or not to draw major gridlines.
gridlines.minor	Logical, indicating whether or not to draw minor gridlines.
borderWidth	Width of the border on the x and y axes.
borderColour	Colour of the border on the x and y axes.
verbose	Boolean (TRUE / FALSE) to print messages to console or not.

Details

Highlight cell-to-cluster assignments across a 2-dimensional reduction / embedding.

Value

A ggplot2 object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat <- jitter(matrix(
  MASS::rnegbin(rexp(1000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat) <- paste0('CD', 1:ncol(mat))
rownames(mat) <- paste0('cell', 1:nrow(mat))

u <- umap::umap(mat)
clusvec <- clusKNN(u$layout)
plotClusters(u$layout, clusvec)
```

plotSignatures *Find enriched markers per identified cluster and visualise these as a custom corrplot.*

Description

Find enriched markers per identified cluster and visualise these as a custom corrplot.

Usage

```
plotSignatures(
  indata,
  assay = "scaled",
  clusterAssign = metadata(indata)[["Cluster"]],
  funcSummarise = function(x) mean(x, na.rm = TRUE),
  col = colorRampPalette(brewer.pal(9, "RdPu"))(100),
  labCex = 1,
  legendPosition = "right",
  legendCex = 1,
  labDegree = 90,
  verbose = TRUE
)
```

Arguments

indata	A data-frame or matrix, or SingleCellExperiment object. If a data-frame or matrix, this should relate to expression data (cells as columns; genes as rows). If a SingleCellExperiment object, data will be extracted from an assay component named by assay.
assay	Name of the assay slot in indata from which data will be taken, assuming indata is a SingleCellExperiment object.
clusterAssign	A vector of cell-to-cluster assignments. This can be from any source but must align with your cells / variables. There is no check to ensure this when indata is not a SingleCellExperiment object.
funcSummarise	A mathematical function used to summarise expression per marker, per cluster.
col	colorRampPalette to be used for shading low-to-high expression.
labCex	cex (size) of the main plot labels.
legendPosition	position of legend. Can be one of 'top', 'right', 'bottom', 'left'
legendCex	cex (size) of the legend labels.
labDegree	Rotation angle of the main plot labels.
verbose	Boolean (TRUE / FALSE) to print messages to console or not.

Details

Find enriched markers per identified cluster and visualise these as a custom corrplot. `plotSignatures` first collapses your input data's expression profiles from the level of cells to the level of clusters based on a mathematical function specified by `funcSummarise`. It then centers and scales the data range to be between -1 and +1 for visualisation purposes.

Value

A `corrplot` object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat <- jitter(matrix(
  MASS::rnegbin(rexp(1000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat) <- paste0('CD', 1:ncol(mat))
rownames(mat) <- paste0('cell', 1:nrow(mat))

u <- umap::umap(mat)$layout
colnames(u) <- c('UMAP1','UMAP2')
rownames(u) <- rownames(mat)
clus <- clusKNN(u)

plotSignatures(t(mat), clusterAssign = clus)
```

---

processFCS *Input, filter, normalise, and transform FCS expression data.*

Description

Input, filter, normalise, and transform FCS expression data.

Usage

```
processFCS(
  files,
  assayname = "scaled",
  metadata = NULL,
  filter = TRUE,
  bgNoiseThreshold = 1,
  euclideanNormThreshold = 1,
  transformation = TRUE,
```

```
  transFun = function(x) asinh(x),
  asinhFactor = 5,
  downsample = 1e+05,
  downsampleVar = 0.1,
  colsDiscard = NULL,
  colsRetain = NULL,
  newColnames = NULL,
  emptyValue = TRUE,
  verbose = TRUE
)
```

Arguments

files	A vector of FCS files.
assayname	Name of the assay slot in which data will be stored.
metadata	Metadata associated with the FCS files specified in 'files'. A strict rule is enforced requiring that rownames(metadata) matches files in both name and order.
filter	Boolean (TRUE / FALSE) to enable filtering (per sample) for background signal / noise.
bgNoiseThreshold	Threshold for background noise. Used when filter == TRUE.
euclideanNormThreshold	Euclidean norm threshold for background noise. Used when filter == TRUE.
transformation	Boolean (TRUE / FALSE) to enable data transformation after filtering.
transFun	The function to apply (per sample) for transformation. Typically, for flow and mass cytometry, this is hyperbolic arc sine (asinh(x)). User can supply any function.
asinhFactor	The factor to apply when transforming via asinh(). For flow cytometry, this is usually 150; for mass cytometry and CyTOF, it is 5. Note that this is not used if the user has supplied their own function to transFun.
downsample	Downsample to this number of random variables. This is performed on the final merged dataset, i.e., after all samples have been bound together. NULL to disable.
downsampleVar	Downsample based on variance. Removes this proportion of cells based on lesser variance. This is applied per sample. If user wishes to apply this globally on the final merged dataset, then set this to 0 and remove based on variance manually.
colsDiscard	Columns to be removed from the final merged data. These names are literal and must match exactly.
colsRetain	Retain these columns only. This is the same as colsDiscard but in reverse. Technically, it is possible to activate both colsDiscard and colsRetain, but colsDiscard will be executed first.
newColnames	A named vector of new marker names to assign to each sample. The values of this vector should be the new marker names; the names of this vector should

	represent the ooriginal marker names. This operation is performed AFTER any operation involving `colsDiscard` and `colsRetain`.
`emptyValue`	boolean (taken from ?flowCore::read.FCS indicating whether or not we allow an empty value for keyword values in TEXT segment.
`verbose`	Boolean (TRUE / FALSE) to print messages to console or not.

Details

Input, filter, normalise, and transform FCS expression data.

Value

A `SingleCellExperiment` object.

Author(s)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
# create random data that follows a negative binomial
mat1 <- jitter(matrix(
  MASS::rnegbin(rexp(50000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat1) <- paste0('CD', 1:ncol(mat1))
rownames(mat1) <- paste0('cell', 1:nrow(mat1))

mat2 <- jitter(matrix(
  MASS::rnegbin(rexp(50000, rate=.1), theta = 4.5),
  ncol = 20))
colnames(mat2) <- paste0('CD', 1:ncol(mat2))
rownames(mat2) <- paste0('cell', 1:nrow(mat2))

metadata <- data.frame(
  group = c('PB1', 'PB2'),
  row.names = c('mat1', 'mat2'),
  stringsAsFactors = FALSE)
```

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