Package 'APackOfTheClones'

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Type Package

Title Visualization of Clonal Expansion for Single Cell Immune
Profiles

Version 1.2.4

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Description Visualize clonal expansion via circle-packing. 'APackOfTheClones' extends 'scRepertoire' to produce a publication-ready visualization of clonal expansion at a single cell resolution, by representing expanded clones as differently sized circles. The method was originally implemented by Murray Christian and Ben Murrell in the following immunology study: Ma et al. (2021) <doi:10.1126/sciimmunol.abg6356>.

BugReports https://github.com/Qile0317/APackOfTheClones/issues

Depends R (>= 4.1.0)

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Suggests cowplot, covr, knitr, rmarkdown, sf, spelling, testthat (>=
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URL https://qile0317.github.io/APackOfTheClones/,
https://github.com/Qile0317/APackOfTheClones,
https://joss.theoj.org/papers/10.21105/joss.06868

LinkingTo Rcpp

VignetteBuilder knitr

NeedsCompilation yes

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Contents

AdjustAPOTC	*Adjust the parameters of the APackOfTheClones reduction in a seurat object*

Description

[Stable]

If the user is unsatisfied with the clonal expansion plot that was generated from [RunAPOTC()](#) and
[APOTCPlot()](#), this function has a range of arguments to modify the data and/or parameters of the
visualization. Note that some of the arguments may conflict with each other.

Usage

```
AdjustAPOTC(
  seurat_obj,
  reduction_base = NULL,
  clonecall = NULL,
  ...,
  extra_filter = NULL,
```

```
    run_id = NULL,
    new_rad_scale_factor = NULL,
    new_clone_scale_factor = NULL,
    repulse = FALSE,
    repulsion_threshold = 1,
    repulsion_strength = 1,
    max_repulsion_iter = 10L,
    relocate_cluster = NULL,
    relocation_coord = NULL,
    nudge_cluster = NULL,
    nudge_vector = NULL,
    recolor_cluster = NULL,
    new_color = NULL,
    rename_label = NULL,
    new_label = NULL,
    relocate_label = NULL,
    label_relocation_coord = NULL,
    nudge_label = NULL,
    label_nudge_vector = NULL,
    verbose = TRUE
)
```

Arguments

seurat_obj	The seurat object to be adjusted.
reduction_base	character. The seurat reduction to base the clonal expansion plotting on. Defaults to `'umap'` but can be any reduction present within the reductions slot of the input seurat object, including custom ones. If ''pca'", the cluster coordinates will be based on PC1 and PC2. However, generally APackOfTheClones is used for displaying UMAP and occasionally t-SNE versions to intuitively highlight clonal expansion.
clonecall	character. The column name in the seurat object metadata to use. See scRepertoire documentation for more information about this parameter that is central to both packages.
...	additional "subsetting" keyword arguments indicating the rows corresponding to elements in the seurat object metadata that should be filtered by. E.g., seurat_clusters = c(1, 9, 10) will filter the cells to those in the seurat_clusters column with any of the values 1, 9, and 10. Unfortunately, column names in the seurat object metadata cannot conflict with the keyword arguments. **MAJOR NOTE** if any subsetting keyword arguments are a *prefix* of any preceding argument names (e.g. a column named reduction is a prefix of the reduction_base argument) R will interpret it as the same argument unless *both* arguments are named. Additionally, this means any subsequent arguments *must* be named.
extra_filter	character. An additional string that should be formatted *exactly* like a statement one would pass into [dplyr::filter](#) that does *additional* filtering to cells in the seurat object - on top of the other keyword arguments - based on the metadata. This means that it will be logically AND'ed with any keyword argument filters. This is a more flexible alternative / addition to the filtering keyword arguments.

	For example, if one wanted to filter by the length of the amino acid sequence of TCRs, one could pass in something like extra_filter = "nchar(CTaa) - 1 > 10". When involving characters, ensure to enclose with single quotes.
run_id	character. This will be the ID associated with the data of a run, and will be used by other important functions like [APOTCPlot()](#) and [AdjustAPOTC](#). Defaults to NULL, in which case the ID will be generated in the following format: reduction_base;clonecall;keyword_arguments;extra_filter
	where if keyword arguments and extra_filter are underscore characters if there was no input for the ... and extra_filter parameters.
new_rad_scale_factor	
	a single numeric in (0, 1). changes the radius scaling factor of all circles.
new_clone_scale_factor	
	a single numeric in (0, 1). changes the clone_scale_factor
repulse	If TRUE, will attempt to push overlapping clusters away from each other.
repulsion_threshold	
	numeric. The radius that clonal circle clusters overlap is acceptable when re-pulsing.
repulsion_strength	
	numeric. The smaller the value the less the clusters repulse each other per itera-tion, and vice versa.
max_repulsion_iter	
	integer. The number of repulsion iterations.
relocate_cluster	
	Numeric or Character. Indicates which cluster(s) based on the index or label to relocate to new coordinates.
relocation_coord	
	numeric of length two or a list of numerics of length two of length of relocate_cluster. If its a list, indicates each coordinate that the clusters in relocate_cluster should move to. If its just a numeric, then will relocate all clusters in relocate_cluster to the input, which is likely not desired behavior, so this should only be conve-nience syntax if relocate_cluster has length 1.
nudge_cluster	Numeric or Character. Indicates which cluster(s) based on the index or label to "nudge"/translate their coordinate(s) by.
nudge_vector	numeric of length two or a list of numerics of length two of length of nudge_cluster. If its a list, indicates each translation vector (in other words, x-y coordinates) that the clusters in nudge_cluster should be translate by. If its just a numeric, then will translate all clusters in nudge_cluster by the input - which mostly is syn-tactic sugar for translating a single cluster if the input of nudge_cluster is of length 1.
recolor_cluster	
	Numeric or Character. Indicates which cluster(s) based on the index or label to change their color by.
new_color	character of arbitrary length. Indicates the corresponding new colors that se-lected clusters in recolor_cluster should be changed to.
rename_label	Numeric or character. Indicates the index or name of label(s) to be renamed.

new_label   Character. Indicates the corresponding new label(s) that selected label(s) in
                   rename_label should be changed to.

relocate_label   Numeric or character. Indicates the index or name of label(s) to be relocated.

label_relocation_coord

                   Numeric of length two or a list of numerics of length two of length of relocate_label.
                   If it's a list, indicates each coordinate that the labels in relocate_label should
                   move to. If it's just a numeric, then will relocate all labels in relocate_label
                   to the input, which is likely not desired behavior, so this should only be conve-
                   nience syntax if relocate_label has length 1.

nudge_label   Numeric or character. Indicates the index or name of label(s) to be "nudged"/translated.

label_nudge_vector

                   Numeric of length two or a list of numerics of length two of length of nudge_label.
                   If it's a list, indicates each translation vector (in other words, x-y coordinates)
                   that the labels in nudge_label should be translated by. If it's just a numeric,
                   then will translate all labels in nudge_label by the input - which mostly is syn-
                   tactic sugar for translating a single label if the input of nudge_label is of length
                   1.

verbose   logical. Decides if visual cues are displayed to the R console of the progress.

Value

The adjusted seurat_obj

Examples

```
# do an APackOfTheClones run
pbmc <- RunAPOTC(get(data("combined_pbmc")), verbose = FALSE)

# adjust the rad_scale_factor, and nudge cluster 1 by x = 1, y = 1
pbmc <- AdjustAPOTC(
    pbmc,
    new_rad_scale_factor = 0.9,
    nudge_cluster = 1,
    nudge_vector = c(1, 1),
    verbose = FALSE
)

# plot the result
APOTCPlot(pbmc)

# rename some labels
pbmc <- AdjustAPOTC(
    pbmc, rename_label = c(2, 5), new_label = c("Foo", "Bar")
)

# perhaps multiple clusters need to be relocated and repulsed
pbmc <- AdjustAPOTC(
    pbmc,
    relocate_cluster = c("Foo", "C10"), # using labels works too
    relocation_coord = list(c(2, 3.5), c(0, 5)),
```

```
    repulse = TRUE,
    verbose = FALSE
)


# plot again to check results
APOTCPlot(pbmc, show_labels = TRUE, verbose = FALSE)
```

APOTCPlot   *Various variations of visualizations of clonal expansion post-*
                             RunAPOTC

Description

[Stable]

Given a seurat object with an 'apotc' (APackOfTheClones) object from running [RunAPOTC()](), this
function will read the information and return a customizable ggplot2 object of the clonal expansion
with a circle size legend. If the user is unhappy about certain aspects of the plot, many parameters
can be adjusted with the [AdjustAPOTC]() function.

The specific APackOfTheClones run to be plotted can be identified in two ways: either by inputting
the run_id associated with the run that was either defined / auto-generated during [RunAPOTC()](), or
by inputting the reduction_base, clonecall, extra_filter and any other keyword arguments
that corresponded to the run. Its heavily recommended to use the run_id. If none of these parame-
ters are inputted, the function defaults to returning the plot of the latest run.

Usage

```
APOTCPlot(
  seurat_obj,
  reduction_base = NULL,
  clonecall = NULL,
  ...,
  extra_filter = NULL,
  run_id = NULL,
  show_shared = NULL,
  only_link = NULL,
  clone_link_width = "auto",
  clone_link_color = "black",
  clone_link_alpha = 0.5,
  res = 360L,
  linetype = "blank",
  use_default_theme = TRUE,
  retain_axis_scales = FALSE,
  alpha = 1,
  show_labels = FALSE,
  label_size = 5,
  add_size_legend = TRUE,
```

```
        legend_sizes = "auto",
        legend_position = "auto",
        legend_buffer = 0.2,
        legend_color = "#808080",
        legend_spacing = "auto",
        legend_label = "Clone sizes",
        legend_text_size = 5,
        add_legend_background = TRUE,
        add_legend_centerspace = 0,
        detail = TRUE,
        verbose = TRUE
    )
```

Arguments

seurat_obj	A seurat object that has been integrated with clonotype data and has had a valid run of [RunAPOTC()]().
reduction_base	character. The seurat reduction to base the clonal expansion plotting on. Defaults to `'umap'` but can be any reduction present within the reductions slot of the input seurat object, including custom ones. If ''pca''', the cluster coordinates will be based on PC1 and PC2. However, generally APackOfTheClones is used for displaying UMAP and occasionally t-SNE versions to intuitively highlight clonal expansion.
clonecall	character. The column name in the seurat object metadata to use. See scRepertoire documentation for more information about this parameter that is central to both packages.
...	additional "subsetting" keyword arguments indicating the rows corresponding to elements in the seurat object metadata that should be filtered by. E.g., `seurat_clusters = c(1, 9, 10)` will filter the cells to those in the `seurat_clusters` column with any of the values 1, 9, and 10. Unfortunately, column names in the seurat object metadata cannot conflict with the keyword arguments. **MAJOR NOTE** if any subsetting keyword arguments are a *prefix* of any preceding argument names (e.g. a column named `reduction` is a prefix of the `reduction_base` argument) R will interpret it as the same argument unless *both* arguments are named. Additionally, this means any subsequent arguments *must* be named.
extra_filter	character. An additional string that should be formatted *exactly* like a statement one would pass into [dplyr::filter]() that does *additional* filtering to cells in the seurat object - on top of the other keyword arguments - based on the metadata. This means that it will be logically AND'ed with any keyword argument filters. This is a more flexible alternative / addition to the filtering keyword arguments. For example, if one wanted to filter by the length of the amino acid sequence of TCRs, one could pass in something like `extra_filter = "nchar(CTaa) - 1 > 10"`. When involving characters, ensure to enclose with single quotes.
run_id	character. This will be the ID associated with the data of a run, and will be used by other important functions like [APOTCPlot()]() and [AdjustAPOTC](). Defaults to NULL, in which case the ID will be generated in the following format: `reduction_base;clonecall;keyword_arguments;extra_filter`

	where if keyword arguments and extra_filter are underscore characters if there was no input for the `...` and `extra_filter` parameters.
show_shared	The output of [getSharedClones](#) can be inputted here, and the resulting plot will overlay lines between clone circles if that clonotype is common between clusters. Note that the input **must** be generated from data in the correct APackOfTheClones run, and the behavior is undefined otherwise and will likely error. The next 4 arguments allow for aesthetic customization of these line links.
only_link	Optional integer indicating to only display clone links originating from this cluster if showing shared clones.
clone_link_width	numeric. The width of the lines that connect shared clones. Defaults to "auto" which will estimate a reasonable value depending on circle sizes.
clone_link_color	character. The color of the lines that connect shared clones. Defaults to "blend" which will use the average colors of the two connected clones. Else, any hex color or valid color string input will work, and the corresponding color will be applied on all links.
clone_link_alpha	numeric. The alpha of the lines that connect shared clones.
res	The number of points on the generated path per full circle. From plot viewers, if circles seem slightly too pixelated, it is recommended to first try to export the plot as an `.svg` before increasing `res` due to increased plotting times from [ggforce::geom_circle](#).
linetype	The type of outline each circle should have. defaults to "blank meaning no outline. More information is in the function documentation of ggforce::geom_circle.
use_default_theme	logical that defaults to TRUE. If TRUE, the resulting plot will have the same theme as the seurat reference reduction plot. Else, the plot will simply have a blank background.
retain_axis_scales	If TRUE, approximately maintains the axis scales of the original reduction plot. However, it will only attempt to extend the axes and never shorten. Users are recommended to set this to TRUE especially if working with subsetted versions of the clonal data to better preserve the geometric relation to the original dimensional reduction.
alpha	numeric. The alpha of the circles in (0, 1]. Defaults to 1.
show_labels	If TRUE, will label each circle cluster at the centroid, defaulting to "C0, C1, ...".
label_size	The text size of labels if shown. Defaults to 5.
add_size_legend	If TRUE, adds a legend to the plot visualizing the relative sizes of clones. Note that it is simply an overlay and not a real ggplot2 legend.
legend_sizes	numeric vector. Indicates the circle sizes to be displayed on the legend, and will always be sorted from smallest to greatest. Defaults to "auto" which estimate a reasonable range of sizes to display.

legend_position

        character or numeric. Can be set to either ″top_left″, ″top_right″, ″bottom_left″, ″bottom_right″ and places the legend roughly in the corresponding position. Otherwise, can be a numeric vector of length 2 indicating the x and y position of the *topmost (smallest) circle* of the legend.

legend_buffer   numeric. Indicates how much to "push" the legend towards the center of the plot from the selected corner. If negative, will push away

legend_color   character. Indicates the hex color of the circles displayed on the legend. Defaults to the hex code for a gray tone

legend_spacing   numeric. Indicates the horizontal distance between each stacked circle on the size legend. Defaults to ″auto″ which will use an estimated value depending on plot size

legend_label   character. The title of the legend, which defaults to ″clone sizes.

legend_text_size

        numeric. The text size of the letters and numbers on the legend

add_legend_background

        logical. If TRUE, will add a border around the legend and fill the background to be white, overlaying anything else.

add_legend_centerspace

        numeric. An additional amount of distance changed between the circle sizes on the left side of the legend and the numbers on the right. Useful to set to around 0.5 (or more / less) when there are particularly large clone sizes that may cover the numbers.

detail   logical. If FALSE, will only plot entire clusters as one large circle, which may be useful in cases where there are a high number of clones resulting in a large number of circles on the resulting ggplot, which has increased plotting times, and certain aspects of the plot needs be finely adjusted with [AdjustAPOTC](#) or simply inspected. This should not be set to FALSE for the actual clonal expansion plot.

verbose   logical. Decides if visual cues are displayed to the R console of the progress.

Value

A ggplot object of the APackOfTheClones clonal expansion plot of the seurat object. There is an additional 10th element in the object named ″APackOfTheClones″ used by other functions in this package and shouldn't interfere with any other ggplot functionality. (As far as currently known)

See Also

[AdjustAPOTC](#)

Examples

```
data("combined_pbmc")

combined_pbmc <- RunAPOTC(
    combined_pbmc, run_id = "run1", verbose = FALSE
```

```
)

# plotting with default arguments will plot the latest "run1"
clonal_packing_plot <- APOTCPlot(combined_pbmc)
```

| combined_pbmc | *Example Multi-sampled T-cell seurat object with integrated TCR library* |

Description

[Stable]

Generated with scRepertoire::combineExpression. To construct this object from scratch, try:

scRepertoire::combineExpression(scRepertoire::combineTCR(get(data("contig_list", , package = "scRepertoire")), samples = c("P17B", "P17L", "P18B", , "P18L", "P19B", "P19L", "P20B", "P20L"), removeNA = FALSE, , removeMulti = FALSE, filterMulti = FALSE), get(data("scRep_example", , package = "scRepertoire")), cloneCall = "gene", proportion = TRUE)

Usage

```
data("combined_pbmc")
```

Format

A Seurat object with the following slots filled

assays Currently only contains one assay ("RNA" - scRNA-seq expression data)

counts - Raw expression data
- data - Normalized expression data
- scale.data - Scaled expression data
- var.features - names of the current features selected as variable
- meta.features - Assay level metadata such as mean and variance

meta.data Cell level metadata with a combined TCR contig list from scRepertoire

active.assay Current default assay

active.ident Current default idents

graphs Neighbor graphs computed, currently stores the SNN

reductions Dimensional reductions: UMAP

version Seurat version used to create the object

commands Command history, including the one used to create this object "combineExpression"

---

containsApotcRun   *Check for the existence of an APackOfTheClones run with its run id*

Description

[Stable]

A convenience function to check for the existence of an APackOfTheClones run with its run id, regardless of if any run has been made

Usage

```
containsApotcRun(seurat_obj, run_id)
```

Arguments

seurat_obj   a seurat object

run_id   character. The id of the associated ApotcRun.

Value

A logical indicating whether the run exists.

Examples

```
pbmc <- RunAPOTC(
    seurat_obj = get(data("combined_pbmc")),
    reduction_base = "umap",
    clonecall = "strict",
    run_id = "run1",
    verbose = FALSE
)

containsApotcRun(pbmc, "run1")
#> [1] TRUE

containsApotcRun(pbmc, "run2")
#> [1] FALSE
```

---

countCloneSizes	*count the number of clonotype sizes in a seurat object combined with a VDJ library overall or by cluster*

Description

[Stable]

Get clonotype frequencies from a seurat object's metadata, either as one whole table, or in a list of tables, based on the current / some custom ident of each cell. Note that depending on the ident (indicated by the by_cluster argument) there may be more or less clonotypes counted based on the number of rows containing NA for that column of that ident if it isn't the active ident.

Usage

```
countCloneSizes(
  seurat_obj,
  clonecall = "strict",
  extra_filter = NULL,
  ...,
  by_cluster = TRUE,
  sort_decreasing = NULL
)
```

Arguments

seurat_obj	a seurat object combined with a VDJ library with scRepertoire.
clonecall	character. The column name in the seurat object metadata to use. See scRepertoire documentation for more information about this parameter that is central to both packages.
extra_filter	character. An additional string that should be formatted *exactly* like a statement one would pass into [dplyr::filter](#) that does *additional* filtering to cells in the seurat object - on top of the other keyword arguments - based on the metadata. This means that it will be logically AND'ed with any keyword argument filters. This is a more flexible alternative / addition to the filtering keyword arguments. For example, if one wanted to filter by the length of the amino acid sequence of TCRs, one could pass in something like extra_filter = "nchar(CTaa) - 1 > 10". When involving characters, ensure to enclose with single quotes.
...	additional "subsetting" keyword arguments indicating the rows corresponding to elements in the seurat object metadata that should be filtered by. E.g., seurat_clusters = c(1, 9, 10) will filter the cells to those in the seurat_clusters column with any of the values 1, 9, and 10. Unfortunately, column names in the seurat object metadata cannot conflict with the keyword arguments. **MAJOR NOTE** if any subsetting keyword arguments are a *prefix* of any preceding argument names (e.g. a column named reduction is a prefix of the reduction_base argument) R will interpret it as the same argument unless *both* arguments are named. Additionally, this means any subsequent arguments *must* be named.

by_cluster	Logical or Character. If TRUE, will output a list of table objects, with the table at each index corresponding to level in Idents(). Each table's names are the clonotype name indicated by clonecall after filtering, while the values are the actual clone sizes. If FALSE, outputs just the aggregate clone sizes for all cells. Note that if FALSE, the output should be identical to that produced by mergeCloneSizes(countCloneSizes(..., by_cluster = TRUE)). Otherwise, this argument can also be a character indicating some column in the seurat object metadata to use a cell identity guiding (e.g. "seurat_clusters").
sort_decreasing	
	a logical or NULL. If TRUE/FALSE, sorts each/the table by clonotype frequency with largest/smallest clones first with a stable sorting algorithm, and if NULL, no order is guaranteed but the output is deterministic.

Value

A list of tables or a single table depending on by_cluster

See Also

mergeCloneSizes

Examples

```
data("combined_pbmc")

countCloneSizes(combined_pbmc)
countCloneSizes(combined_pbmc, "aa")
countCloneSizes(combined_pbmc, "nt", orig.ident = c("P17B", "P17L"))
```

---

deleteApotcData	*Delete the results of an APackOfTheClones run*

Description

[Stable]

A convenience function to erase all data associated with a particular run, including the ApotcData and command in seurat_obj@command. The run_id would be no longer accessible afterwards.

Usage

```
deleteApotcData(seurat_obj, run_id)
```

Arguments

seurat_obj	a seurat object that has had RunAPOTC ran on it before in order of the functions being called.
run_id	character. The id of the associated ApotcRun.

Value

The modified input seurat object

Examples

```
pbmc <- RunAPOTC(
    seurat_obj = get(data("combined_pbmc")),
    reduction_base = "umap",
    clonecall = "strict",
    run_id = "run1",
    verbose = FALSE
)

getApotcDataIds(pbmc)
#> [1] "run1"

# delete the data
pbmc <- deleteApotcData(pbmc, "run1")

getApotcDataIds(pbmc)
#> NULL
```

---

getApotcDataIds   *Get all run ids of previous RunAPOTC runs on a seurat object*

Description

[Stable]

A convenience function to get all run ids of previous RunAPOTC run IDs

Usage

```
getApotcDataIds(seurat_obj)
```

Arguments

seurat_obj   a seurat object that has had RunAPOTC ran on it before in order of the functions
                being called.

Value

a character vector of all run ids of previous RunAPOTC runs, in the order they were ran in. If there
are no runs on the object, it returns NULL.

Examples

```
pbmc <- RunAPOTC(
    seurat_obj = get(data("combined_pbmc")),
    reduction_base = "umap",
    clonecall = "strict",
    verbose = FALSE
)

getApotcDataIds(pbmc)
#> [1] "umap;CTstrict;_;_"

pbmc <- RunAPOTC(
    seurat_obj = pbmc,
    reduction_base = "umap",
    clonecall = "gene",
    verbose = FALSE
)

getApotcDataIds(pbmc)
#> [1] "umap;CTstrict;_;_" "umap;CTgene;_;_"
```

---

getLastApotcDataId   *Get the object id of the most recent RunAPOTC run on a seurat object*

Description

[Stable]

A convenience function to get the object id of the most recent valid [RunAPOTC()](#) run, to be used by [APOTCPlot()](#) and [AdjustAPOTC](#)

Usage

```
getLastApotcDataId(seurat_obj)
```

Arguments

seurat_obj   a seurat object that has had RunAPOTC ran on it before in order of the functions being called.

Value

a character of the object id of the last [RunAPOTC()](#) call

Examples

```
# first run
pbmc <- RunAPOTC(
    seurat_obj = get(data("combined_pbmc")),
    reduction_base = "umap",
    clonecall = "strict",
    verbose = FALSE
)

getLastApotcDataId(pbmc)
#> [1] "umap;CTstrict;_;_"

# second run with a different clonecall
pbmc <- RunAPOTC(
    seurat_obj = pbmc,
    reduction_base = "umap",
    clonecall = "gene",
    verbose = FALSE
)

getLastApotcDataId(pbmc)
#> [1] "umap;CTgene;_;_"
```

---

getReductionCentroids   *Calculate seurat cluster centroids based on a Dimensional reduction*

Description

[Stable]

Utility function to calculate the physical xy coordinates of each seurat cluster based on a dimensional reduction already present in the object. The results are returned in a list with the length of the number of distinct seurat clusters based on the seurat_obj `meta.data`.

Usage

```
getReductionCentroids(seurat_obj, reduction)
```

Arguments

seurat_obj   input seurat object with the dimensional reduction of choice already present, and
                seurat clusters computed.
reduction   character. The reduction that the centroid calculation should be based on.

Value

A list of the length of the number of distinct clusters in the seurat object metadata, where each element of the list is a numeric vector of length 2, with the numbers corresponding to the x and y coordinate respectively of the seurat cluster with the corresponding index.

Examples

```
data("combined_pbmc")
getReductionCentroids(combined_pbmc, reduction = "umap")
```

---

getSharedClones   *Compute a list of clonotypes that are shared between seurat clusters*

Description

[Stable]

This function allows users to get a list of clonotypes that are shared between clusters based on the levels of the active cell identities / some custom identity based on the alt_ident. A list is returned with its **names** being the shared clonotypes, and the values are numeric vectors indicating the index of the clusters that clonotype is found in. The index corresponds to the index in the default levels of the factored identities.

If run_id is inputted, then the function will attempt to get the shared clonotypes from the corresponding APackOfTheClones run generated from [RunAPOTC()](). Otherwise, it will use the filtering / subsetting parameters to generate the shared clones.

Usage

```
getSharedClones(
  seurat_obj,
  reduction_base = "umap",
  clonecall = "strict",
  ...,
  extra_filter = NULL,
  alt_ident = NULL,
  run_id = NULL,
  top = NULL,
  top_per_cl = NULL,
  intop = NULL,
  intop_per_cl = NULL,
  publicity = c(2L, Inf)
)
```

Arguments

seurat_obj	Seurat object with one or more dimension reductions and already have been integrated with a TCR/BCR library with scRepertoire::combineExpression.
reduction_base	character. The seurat reduction to base the clonal expansion plotting on. Defaults to 'umap' but can be any reduction present within the reductions slot of the input seurat object, including custom ones. If ''pca'", the cluster coordinates will be based on PC1 and PC2. However, generally APackOfTheClones is used for displaying UMAP and occasionally t-SNE versions to intuitively highlight clonal expansion.

clonecall   character. The column name in the seurat object metadata to use. See scRepertoire
                    documentation for more information about this parameter that is central to both
                    packages.

...   additional "subsetting" keyword arguments indicating the rows corresponding to
                    elements in the seurat object metadata that should be filtered by. E.g., seurat_clusters
                    = c(1, 9, 10) will filter the cells to those in the seurat_clusters column with
                    any of the values 1, 9, and 10. Unfortunately, column names in the seurat object
                    metadata cannot conflict with the keyword arguments. **MAJOR NOTE** if any
                    subsetting keyword arguments are a *prefix* of any preceding argument names
                    (e.g. a column named reduction is a prefix of the reduction_base argument)
                    R will interpret it as the same argument unless *both* arguments are named. Ad-
                    ditionally, this means any subsequent arguments *must* be named.

extra_filter   character. An additional string that should be formatted *exactly* like a statement
                    one would pass into [dplyr::filter](#) that does *additional* filtering to cells in the
                    seurat object - on top of the other keyword arguments - based on the metadata.
                    This means that it will be logically AND'ed with any keyword argument filters.
                    This is a more flexible alternative / addition to the filtering keyword arguments.
                    For example, if one wanted to filter by the length of the amino acid sequence of
                    TCRs, one could pass in something like extra_filter = "nchar(CTaa) - 1 >
                    10". When involving characters, ensure to enclose with single quotes.

alt_ident   character. By default, cluster identity is assumed to be whatever is in Idents(seurat_obj),
                    and clones will be grouped by the active ident. However, alt_ident could be set
                    as the name of some column in the meta data of the seurat object to be grouped
                    by. This column is meant to have been a product of Seurat::StashIdent or
                    manually added.

run_id   character. This will be the ID associated with the data of a run, and will be used
                    by other important functions like [APOTCPlot()](#) and [AdjustAPOTC](#). Defaults to
                    NULL, in which case the ID will be generated in the following format:
                    reduction_base;clonecall;keyword_arguments;extra_filter

                    where if keyword arguments and extra_filter are underscore characters if there
                    was no input for the ... and extra_filter parameters.

top   integer or numeric in (0, 1) - if not null, filters the output clones so that only
                    the shared clonotypes with counts the top top count / proportion (for numeric
                    in (0, 1) input) shared clones are kept. For cases where several clonotypes tie in
                    size, the clonotype(s) added are not guaranteed but deterministic given the other
                    arguments are identical.

top_per_cl   integer or numeric in (0, 1) - if not null, filters the output clones so that for each
                    seurat cluster, only the clonotypes with the top_per_cl frequency/count is pre-
                    served when aggregating shared clones, in the same way as the above. Note that
                    if inputted in conjunction with top, it will get the *intersection* of the clonotypes
                    filtered each way. For cases where several clonotypes tie in size, the clono-
                    type(s) added are not guaranteed but deterministic given the other arguments are
                    identical.

intop   integer or numeric in (0, 1) - if not null, filters the raw clone sizes before com-
                    puting the shared clonotypes so that only the clonotypes that have their overall
                    size in the top intop largest sizes (if it is integer, else the intop proportion) are

kept. To emphasize, this argument **does not necessarily** return the top shared clones and likely a little less, because this filters the raw clone sizes, of which, its very likely that not all those clones end up being shared.

intop_per_cl   integer or numeric in (0, 1) - if not null, filters the raw *clustered* clone sizes before computing shared clones, so that for every clone in a seurat cluster, the top intop_per_cl count / proportion (for numeric in (0, 1) input) clones are kept.

publicity   numeric pair. A simple filter range of c(lowerbound, upperbound) to retain only shared clones with their "publicity" - number of clusters they are present in - within this range.

Value

a named list where each name is a clonotype, each element is a numeric indicating which seurat cluster(s) its in, in no particular order. If no shared clones are present, the output is an empty list.

Examples

```
data("combined_pbmc")

getSharedClones(combined_pbmc)

getSharedClones(
    combined_pbmc,
    orig.ident = c("P17B", "P18B"), # a named subsetting parameter
    clonecall = "aa"
)

# extract shared clones from a past RunAPOTC run
combined_pbmc <- RunAPOTC(
    combined_pbmc, run_id = "foo", verbose = FALSE
)

getSharedClones(
    combined_pbmc, run_id = "foo", top = 5
)

# doing a run and then getting the clones works too
combined_pbmc <- RunAPOTC(combined_pbmc, run_id = "run1", verbose = FALSE)
getSharedClones(combined_pbmc, run_id = "run1")
```

---

mergeCloneSizes   *Merge a list of Clustered Clonotype Frequency Tables*

Description

The list of clustered clonotype frequencies from countCloneSizes can be merged by this function to a frequency table of all clonotypes similar to the data that can be seen in the seurat object metadata. By default, this function sorts the table with largest clonotypes first, and this may be useful for quickly gauging which clonotypes are the most expanded overall.

Usage

```
mergeCloneSizes(clustered_clone_sizes, sort_decreasing = TRUE)
```

Arguments

clustered_clone_sizes

the output of countCloneSizes.

sort_decreasing

a logical or NULL. If TRUE/FALSE, sorts the table by clonotype frequency with largest/smallest clones first, and if NULL, no order is guaranteed but the output is deterministic.

Value

a table object

See Also

countCloneSizes

Examples

```
clustered_clone_sizes <- countCloneSizes(get(data("combined_pbmc")))
mergeCloneSizes(clustered_clone_sizes)
```

---

overlayLegend   *overlay a clone size legend on an APackOfTheClones plot*

Description

[Stable]

This function has most of the parameters related to legend in APOTCPlot(), and can plot a new / override the current legend. However, it is very important that the input plot to the function is a plot generated solely by APOTCPlot() or vizAPOTC() due to it being a custom ggplot object. It will not override or erase any additional layers that the user/other functions have added. To just remove the legend, see removeLegend.

Usage

```
overlayLegend(
  apotc_ggplot,
  legend_sizes = "auto",
  legend_position = "auto",
  legend_buffer = 0.2,
  legend_color = "#808080",
  legend_spacing = "auto",
  legend_label = "Clone sizes",
  legend_text_size = 5,
  add_legend_background = TRUE,
  add_legend_centerspace = 0,
  linetype = "blank",
  res = 360L
)
```

Arguments

apotc_ggplot	a ggplot object that is the output of [APOTCPlot()](#) or [vizAPOTC()](#)
legend_sizes	numeric vector. Indicates the circle sizes to be displayed on the legend, and will always be sorted from smallest to greatest. Defaults to "auto" which estimate a reasonable range of sizes to display.
legend_position	character or numeric. Can be set to either "top_left", "top_right", "bottom_left", "bottom_right" and places the legend roughly in the corresponding position. Otherwise, can be a numeric vector of length 2 indicating the x and y position of the *topmost (smallest) circle* of the legend.
legend_buffer	numeric. Indicates how much to "push" the legend towards the center of the plot from the selected corner. If negative, will push away
legend_color	character. Indicates the hex color of the circles displayed on the legend. Defaults to the hex code for a gray tone
legend_spacing	numeric. Indicates the horizontal distance between each stacked circle on the size legend. Defaults to "auto" which will use an estimated value depending on plot size
legend_label	character. The title of the legend, which defaults to "clone sizes.
legend_text_size	numeric. The text size of the letters and numbers on the legend
add_legend_background	logical. If TRUE, will add a border around the legend and fill the background to be white, overlaying anything else.
add_legend_centerspace	numeric. An additional amount of distance changed between the circle sizes on the left side of the legend and the numbers on the right. Useful to set to around 0.5 (or more / less) when there are particularly large clone sizes that may cover the numbers.

| linetype | The type of outline each circle should have. defaults to "blank meaning no out-line. More information is in the function documentation of `ggforce::geom_circle`. |
| res | The number of points on the generated path per full circle. From plot viewers, if circles seem slightly too pixelated, it is recommended to first try to export the plot as an `.svg` before increasing `res` due to increased plotting times from [ggforce::geom_circle](#). |

Details

The size legend on APackOfTheClones plots are simply a collection of annotation layers of the `rect`, `text`, `circle` geoms. Therefore it isn't quite a ggplot legend. In the actual ggplot object, the `$layers` element should all be named with an empty character `""`, and those that comprise the annotation layers of the legend should be named whatever `APackOfTheClones:::.ApotcLegendLayerName` is. Note that this is simply an implementation detail that the user should not interfere with.

Value

A ggplot object of the APackOfTheClones clonal expansion plot of the seurat object. There is an additional 10th element in the object named `"APackOfTheClones"` used by other functions in this package and shouldn't interfere with any other ggplot functionality. (As far as currently known)

See Also

[removeLegend](#)

Examples

```
library(dplyr)

# create a plot with a legend
apotc_plot <- vizAPOTC(get(data("combined_pbmc")), verbose = FALSE)

# reposition the legend to top right
overlayLegend(apotc_plot, legend_position = "top right")

# use different sizes and label - may be nice to use the pipe
apotc_plot %>% overlayLegend(
    legend_sizes = c(1, 3, 7, 9),
    legend_label = "odd sizes"
)
```

| removeLegend | *Remove current APackOfTheClones legend* |

Description

[Stable]

Removes the clone size legend on an APackOfTheClones plot, if one is present. Will preserve any additional ggplot layers.

Usage

```
removeLegend(apotc_ggplot)
```

Arguments

apotc_ggplot   a ggplot object that is the output of `APOTCPlot()` or `vizAPOTC()`

Value

A ggplot object of the APackOfTheClones clonal expansion plot of the seurat object. There is an additional 10th element in the object named "APackOfTheClones" used by other functions in this package and shouldn't interfere with any other ggplot functionality. (As far as currently known)

See Also

overlayLegend

Examples

```
# create an APackOfTheClones plot with a legend
apotc_plot <- vizAPOTC(
    get(data("combined_pbmc")),
    add_size_legend = TRUE,
    verbose = FALSE
)

# remove the legend
apotc_plot <- removeLegend(apotc_plot)
apotc_plot
```

---

renameApotcRun   *Rename an APackOfTheClones run*

Description

[Stable]

A function to rename an APackOfTheClones run identified by its run id in a Seurat object.

Usage

```
renameApotcRun(seurat_obj, old_run_id, new_run_id)
```

Arguments

seurat_obj   A Seurat object containing APackOfTheClones data - the output of `RunAPOTC()`

old_run_id   Character. The current id of the APackOfTheClones run to be renamed.

new_run_id   Character. The new id to assign to the APackOfTheClones run.

Value

A Seurat object with the APackOfTheClones run renamed.

Examples

```
pbmc <- RunAPOTC(
    seurat_obj = get(data("combined_pbmc")),
    reduction_base = "umap",
    clonecall = "strict",
    run_id = "run1",
    verbose = FALSE
)

pbmc <- renameApotcRun(pbmc, "run1", "new_run")
# Now "run1" has been renamed to "new_run"
```

RunAPOTC   *Run the APackOfTheClones method on a combined Seurat object for*
                                   downstream visualization of clonal expansion

---

Description

[Stable]

Computes necessary information for an APackOfTheClones clonal expansion plot ([APOTCPlot()](#))
and stores it in the seurat object. Gets sizes of unique clones and utilizes a circle-packing algorithm
to pack circles representing individual clones in approximately the same dimensional reduction
(reduction_base) coordinates based on some cell ident (defaults to the active ident).

The parameter extra_filter along with an unlimited number of additional keyword arguments
can be used to filter the cells by certain conditions in the metadata, and new results will be stored in
addition to other runs the users may have done.

Each APackOfTheClones run is uniquely identified by the parameters reduction_base, clonecall,
extra_filter, and any additional keywords passed to filter the metadata. Each distinct run result is
stored in the seurat object and has an associated Id generated from the aforementioned parameters.
To view the id of the latest run, call [getLastApotcDataId](#). To view all the ids of previous runs, call
[getApotcDataIds.](#) To work further with a specific run (most importantly, plotting), the user can use
this id in the arguments with is slightly more convenient than passing in the original RunAPOTC
parameters again but both ways work.

If the user wishes to manually customize/fix the expansion plot generated, the circular packing
information can be modified with the [AdjustAPOTC](#) function.

Usage

```
RunAPOTC(
  seurat_obj,
  reduction_base = "umap",
```

```
    clonecall = "strict",
    ...,
    extra_filter = NULL,
    alt_ident = NULL,
    run_id = NULL,
    clone_scale_factor = "auto",
    rad_scale_factor = 0.95,
    order_clones = TRUE,
    try_place = FALSE,
    repulse = TRUE,
    repulsion_threshold = 1,
    repulsion_strength = 1,
    max_repulsion_iter = 20L,
    override = FALSE,
    verbose = TRUE
)
```

Arguments

seurat_obj   Seurat object with one or more dimension reductions and already have been
                  integrated with a TCR/BCR library with scRepertoire::combineExpression.

reduction_base   character. The seurat reduction to base the clonal expansion plotting on. De-
                  faults to 'umap' but can be any reduction present within the reductions slot of
                  the input seurat object, including custom ones. If ''pca'", the cluster coordinates
                  will be based on PC1 and PC2. However, generally APackOfTheClones is used
                  for displaying UMAP and occasionally t-SNE versions to intuitively highlight
                  clonal expansion.

clonecall   character. The column name in the seurat object metadata to use. See scRepertoire
                  documentation for more information about this parameter that is central to both
                  packages.

...   additional "subsetting" keyword arguments indicating the rows corresponding to
                  elements in the seurat object metadata that should be filtered by. E.g., seurat_clusters
                  = c(1, 9, 10) will filter the cells to those in the seurat_clusters column with
                  any of the values 1, 9, and 10. Unfortunately, column names in the seurat object
                  metadata cannot conflict with the keyword arguments. **MAJOR NOTE** if any
                  subsetting keyword arguments are a *prefix* of any preceding argument names
                  (e.g. a column named reduction is a prefix of the reduction_base argument)
                  R will interpret it as the same argument unless *both* arguments are named. Ad-
                  ditionally, this means any subsequent arguments *must* be named.

extra_filter   character. An additional string that should be formatted *exactly* like a statement
                  one would pass into [dplyr::filter](#) that does *additional* filtering to cells in the
                  seurat object - on top of the other keyword arguments - based on the metadata.
                  This means that it will be logically AND'ed with any keyword argument filters.
                  This is a more flexible alternative / addition to the filtering keyword arguments.
                  For example, if one wanted to filter by the length of the amino acid sequence of
                  TCRs, one could pass in something like extra_filter = "nchar(CTaa) - 1 >
                  10". When involving characters, ensure to enclose with single quotes.

alt_ident   character. By default, cluster identity is assumed to be whatever is in Idents(seurat_obj),
                   and clones will be grouped by the active ident. However, alt_ident could be set
                   as the name of some column in the meta data of the seurat object to be grouped
                   by. This column is meant to have been a product of Seurat::StashIdent or
                   manually added.

run_id   character. This will be the ID associated with the data of a run, and will be used
                   by other important functions like APOTCPlot() and AdjustAPOTC. Defaults to
                   NULL, in which case the ID will be generated in the following format:
                   reduction_base;clonecall;keyword_arguments;extra_filter

                   where if keyword arguments and extra_filter are underscore characters if there
                   was no input for the ... and extra_filter parameters.

clone_scale_factor

                   Dictates how much to scale each circle(between 0,1) radius when converting
                   from clonotype counts into circles that represent individual clonotypes. The
                   argument defaults to the character "auto", and if so, the most visually pleasing
                   factor will be estimated.

rad_scale_factor

                   numeric between 0 and 1. This value decreases the radius of the smallest clones
                   by this scale factor. And the absolute value of this decrease will be applied to all
                   packed circles, effectively shrinking all circles on the spot, and introduce more
                   constant spacing in between.

order_clones   logical. Decides if the largest clone circles should be near cluster centroids.
                   This is highly recommended to be set to TRUE for increased intuitiveness of
                   the visualization, as resulting plots tend to give an improved impression of the
                   proportion of expanded clones. If FALSE, will randomly scramble the positions
                   of each circle. For the sake of being replicable, a random seed is recommended
                   to be set with set.seed.

try_place   If TRUE, always minimizes distance from a newly placed circle to the origin in
                   the circle packing algorithm.

repulse   If TRUE, will attempt to push overlapping clusters away from each other.

repulsion_threshold

                   numeric. The radius that clonal circle clusters overlap is acceptable when re-
                   pulsing.

repulsion_strength

                   numeric. The smaller the value the less the clusters repulse each other per itera-
                   tion, and vice versa.

max_repulsion_iter

                   integer. The number of repulsion iterations.

override   logical. If TRUE, will override any existing APackOfTheClones run data with
                   the same run_id.

verbose   logical. Decides if visual cues are displayed to the R console of the progress.

Details

Note that the subsetting arguments ... and extra_filter are only a quick convenience to subset
based on metadata, and the subset S3 method defined in Seurat is much more mature are has more

features. Additionally, users need to work with data subsets are recommended to and likely already are working with seurat objects subsetted/split with `Seurat::SplitObject`.

All APackOfTheClones run data is stored in the Seurat object under `seurat_object@misc$APackOfTheClones`, which is a list of S4 objects of the type "ApotcData", with each element corresponding to a unique run. The id of each run is the name of each element in the list. The user **really shouldn't** manually modify anything in the list as it may cause unexpected behavior with many other functions.

Additionally, it logs a seurat command associated with the run in the `seurat_object@commands` slot as a "SeuratCommand" object (from Seurat), where the name of the object in the list is formatted as `RunAPOTC.run_id`.

Value

A modified version of the input seurat object, which harbors data necessary for visualizing the clonal expansion of the cells with [APOTCPlot()](#) and has a friendly user interface to modify certain attributes with [AdjustAPOTC](#).

Cluster labelling

For the ident that was used to cluster the clones, labels for each cluster are inferred and stored in the run so that they can be used by other functions and optionally overlaid on the plot over clusters. If the levels of the ident used is a naturally ordered integer sequence, then the labels generated would be `"C1"`, `"C2"`, `"C3"` ... , else they would be the actual ident levels themselves.

See Also

[APOTCPlot()](#), [AdjustAPOTC](#), [getApotcDataIds](#)

Examples

```
data("combined_pbmc")

# this is the recommended approach to use a custom run_id with default params
combined_pbmc <- RunAPOTC(combined_pbmc, run_id = "default", verbose = FALSE)

# here's a seperate run with some filters to the meta data, where
# `orig.ident` is a custom column in the example data. Notice that it is not
# a `RunAPOTC()` parameter but a user keyword argument
combined_pbmc <- RunAPOTC(
    combined_pbmc, run_id = "sample17", orig.ident = c("P17B", "P17L"),
    verbose = FALSE
)

# the exact same thing can be achieved with the `extra_filter` parameter
combined_pbmc <- RunAPOTC(
    combined_pbmc,
    run_id = "sample17",
    extra_filter = "substr(orig.ident, 2, 3) == '17'",
    override = TRUE,
    verbose = FALSE
)
```

---

showCloneHighlight   *Highlight specific clones on an APackOfTheClones ggplot*

Description

[Experimental]

This is an analogue for scRepertoire::highlightClones that can highlight certain clonotypes on an APackOfTheClones clonal expansion plot. For most combinations of the arguments, there will be a ggplot fill legend on the right side that correspond to each (existing) clonotype.

Usage

```
showCloneHighlight(
  apotc_ggplot,
  clonotype,
  color_each = TRUE,
  default_color = "#808080",
  scale_bg = 1,
  fill_legend = TRUE
)
```

Arguments

apotc_ggplot	A ggplot object that is the output of APOTCPlot() or vizAPOTC() of an APack-OfTheClones plot to be highlighted on.
clonotype	character vector of the sequence(s) to highlight. Note that it must be of the clonecall of the code that created the plot. A warning will be shown if any of the sequences are not present.
color_each	Either a logical of length 1, or a character(s). It is TRUE by default, which assigns a unique default ggplot color to each highlighted clone. If FALSE, each highlighted clone will retain its current color and no legend based on color is shown. A possible application here is to simply gauge the distribution of any shared clone. It can also indicate the uniform color of each highlighted clone: if it is a character of length 1, all highlighted clones will be of that color. Else it must be a character vector of the same length as clonotype, with each color corresponding to the clone. Here is a suitable place to use any palette function from the many other CRAN palette packages such as "viridis" or "RColorBrewer". Note that currently, the user must ensure clonotype contains only unique characters.
default_color	A character of length 1 or NULL indicating the color of non-highlighted clones. If NULL, all un-highlighted sequences will retain their original color in sc.data. Else, if it is a character, it should be a valid color that all un-highlighted clones are. Defaults to the hex code for gray.
scale_bg	A positive numeric. Scales the brightness value of each color of the non-highlighted clones by itself as a scaling factor. Defaults to 1 which will not alter the current

brightness. Note that if color_each = FALSE and default_color = NULL, this is equivalent to not highlighting any clones - in this case, it may be useful to alter scale_bg slightly so that the non-highlighted clones are darkened/brightened.

fill_legend   logical indicating whether a ggplot legend of the "fill" of each clonotype should be displayed.

Details

Under the hood, this function simply mutates the plotting data.frame under $data in the ggplot object, and operates on a column named color.

Note that if color_each = FALSE and default_color = NULL, this is equivalent to simply not highlighting anything and a warning will be shown.

Value

A ggplot object with the data modified to the highlighted colors

Examples

```
library(dplyr)
data("combined_pbmc")

# piping the plot can be nice to read syntactically -
# By default, assigns unique colors to highlights and everything else is gray
vizAPOTC(combined_pbmc, clonecall = "aa", verbose = FALSE) %>%
    showCloneHighlight("CASLSGSARQLTF_CASSSTVAGEQYF")

# one useful application is to highlight shared clones - beware that the
# clonotype sequences may get extremely long in the legend
shared_aa_clones <- names(getSharedClones(combined_pbmc, clonecall = "aa"))
vizAPOTC(combined_pbmc, clonecall = "aa", verbose = FALSE) %>%
    showCloneHighlight(shared_aa_clones)
```

---

vizAPOTC   *Directly visualize clonal expansion of a combined seurat object*

Description

[Stable]

This function combines the functionality of both RunAPOTC() and APOTCPlot(). Given a Seurat object, it first runs the APackOfTheClones method (RunAPOTC()) to compute clonal expansion information, and then generates a ggplot2 object of the clonal expansion plot with a circle size legend. (APOTCPlot())

Usage

```
vizAPOTC(
  seurat_obj,
  reduction_base = "umap",
  clonecall = "strict",
  ...,
  extra_filter = NULL,
  alt_ident = NULL,
  clone_scale_factor = "auto",
  rad_scale_factor = 0.95,
  order_clones = TRUE,
  try_place = FALSE,
  repulse = TRUE,
  repulsion_threshold = 1,
  repulsion_strength = 1,
  max_repulsion_iter = 20L,
  show_shared = NULL,
  only_link = NULL,
  clone_link_width = "auto",
  clone_link_color = "black",
  clone_link_alpha = 0.5,
  res = 360L,
  linetype = "blank",
  use_default_theme = TRUE,
  retain_axis_scales = FALSE,
  alpha = 1,
  show_labels = FALSE,
  label_size = 5,
  add_size_legend = TRUE,
  legend_sizes = "auto",
  legend_position = "auto",
  legend_buffer = 0.2,
  legend_color = "#808080",
  legend_spacing = "auto",
  legend_label = "Clone sizes",
  legend_text_size = 5,
  add_legend_background = TRUE,
  add_legend_centerspace = 0,
  detail = TRUE,
  verbose = TRUE
)
```

Arguments

seurat_obj   A seurat object that has been integrated with clonotype data with `scRepertoire::combineExpression`.

reduction_base character. The seurat reduction to base the clonal expansion plotting on. De-
                faults to `'umap'` but can be any reduction present within the reductions slot of
                the input seurat object, including custom ones. If ''pca''', the cluster coordinates

will be based on PC1 and PC2. However, generally APackOfTheClones is used for displaying UMAP and occasionally t-SNE versions to intuitively highlight clonal expansion.

clonecall   character. The column name in the seurat object metadata to use. See scRepertoire documentation for more information about this parameter that is central to both packages.

...   additional "subsetting" keyword arguments indicating the rows corresponding to elements in the seurat object metadata that should be filtered by. E.g., seurat_clusters = c(1, 9, 10) will filter the cells to those in the seurat_clusters column with any of the values 1, 9, and 10. Unfortunately, column names in the seurat object metadata cannot conflict with the keyword arguments. **MAJOR NOTE** if any subsetting keyword arguments are a *prefix* of any preceding argument names (e.g. a column named reduction is a prefix of the reduction_base argument) R will interpret it as the same argument unless *both* arguments are named. Additionally, this means any subsequent arguments *must* be named.

extra_filter   character. An additional string that should be formatted *exactly* like a statement one would pass into [dplyr::filter](#) that does *additional* filtering to cells in the seurat object - on top of the other keyword arguments - based on the metadata. This means that it will be logically AND'ed with any keyword argument filters. This is a more flexible alternative / addition to the filtering keyword arguments. For example, if one wanted to filter by the length of the amino acid sequence of TCRs, one could pass in something like extra_filter = "nchar(CTaa) - 1 > 10". When involving characters, ensure to enclose with single quotes.

alt_ident   character. By default, cluster identity is assumed to be whatever is in Idents(seurat_obj), and clones will be grouped by the active ident. However, alt_ident could be set as the name of some column in the meta data of the seurat object to be grouped by. This column is meant to have been a product of Seurat::StashIdent or manually added.

clone_scale_factor
                 Dictates how much to scale each circle(between 0,1) radius when converting from clonotype counts into circles that represent individual clonotypes. The argument defaults to the character "auto", and if so, the most visually pleasing factor will be estimated.

rad_scale_factor
                 numeric between 0 and 1. This value decreases the radius of the smallest clones by this scale factor. And the absolute value of this decrease will be applied to all packed circles, effectively shrinking all circles on the spot, and introduce more constant spacing in between.

order_clones   logical. Decides if the largest clone circles should be near cluster centroids. This is highly recommended to be set to TRUE for increased intuitiveness of the visualization, as resulting plots tend to give an improved impression of the proportion of expanded clones. If FALSE, will randomly scramble the positions of each circle. For the sake of being replicable, a random seed is recommended to be set with [set.seed](#).

try_place   If TRUE, always minimizes distance from a newly placed circle to the origin in the circle packing algorithm.

repulse   If TRUE, will attempt to push overlapping clusters away from each other.

repulsion_threshold

                numeric. The radius that clonal circle clusters overlap is acceptable when repulsing.

repulsion_strength

                numeric. The smaller the value the less the clusters repulse each other per iteration, and vice versa.

max_repulsion_iter

                integer. The number of repulsion iterations.

show_shared   The output of [getSharedClones](#) can be inputted here, and the resulting plot will overlay lines between clone circles if that clonotype is common between clusters. Note that the input **must** be generated from data in the correct APackOfTheClones run, and the behavior is undefined otherwise and will likely error. The next 4 arguments allow for aesthetic customization of these line links.

only_link   Optional integer indicating to only display clone links originating from this cluster if showing shared clones.

clone_link_width

                numeric. The width of the lines that connect shared clones. Defaults to "auto" which will estimate a reasonable value depending on circle sizes.

clone_link_color

                character. The color of the lines that connect shared clones. Defaults to "blend" which will use the average colors of the two connected clones. Else, any hex color or valid color string input will work, and the corresponding color will be applied on all links.

clone_link_alpha

                numeric. The alpha of the lines that connect shared clones.

res   The number of points on the generated path per full circle. From plot viewers, if circles seem slightly too pixelated, it is recommended to first try to export the plot as an .svg before increasing res due to increased plotting times from [ggforce::geom_circle](#).

linetype   The type of outline each circle should have. defaults to "blank meaning no outline. More information is in the function documentation of ggforce::geom_circle.

use_default_theme

                logical that defaults to TRUE. If TRUE, the resulting plot will have the same theme as the seurat reference reduction plot. Else, the plot will simply have a blank background.

retain_axis_scales

                If TRUE, approximately maintains the axis scales of the original reduction plot. However, it will only attempt to extend the axes and never shorten. Users are recommended to set this to TRUE especially if working with subsetted versions of the clonal data to better preserve the geometric relation to the original dimensional reduction.

alpha   numeric. The alpha of the circles in (0, 1]. Defaults to 1.

show_labels   If TRUE, will label each circle cluster at the centroid, defaulting to "C0, C1, ...".

label_size   The text size of labels if shown. Defaults to 5.

add_size_legend

> If TRUE, adds a legend to the plot visualizing the relative sizes of clones. Note that it is simply an overlay and not a real ggplot2 legend.

legend_sizes   numeric vector. Indicates the circle sizes to be displayed on the legend, and will always be sorted from smallest to greatest. Defaults to "auto" which estimate a reasonable range of sizes to display.

legend_position

> character or numeric. Can be set to either "top_left", "top_right", "bottom_left", "bottom_right" and places the legend roughly in the corresponding position. Otherwise, can be a numeric vector of length 2 indicating the x and y position of the *topmost (smallest) circle* of the legend.

legend_buffer   numeric. Indicates how much to "push" the legend towards the center of the plot from the selected corner. If negative, will push away

legend_color   character. Indicates the hex color of the circles displayed on the legend. Defaults to the hex code for a gray tone

legend_spacing   numeric. Indicates the horizontal distance between each stacked circle on the size legend. Defaults to "auto" which will use an estimated value depending on plot size

legend_label   character. The title of the legend, which defaults to "clone sizes.

legend_text_size

> numeric. The text size of the letters and numbers on the legend

add_legend_background

> logical. If TRUE, will add a border around the legend and fill the background to be white, overlaying anything else.

add_legend_centerspace

> numeric. An additional amount of distance changed between the circle sizes on the left side of the legend and the numbers on the right. Useful to set to around 0.5 (or more / less) when there are particularly large clone sizes that may cover the numbers.

detail   logical. If FALSE, will only plot entire clusters as one large circle, which may be useful in cases where there are a high number of clones resulting in a large number of circles on the resulting ggplot, which has increased plotting times, and certain aspects of the plot needs to be finely adjusted with AdjustAPOTC or simply inspected. This should not be set to FALSE for the actual clonal expansion plot.

verbose   logical. Decides if visual cues are displayed to the R console of the progress.

Details

Note that the subsetting arguments ... and extra_filter are only a quick convenience to subset based on metadata, and the subset S3 method defined in Seurat is much more mature are has more features. Additionally, users need to work with data subsets are recommended to and likely already are working with seurat objects subsetted/split with Seurat::SplitObject.

Value

A ggplot object of the APackOfTheClones clonal expansion plot of the seurat object. There is an additional 10th element in the object named "APackOfTheClones" used by other functions in this package and shouldn't interfere with any other ggplot functionality. (As far as currently known)

Cluster labelling

For the ident that was used to cluster the clones, labels for each cluster are inferred and stored in the run so that they can be used by other functions and optionally overlaid on the plot over clusters. If the levels of the ident used is a naturally ordered integer sequence, then the labels generated would be "C1", "C2", "C3" ... , else they would be the actual ident levels themselves.

See Also

[AdjustAPOTC](#)

Examples

```
data("combined_pbmc")

# plot with default parameters
vizAPOTC(combined_pbmc, verbose = FALSE)

# use arguments from RunAPOTC and APOTCPlot
vizAPOTC(
    combined_pbmc, try_place = TRUE, show_labels = TRUE, verbose = FALSE
)
```

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