

Package ‘barcodetrackR’

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Type Package

Title Functions for Analyzing Cellular Barcoding Data

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Description barcodetrackR is an R package developed for the analysis and visualization of clonal tracking data. Data required is samples and tag abundances in matrix form. Usually from cellular barcoding experiments, integration site retrieval analyses, or similar technologies.

URL <https://github.com/dunbarlabNIH/barcodetrackR>

License file LICENSE

LazyData FALSE

Encoding UTF-8

biocViews Software, Visualization, Sequencing

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barcode_binary_heatmap

Barcode Binary Heatmap

Description

Creates a binary heatmap showing the absence or presence of new clones in samples ordered from L to R in the SummarizedExperiment.

Usage

```
barcode_binary_heatmap(
  your_SE,
  plot_labels = NULL,
  threshold = 0,
  your_title = NULL,
```

```

    label_size = 12,
    return_table = FALSE
  )

```

Arguments

| | |
|---------------------------|---|
| <code>your_SE</code> | A Summarized Experiment object. |
| <code>plot_labels</code> | Vector of x axis labels. Defaults to <code>colnames(your_SE)</code> . |
| <code>threshold</code> | Clones with a proportion below this threshold will be set to 0. |
| <code>your_title</code> | The title for the plot. |
| <code>label_size</code> | The size of the column labels. |
| <code>return_table</code> | Logical. Whether or not to return table of barcode sequences with their presence or absence in each sample indicated as a 1 or 0 respectively in the value column column. |

Value

Displays a binary heat map in the current plot window. Or if `return_table` is set to `TRUE`, returns a dataframe indicating the presence or absence of each barcode in each sample.

Examples

```

data(wu_subset)
barcode_binary_heatmap(your_SE = wu_subset[, 1:4])

```

| | |
|--------------------------------|--------------------------|
| <code>barcode_ggheatmap</code> | <i>barcode_ggheatmap</i> |
|--------------------------------|--------------------------|

Description

Creates a heatmap displaying the log abundance of the top 'n' clones from each sample in the Summarized Experiment object, using `ggplot2`. Clones are on the y-axis and samples are on the x-axis. The ordering and clustering of clones on the y-axis as well as all aesthetics of the plot can be controlled through the arguments described below.

Usage

```

barcode_ggheatmap(
  your_SE,
  plot_labels = NULL,
  n_clones = 10,
  cellnote_assay = "stars",
  your_title = NULL,
  grid = TRUE,
  label_size = 12,
  dendro = FALSE,

```

```

cellnote_size = 4,
distance_method = "Euclidean",
minkowski_power = 2,
hclust_linkage = "complete",
row_order = "hierarchical",
clusters = 0,
percent_scale = c(0, 2.5e-05, 0.001, 0.01, 0.1, 1),
color_scale = c("#4575B4", "#4575B4", "lightblue", "#fefeb9", "#D73027", "red4"),
return_table = FALSE
)

```

Arguments

| | |
|------------------------------|--|
| <code>your_SE</code> | A Summarized Experiment object. |
| <code>plot_labels</code> | Vector of x axis labels. Defaults to <code>colnames(your_SE)</code> . |
| <code>n_clones</code> | The top 'n' clones to plot. |
| <code>cellnote_assay</code> | Character. One of "stars", "counts", or "proportions." To have no cellnote, set <code>cellnote_size</code> to 0. |
| <code>your_title</code> | The title for the plot. |
| <code>grid</code> | Logical. Include a grid or not in the heatmap. |
| <code>label_size</code> | The size of the column labels. |
| <code>dendro</code> | Logical. Whether or not to show row dendrogram when hierarchical clustering. |
| <code>cellnote_size</code> | The numerical size of the cell note labels. To have no cellnote, set <code>cellnote_size</code> to 0. |
| <code>distance_method</code> | Character. Use <code>summary(proxy::pr_DB)</code> to see all possible options for distance metrics in clustering. |
| <code>minkowski_power</code> | The power of the Minkowski distance (if <code>minkowski</code> is the distance method used). |
| <code>hclust_linkage</code> | Character. One of "ward.D", "ward.D2", "single", "complete", "average" (= UPGMA), "mcquitty" (= WPGMA), "median" (= WPGMC) or "centroid" (= UPGMC). |
| <code>row_order</code> | Character; "hierarchical" to perform hierarchical clustering on the output and order in that manner, "emergence" to organize rows by order of presence in data (from left to right), or a character vector of rows within the summarized experiment to plot. |
| <code>clusters</code> | How many clusters to cut hierarchical tree into for display when <code>row_order</code> is "hierarchical". |
| <code>percent_scale</code> | A numeric vector through which to spread the color scale (values inclusive from 0 to 1). Must be same length as <code>color_scale</code> . |
| <code>color_scale</code> | A character vector which indicates the colors of the color scale. Must be same length as <code>percent_scale</code> . |
| <code>return_table</code> | Logical. Whether or not to return table of barcode sequences with their log abundance in the 'value' column and cellnote for each sample instead of displaying a plot. |

Value

Displays a heatmap in the current plot window. Or if `return_table` is set to `TRUE`, returns a dataframe of the barcode sequences, log abundances, and cellnotes for each sample.

Examples

```
data(wu_subset)
barcode_ggheatmap(
  your_SE = wu_subset, n_clones = 10,
  grid = TRUE, label_size = 6
)
```

`barcode_ggheatmap_stat`*Barcode Top Clone Heatmap*

Description

Creates a heatmap from the columns of data in the Summarized Experiment object, with the option to label based on statistical analysis. Uses `ggplot2`.

Usage

```
barcode_ggheatmap_stat(
  your_SE,
  sample_size,
  stat_test = "chi-squared",
  stat_option = "subsequent",
  reference_sample = NULL,
  stat_display = "top",
  show_all_significant = FALSE,
  p_threshold = 0.05,
  p_adjust = "none",
  bc_threshold = 0,
  plot_labels = NULL,
  n_clones = 10,
  cellnote_assay = "stars",
  your_title = NULL,
  grid = TRUE,
  label_size = 12,
  dendro = FALSE,
  cellnote_size = 4,
  distance_method = "Euclidean",
  minkowski_power = 2,
  hclust_linkage = "complete",
  row_order = "hierarchical",
  clusters = 0,
```

```

percent_scale = c(0, 2.5e-05, 0.001, 0.01, 0.1, 1),
color_scale = c("#4575B4", "#4575B4", "lightblue", "#fefeb9", "#D73027", "red4"),
return_table = FALSE
)

```

Arguments

| | |
|-----------------------------------|---|
| <code>your_SE</code> | A Summarized Experiment object. |
| <code>sample_size</code> | A numeric vector providing the sample size of each column of the Summarized-Experiment passed to the function. This sample size describes the samples that the barcoding data is meant to approximate. |
| <code>stat_test</code> | The statistical test to use on the constructed contingency table for each barcode. Options are "chi-squared" and "fisher." |
| <code>stat_option</code> | For "subsequent" statistical testing is performed on each column of data compared to the column before it. For "reference," all other columns of data are compared to a reference column. |
| <code>reference_sample</code> | Provide the column name of the reference column if <code>stat_option</code> is set to "reference." Defaults to the first column in the SummarizedExperiment. |
| <code>stat_display</code> | Choose which clones to display on the heatmap. If set to "top," the top <code>n_clones</code> ranked by abundance for each sample will be displayed. If set to "change," the top <code>n_clones</code> with the lowest p-value from statistical testing will be shown for each sample. If set to "increase," the top <code>n_clones</code> (ranked by p-value) which increase in abundance for each sample will be shown. And if set to "decrease," the top <code>n_clones</code> (ranked by lowest p-value) which decrease in abundance will be shown. |
| <code>show_all_significant</code> | Logical. If set to TRUE when <code>stat_display</code> = "change," "increase," or "decrease" then the <code>n_clones</code> argument will be overridden and all clones with a statistically significant change, increase, or decrease in proportion will be shown. |
| <code>p_threshold</code> | The <code>p_value</code> threshold to use for statistical testing |
| <code>p_adjust</code> | Character, default = "none". To correct p-values for multiple comparisons, set to any of the p value adjustment methods in the <code>p.adjust</code> function in R stats, which includes "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", and "fdr". |
| <code>bc_threshold</code> | Clones must be above this proportion in at least one sample to be included in statistical testing. |
| <code>plot_labels</code> | Vector of x axis labels. Defaults to <code>colnames(your_SE)</code> . |
| <code>n_clones</code> | The top 'n' clones to plot. |
| <code>cellnote_assay</code> | Character. One of "stars", "reads", "proportions" or "p_val" |
| <code>your_title</code> | The title for the plot. |
| <code>grid</code> | Logical. Include a grid or not in the heatmap. |
| <code>label_size</code> | The size of the column labels. |
| <code>dendro</code> | Logical. Whether or not to show row dendrogram when hierarchical clustering. |

| | |
|-----------------|--|
| cellnote_size | The numerical size of the cell note labels. |
| distance_method | Character. Use <code>summary(proxy::pr_DB)</code> to see all possible options for distance metrics in clustering. |
| minkowski_power | The power of the Minkowski distance (if <code>minkowski</code> is the distance method used). |
| hclust_linkage | Character. One of "ward.D", "ward.D2", "single", "complete", "average" (= UPGMA), "mcquitty" (= WPGMA), "median" (= WPGMC) or "centroid" (= UPGMC). |
| row_order | Character; "hierarchical" to perform hierarchical clustering on the output and order in that manner, "emergence" to organize rows by order of presence in data (from left to right), or a character vector of rows within the summarized experiment to plot. |
| clusters | How many clusters to cut hierarchical tree into for display when <code>row_order</code> is "hierarchical". |
| percent_scale | A numeric vector through which to spread the color scale (values inclusive from 0 to 1). Must be same length as <code>color_scale</code> . |
| color_scale | A character vector which indicates the colors of the color scale. Must be same length as <code>percent_scale</code> . |
| return_table | Logical. Whether or not to return table of barcode sequences with their log abundance in the 'value' column and cellnote (* indicating statistical significant change, for example) for each sample instead of displaying a plot. Note, for more in-depth statistical analysis, use the " <code>barcode_stat_test</code> " function. |

Value

Displays a heatmap in the current plot window. Or if `return_table` is set to TRUE, returns a dataframe of the barcode sequences, log abundances, and cellnote for each sample.

Examples

```
data(wu_subset)
barcode_ggheatmap_stat(
  your_SE = wu_subset[, 1:4], sample_size = rep(5000, 4),
  stat_test = "chi-squared", stat_option = "subsequent",
  p_threshold = 0.05, n_clones = 10,
  cellnote_assay = "stars", bc_threshold = 0.005
)
```

Description

Carries out a specific instance of statistical testing relevant to clonal tracking experiments. For longitudinal observations (of barcode abundances) in the provided SE object, use a Chi-squared or Fisher exact test whether each barcode proportion has changed between samples.

Each column in the provided SE will be "tested" against the reference sample. If the 'stat_option' argument is set to its default of "subsequent" then each sample will be compared to the sample before it. If this argument is set to "reference" the reference sample column name must be provided and each column will be tested against that reference sample.

Usage

```
barcode_stat_test(
  your_SE,
  sample_size,
  stat_test = "chi-squared",
  stat_option = "subsequent",
  reference_sample = NULL,
  p_adjust = "none",
  bc_threshold = 0
)
```

Arguments

| | |
|------------------|--|
| your_SE | A Summarized Experiment object containing clonal tracking data as created by the barcodetrackR 'create_SE' function. |
| sample_size | A numeric vector providing the sample size of each column of the Summarized-Experiment passed to the function. This sample size describes the samples that the barcoding data is meant to approximate, for example the number of cells barcodes were extracted from. |
| stat_test | The statistical test to use on the constructed contingency table for each barcode. Options are "chi-squared" and "fisher." For information, see [chisq.test](https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/chisq.test) [fisher.test](https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/fisher.test) |
| stat_option | For "subsequent" statistical testing is performed on each column of data compared to the column before it. For "reference," all other columns of data are compared to a reference column specified in the 'reference_sample' argument. |
| reference_sample | Provide the column name of the reference column if stat_option is set to "reference." Defaults to the first column in the SummarizedExperiment. |
| p_adjust | Character, default = "none". To correct p-values for multiple comparisons, set to any of the p value adjustment methods in the p.adjust function in R stats, which includes "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", and "fdr". |
| bc_threshold | Clones must be above this proportion in at least one sample to be included in statistical testing. Default is 0. Use this to ignore low-abundance clones which are more likely to be noise or artifact. |

Value

Returns a list of 3 dataframes containing the following information for each observation (or barcode) which passed the provided `bc_threshold`:

[["FC"]], Fold Change of barcode abundance for each sample relative to the previous sample or to the specified reference sample. Please note that for maximal user control over results, the FC dataframe will contain 0 for barcodes where the test sample has an abundance of 0, Inf for barcodes where the reference sample had an abundance of 0 and NaN for a barcode where both the test and reference sample have an abundance of 0;

[["log_FC"]], same as previous but the log Fold Change. Please note that again for maximal user control, the `log_FC` dataframe will contain NaN values when the FC was Nan, -Inf values when the FC was 0, and Inf values when the FC was Inf;

[["p_val"]], the p-value returned from either the Chi-squared or Fisher exact test indicating whether each barcode changed in proportion between the test sample and the reference sample. Please note that the p value will be NaN if both abundances are 0, otherwise a p-value will be assigned.

Also, note that one column of each resulting dataframe will contain all NAs - in the case where the `'stat_option'` argument is set to "subsequent" then this will be the first sample since there is no subsequent sample to compare to. In the case where the `'stat_option'` argument is set to "reference" then the reference sample will contain NAs.

Examples

```
data(wu_subset)
barcode_stat_test(
  your_SE = wu_subset[, 1:4], sample_size = rep(5000, 4),
  stat_test = "chi-squared", stat_option = "subsequent",
  bc_threshold = 0.0001
)
```

bias_histogram

Bias histogram

Description

Given a summarized experiment, gives histogram of log biases for 2 cell types. Each stacked bar in the histogram represents a clone binned by log bias defined as the log₂ of the percentage abundance in the sample specified in "bias_1" divided by the percentage abundance in "bias_2."

Usage

```
bias_histogram(
  your_SE,
  split_bias_on,
  bias_1,
  bias_2,
  split_bias_over,
  bias_over = NULL,
  remove_unique = FALSE,
```

```

breaks = c(10, 2, 1, 0.5),
text_size = 10,
linesize = 0.4,
ncols = 1,
scale_all_y = TRUE,
return_table = FALSE
)

```

Arguments

| | |
|------------------------------|--|
| <code>your_SE</code> | Your SummarizedExperiment of barcode data and associated metadata |
| <code>split_bias_on</code> | The column in <code>'colData(your_SE)'</code> from which <code>'bias_1'</code> and <code>'bias_2'</code> will be chosen |
| <code>bias_1</code> | The first cell type (or other factor) to be compared. Must be a possible value of the <code>split_bias_on</code> column of your metadata. Will be on the RIGHT side of the histogram. |
| <code>bias_2</code> | The second cell type (or other factor) to be compared. Must be a possible value of the <code>split_bias_on</code> column of your metadata. Will be on the LEFT side of the ridge plot |
| <code>split_bias_over</code> | The column in <code>'colData(your_SE)'</code> that you wish to observe the bias split for. The output will contain a faceted plot: one facet for each value of <code>'split_bias_over'</code> comparing the samples matching <code>'bias_1'</code> and <code>'bias_2'</code> from the <code>'split_bias_on'</code> argument. |
| <code>bias_over</code> | Choice(s) from the column designated in <code>'split_bias_over'</code> that will be used for plotting. Defaults to all. |
| <code>remove_unique</code> | If set to true, only clones present in both samples will be considered. |
| <code>breaks</code> | Numeric. The breaks specified for bins on the x-axis (how biased the clones are towards one factor or the other). |
| <code>text_size</code> | The size of the text in the plot. |
| <code>linesize</code> | The linewidth of the stacked bars which represent individual barcodes |
| <code>ncols</code> | Numeric. Number of columns to plot on using <code>plot_grid</code> from <code>cowplot</code> . |
| <code>scale_all_y</code> | Logical. Whether or not to plot all plots on the same y axis limits. |
| <code>return_table</code> | Logical. If set to TRUE, instead of a plot, the function will return a list containing a dataframe for each sample-sample log bias combination containing each barcode sequence and its bias between the samples. |

Value

Histogram of log bias for two factors faceted over another set of factors. Or, if `return_table` is set to TRUE, a list of dataframes containing the log bias data for each bias comparison passed to the function.

Examples

```

data(wu_subset)
bias_histogram(
  your_SE = wu_subset, split_bias_on = "celltype",
  bias_1 = "B", bias_2 = "T",
  split_bias_over = "months", ncols = 2
)

```

bias_lineplot

Bias line plot

Description

Given a summarized experiment and a specified factor to compare bias between "split_bias_on", shows the value of that bias plotted against another specified factor "split_bias_over" where each clone is represented by a line shaded by its overall abundance in the two samples being compared.

Usage

```

bias_lineplot(
  your_SE,
  split_bias_on,
  bias_1,
  bias_2,
  split_bias_over,
  bias_over = NULL,
  remove_unique = FALSE,
  text_size = 16,
  keep_numeric = TRUE,
  return_table = FALSE
)

```

Arguments

| | |
|-----------------|--|
| your_SE | SummarizedExperiment of barcode data and associated metadata |
| split_bias_on | The column of metadata corresponding to cell types (or other factor to be compared.) |
| bias_1 | The first cell type (or other factor) to be compared. Must be a possible value of the split_bias_on column of your metadata. Will be on the UPPER side of the line plot |
| bias_2 | The second cell type (or other factor) to be compared. Must be a possible value of the split_bias_on column of your metadata. Will be on the LOWER side of the line plot |
| split_bias_over | The column of metadata to plot by. If numeric, y axis will be in increasing order. If categorical, it will follow order of metadata. |

| | |
|---------------|--|
| bias_over | Choice(s) from the column designated in ‘split_bias_over‘ that will be used for plotting. Defaults to all. |
| remove_unique | Logical. If set to true, only clones present in both samples will be considered. |
| text_size | Numeric. The size of the text in the plot. |
| keep_numeric | Logical. Whether to keep the numeric spacing within split_bias_over or switch to discrete x scale. |
| return_table | Logical. If set to TRUE, rather than returnign a plot, the function will return a dataframe containing for each barcode sequence and each point of comparison: the bias value, the added proportion between the two factors at that point (cumul_sum), and the maximum cumul_sum (peak_abundance) of that barcode sequence at any point of comparison. |

Value

Bias line plot for two lineages over time. Or if return_table is set to TRUE, a dataframe containing the bias values for each barcode sequence between the two samples at all points of comparison.

Examples

```
data(wu_subset)
bias_lineplot(
  your_SE = wu_subset, split_bias_on = "celltype",
  bias_1 = "B", bias_2 = "T", split_bias_over = "months"
)
```

| | |
|-----------------|------------------------|
| bias_ridge_plot | <i>Bias Ridge plot</i> |
|-----------------|------------------------|

Description

Given a summarized experiment and a specified factor to compare bias between, gives ridge plots which show the density of clones at each value of log bias where log bias is calculated as $\log((\text{normalized abundance in sample 1} + 1)/(\text{normalized abundance in sample 2} + 1))$. If the weighted option is set to TRUE, the density estimator will weight the estimation by the added proportion of the clone between the two samples.

Usage

```
bias_ridge_plot(
  your_SE,
  split_bias_on,
  bias_1,
  bias_2,
  split_bias_over,
  bias_over = NULL,
  remove_unique = FALSE,
  weighted = FALSE,
```

```

    text_size = 16,
    add_dots = FALSE,
    return_table = FALSE
  )

```

Arguments

| | |
|------------------------------|--|
| <code>your_SE</code> | Your SummarizedExperiment of barcode data and associated metadata |
| <code>split_bias_on</code> | The column of metadata corresponding to cell types (or whatever factors you want to compare the bias between). |
| <code>bias_1</code> | The first cell type (or other factor) to be compared. Must be a possible value of the <code>split_bias_on</code> column of your metadata. Will be on the RIGHT side of the ridge plot |
| <code>bias_2</code> | The second cell type (or other factor) to be compared. Must be a possible value of the <code>split_bias_on</code> column of your metadata. Will be on the LEFT side of the ridge plot |
| <code>split_bias_over</code> | The column of metadata to plot by. If numeric, y axis will be in increasing order. If categorical, it will follow order of metadata. |
| <code>bias_over</code> | Choice(s) from the column designated in ‘ <code>split_bias_over</code> ’ that will be used for plotting. Defaults to all. |
| <code>remove_unique</code> | If set to true, only clones present in both samples will be considered. |
| <code>weighted</code> | If true, the density estimation will be weighted by the overall contribution of each barcode to the two samples being compared. |
| <code>text_size</code> | Numeric. The size of the text in the plot. |
| <code>add_dots</code> | Logical. Whether or not to add dots underneath the density plots. Dot size is proportion to the added proportion of the clone in the two samples. |
| <code>return_table</code> | Logical. If true, rather than returning a plot, the function will return a dataframe containing the calculated bias and <code>cumul_sum</code> which contains the added proportion between the two samples, for each barcode sequence across each sample considered. |

Value

Bias plot for two lineages over time. Or a dataframe containing the bias value and added proportion of each barcode if `return_table` is set to TRUE.

Examples

```

data(wu_subset)
bias_ridge_plot(
  your_SE = wu_subset, split_bias_on = "celltype",
  bias_1 = "B", bias_2 = "T", split_bias_over = "months",
  add_dots = TRUE
)

```

| | |
|------------------|-------------------|
| build_index_html | <i>Build html</i> |
|------------------|-------------------|

Description

Build html for vignette to index.html in docs

Usage

```
build_index_html(  
  target = "vignettes/Introduction_to_barcodetrackR.Rmd",  
  output = "index.html"  
)
```

Arguments

| | |
|--------|------------------------------------|
| target | the vignette to build |
| output | the target for the vignette output |

Value

Writes the vignette to docs/index.html. Only for internal use (get outta here!).

| | |
|---------------|------------------------------|
| chord_diagram | <i>Barcode Chord Diagram</i> |
|---------------|------------------------------|

Description

Creates a chord diagram showing each cell type (or other factor) as a region around a circle and shared clones between these cell types as links between the regions. The space around the regions which is not connected to a chord indicates clones unique to that sample, not shared with other samples.

Usage

```
chord_diagram(  
  your_SE,  
  weighted = FALSE,  
  plot_label = "SAMPLENAME",  
  alpha = 1,  
  your_title = NULL,  
  text_size = 12,  
  return_table = FALSE  
)
```

Arguments

| | |
|---------------------------|---|
| <code>your_SE</code> | Summarized Experiment object containing clonal tracking data as created by the <code>barcodetrackR</code> <code>'create_SE'</code> function. |
| <code>weighted</code> | Logical. <code>weighted = F</code> which is default will make links based on the number of shared clones between the factors. <code>Weighted = TRUE</code> will make the link width based on the clone's proportion in the samples. |
| <code>plot_label</code> | Character. Name of <code>colData</code> variable to use as labels for regions. Defaults to <code>SAMPLENAME</code> |
| <code>alpha</code> | Numeric. Transparency of links. Default = 1 is opaque. 0 is completely translucent |
| <code>your_title</code> | Character. The title for the plot. |
| <code>text_size</code> | Numeric. Size of region labels |
| <code>return_table</code> | Logical. If set to <code>TRUE</code> , in addition to plotting a chord diagram in the plot window, the function will return a dataframe of the shared clonality used to make the chord diagram. If <code>Weighted</code> is <code>FALSE</code> , the dataframe will contain a row for each set of clones and values of 1 or 0 indicating the samples which share that set of clones, and a <code>freq</code> column which is the number of clones in that set. If <code>weighted</code> is set to <code>TRUE</code> , each row will contain a set of clones and the data will show the proportion that set of clones comprises in each sample. The proportions of 0 indicate which samples do not share that set of clones. |

Value

Displays a chord diagram in the current plot window depicting shared clonality between samples (regions) as chords or links between the regions. Or,

Examples

```
data(wu_subset)
chord_diagram(your_SE = wu_subset[, c(4, 8, 12)], plot_label = "celltype")
```

`clonal_contribution` *Clonal contribution plot*

Description

Bar or line plot of percentage contribution of the top clones from a selected sample or all clones across samples matching the specified filter within the `SummarizedExperiment` object. Usually used for tracking a cell lineage's top clones over time.

Usage

```

clonal_contribution(
  your_SE,
  SAMPLENAME_choice = NULL,
  filter_by,
  filter_selection,
  plot_over,
  plot_over_display_choices = NULL,
  clone_sequences = NULL,
  n_clones = 10,
  graph_type = "bar",
  keep_numeric = TRUE,
  plot_non_selected = TRUE,
  linesize = 0.2,
  text_size = 15,
  your_title = "",
  y_limit = NULL,
  return_table = FALSE
)

```

Arguments

| | |
|--|--|
| <code>your_SE</code> | A Summarized Experiment object. |
| <code>SAMPLENAME_choice</code> | The identifying <code>SAMPLENAME</code> from which to obtain the top " <code>n_clones</code> " clones to color. If <code>NULL</code> and <code>clone_sequences</code> is <code>NULL</code> , all clones will be shown as gray. |
| <code>filter_by</code> | Name of metadata column to filter by e.g. Lineage |
| <code>filter_selection</code> | The value of the filter column to display e.g. "T" (within Lineage) |
| <code>plot_over</code> | The column of metadata that you want to be the x-axis of the plot. e.g. Month. For numeric metadata, the x-axis will be ordered in ascending fashion. For categorical metadata, the sample order will be followed. |
| <code>plot_over_display_choices</code> | Choice(s) from the column designated in <code>plot_over</code> that will be used for plotting. Defaults to all. |
| <code>clone_sequences</code> | The identifying rownames within <code>your_SE</code> for which to plot. <code>SAMPLENAME_choice</code> should be set to <code>NULL</code> or not specified if <code>clone_sequences</code> is specified. |
| <code>n_clones</code> | Numeric. Number of top clones from <code>SAMPLENAME_choice</code> that should be assigned a unique color. |
| <code>graph_type</code> | Choice of "bar" or "line" for how to display the clonal contribution data |
| <code>keep_numeric</code> | If <code>plot_over</code> is numeric, whether to space the x-axis appropriately according to the numerical values. |
| <code>plot_non_selected</code> | Plot clones NOT found within the top clones in <code>SAMPLENAME_choice</code> or the specified clones passed to <code>clone_sequences</code> . These clones are colored gray. If |

| | |
|--------------|---|
| | both SAMPLENAME_choice and clone_sequences are NULL, this argument must be set to TRUE. Otherwise, there will be no data to show. |
| linesize | Numeric. Thickness of the lines. |
| text_size | Numeric. Size of text in plot. |
| your_title | Title string for your plot. |
| y_limit | Numeric. What the max value of the y scale should be for the "proportions" assay. |
| return_table | Logical. If set to TRUE, the function will return a dataframe with each sequence that is selected and its percentage contribution to each selected sample rather than a plot. |

Value

Displays a stacked area line or bar plot (made by ggplot2) of the samples' top clones. Or, if return_table is set to TRUE, returns a dataframe of the percentage abundances in each sample.

Examples

```
data(wu_subset)
clonal_contribution(
  your_SE = wu_subset, graph_type = "bar",
  SAMPLENAME_choice = "ZJ31_20m_T",
  filter_by = "celltype", filter_selection = "T",
  plot_over = "months", n_clones = 10
)
```

clonal_count

Clonal count plot

Description

A line plot that tracks the total number of clones or the cumulative number of clones from selected samples of the SummarizedExperiment object plotted over a specified variable.

Usage

```
clonal_count(
  your_SE,
  percent_threshold = 0,
  plot_over,
  plot_over_display_choices = NULL,
  keep_numeric = TRUE,
  group_by,
  group_by_choices = NULL,
  cumulative = FALSE,
  point_size = 3,
```

```

    line_size = 2,
    text_size = 12,
    your_title = NULL,
    return_table = FALSE
  )

```

Arguments

| | |
|--|---|
| <code>your_SE</code> | Summarized Experiment object containing clonal tracking data as created by the <code>barcodetrackR</code> 'create_SE' function. |
| <code>percent_threshold</code> | Numeric. The percent threshold for which to count barcodes as present or not present. Set to 0 by default. |
| <code>plot_over</code> | The column of metadata that you want to be the x-axis of the plot. e.g. <code>timepoint</code> |
| <code>plot_over_display_choices</code> | Choice(s) from the column designated in <code>plot_over</code> that will be used for plotting. Defaults to all if left as <code>NULL</code> . |
| <code>keep_numeric</code> | If <code>plot_over</code> is numeric, whether to space the x-axis appropriately according to the numerical values. |
| <code>group_by</code> | The column of metadata you want to group by e.g. <code>cell_type</code> . |
| <code>group_by_choices</code> | Choice(s) from the column designated in <code>group_by</code> that will be used for plotting. Defaults to all if left as <code>NULL</code> . |
| <code>cumulative</code> | Logical. If <code>TRUE</code> , will plot cumulative counts over the 'plot_over' argument rather than unique counts per sample (the default, which is <code>FALSE</code>). |
| <code>point_size</code> | Numeric. Size of points. |
| <code>line_size</code> | Numeric. Size of lines. |
| <code>text_size</code> | Numeric. Size of text in plot. |
| <code>your_title</code> | The title for the plot. |
| <code>return_table</code> | Logical. If set to <code>true</code> , rather than returning a plot, the function will return the clonal count or cumulative count of each sample in a dataframe. |

Value

Outputs plot of a diversity measure tracked for groups over a factor. Or if `return_table` is set to `TRUE`, a dataframe of the number of clones (or cumulative clones) for each sample.

Examples

```

data(wu_subset)
clonal_count(your_SE = wu_subset, cumulative = FALSE, plot_over = "months", group_by = "celltype")

```

| | |
|------------------|------------------------------|
| clonal_diversity | <i>Clonal diversity plot</i> |
|------------------|------------------------------|

Description

A line plot that tracks a diversity measure from selected samples of the SummarizedExperiment object plotted over a specified variable.

Usage

```
clonal_diversity(
  your_SE,
  plot_over,
  plot_over_display_choices = NULL,
  keep_numeric = TRUE,
  group_by,
  group_by_choices = NULL,
  index_type = "shannon",
  point_size = 3,
  line_size = 2,
  text_size = 12,
  your_title = NULL,
  return_table = FALSE
)
```

Arguments

| | |
|--|---|
| <code>your_SE</code> | Summarized Experiment object containing clonal tracking data as created by the <code>barcodetrackR</code> <code>'create_SE'</code> function. |
| <code>plot_over</code> | The column of metadata that you want to be the x-axis of the plot. e.g. <code>timepoint</code> |
| <code>plot_over_display_choices</code> | Choice(s) from the column designated in <code>plot_over</code> that will be used for plotting. Defaults to all if left as <code>NULL</code> . |
| <code>keep_numeric</code> | If <code>plot_over</code> is numeric, whether to space the x-axis appropriately according to the numerical values. |
| <code>group_by</code> | The column of metadata you want to group by e.g. <code>cell_type</code> |
| <code>group_by_choices</code> | Choice(s) from the column designated in <code>group_by</code> that will be used for plotting. Defaults to all if left as <code>NULL</code> . |
| <code>index_type</code> | Character. One of "shannon", "shannon_count", "simpson", or "invsimpson". |
| <code>point_size</code> | Numeric. Size of points. |
| <code>line_size</code> | Numeric. Size of lines. |
| <code>text_size</code> | Numeric. Size of text in plot. |
| <code>your_title</code> | Character. The title for the plot. |

`return_table` Logical. IF set to TRUE, rather than returning the plot of clonal diversity, the function will return a dataframe containing the diversity index values for each specified sample.

Value

Outputs plot of a diversity measure tracked for groups over a factor. Or if `return_table` is set to true, a dataframe will be returned instead.

Examples

```
data(wu_subset)
clonal_diversity(
  your_SE = wu_subset, index_type = "shannon",
  plot_over = "months", group_by = "celltype"
)
```

`cor_plot`

Correlation Plot

Description

Plots the pairwise correlation between the specified assay of each sample-sample pair in the provided SummarizedExperiment.

Usage

```
cor_plot(
  your_SE,
  assay = "proportions",
  plot_labels = colnames(your_SE),
  method_corr = "pearson",
  your_title = "",
  grid = TRUE,
  label_size = 8,
  plot_type = "color",
  no_negatives = FALSE,
  return_table = FALSE,
  color_scale = "default",
  number_size = 3,
  point_scale = 1
)
```

Arguments

`your_SE` A Summarized Experiment object.

`assay` The choice of assay to use for the correlation calculation. Set to "proportions" by default.

| | |
|--------------|---|
| plot_labels | Vector of x axis labels. Defaults to colnames(your_SE). |
| method_corr | Character. One of "pearson", "spearman", or "kendall". |
| your_title | Character. The title for the plot. |
| grid | Logical. Include a grid or not in the correlation plot |
| label_size | Numeric. The size of the column labels. |
| plot_type | Character. One of "color", "circle", or "number". |
| no_negatives | Logical. Whether to make negative correlations = 0. |
| return_table | Logical. Whether or not to return table of p-values, confidence intervals, and R values instead of displaying a plot. |
| color_scale | Character. Either "default" or an odd-numbered color scale where the lowest value will correspond to -1, the median value to 0, and the highest value to 1. |
| number_size | Numeric. Size of the text label when plot_type is "number". |
| point_scale | Numeric. The size of the largest point if the plot_type is "circle" |

Value

Plots pairwise correlation plot for the samples in your_SE.

Examples

```
data(wu_subset)
cor_plot(your_SE = wu_subset, plot_type = "color")
# "
```

create_SE

create_SE

Description

Creates a SummarizedExperiment object from a data frame containing clonal tracking counts ('your_data') with rows as observations and columns as samples, and the associated metadata ('meta_data') with rows as samples and columns of information describing those samples.

Usage

```
create_SE(
  your_data = NULL,
  meta_data = NULL,
  threshold = 0,
  threshold_type = "relative",
  log_base = exp(1),
  scale_factor = 1e+06
)
```

Arguments

| | |
|----------------|--|
| your_data | A data frame. For clonal tracking data, this will be individual barcodes or lineage tracing elements in rows and samples in columns. |
| meta_data | A data frame containing all meta-data. Must, at the very least, include a column called "SAMPLENAME" that contains all of the colnames within the data frame passed as 'your_data' and only those colnames. |
| threshold | Numeric. The minimum threshold abundance for a barcode to be maintained in the SE. If 'threshold_type' is relative, this parameter should be between 0 and 1. If 'threshold_type' is absolute, this parameter should be greater than 1. |
| threshold_type | Character. One of "relative" or "absolute" relative. If a relative threshold is specified, only those rows which have higher than 'threshold' proportion of reads within at least one sample will be kept as non-zero. If an absolute threshold is specified, only those rows which have an absolute read count higher than 'threshold' in at least one sample will be kept as non-zero. |
| log_base | A numeric indicating which base to use when logging the normalized data |
| scale_factor | A numeric indicating what scaling factor to use in normalization. For the default value of 1 million, barcode proportions on a per sample basis will be multiplied by 1 million before log+1 normalization. |

Value

Returns a SummarizedExperiment holding your clonal tracking data and the associated metadata.

Examples

```
count_path <- system.file("extdata",
  "/WuC_etal_appdata/sample_data_ZJ31.txt",
  package = "barcodetrackR"
)
wu_dataframe <- read.delim(count_path, row.names = 1)
metadata_path <- system.file("extdata",
  "/WuC_etal_appdata/sample_metadata_ZJ31.txt",
  package = "barcodetrackR"
)
wu_metadata <- read.delim(metadata_path)
wu_SE <- create_SE(
  your_data = wu_dataframe, meta_data = wu_metadata,
  threshold = 0
)
```

dist_plot

Pairwise Distance Plot

Description

Plots the pairwise distances of the specified assay between each sample-sample pair in the provided SummarizedExperiment.

Usage

```

dist_plot(
  your_SE,
  assay = "proportions",
  plot_labels = colnames(your_SE),
  dist_method = "euclidean",
  cluster_tree = FALSE,
  your_title = "",
  grid = TRUE,
  label_size = 10,
  plot_type = "color",
  no_negatives = FALSE,
  return_table = FALSE,
  color_pal = "Blues",
  number_size = 3,
  point_scale = 5,
  minkowski_p = 2
)

```

Arguments

| | |
|--------------|---|
| your_SE | A Summarized Experiment object. |
| assay | The choice of assay to use for the correlation calculation. Set to "proportions" by default. |
| plot_labels | Vector of x axis labels. Defaults to colnames(your_SE). |
| dist_method | Character. Distance OR similarity measure from the 'proxy' package. Full list of distance and similarity measures can be found using 'summary(proxy::pr_DB)'. Default is "euclidean". Distances will be calculated for distance measures, while similarities will be calculated for similarity measures. Distance OR similarity measure will be calculated using the 'assay' specified. |
| cluster_tree | Logical. Whether to cluster samples and plot a hierarchical tree calculated from the distance or similarity measure used. Default is FALSE. |
| your_title | Character. The title for the plot. |
| grid | Logical. Include a grid or not in the resulting plot. |
| label_size | Numeric. The size of the column labels. |
| plot_type | Character. One of "color", "circle", or "number". |
| no_negatives | Logical. Whether to make negative correlations = 0. |
| return_table | Logical. Whether or not to return table of p-values, confidence intervals, and R values instead of displaying a plot. |
| color_pal | Character. One of 'Reds', 'Purples', 'Oranges', 'Greys', 'Greens', or 'Blues' that designates the brewer.pal color scale to use. |
| number_size | Numeric. size of the text label when plot_type is "number". |
| point_scale | Numeric. The size of the largest point if the plot_type is "circle". |
| minkowski_p | Numeric. If 'Minkowski' is chosen, the 'p' used to calculate the Minkowski distance. |

Value

Plots pairwise correlation plot for the samples in your_SE.

Examples

```
data(wu_subset)
dist_plot(your_SE = wu_subset, plot_type = "color")
# "
```

estimate_barcode_threshold

Estimate Barcode Threshold

Description

Estimates an appropriate minimum abundance threshold for reliably detected barcodes in a clonal tracking dataset.

For a specified capture efficiency C , the minimum clone size N that we can expect to detect with confidence level P is calculated from:

$$P = 1 - (1 - C)^N$$

The proportional abundance of a clonal tag of size N is

$$N / (T * F)$$

where T is the total population size of cells or genomes and F is the frequency or proportion of the total population which is labeled or genetically modified with the clonal tag.

The population size and proportion labeled must be determined experimentally. The capture efficiency should be estimated for a given clonal tracking technique by simulating the barcode retrieval process in silico and finding the capture efficiency which leads to a total # of detected barcodes matching the experimentally determined number. Adair et al (PMID: 32355868) performed this analysis for viral integration site analysis and DNA barcode sequencing and determined good estimates for the capture efficiencies of these two technologies to be 0.05 and 0.4 respectively.

Usage

```
estimate_barcode_threshold(
  capture_efficiency = NULL,
  population_size,
  proportion_labeled,
  confidence_level = 0.95,
  verbose = TRUE
)
```

Arguments

| | |
|--------------------|--|
| capture_efficiency | Numeric. The capture efficiency of the clonal tracking method to detect a given clone. Must be between 0 and 1. See the description for details on how to estimate this value for a given experiment. |
| population_size | Numeric. The total number of cells/genomes within each sample analyzed in the clonal tracking study. This is an experimentally determined value. |
| proportion_labeled | Numeric. The proportion of the 'population_size' which is genetically modified or contains a clonal tracking index. This is an experimentally determined value. |
| confidence_level | Numeric. The confidence level for estimating the minimum abundance threshold. Must be between 0 and 1. Default is 0.95 for 95 percent confidence that a clone with proportion 'relative_threshold' will be detected. Increasing this parameter closer to one will result in a more stringent abundance threshold and decreasing this parameter will result in a more permissive abundance threshold. |
| verbose | Logical. Whether to print the calculated threshold. |

Value

Returns a single numeric 'relative_threshold' describing the proportional abundance above which clones can be considered reliable given the provided capture efficiency and labeled population size. Pass this value into the function 'threshold_SE' to threshold an existing SummarizedExperiment object or the function 'create_SE' to threshold a SummarizedExperiment object upon creation from dataframes of counts and metadata.

Examples

```
estimate_barcode_threshold(  
  capture_efficiency = 0.4,  
  population_size = 500000,  
  proportion_labeled = 0.3,  
  confidence_level = 0.95,  
  verbose = TRUE  
)
```

| | |
|----------------|---|
| get_top_clones | <i>get_top_clones (helper function)</i> |
|----------------|---|

Description

Retrieves the sequence(s) (row-identifier(s)) of the top "n_clones" within the specified sample from a SummarizedExperiment object.

Usage

```
get_top_clones(your_SE, SAMPLENAME_choice, n_clones = 10)
```

Arguments

| | |
|--------------------------------|--|
| <code>your_SE</code> | A summarized experiment. |
| <code>SAMPLENAME_choice</code> | Name of the <code>SAMPLENAME</code> identifier within <code>your_SE</code> from which to retrieve the top clones from. |
| <code>n_clones</code> | Numeric. Number of top clones from the specified sample that should be retrieved. |

Value

The row indices for the top `n_clones` in the dataset, using the 'ranks' assay.

Examples

```
data(wu_subset)
get_top_clones(wu_subset, "ZJ31_6m_T", n_clones = 10)
```

launchApp

Launch Barcode App

Description

Launches the Shiny Barcode App.

Usage

```
launchApp(x = NULL)
```

Arguments

| | |
|----------------|------|
| <code>x</code> | NULL |
|----------------|------|

Value

Page launching the Shiny Barcode App

Examples

```
if (interactive()) launchApp()
```

mds_plot

*MDS Plot***Description**

Calculates a similarity/dissimilarity index or matrix for each sample-sample pair and reduces the resulting dist matrix into two dimensions

Usage

```
mds_plot(
  your_SE,
  group_by = "SAMPLENAME",
  method_dist = "bray",
  assay = "proportions",
  your_title = NULL,
  point_size = 3,
  text_size = 12,
  return_table = FALSE,
  kmeans_cluster = FALSE,
  k.param = 3,
  draw_ellipses = FALSE
)
```

Arguments

| | |
|----------------|--|
| your_SE | Summarized Experiment object containing clonal tracking data as created by the barcodetrackR 'create_SE' function. |
| group_by | Column of metadata to color samples by. Can also specify "kmeans_cluster" if kmeans_cluster argument is set to TRUE, and then the grouping variables will be the clustering result. |
| method_dist | Dissimilarity index from vegan. One of "manhattan", "euclidean", "canberra", "clark", "bray", "kulczynski", "jaccard", "gower", "altGower", "morisita", "horn", "mountford", "raup", "binomial", "chao", or "cao". |
| assay | The assay to calculate the index on |
| your_title | Character. The title for the plot. |
| point_size | Numeric. The size of the points. |
| text_size | Numeric. Size of text in plot. |
| return_table | Logical. If set to true, the function will return a dataframe containing each samples reduced measure of dissimilarity coordinates. |
| kmeans_cluster | Logical. If set to true, each sample will be assigned a cluster computed by kmeans on the chosen assay. |
| k.param | Numeric. If kmeans_cluster is TRUE, provide the number of kmeans clusters to identify. |
| draw_ellipses | Logical. If kmeans_cluster is TRUE, draw ellipses around the different kmeans clusters. |

Value

Plots dissimilarity indices between samples in your_SE. Or if return table is set to TRUE, returns a dataframe of each sample's reduced measures of dissimilarity coordinates.

Examples

```
data(wu_subset)
mds_plot(your_SE = wu_subset, method_dist = "bray", group_by = "celltype")
# "
```

rank_abundance_plot *Rank Abundance Plot*

Description

Create a rank abundance plot of the barcodes in the chosen samples provided in 'your_SE'. Use this function to visualize the distribution of barcode abundances within sample(s). Note: If comparing the visualization to the statistical testing results from 'rank_abundance_stat_test' function in barcodetrackR, please set the 'scale_rank' to TRUE. The K-S test is agnostic to number of samples so it is directly comparable to the visualization produced when the barcode ranks are scaled between 0 and 1.

Usage

```
rank_abundance_plot(
  your_SE,
  scale_rank = FALSE,
  point_size = 3,
  your_title = NULL,
  text_size = 12,
  plot_labels = NULL,
  return_table = FALSE
)
```

Arguments

| | |
|-------------|---|
| your_SE | Summarized Experiment object containing clonal tracking data as created by the barcodetrackR 'create_SE' function. |
| scale_rank | Logical. Whether or not to scale all ranks from 0 to 1 or keep barcode ranks as their actual integer values. When 'scale_rank' is set to FALSE, all samples will not necessarily have the same x maximum. |
| point_size | Numeric. Size of the points for the plot. |
| your_title | Character. Specify a title for the plot. |
| text_size | Numeric. Size of text in plot. |
| plot_labels | Vector of labels for each sample. If not specified, the colnames(your_SE) will be used. |

`return_table` Logical. If set to TRUE, rather than a plot, the function will return a dataframe containing for each sample, each barcode in rank order with its abundance in that sample, its scaled rank (0 to 1), and the cumulative sum of abundance for all barcodes with rank \leq the rank of that barcode.

Value

Displays a rank-abundance plot (made by ggplot2) of the samples chosen. Each point represents a single barcode with the x-value describing its rank in abundance with 1 being the most abundant barcode. The y-value representing the cumulative abundance of all barcodes with rank less than or equal to the x-axis value. If the `return_table` is set to TRUE, instead of a plot, a dataframe with the rank abundance data will be returned.

Examples

```
data(wu_subset)
rank_abundance_plot(your_SE = wu_subset[, 1:4], point_size = 2)
```

rank_abundance_stat_test

Rank Abundance Statistical Test

Description

Carries out a specific instance of statistical testing relevant to clonal tracking experiments. For the provided SummarizedExperiment, compare the rank-abundance distribution which is described by the increase in cumulative abundance within that sample as barcode abundances are added, starting with the most abundant barcode. The two-sided Kolmogorov-Smirnov statistical test is carried out comparing each pair of samples using the R function `ks.test`: <https://www.rdocumentation.org/packages/dgof/versions/1.2/topi>. Note that this test compares rank-abundance distribution regardless of whether the samples share the same barcodes or lineage tracing elements. The test could be employed on two samples with no barcode sequence overlap, simply to compare whether the rank abundance distribution of barcodes is drawn from the same distribution.

Usage

```
rank_abundance_stat_test(your_SE, statistical_test = "ks")
```

Arguments

`your_SE` Summarized Experiment object containing clonal tracking data as created by the `barcodetrackR` 'create_SE' function.

`statistical_test` The statistical test used to compare distributions. For now, the only implemented test is the Kolmogorov-Smirnov test.

Value

Returns a list containing two dataframes

[[`"D_statistic"`]] is a dataframe containing pairwise D-statistics between each pair of samples in `your_SE`. The D statistic represents the maximal difference between the two rank abundance distributions.

[[`"p_value"`]] A dataframe containing the p-value computed by the KS test for each pair of samples. The null hypothesis is that the two rank-abundance profiles come from the same distribution.

Examples

```
data(wu_subset)
rank_abundance_stat_test(your_SE = wu_subset, statistical_test = "ks")
```

scatter_plot

Scatter Plot

Description

Plots a scatter plot of two samples in the Summarized Experiment object

Usage

```
scatter_plot(
  your_SE,
  assay = "proportions",
  plot_labels = colnames(your_SE),
  method_corr = "pearson",
  display_corr = TRUE,
  point_size = 0.5,
  your_title = "",
  text_size = 12
)
```

Arguments

| | |
|---------------------------|--|
| <code>your_SE</code> | A Summarized Experiment object of two samples. |
| <code>assay</code> | The choice of assay to plot on the scatter plot. Set to "proportions" by default. |
| <code>plot_labels</code> | The labels for the X and Y axis of the plot |
| <code>method_corr</code> | Character. One of "pearson", "spearman", or "kendall". Can also use "manhattan" to compute manhattan distance instead. |
| <code>display_corr</code> | Logical. Whether to display the computer correlation or not. |
| <code>point_size</code> | Numeric. The size of the points being plotted. |
| <code>your_title</code> | Logical. The title for the plot. |
| <code>text_size</code> | Numeric. Size of text in plot. |

Value

Displays a scatter plot of the specified assay for the specified samples in your_SE with correlation value optionally displayed.

Examples

```
data(wu_subset)
scatter_plot(your_SE = wu_subset[, c(4, 8)])
# "
```

stat_hist

Stat histogram

Description

Given a summarized experiment, gives a histogram of the acc assay or choice of metadata.

Usage

```
stat_hist(
  your_SE,
  data_choice = "assay stats",
  assay_choice = "counts",
  metadata_stat = NULL,
  group_meta_by = NULL,
  scale_all_y = FALSE,
  y_log_axis = FALSE,
  text_size = 12,
  n_bins = 30,
  n_cols = NULL,
  your_title = NULL
)
```

Arguments

| | |
|---------------|---|
| your_SE | Your SummarizedExperiment of barcode data and associated metadata. |
| data_choice | Either "assay stats" which allows you to view the distribution of values in the 'assay_choice' assay, or "metadata stats" which allows you to view the distribution of metadata values in your SummarizedExperiment object. |
| assay_choice | When data_choice is set to "assay stats", designates which assay will be used. |
| metadata_stat | When data_choice is set to "metadata stats", The metadata values that will be used. |
| group_meta_by | When data_choice is set to "metadata stats", facet the histogram using this column of metadata. If NULL, no grouping or faceting applied |
| scale_all_y | Logical. Whether or not to plot all plots on the same y axis limits. |

| | |
|-------------------------|--|
| <code>y_log_axis</code> | Logical. Whether or not to put y axis on log scale |
| <code>text_size</code> | Size of text. |
| <code>n_bins</code> | Number of bins for histograms. Default is 30. |
| <code>n_cols</code> | Number of columns for faceted histograms. If NULL (default) will automatically choose <code>n_cols</code> for facetting. |
| <code>your_title</code> | Character. The title for the plot. |

Value

Histogram of chosen statistics

Examples

```
data(wu_subset)
stat_hist(
  your_SE = wu_subset[, 1], data_choice = "assay stats",
  assay_choice = "counts"
)
```

subset_SE

subset_SE

Description

Subsets an existing SummarizedExperiment object.

Usage

```
subset_SE(your_SE, ...)
```

Arguments

| | |
|----------------------|---|
| <code>your_SE</code> | A SummarizedExperiment object. |
| <code>...</code> | Arguments passed to <code>subset_SE</code> in the form of ‘X = keys’ where ‘X’ is a column from SE’s <code>colData</code> and ‘keys’ are entries in the <code>colData</code> to subset. |

Value

Returns a subsetted SummarizedExperiment object.

Examples

```
data(wu_subset)
wu_B.5month <- subset_SE(wu_subset, celltype = "B", timepoint = "6.0")
```

| | |
|-----------|------------------|
| threshold | <i>Threshold</i> |
|-----------|------------------|

Description

This is a helper which function takes in sequence data in table form, along with a threshold, to each column (e.g. if threshold is set as 0.0005, only rows in which an element is above 0.05 its column will be kept).

Usage

```
threshold(your_data, thresh = 5e-04, thresh_type = "relative")
```

Arguments

| | |
|-------------|---|
| your_data | A data frame. Usually individual barcodes in rows and samples in columns. |
| thresh | Numeric. |
| thresh_type | Character. One of "relative" or "absolute" |

Value

A data frame where all rows (barcodes) that did not have at least one element meet the threshold have been discarded.

Examples

```
data(wu_subset)
threshold(SummarizedExperiment::assay(wu_subset, assay = "counts"),
  thresh = 0.0005
)
```

| | |
|--------------|---------------------|
| threshold_SE | <i>Threshold SE</i> |
|--------------|---------------------|

Description

Removes barcodes from a SummarizedExperiment object which have an abundance lower than the provided relative or absolute threshold. See the function 'estimate_barcode_threshold' to estimate an appropriate threshold for an SE.

Usage

```
threshold_SE(
  your_SE,
  threshold_value,
  threshold_type = "relative",
  verbose = TRUE
)
```

Arguments

| | |
|------------------------------|--|
| <code>your_SE</code> | A Summarized Experiment object. |
| <code>threshold_value</code> | Numeric. The minimum threshold abundance for a barcode to be maintained in the SE. If 'threshold_type' is relative, this parameter should be between 0 and 1. If 'threshold_type' is absolute, this parameter should be greater than 1. |
| <code>threshold_type</code> | Character. One of "relative" or "absolute" relative. If a relative threshold is specified, only those rows which have higher than 'threshold_value' proportion of reads within at least one sample will be kept as non-zero. If an absolute threshold is specified, only those rows which have an absolute read count higher than 'threshold_value' in at least one sample will be kept as non-zero. |
| <code>verbose</code> | Logical. If TRUE, print the total number of barcodes removed from the SE. |

Value

Returns a SummarizedExperiment containing only barcodes which passed the supplied threshold in at least one sample. All of the default assays are re-calculated after thresholding is applied. Note that since the SE is re-instantiated, any custom assays should be recalculated after thresholding.

Examples

```
data(wu_subset)
threshold_SE(
  your_SE = wu_subset, threshold_value = 0.005,
  threshold_type = "relative", verbose = TRUE
)
```

wu_subset

Small subset of Wu barcoding dataset

Description

A SummarizedExperiment object containing a subset of the Wu barcoding dataset. It includes peripheral blood T, B, Gr, NK_56, and NK-16 samples from the first 4 times points of macaque ZJ31.

Usage

```
data(wu_subset)
```

Format

A SummarizedExperiment object with 215 features rows and 20 samples:

assays includes the counts, proportions, ranks, normalized, and logs assays

colData includes the accompanying metadata for the samples

metadata includes the scale_factor used and the log_base used in the log assay ...

Source

```
system.file("sample_data/WuC_etal/monkey_ZJ31.txt", package = "barcodetrackR") system.file("sample_data/WuC_etal/mo
package = "barcodetrackR") wu_SE <- create_SE(your_data = wu_dataframe, meta_data = wu_metadata,
threshold = 0.005) wu_subset <- wu_SE[,1:20] http://dx.doi.org/10.1126/sciimmunol.aat9781
```

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