Package 'scRepertoire'

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`scRepertoire-package` *scRepertoire: A toolkit for single-cell immune receptor profiling*

Description

scRepertoire is a toolkit for processing and analyzing single-cell T-cell receptor (TCR) and immunoglobulin (Ig). The scRepertoire framework supports use of 10x, AIRR, BD, MiXCR, TRUST4, and WAT3R single-cell formats. The functionality includes basic clonal analyses, repertoire summaries, distance-based clustering and interaction with the popular Seurat and SingleCellExperiment/Bioconductor R single-cell workflows.

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See Also

Useful links:

- https://www.borch.dev/uploads/scRepertoire/
- Report bugs at https://github.com/BorchLab/scRepertoire/issues

._bind_contig_list *Bind a List of Contig Data Frames and Add Grouping Variable*

Description

Bind a List of Contig Data Frames and Add Grouping Variable

Usage

```
._bind_contig_list(contig_list)
```

._split_and_pad *Split String and Pad to a Fixed-Width Matrix*

Description

Split String and Pad to a Fixed-Width Matrix

Usage

```
._split_and_pad(x, split, n_cols)
```

addVariable *Adding Variables After combineTCR() or combineBCR()*

Description

This function adds variables to the product of `combineTCR()`, or `combineBCR()` to be used in later visualizations. For each element, the function will add a column (labeled by `variable.name`) with the variable. The length of the `variables` parameter needs to match the length of the combined object.

Usage

```
addVariable(input.data, variable.name = NULL, variables = NULL)
```

Arguments

`input.data`	The product of `combineTCR()` or `combineBCR()`.
`variable.name`	A character string that defines the new variable to add.
`variables`	A character vector defining the desired column value for each list element. Must match the length of the product of `combineTCR()` or `combineBCR()`.

Value

input.data list with the variable column added to each element.

Examples

```
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))
combined <- addVariable(combined,
                        variable.name = "Type",
                        variables = rep(c("B", "L"), 4))
```

`alluvialClones` *Alluvial Plotting for Single-Cell Object*

Description

View the proportional contribution of clones by Seurat or SCE object meta data after `combineExpression()`. The visualization is based on the ggalluvial package, which requires the aesthetics to be part of the axes that are visualized. Therefore, alpha, facet, and color should be part of the the axes you wish to view or will add an additional stratum/column to the end of the graph.

Usage

```
alluvialClones(
  sc.data,
  clone.call = NULL,
  chain = "both",
  y.axes = NULL,
  color = NULL,
  facet = NULL,
  alpha = NULL,
  top.clones = NULL,
  min.freq = 0,
  highlight.clones = NULL,
  highlight.color = "red",
  stratum.width = 0.2,
  flow.alpha = 0.5,
  show.labels = TRUE,
  label.size = 2,
  order.strata = NULL,
  export.table = NULL,
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

sc.data	The product of combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
y.axes	The columns that will separate the proportional visualizations.
color	The column header or clone(s) to be highlighted.
facet	The column label to separate.
alpha	The column header to have gradated opacity.
top.clones	Show only the top N clones by frequency. If NULL (default), show all clones.
min.freq	Minimum frequency threshold for displaying flows. Clones appearing fewer than this many times are filtered out.
highlight.clones	Character vector of specific clone sequences to highlight. These clones will be colored distinctly while others are shown in gray.
highlight.color	Color to use for highlighted clones (default: "red").
stratum.width	Width of the stratum bars (default: 0.2).
flow.alpha	Transparency of the flows (default: 0.5). Highlighted clones use full opacity.
show.labels	If TRUE (default), display stratum labels.
label.size	Text size for stratum labels (default: 2).
order.strata	Named list specifying the order of levels within each stratum. Names should match column names in y.axes.
export.table	If TRUE, returns a data frame of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals.
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

A ggplot object visualizing categorical distribution of clones, or a data.frame if export.table = TRUE.

Examples

```
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))
```

```
# Getting a sample of a Seurat object
scRep_example <- get(data("scRep_example"))

# Using combineExpresion()
scRep_example <- combineExpression(combined, scRep_example)
scRep_example$Patient <- substring(scRep_example$orig.ident, 1,3)

# Using alluvialClones()
alluvialClones(scRep_example,
                   clone.call = "gene",
                   y.axes = c("Patient", "ident"),
                   color = "ident")

# Show only top 50 most frequent clones
alluvialClones(scRep_example,
                   clone.call = "aa",
                   y.axes = c("Patient", "ident"),
                   top.clones = 50)

# Highlight specific clones
alluvialClones(scRep_example,
                   clone.call = "aa",
                   y.axes = c("Patient", "ident"),
                   highlight.clones = c("CVVSDNTGGFKTIF_CASSVRRERANTGELFF"))
```

`annotateInvariant` *Annotate invariant T cells (MAIT or iNKT) in single-cell TCR data*

Description

The `annotateInvariant()` function identifies potential mucosal-associated invariant T (`MAIT``) cells or invariant nat cells from single-cell sequencing datasets based on their characteristic TCR usage. It extracts TCR chain information from the provided single-cell data, checks it against known invariant T-cell receptor criteria for either MAIT or iNKT cells, and returns a score indicating the presence (1) or absence (0) of these invariant cell populations for each individual cell. The function supports data from mouse and human samples, providing a convenient method to annotate specialized T-cell subsets within single-cell analyses.

Usage

```
annotateInvariant(
  input.data,
  type = c("MAIT", "iNKT"),
  species = c("mouse", "human")
)
```

Arguments

input.data	The product of combineTCR() or combineExpression().
type	Character specifying the type of invariant cells to annotate (MAIT or iNKT).
species	Character specifying the species ('mouse' or 'human').

Value

A single-cell object or list with the corresponding annotation scores (0 or 1) added.

Examples

```
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Getting a sample of a Seurat object
scRep_example <- get(data("scRep_example"))

# Using combineExpresion()
scRep_example <- combineExpression(combined, scRep_example)

# Using annotateInvariant()
annotateInvariant(input.data = scRep_example, type = "MAIT", species = "human")
annotateInvariant(input.data = scRep_example, type = "iNKT", species = "human")
```

---

clonalAbundance *Plot the Relative Abundance of Clones*

Description

Displays the number of clones at specific frequencies by sample or group. Visualization can either be a line graph (scale = FALSE) using calculated numbers or density plot (scale = TRUE). Multiple sequencing runs can be group together using the group parameter. If a matrix output for the data is preferred, set export.table = TRUE.

Usage

```
clonalAbundance(
  input.data,
  clone.call = NULL,
  chain = "both",
  scale = FALSE,
  group.by = NULL,
  order.by = NULL,
  export.table = NULL,
```

```
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
scale	Converts the graphs into density plots in order to show relative distributions.
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals.
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

A ggplot object visualizing clonal abundance by group, or a data.frame if export.table = TRUE.

Author(s)

Nick Borcherding, Justin Reimertz

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Using clonalAbundance()
clonalAbundance(combined,
                clone.call = "gene",
                scale = FALSE)
```

clonalBias *Calculate Clonal Bias Towards a Cluster or Compartment*

Description

The metric seeks to quantify how individual clones are skewed towards a specific cellular compartment or cluster. A clone bias of 1 - indicates that a clone is composed of cells from a single compartment or cluster, while a clone bias of 0 - matches the background subtype distribution. Please read and cite the following manuscript if using clonalBias().

Usage

```
clonalBias(
  sc.data,
  clone.call = NULL,
  split.by = NULL,
  group.by = NULL,
  n.boots = 20,
  min.expand = 10,
  export.table = NULL,
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

sc.data	The single-cell object after combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
split.by	The variable to use for calculating the baseline frequencies. For example, "Type" for lung vs peripheral blood comparison
group.by	A column header in the metadata that bias will be based on.
n.boots	number of bootstraps to downsample.
min.expand	clone frequency cut off for the purpose of comparison.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals.
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

ggplot scatter plot with clone bias

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Getting a sample of a Seurat object
scRep_example <- get(data("scRep_example"))

# Using combineExpresion()
scRep_example <- combineExpression(combined, scRep_example)
scRep_example$Patient <- substring(scRep_example$orig.ident,1,3)

# Using clonalBias()
clonalBias(scRep_example,
              clone.call = "aa",
              split.by = "Patient",
              group.by = "seurat_clusters",
              n.boots = 5,
              min.expand = 2)
```

---

clonalBin *Bin Clones by Frequency or Proportion*

Description

This function adds a clonal grouping variable (`cloneSize`) to the output of `combineTCR()`, `combineBCR()`, or `combineExpression()`. It calculates the clonal frequency and proportion, then bins clones into categories based on customizable thresholds. This is useful for categorizing clones prior to downstream analysis or visualization.

Usage

```
clonalBin(
  input.data,
  clone.call = NULL,
  chain = "both",
  group.by = NULL,
  proportion = TRUE,
  clone.size = NULL,
  cloneCall = NULL,
  cloneSize = NULL
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL (for both light chains), both.
group.by	A column header in the metadata to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element.
proportion	Whether to use proportion (TRUE) or total frequency (FALSE) of the clone for binning.
clone.size	The bins for the grouping based on proportion or frequency. If proportion is FALSE and the clone.size values are not set high enough based on frequency, the upper limit of clone.size will be automatically updated.
cloneCall	**[Deprecated]** Use clone.call instead.
cloneSize	**[Deprecated]** Use clone.size instead.

Value

A list of data frames with clonal frequency, clonal proportion, and cloneSize columns added.

Author(s)

Nick Borcherding

Examples

```
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Adding clonal bins with default settings (proportion-based)
combined <- clonalBin(combined)

# Adding clonal bins based on frequency
combined <- clonalBin(combined,
                      proportion = FALSE,
                      clone.size = c(Rare = 1, Small = 5, Medium = 20,
                                     Large = 100, Hyperexpanded = 500))

# Using a custom grouping variable
combined <- addVariable(combined,
                        variable.name = "Type",
                        variables = rep(c("B", "L"), 4))
combined <- clonalBin(combined, group.by = "Type")
```

clonalCluster *Cluster clones by sequence similarity*

Description

This function clusters TCRs or BCRs based on the edit distance or alignment score of their CDR3 sequences. It can operate on either nucleotide (`nt`) or amino acid (`aa`) sequences and can optionally enforce that clones share the same V and/or J genes. The output can be the input object with an added metadata column for cluster IDs, a sparse adjacency matrix, or an `igraph` graph object representing the cluster network.

Usage

```
clonalCluster(
  input.data,
  chain = "TRB",
  sequence = "aa",
  threshold = 0.85,
  group.by = NULL,
  dist.type = NULL,
  dist.mat = NULL,
  normalize = "length",
  gap.open = NULL,
  gap.extend = NULL,
  cluster.method = "components",
  cluster.prefix = "cluster.",
  use.V = TRUE,
  use.J = FALSE,
  export.adj.matrix = NULL,
  export.graph = NULL,
  dist_type = NULL,
  dist_mat = NULL,
  gap_open = NULL,
  gap_extend = NULL,
  exportAdjMatrix = NULL,
  exportGraph = NULL
)
```

Arguments

`input.data`	The product of `combineTCR()`, `combineBCR()` or `combineExpression()`.
`chain`	The TCR/BCR chain to use. Use `both` to include both chains (e.g., TRA/TRB). Accepted values: `TRA`, `TRB`, `TRG`, `TRD`, `IGH`, `IGL`, `IGK`, `Light` (for both light chains), or `both` (for TRA/B and Heavy/Light).
`sequence`	Clustering based on either `aa` or `nt` sequences.

threshold	The similarity threshold. If < 1, treated as normalized similarity (higher is stricter). If >= 1, treated as raw edit distance (lower is stricter).
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, clusters will be calculated across all sequences.
dist.type	The distance metric to use. Options: "levenshtein" (default), "hamming", "damerau" (allows transpositions), "nw" (Needleman-Wunsch), or "sw" (Smith-Waterman).
dist.mat	The substitution matrix to use for alignment-based metrics ("nw" or "sw"). Options: "BLOSUM45", "BLOSUM50", "BLOSUM62", "BLOSUM80" (default), "BLOSUM100", "PAM30", "PAM40", "PAM70", "PAM120", "PAM250", or "identity".
normalize	Method for normalizing distances. Options: "none", "maxlen" (divide by max sequence length), or "length" (default, divide by mean sequence length). If threshold < 1, this controls how the similarity is calculated.
gap.open	Penalty for opening a gap in alignment metrics (default: -10).
gap.extend	Penalty for extending a gap in alignment metrics (default: -1).
cluster.method	The clustering algorithm to use. Defaults to "components", which finds connected subgraphs.
cluster.prefix	A character prefix to add to the cluster names (e.g., "cluster.").
use.V	If TRUE, sequences must share the same V gene to be clustered together.
use.J	If TRUE, sequences must share the same J gene to be clustered together.
export.adj.matrix	If TRUE, the function returns a sparse adjacency matrix (dgCMatrix) of the network.
export.graph	If TRUE, the function returns an igraph object of the sequence network.
dist_type	**[Deprecated]** Use dist.type instead.
dist_mat	**[Deprecated]** Use dist.mat instead.
gap_open	**[Deprecated]** Use gap.open instead.
gap_extend	**[Deprecated]** Use gap.extend instead.
exportAdjMatrix	**[Deprecated]** Use export.adj.matrix instead.
exportGraph	**[Deprecated]** Use export.graph instead.

Details

The clustering process is as follows:

1. The function retrieves the relevant chain data from the input object.
2. It calculates the distance between all sequences within each group (or across the entire dataset if group.by is NULL).
3. An edge list is constructed, connecting sequences that meet the similarity threshold.
4. The threshold parameter behaves differently based on its value:
    - threshold **< 1 (e.g., 0.85):** Interpreted as a *normalized* distance. A higher value means greater similarity is required.

- `threshold` **>= 1 (e.g., 2):** Interpreted as a maximum *raw* edit distance. A lower value means greater similarity is required.

5. **Distance Metrics:**
   - **Levenshtein/Hamming/Damerau:** Standard edit distance calculations.
   - **Alignment (NW/SW):** If `dist.type` is "nw" (Needleman-Wunsch) or "sw" (Smith-Waterman), alignment scores are calculated using the specified substitution matrix (`dist.mat`). These scores are converted to a distance-like metric for clustering.
6. An `igraph` graph is built from the edge list.
7. A clustering algorithm is run on the graph (default: connected components).

Value

Depending on the export parameters, one of the following:

- An amended `input.data` object with a new metadata column containing cluster IDs (default).
- An `igraph` object if `export.graph = TRUE`.
- A sparse `dgCMatrix` object if `export.adj.matrix = TRUE`.

Examples

```
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Standard Levenshtein clustering (85% similarity)
sub_combined <- clonalCluster(combined[c(1,2)],
                              chain = "TRA",
                              sequence = "aa",
                              threshold = 0.85)

# Alignment-based clustering using BLOSUM80
sub_combined_nw <- clonalCluster(combined[c(1,2)],
                                 chain = "TRA",
                                 dist.type = "nw",
                                 dist.mat = "BLOSUM80",
                                 threshold = 0.85)

# Export the graph object instead
graph_obj <- clonalCluster(combined[c(1,2)],
                           chain = "TRA",
                           export.graph = TRUE)
```

clonalCompare *Compare Clonal Abundance Across Variables*

Description

This function visualizes the relative abundance of specific clones across different samples or groups. It is useful for tracking how the proportions of top clones change between conditions. The output can be an alluvial plot to trace clonal dynamics or an area plot to show compositional changes.

Usage

```
clonalCompare(
  input.data,
  clone.call = NULL,
  chain = "both",
  samples = NULL,
  clones = NULL,
  top.clones = NULL,
  highlight.clones = NULL,
  relabel.clones = FALSE,
  group.by = NULL,
  order.by = NULL,
  graph = "alluvial",
  proportion = TRUE,
  export.table = NULL,
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR, combineBCR, or combineExpression.
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
samples	The specific samples to isolate for visualization.
clones	The specific clonal sequences of interest
top.clones	The top number of clonal sequences per group. (e.g., top.clones = 5)
highlight.clones	Clonal sequences to highlight, if present, all other clones returned will be grey

relabel.clones	Simplify the legend of the graph by returning clones that are numerically indexed
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
graph	The type of plot to generate. Accepted values are alluvial (default) or area
proportion	If TRUE (default), the y-axis will represent the proportional abundance of clones. If FALSE, the y-axis will represent raw clone counts.‘
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

A ggplot object visualizing proportions of clones by groupings, or a data.frame if export.table = TRUE.

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Using clonalCompares()
clonalCompare(combined,
              top.clones = 5,
              samples = c("P17B", "P17L"),
              clone.call = "aa")
```

---

clonalDiversity *Calculate Clonal Diversity*

Description

This function calculates a specified diversity metric for samples or groups within a dataset. To control for variations in library size, the function can perform bootstrapping with downsampling. It resamples each group to the size of the smallest group and calculates the diversity metric across multiple iterations, returning the mean value.

Usage

```
clonalDiversity(
  input.data,
  clone.call = NULL,
  metric = "shannon",
  chain = "both",
  group.by = NULL,
  order.by = NULL,
  x.axis = NULL,
  export.table = NULL,
  palette = "inferno",
  n.boots = 100,
  return.boots = FALSE,
  skip.boots = FALSE,
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
metric	The diversity metric to calculate. Must be a single string from the list of available metrics (see Details).
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
x.axis	An additional metadata variable to group samples along the x-axis.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals.
n.boots	The number of bootstrap iterations to perform (default is 100).
return.boots	If TRUE, returns all bootstrap values instead of the mean. Automatically enables export.table.
skip.boots	If TRUE, disables downsampling and bootstrapping. The metric will be calculated on the full dataset for each group. Defaults to FALSE.
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Details

The function operates by first splitting the dataset by the specified `group.by` variable.

Downsampling and Bootstrapping: To make a fair comparison between groups of different sizes, diversity metrics often require normalization. This function implements this by downsampling.

1. It determines the number of clones in the smallest group.
2. For each group, it performs `n.boots` iterations (default = 100).
3. In each iteration, it randomly samples the clones (with replacement) down to the size of the smallest group.
4. It calculates the selected diversity metric on this downsampled set.
5. The final reported diversity value is the mean of the results from all bootstrap iterations.

This process can be disabled by setting `skip.boots = TRUE`.

Available Diversity Metrics (metric): The function uses a registry of metrics imported from the immApex package. You can select one of the following:

- `"shannon"`: Shannon's Entropy. See `shannon_entropy`.
- `"inv.simpson"`: Inverse Simpson Index. See `inv_simpson`.
- `"gini.simpson"`: Gini-Simpson Index. See `gini_simpson`.
- `"norm.entropy"`: Normalized Shannon Entropy. See `norm_entropy`.
- `"pielou"`: Pielou's Evenness (same as norm.entropy). See `pielou_evenness`.
- `"ace"`: Abundance-based Coverage Estimator. See `ace_richness`.
- `"chao1"`: Chao1 Richness Estimator. See `chao1_richness`.
- `"gini"`: Gini Coefficient for inequality. See `gini_coef`.
- `"d50"`: The number of top clones making up 50% of the library. See `d50_dom`.
- `"hill0"`, `"hill1"`, `"hill2"`: Hill numbers of order 0, 1, and 2. See `hill_q`.

Value

A ggplot object visualizing the diversity metric, or a data.frame if `export.table = TRUE`.

Author(s)

Andrew Malone, Nick Borcherding, Nathan Vanderkraan

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Calculate Shannon diversity, grouped by sample
clonalDiversity(combined,
                clone.call = "gene",
                metric = "shannon")
```

```
# Calculate Inverse Simpson without bootstrapping
clonalDiversity(combined,
                clone.call = "aa",
                metric = "inv.simpson",
                skip.boots = TRUE)
```

`clonalHomeostasis` *Plot Clonal Homeostasis of the Repertoire*

Description

This function calculates the space occupied by clone proportions. The grouping of these clones is based on the parameter `clone.size`, at default, `clone.size` will group the clones into bins of Rare = 0 to 0.0001, Small = 0.0001 to 0.001, etc. To adjust the proportions, change the number or labeling of the clone.size parameter. If a matrix output for the data is preferred, set `export.table` = TRUE.

Usage

```
clonalHomeostasis(
  input.data,
  clone.size = NULL,
  clone.call = NULL,
  chain = "both",
  group.by = NULL,
  order.by = NULL,
  export.table = NULL,
  palette = "inferno",
  cloneSize = NULL,
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

`input.data`	The product of `combineTCR()`, `combineBCR()`, or `combineExpression()`.
`clone.size`	The cut points of the proportions.
`clone.call`	Defines the clonal sequence grouping. Accepted values are: `gene` (VDJC genes), `nt` (CDR3 nucleotide sequence), `aa` (CDR3 amino acid sequence), or `strict` (VDJC + nt). A custom column header can also be used.
`chain`	The TCR/BCR chain to use. Use `both` to include both chains (e.g., TRA/TRB). Accepted values: `TRA`, `TRB`, `TRG`, `TRD`, `IGH`, `IGL`, `IGK`, `Light` (for both light chains), or `both` (for TRA/B and Heavy/Light).

group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals.
cloneSize	**[Deprecated]** Use clone.size instead.
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

A ggplot object visualizing clonal homeostasis, or a data.frame if export.table = TRUE.

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))
clonalHomeostasis(combined, clone.call = "gene")
```

---

clonalLength *Plot the Distribution of Sequence Lengths*

Description

This function displays either the nucleotide nt or amino acid aa sequence length. The sequence length visualized can be selected using the chains parameter, either the combined clone (both chains) or across all single chains. Visualization can either be a histogram or if scale = TRUE, the output will be a density plot. Multiple sequencing runs can be group together using the group.by parameter.

Usage

```
clonalLength(
  input.data,
  clone.call = NULL,
  chain = "both",
  group.by = NULL,
  order.by = NULL,
  scale = FALSE,
  export.table = NULL,
```

```
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression()
clone.call	Defines the clonal sequence grouping. Accepted values are: nt (CDR3 nucleotide sequence) or aa (CDR3 amino acid sequence)
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
scale	Converts the graphs into density plots in order to show relative distributions.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

A ggplot object visualizing the distributions by length, or a data.frame if export.table = TRUE.

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))
clonalLength(combined, clone.call = "aa", chain = "both")
```

clonalNetwork *Visualize Clonal Network in Dimensional Reductions*

Description

This function generates a network based on clonal proportions of an indicated identity and then superimposes the network onto a single-cell object dimensional reduction plot.

Usage

```
clonalNetwork(
  sc.data,
  clone.call = NULL,
  chain = "both",
  reduction = "umap",
  group.by = "ident",
  filter.clones = NULL,
  filter.identity = NULL,
  filter.proportion = NULL,
  filter.graph = FALSE,
  export.clones = NULL,
  export.table = NULL,
  palette = "inferno",
  cloneCall = NULL,
  exportClones = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

sc.data	The single-cell object after combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
reduction	The name of the dimensional reduction of the single-cell object.
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). This will be the nodes overlaid onto the graph.
filter.clones	Use to select the top n clones (e.g., filter.clones`**` = 2000) or n of clones based on the minimu = "min").
filter.identity	Display the network for a specific level of the indicated identity.

`filter.proportion`
: Remove clones from the network below a specific proportion.

`filter.graph`
: Remove the reciprocal edges from the half of the graph, allowing for cleaner visualization.

`export.clones`
: Exports a table of clones that are shared across multiple identity groups and ordered by the total number of clone copies.

`export.table`
: If `TRUE`, returns a data frame or matrix of the results instead of a plot.

`palette`
: Colors to use in visualization - input any hcl.pals.

`cloneCall`
: **[Deprecated]** Use `clone.call` instead.

`exportClones`
: **[Deprecated]** Use `export.clones` instead.

`exportTable`
: **[Deprecated]** Use `export.table` instead.

`...`
: Additional arguments passed to the ggplot theme

Value

ggplot object

Examples

```
## Not run:
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Getting a sample of a Seurat object
scRep_example  <- get(data("scRep_example"))

# Using combineExpresion()
scRep_example  <- combineExpression(combined, scRep_example)

# Using clonalNetwork()
clonalNetwork(scRep_example,
              reduction = "umap",
              group.by = "seurat_clusters")

## End(Not run)
```

---

clonalOccupy *Plot cloneSize by Variable in Single-Cell Objects*

Description

View the count of clones frequency group in Seurat or SCE object meta data after `combineExpression()`. The visualization will take the new meta data variable `cloneSize` and plot the number of cells with each designation using a secondary variable, like cluster. Credit to the idea goes to Drs. Carmona and Andreatta and their work with ProjectTIL.

Usage

```
clonalOccupy(
  sc.data,
  x.axis = "ident",
  label = TRUE,
  facet.by = NULL,
  order.by = NULL,
  proportion = FALSE,
  na.include = FALSE,
  export.table = NULL,
  palette = "inferno",
  exportTable = NULL,
  ...
)
```

Arguments

sc.data	The single-cell object after combineExpression()
x.axis	The variable in the meta data to graph along the x.axis.
label	Include the number of clone in each category by x.axis variable
facet.by	The column header used for faceting the graph
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
proportion	Convert the stacked bars into relative proportion
na.include	Visualize NA values or not
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

Stacked bar plot of counts of cells by clone frequency group

Examples

```
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Getting a sample of a Seurat object
scRep_example <- get(data("scRep_example"))

# Using combineExpresion()
scRep_example <- combineExpression(combined, scRep_example)
```

```
# Using clonalOccupy
clonalOccupy(scRep_example, x.axis = "ident")
table <- clonalOccupy(scRep_example, x.axis = "ident", export.table = TRUE)
```

---

clonalOverlap *Examining the clonal overlap between groups or samples*

Description

This functions allows for the calculation and visualizations of various overlap metrics for clones. The methods include overlap coefficient (`overlap`), Morisita's overlap index (`morisita`), Jaccard index (`jaccard`), cosine similarity (`cosine`) or the exact number of clonal overlap (`raw`).

Usage

```
clonalOverlap(
  input.data,
  clone.call = NULL,
  method = c("overlap", "morisita", "jaccard", "cosine", "raw"),
  chain = "both",
  group.by = NULL,
  order.by = NULL,
  export.table = NULL,
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

`input.data`	The product of `combineTCR()`, `combineBCR()`, or `combineExpression()`
`clone.call`	Defines the clonal sequence grouping. Accepted values are: `gene` (VDJC genes), `nt` (CDR3 nucleotide sequence), `aa` (CDR3 amino acid sequence), or `strict` (VDJC + nt). A custom column header can also be used.
`method`	The method to calculate the `overlap`, `morisita`, `jaccard`, `cosine` indices or `raw` for the base numbers
`chain`	The TCR/BCR chain to use. Use `both` to include both chains (e.g., TRA/TRB). Accepted values: `TRA`, `TRB`, `TRG`, `TRD`, `IGH`, `IGL`, `IGK`, `Light` (for both light chains), or `both` (for TRA/B and Heavy/Light).
`group.by`	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If `NULL`, data will be analyzed by list element or active identity in the case of single-cell objects.

order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Details

The formulas for the indices are as follows:

Overlap Coefficient:

$$overlap = \frac{\sum \min(a, b)}{\min(\sum a, \sum b)}$$

Raw Count Overlap:

$$raw = \sum \min(a, b)$$

Morisita Index:

$$morisita = \frac{\sum ab}{(\sum a)(\sum b)}$$

Jaccard Index:

$$jaccard = \frac{\sum \min(a, b)}{\sum a + \sum b - \sum \min(a, b)}$$

Cosine Similarity:

$$cosine = \frac{\sum ab}{\sqrt{(\sum a^2)(\sum b^2)}}$$

Where:

- a and b are the abundances of species i in groups A and B, respectively.

Value

A ggplot object visualizing clonal overlap or a data.frame if exportTable = TRUE.

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Using clonalOverlap()
clonalOverlap(combined,
              clone.call = "aa",
              method = "jaccard")
```

clonalOverlay *Visualize Distribution of Clonal Frequency*

Description

This function allows the user to visualize the clonal expansion by overlaying the cells with specific clonal frequency onto the dimensional reduction plots in Seurat. Credit to the idea goes to Drs Andreatta and Carmona and their work with ProjectTIL.

Usage

```
clonalOverlay(
  sc.data,
  reduction = NULL,
  cut.category = "clonalFrequency",
  cutpoint = 30,
  bins = 25,
  pt.size = 0.5,
  pt.alpha = 1,
  facet.by = NULL,
  ...
)
```

Arguments

sc.data	The single-cell object after combineExpression().
reduction	The dimensional reduction to visualize.
cut.category	Meta data variable of the single-cell object to use for filtering.
cutpoint	The overlay cut point to include, this corresponds to the cut.category variable in the meta data of the single-cell object.
bins	The number of contours to the overlay
pt.size	The point size for plotting (default is 0.5)
pt.alpha	The alpha value for plotting (default is 1)
facet.by	meta data variable to facet the comparison
...	Additional arguments passed to the ggplot theme

Value

A ggplot object visualizing distributions of clones along a dimensional reduction within the single-cell object

Author(s)

Francesco Mazziotta, Nick Borcherding

Examples

```
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))
# Getting a sample of a Seurat object
scRep_example <- get(data("scRep_example"))

# Using combineExpresion()
scRep_example <- combineExpression(combined,
                                   scRep_example)

# Using clonalOverlay()
clonalOverlay(scRep_example,
              reduction = "umap",
              cutpoint = 3,
              bins = 5)
```

`clonalProportion` *Plot the Clonal Space Occupied by Specific Clones*

Description

This function calculates the relative clonal space occupied by the clones. The grouping of these clones is based on the parameter `clonalSplit`, at default, `clonalSplit` will group the clones into bins of 1:10, 11:100, 101:1001, etc. To adjust the clones selected, change the numbers in the variable split. If a matrix output for the data is preferred, set `exportTable` = TRUE.

Usage

```
clonalProportion(
  input.data,
  clonal.split = NULL,
  clone.call = NULL,
  chain = "both",
  group.by = NULL,
  order.by = NULL,
  export.table = NULL,
  palette = "inferno",
  clonalSplit = NULL,
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression().
clonal.split	The cut points for the specific clones, default = c(10, 100, 1000, 10000, 30000, 1e+05)
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals
clonalSplit	**[Deprecated]** Use clonal.split instead.
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

A ggplot object dividing space occupied by ranks of clones or a data.frame if exportTable = TRUE.

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))
# Using clonalProportion()
clonalProportion(combined, clone.call = "gene")
```

---

clonalQuant *Plot Number or Proportions of Clones*

Description

This function quantifies unique clones. The unique clones can be either reported as a raw output or scaled to the total number of clones recovered using the scale parameter.

Usage

```
clonalQuant(
  input.data,
  clone.call = NULL,
  chain = "both",
  scale = FALSE,
  group.by = NULL,
  order.by = NULL,
  export.table = NULL,
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
scale	Converts the graphs into percentage of unique clones
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

A ggplot object visualizing the total or relative number of clones or a data.frame if exportTable = TRUE.

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
```

```
                                    "P19B","P19L", "P20B", "P20L"))

# Using clonalQuant()
clonalQuant(combined,
            clone.call="strict",
            scale = TRUE)
```

---

clonalRarefaction *Calculate rarefaction based on the abundance of clones*

Description

This functions uses the Hill numbers of order q: species richness (q = 0), Shannon diversity (q = 1), the exponential of Shannon entropy and Simpson diversity (q = 2, the inverse of Simpson concentration) to compute diversity estimates for rarefaction and extrapolation. The function relies on the iNEXT::iNEXT() R package. Please read and cite the manuscript if using this function. The input into the iNEXT calculation is abundance, incidence-based calculations are not supported.

Usage

```
clonalRarefaction(
  input.data,
  clone.call = NULL,
  chain = "both",
  group.by = NULL,
  plot.type = 1,
  hill.numbers = 0,
  n.boots = 20,
  export.table = NULL,
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element or active identity in the case of single-cell objects.

plot.type	sample-size-based rarefaction/extrapolation curve (type = 1); sample completeness curve (type = 2); coverage-based rarefaction/extrapolation curve (type = 3).
hill.numbers	The Hill numbers to be plotted out (0 - species richness, 1 - Shannon, 2 - Simpson)
n.boots	The number of bootstrap replicates used to derive confidence intervals for the diversity estimates. More replicates can provide a more reliable measure of statistical variability.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals.
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Using clonalRarefaction()
clonalRarefaction(combined[c(1,2)], clone.call = "gene", n.boots = 3)
```

---

clonalScatter *Scatter Plot Comparing Clones Across Two Samples*

Description

This function produces a scatter plot directly comparing the specific clones between two samples. The clones will be categorized by counts into singlets or expanded, either exclusive or shared between the selected samples.

Usage

```
clonalScatter(
  input.data,
  clone.call = NULL,
  x.axis = NULL,
  y.axis = NULL,
  chain = "both",
  dot.size = "total",
  group.by = NULL,
  graph = "proportion",
```

```
  export.table = NULL,
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
x.axis	name of the list element to appear on the x.axis.
y.axis	name of the list element to appear on the y.axis.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
dot.size	either total or the name of the list element to use for size of dots.
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element or active identity in the case of single-cell objects.
graph	graph either the clonal "proportion" or "count".
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals.
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

A ggplot object visualizing clonal dynamics between two groupings or a data.frame if exportTable = TRUE.

Examples

```
#Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Using clonalScatter()
clonalScatter(combined,
              x.axis = "P17B",
              y.axis = "P17L",
              graph = "proportion")
```

clonalSizeDistribution *Plot powerTCR Clustering Based on Clonal Size*

Description

This function produces a hierarchical clustering of clones by sample using discrete gamma-GPD spliced threshold model. If using this model please read and cite powerTCR (more info available at PMID: 30485278).

Usage

```
clonalSizeDistribution(
  input.data,
  clone.call = NULL,
  chain = "both",
  method = "ward.D2",
  threshold = 1,
  group.by = NULL,
  export.table = NULL,
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
method	The clustering parameter for the dendrogram.
threshold	Numerical vector containing the thresholds the grid search was performed over.
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed as by list element or active identity in the case of single-cell objects.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals.
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Details

The probability density function (pdf) for the **Generalized Pareto Distribution (GPD)** is given by:

$$f(x|\mu,\sigma,\xi) = \frac{1}{\sigma}\left(1+\xi\left(\frac{x-\mu}{\sigma}\right)\right)^{-\left(\frac{1}{\xi}+1\right)}$$

Where:

- μ is a location parameter
- $\sigma > 0$ is a scale parameter
- ξ is a shape parameter
- $x \geq \mu$ if $\xi \geq 0$ and $\mu \leq x \leq \mu - \sigma/\xi$ if $\xi < 0$

The probability density function (pdf) for the **Gamma Distribution** is given by:

$$f(x|\alpha,\beta) = \frac{x^{\alpha-1}e^{-x/\beta}}{\beta^{\alpha}\Gamma(\alpha)}$$

Where:

- $\alpha > 0$ is the shape parameter
- $\beta > 0$ is the scale parameter
- $x \geq 0$
- $\Gamma(\alpha)$ is the gamma function of α

Value

A ggplot object visualizing dendrogram of clonal size distribution or a data.frame if `exportTable = TRUE`.

Author(s)

Hillary Koch

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Using clonalSizeDistribution()
clonalSizeDistribution(combined,
                       clone.call = "strict",
                       method="ward.D2")
```

combineBCR *Combine B Cell Receptor Contig Data*

Description

This function consolidates a list of BCR sequencing results to the level of the individual cell barcodes. Using the samples and ID parameters, the function will add the strings as prefixes to prevent issues with repeated barcodes. The resulting new barcodes will need to match the Seurat or SCE object in order to use, `combineExpression()`. Unlike `combineTCR()`, combineBCR produces a column `CTstrict` based on the edit distance clustering from `clonalCluster()`. The `CTstrict` column is formatted as `Heavy_Light` (underscore-separated) for downstream compatibility. Connected clones are labeled with `cluster.X`, while unconnected clones (singlets) are labeled with the V gene and CDR3 sequence (e.g., `IGHV3-64.CAKSYS..._IGKV3-15.CQQYSN...`).

Usage

```
combineBCR(
  input.data,
  samples = NULL,
  ID = NULL,
  chain = "IGH",
  sequence = "nt",
  dist.type = NULL,
  dist.mat = NULL,
  normalize = "length",
  gap.open = NULL,
  gap.extend = NULL,
  call.related.clones = TRUE,
  group.by = NULL,
  threshold = 0.85,
  cluster.method = "components",
  use.V = TRUE,
  use.J = TRUE,
  remove.na = NULL,
  remove.multi = NULL,
  filter.multi = NULL,
  filter.nonproductive = NULL,
  removeNA = NULL,
  removeMulti = NULL,
  filterMulti = NULL,
  filterNonproductive = NULL,
  dist_type = NULL,
  dist_mat = NULL,
  gap_open = NULL,
  gap_extend = NULL
)
```

Arguments

input.data	List of filtered contig annotations or outputs from loadContigs().
samples	A character vector of sample labels. Must be the same length as the input list.
ID	An optional character vector for additional sample identifiers.
chain	The chain to use for clustering when call.related.clones = TRUE. Passed to clonalCluster(). Default is "IGH".
sequence	The sequence type ("nt" or "aa") to use for clustering. Passed to clonalCluster(). Default is "nt".
dist.type	The distance metric to use. Options: "levenshtein" (default), "hamming", "damerau", "nw" (Needleman-Wunsch), or "sw" (Smith-Waterman).
dist.mat	The substitution matrix to use for alignment-based metrics ("nw" or "sw"). Options include "BLOSUM62", "PAM30", etc.
normalize	Method for normalizing distances. Options: "none" (default), "maxlen", or "length".
gap.open	Penalty for opening a gap in alignment metrics (default: -10).
gap.extend	Penalty for extending a gap in alignment metrics (default: -1).
call.related.clones	Logical. If TRUE, uses clonalCluster() to identify related clones based on sequence similarity. If FALSE, defines clones by the exact V-gene and CDR3 amino acid sequence.
group.by	The column header used for to group clones. If ('NULL'), clusters will be calculated across samples.
threshold	The similarity threshold passed to clonalCluster() if call.related.clones = TRUE. See ?clonalCluster for details.
cluster.method	The clustering algorithm to use. Defaults to "components", which finds connected subgraphs.
use.V	Logical. If TRUE, sequences must share the same V gene to be clustered together.
use.J	Logical. If TRUE, sequences must share the same J gene to be clustered together.
remove.na	This will remove any chain without values.
remove.multi	Logical. If TRUE, removes cells that have more than one distinct heavy or light chain after processing.
filter.multi	Logical. If TRUE, filters multi-chain cells to retain only the most abundant IGH and IGL/IGK chains.
filter.nonproductive	Logical. If TRUE, removes non-productive contigs from the analysis.
removeNA	**[Deprecated]** Use remove.na instead.
removeMulti	**[Deprecated]** Use remove.multi instead.
filterMulti	**[Deprecated]** Use filter.multi instead.
filterNonproductive	**[Deprecated]** Use filter.nonproductive instead.
dist_type	**[Deprecated]** Use dist.type instead.
dist_mat	**[Deprecated]** Use dist.mat instead.
gap_open	**[Deprecated]** Use gap.open instead.
gap_extend	**[Deprecated]** Use gap.extend instead.

Value

A list of data frames, where each data frame represents a sample. Each row corresponds to a unique cell barcode, with columns detailing the BCR chains and the assigned clone ID.

Examples

```
# Data derived from the 10x Genomics intratumoral NSCLC B cells
BCR <- read.csv("https://www.borch.dev/uploads/contigs/b_contigs.csv")
combined <- combineBCR(BCR,
                       samples = "Patient1",
                       threshold = 0.85)
```

---

combineExpression *Adding Clonal Information to Single-Cell Object*

Description

This function adds the immune receptor information to the Seurat or SCE object to the meta data. By default this function also calculates the frequencies and proportion of the clones by sequencing run (group.by = NULL). To change how the frequencies/proportions are calculated, select a column header for the group.by variable. Importantly, before using combineExpression() ensure the barcodes of the single-cell object object match the barcodes in the output of the combineTCR() or combineBCR().

Usage

```
combineExpression(
  input.data,
  sc.data,
  clone.call = NULL,
  chain = "both",
  group.by = NULL,
  proportion = TRUE,
  filter.na = NULL,
  clone.size = NULL,
  add.label = NULL,
  cloneCall = NULL,
  cloneSize = NULL,
  filterNA = NULL,
  addLabel = NULL
)
```

Arguments

input.data	The product of combineTCR(), combineBCR() or a list of both c(combineTCR(), combineBCR()).

sc.data	The Seurat or Single-Cell Experiment (SCE) object to attach
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL (for both light chains), both.
group.by	A column header in lists to group the analysis by (e.g., "sample", "treatment"). If NULL, will be based on the list element.
proportion	Whether to proportion (TRUE) or total frequency (FALSE) of the clone based on the group.by variable.
filter.na	Method to subset Seurat/SCE object of barcodes without clone information
clone.size	The bins for the grouping based on proportion or frequency. If proportion is FALSE and the clone.sizes are not set high enough based on frequency, the upper limit of clone.sizes will be automatically updated.
add.label	This will add a label to the frequency header, allowing the user to try multiple group.by variables or recalculate frequencies after subsetting the data.
cloneCall	**[Deprecated]** Use clone.call instead.
cloneSize	**[Deprecated]** Use clone.size instead.
filterNA	**[Deprecated]** Use filter.na instead.
addLabel	**[Deprecated]** Use add.label instead.

Value

Single-cell object with clone information added to meta data information

Examples

```
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Getting a sample of a Seurat object
scRep_example <- get(data("scRep_example"))

# Using combineExpresion()
scRep_example <- combineExpression(combined, scRep_example)
```

combineTCR *Combine T Cell Receptor Contig Data*

Description

This function consolidates a list of TCR sequencing results to the level of the individual cell barcodes. Using the `samples` and `ID` parameters, the function will add the strings as prefixes to prevent issues with repeated barcodes. The resulting new barcodes will need to match the Seurat or SCE object in order to use, `combineExpression()`. Several levels of filtering exist - `remove.na`, `remove.multi`, or `filter.multi` are parameters that control how the function deals with barcodes with multiple chains recovered.

Usage

```
combineTCR(
  input.data,
  samples = NULL,
  ID = NULL,
  remove.na = NULL,
  remove.multi = NULL,
  filter.multi = NULL,
  filter.nonproductive = NULL,
  removeNA = NULL,
  removeMulti = NULL,
  filterMulti = NULL,
  filterNonproductive = NULL
)
```

Arguments

`input.data`	List of filtered contig annotations or outputs from `loadContigs()`.
`samples`	The labels of samples (recommended).
`ID`	The additional sample labeling (optional).
`remove.na`	This will remove any chain without values.
`remove.multi`	This will remove barcodes with greater than 2 chains.
`filter.multi`	This option will allow for the selection of the 2 corresponding chains with the highest expression for a single barcode.
`filter.nonproductive`	This option will allow for the removal of nonproductive chains if the variable exists in the contig data. Default is set to TRUE to remove nonproductive contigs.
`removeNA`	**[Deprecated]** Use `remove.na` instead.
`removeMulti`	**[Deprecated]** Use `remove.multi` instead.
`filterMulti`	**[Deprecated]** Use `filter.multi` instead.
`filterNonproductive`	**[Deprecated]** Use `filter.nonproductive` instead.

Value

List of clones for individual cell barcodes

Examples

```
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))
```

---

contig_list *A List of Eight Single-cell TCR Sequencing Runs.*

Description

A list of 8 `filtered_contig_annotations.csv` files outputted from 10X Cell Ranger. More information on the data can be found in the following manuscript.

---

createHTOContigList *Deconvolute Contig Information from Multiplexed Experiments*

Description

This function reprocess and forms a list of contigs for downstream analysis in scRepertoire, `createHTOContigList()` take the filtered contig annotation output and the single-cell RNA object to create the list. If using an integrated single-cell object, it is recommended to split the object by sequencing run and remove extra prefixes and suffixes on the barcode before using `createHTOContigList()`. Alternatively, the variable `multi.run` can be used to separate a list of contigs by a meta data variable. This may have issues with the repeated barcodes.

Usage

```
createHTOContigList(contig, sc.data, group.by = NULL, multi.run = NULL)
```

Arguments

`contig`	The filtered contig annotation file from multiplexed experiment
`sc.data`	The Seurat or Single-Cell Experiment object.
`group.by`	One or more meta data headers to create the contig list based on. If more than one header listed, the function combines them into a single variable.
`multi.run`	If using integrated single-cell object, the meta data variable that indicates the sequencing run.

Value

Returns a list of contigs as input for combineBCR() or combineTCR()

Examples

```
## Not run:
filtered.contig <- read.csv(".../Sample/outs/filtered_contig_annotations.csv")

contig.list <- createHTOContigList(contig = filtered.contig,
                                   sc.data = Seurat.Obj,
                                   group.by = "HTO_maxID")

## End(Not run)
```

---

exportClones *Export Clonal Data in Various Formats*

Description

Exports clonal information (gene sequences, amino acids, nucleotides) from scRepertoire objects into a file or a data frame. The output format can be tailored for compatibility with different analysis workflows.

Usage

```
exportClones(
  input.data,
  format = "paired",
  group.by = NULL,
  write.file = TRUE,
  dir = NULL,
  file.name = "clones.csv"
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression().
format	The format for exporting clones. Options are: paired, airr, TCRMatch, tcrpheno, immunarch.
group.by	The variable in the metadata to use for grouping. If NULL, data will be grouped by the sample names.
write.file	If TRUE (default), saves the output to a CSV file. If FALSE, returns the data frame or list to the R environment.
dir	The directory where the output file will be saved. Defaults to the current working directory.
file.name	The name of the file to be saved.

Details

The `format` parameter determines the structure of the output:

- `paired`: Exports a data frame where each row represents a barcode, with paired chain information (amino acid, nucleotide, genes) in separate columns.
- `airr`: Exports a data frame that adheres to the Adaptive Immune Receptor Repertoire (AIRR) Community format, with each row representing a single receptor chain.
- `TCRMatch`: Exports a data frame specifically for the TCRMatch algorithm, containing the TRB chain amino acid sequence and clonal frequency.
- `tcrpheno`: Exports a data frame compatible with the `tcrpheno` pipeline, with TRA and TRB chains in separate columns.
- `immunarch`: Exports a list containing a data frame and metadata formatted for use with the `immunarch` package.

Value

A data frame or list in the specified format, either returned to the R environment or saved as a CSV file.

Author(s)

Jonathan Noonan, Nick Borcherding

Examples

```
## Not run:
#Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B", "P19L", "P20B", "P20L"))

# Export as a paired data frame and save to a file
exportClones(combined, format = "paired", file.name = "paired_clones.csv")

# Return an AIRR-formatted data frame to the environment
airr_df <- exportClones(combined, format = "airr", write.file = FALSE)

## End(Not run)
```

`expression2List` **DEPRECATED** *Take the meta data in seurat/SCE and place it into a list*

Description

[Deprecated]

Allows users to perform more fundamental measures of clonotype analysis using the meta data from the seurat or SCE object. For Seurat objects the active identity is automatically added as "cluster". Remaining grouping parameters or SCE or Seurat objects must appear in the meta data.

This function is deprecated as of version 2 due to the confusion it caused to many users. Users are encouraged to remain with the abstraction barrier of combined single cell objects and the outputs of `combineTCR()` and `combineBCR()` for all functions.

We discourage the use of this function, but if you have to use it, set the `force` argument to `TRUE`.

Usage

```
expression2List(sc, ..., force = FALSE)
```

Arguments

`sc`	output of `combineExpression()`.
`...`	previously the `group` or `split.by` argument, indicating the column header to group the new list by. This should strictly be one argument and is an ellipsis for backwards compatibility. Everything after the first argument is ignored.
`force`	logical. If not `TRUE` (default), a deprecation error will be thrown. Otherwise the function will run but not guaranteed to be stable.

Value

list derived from the meta data of single-cell object with elements divided by the group parameter

---

getCirclize *Generate Data Frame to Plot Chord Diagram*

Description

This function will take the meta data from the product of `combineExpression()` and generate a relational data frame to be used for a chord diagram. Each chord will represent the number of clones unique and shared across the multiple `group.by` variable. If using the downstream circlize R package, please read and cite the following manuscript. If looking for more advanced ways for circular visualizations, there is a great cookbook for the circlize package.

Usage

```
getCirclize(
  sc.data,
  clone.call = NULL,
  group.by = NULL,
  method = c("unique", "abundance", "jaccard", "overlap"),
```

```
  proportion = FALSE,
  symmetric = TRUE,
  include.self = TRUE,
  include.metadata = FALSE,
  min.shared = 0,
  top.links = NULL,
  filter.sectors = NULL,
  palette = "inferno",
  cloneCall = NULL
)
```

Arguments

sc.data	The single-cell object after combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
group.by	A column header (or vector of column headers for hierarchical grouping) in the metadata to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by active identity. When multiple columns are provided, they are combined with "_" separator for multi-level annotations.
method	The method for calculating link values: "unique" (default) counts unique shared clones, "abundance" sums clone frequencies, "jaccard" calculates Jaccard similarity, "overlap" calculates overlap coefficient.
proportion	Calculate the relationship by unique clones (FALSE, default) or normalized by proportion (TRUE).
symmetric	If TRUE (default), returns symmetric relationships. If FALSE, returns directional flow showing proportion of source's clones found in destination.
include.self	Include counting the clones within a single group.by comparison.
include.metadata	If TRUE, returns a list with links data frame and sector-level metadata including cell counts, clone counts, and expansion metrics.
min.shared	Minimum number of shared clones to include a link (default 0).
top.links	Keep only the top N links by value. If NULL (default), keep all.
filter.sectors	Character vector of sectors to include. If NULL, include all.
palette	Colors to use for sector color suggestions - input any hcl.pals.
cloneCall	**[Deprecated]** Use clone.call instead.

Value

A data frame of shared clones between groups formatted for chordDiagram. If include.metadata = TRUE, returns a list with links (the edge data frame), sectors (sector-level statistics), and colors (suggested colors for each sector).

Author(s)

Dillon Corvino, Nick Borcherding

Examples

```
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Getting a sample of a Seurat object
scRep_example <- get(data("scRep_example"))
scRep_example <- combineExpression(combined,
                                   scRep_example)

# Getting data frame output for Circlize
circles <- getCirclize(scRep_example,
                       group.by = "seurat_clusters")

# Multi-level grouping for hierarchical chord diagrams
scRep_example$Patient <- substring(scRep_example$orig.ident, 1, 3)
circles <- getCirclize(scRep_example,
                       group.by = c("Patient", "seurat_clusters"))

# Get rich output with sector metadata
result <- getCirclize(scRep_example,
                      group.by = "seurat_clusters",
                      include.metadata = TRUE)
```

`getContigDoublets` *Get Contig Doublets*

Description

[Experimental]

This function identifies potential doublets by finding common barcodes between TCR and BCR outputs. It extracts unique barcodes from each list of dataframes, finds the intersection of the barcodes, and joins the resulting data.

Usage

```
getContigDoublets(tcrOutput, bcrOutput)
```

Arguments

`tcrOutput`	Output of `combineTCR()`. A list of data.frames containing TCR contig information, each dataframe must have a `barcode` column.
`bcrOutput`	Output of `combineBCR()`. A list of data.frames containing BCR contig information, each dataframe must have a `barcode` column.

Value

A dataframe of barcodes that exist in both the TCR and BCR data, with columns from both sets of data. There will be an additional column `contigType` of type factor with levels 'TCR' and 'BCR' indicating the origin of the contig - this will be the new first column.

If there are no doublets, the returned data.frame will have the same colnames but no rows.

`getHumanIgPseudoGenes` *Get Human Immunoglobulin pseudogenes*

Description

This function returns a character vector of human immunoglobulin pseudogenes. These are also the genes that are removed from the variable gene list in `quietVDJgenes()`.

Usage

```
getHumanIgPseudoGenes()
```

Value

Character vector of human immunoglobulin pseudogenes.

`highlightClones` *Highlighting Specific Clones*

Description

Use a specific clonal sequence to highlight on top of the dimensional reduction in single-cell object.

Usage

```
highlightClones(sc.data, clone.call = NULL, sequence = NULL, cloneCall = NULL)
```

Arguments

`sc.data`	The single-cell object to attach after `combineExpression()`
`clone.call`	Defines the clonal sequence grouping. Accepted values are: `gene` (VDJC genes), `nt` (CDR3 nucleotide sequence), `aa` (CDR3 amino acid sequence), or `strict` (VDJC + nt). A custom column header can also be used.
`sequence`	The specific sequence or sequence to highlight
`cloneCall`	**[Deprecated]** Use `clone.call` instead.

Value

Single-cell object object with new meta data column for indicated clones

Examples

```
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Getting a sample of a Seurat object
scRep_example  <- get(data("scRep_example"))

# Using combineExpresion()
scRep_example  <- combineExpression(combined,
                                    scRep_example)

# Using highlightClones()
scRep_example   <- highlightClones(scRep_example,
                                   clone.call= "aa",
                                   sequence = c("CVVSDNTGGFKTIF_CASSVRRERANTGELFF"))
```

`loadContigs` *Load Immune Receptor Sequencing Contigs*

Description

This function loads and processes contig data from various single-cell immune receptor sequencing formats. It reads data from a directory (recursively) or from an already loaded list/data frame, transforms it to a common structure, and returns a list of contigs ready for downstream analysis with `combineTCR()` or `combineBCR()`.

Supported file formats and their expected file names:

- `10X`: "filtered_contig_annotations.csv"
- `AIRR`: "airr_rearrangement.tsv"
- `BD`: "Contigs_AIRR.tsv"
- `Dandelion`: "all_contig_dandelion.tsv"
- `Immcantation`: "_data.tsv" (or similar)
- "JSON": ".json"
- `ParseBio`: "barcode_report.tsv"
- `MiXCR`: "clones.tsv"
- `TRUST4`: "barcode_report.tsv"
- `WAT3R`: "barcode_results.csv"

Usage

```
loadContigs(input, format = "10X")
```

Arguments

input	A directory path containing contig files or a list/data frame of pre-loaded contig data.
format	A string specifying the data format. Must be one of: auto, 10X, AIRR, BD, Dandelion, JSON, MiXCR, ParseBio, TRUST4, WAT3R, or Immcantation. If "auto", the function attempts automatic format detection.

Value

A list of contigs formatted for use with combineTCR() or combineBCR(). Rows containing only NA values (aside from the barcode) are dropped.

Examples

```
TRUST4 <- read.csv("https://www.borch.dev/uploads/contigs/TRUST4_contigs.csv")
contig.list <- loadContigs(TRUST4, format = "TRUST4")

BD <- read.csv("https://www.borch.dev/uploads/contigs/BD_contigs.csv")
contig.list <- loadContigs(BD, format = "BD")

WAT3R <- read.csv("https://www.borch.dev/uploads/contigs/WAT3R_contigs.csv")
contig.list <- loadContigs(WAT3R, format = "WAT3R")
```

---

percentAA *Plot Relative Amino Acid Composition by Position*

Description

This function the proportion of amino acids along the residues of the CDR3 amino acid sequence.

Usage

```
percentAA(
  input.data,
  chain = "TRB",
  group.by = NULL,
  order.by = NULL,
  aa.length = 20,
  export.table = NULL,
  palette = "inferno",
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression().
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
aa.length	The maximum length of the CDR3 amino acid sequence.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

A ggplot object visualizing amino acid by proportion or a data.frame if exportTable = TRUE.

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Using percentAA()
percentAA(combined,
          chain = "TRB",
          aa.length = 20)
```

---

percentGeneUsage *Summarizes and Visualizes Gene Usage*

Description

This function quantifies and visualizes the usage of V, D, or J genes, or gene pairings across T or B cell clones.

Usage

```
percentGeneUsage(
  input.data,
  chain = "TRB",
  genes = "TRBV",
  group.by = NULL,
  order.by = NULL,
  summary.fun = c("percent", "proportion", "count"),
  plot.type = "heatmap",
  export.table = NULL,
  palette = "inferno",
  exportTable = NULL,
  ...
)

vizGenes(
  input.data,
  x.axis = "TRBV",
  y.axis = NULL,
  group.by = NULL,
  plot = "heatmap",
  order.by = NULL,
  summary.fun = c("percent", "proportion", "count"),
  export.table = NULL,
  palette = "inferno",
  exportTable = NULL
)

percentGenes(
  input.data,
  chain = "TRB",
  gene = "Vgene",
  group.by = NULL,
  order.by = NULL,
  export.table = NULL,
  summary.fun = c("percent", "proportion", "count"),
  palette = "inferno",
  exportTable = NULL
)

percentVJ(
  input.data,
  chain = "TRB",
  group.by = NULL,
  order.by = NULL,
  summary.fun = c("percent", "proportion", "count"),
  export.table = NULL,
  palette = "inferno",
```

```
  exportTable = NULL
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression().
chain	The TCR/BCR chain to use. Accepted values: TRA, TRB, TRG, TRD, IGH, IGL (for both light chains)
genes	A character vector specifying the gene loci to analyze. Can be a single gene e.g., "TRBV" or "IGHJ" or a pair for genes analysis (e.g., c("TRBV", "TRAV"), or "TRBV", "TRBJ").
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed as by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
summary.fun	Character string choosing the summary statistic - "percent" (default), "proportion", or "count".
plot.type	The type of plot to return: "heatmap" (default for paired loci, also available for single loci), or "barplot" (for single loci).
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any
exportTable	**[Deprecated]** Use export.table instead. hcl.pals.
...	Additional arguments passed to the ggplot theme
x.axis	Gene segments to separate the x-axis, such as TRAV, TRBD, IGKJ.
y.axis	Variable to separate the y-axis, can be both categorical or other gene gene segments, such as TRAV, TRBD, IGKJ.
plot	The type of plot to return - heatmap or barplot.
gene	Vgene, Dgene or Jgene

Value

A ggplot object displaying a heatmap or bar plot of gene usage. If exportTable = TRUE, a matrix or data frame of the raw data is returned.

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Visualize single gene (TRBV) usage as a heatmap, grouped by sample
percentGeneUsage(combined,
                 genes = "TRBV",
                 group.by = "sample",
```

```
                plot.type = "heatmap",
                summary.fun = "percent")

# Visualize single gene (TRBV) usage as a barplot, grouped by sample
percentGeneUsage(combined,
                genes = "TRBV",
                group.by = "sample",
                plot.type = "barplot",
                summary.fun = "count")

# Visualize paired gene (TRBV-TRBJ) usage as a heatmap
percentGeneUsage(combined[1:2],
                genes = c("TRBV", "TRBJ"),
                group.by = "sample",
                plot.type = "heatmap",
                summary.fun = "proportion")

# Export the raw data table for single gene usage
trbv_usage_table <- percentGeneUsage(combined,
                                     genes = "TRBV",
                                     group.by = "sample",
                                     export.table = TRUE,
                                     summary.fun = "count")

# Export the raw data table for paired gene usage
trbv_trbj_usage_table <- percentGeneUsage(combined,
                                          genes = c("TRBV", "TRBJ"),
                                          group.by = "sample",
                                          export.table = TRUE,
                                          summary.fun = "percent")


# Visualize paired gene (TRAV-TRAJ) usage as a heatmap
vizGenes(combined[1:2],
         x.axis = "TRAV",
         y.axis = "TRAJ",
         group.by = "sample",
         summary.fun = "count")

# Visualize cross-chain gene pairing (TRBV-TRAV)
vizGenes(combined[1:2],
         x.axis = "TRBV",
         y.axis = "TRAV",
         group.by = "sample",
         summary.fun = "percent")


# Quantify and visualize TRA V-gene usage as a heatmap
percentGenes(combined,
             chain = "TRA",
             gene = "Vgene",
             group.by = "sample",
             summary.fun = "percent")
```

```
# Quantify TRA J-gene usage and export the table
traj_usage_table <- percentGenes(combined,
                                 chain = "TRA",
                                 gene = "Jgene",
                                 group.by = "sample",
                                 export.table = TRUE,
                                 summary.fun = "count")

# Quantify and visualize TRB V-J gene pairings as a heatmap
percentVJ(combined[1:2],
          chain = "TRB",
          group.by = "sample",
          summary.fun = "percent")

# 2. Quantify TRA V-J gene pairings and export the table
trav_traj_table <- percentVJ(combined,
                             chain = "TRA",
                             group.by = "sample",
                             export.table = TRUE,
                             summary.fun = "proportion")
```

`percentKmer` *Analyze K-mer Motif Composition*

Description

This function calculates and visualizes the frequency of k-mer motifs for either nucleotide (nt) or amino acid (aa) sequences. It produces a heatmap showing the relative composition of the most variable motifs across samples or groups.

Usage

```
percentKmer(
  input.data,
  chain = "TRB",
  clone.call = NULL,
  group.by = NULL,
  order.by = NULL,
  motif.length = 3,
  min.depth = 3,
  top.motifs = 30,
  export.table = NULL,
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression()
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
clone.call	Defines the clonal sequence grouping. Accepted values are: nt (CDR3 nucleotide sequence) or aa (CDR3 amino acid sequence).
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed as by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
motif.length	The length of the kmer to analyze
min.depth	Minimum count a motif must reach to be retained in the output (>= 1). **Default:** 3.
top.motifs	Return the n most variable motifs as a function of median absolute deviation
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Details

The function first calculates k-mer frequencies for each sample/group. By default, it then identifies the 30 most variable motifs based on the Median Absolute Deviation (MAD) across all samples and displays their frequencies in a heatmap.

Value

A ggplot object displaying a heatmap of motif percentages. If exportTable = TRUE, a matrix of the raw data is returned.

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Using percentKmer()
percentKmer(combined,
            chain = "TRB",
            motif.length = 3)
```

positionalEntropy *Examining the Diversity of Amino Acids by Position*

Description

This function the diversity amino acids along the residues of the CDR3 amino acid sequence. Please see clonalDiversity() for more information on the underlying methods for diversity/entropy calculations. Positions without variance will have a value reported as 0 for the purposes of comparison.

Usage

```
positionalEntropy(
  input.data,
  chain = "TRB",
  group.by = NULL,
  order.by = NULL,
  aa.length = 20,
  method = "norm.entropy",
  export.table = NULL,
  palette = "inferno",
  exportTable = NULL,
  ...
)
```

Arguments

input.data	The product of combineTCR(), combineBCR(), or combineExpression()
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed as by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
aa.length	The maximum length of the CDR3 amino acid sequence.
method	The method to calculate the entropy/diversity - "shannon", "inv.simpson", "gini.simpson", "norm.entropy", "pielou", "hill0", "hill1", "hill2"
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Value

A ggplot object displaying entropy or diversity by amino acid position. If `exportTable = TRUE`, a matrix of the raw data is returned.

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Using positionalEntropy()
positionalEntropy(combined,
                  chain = "TRB",
                  aa.length = 20)
```

`positionalProperty` *Plot Positional Physicochemical Property Analysis*

Description

This function analyzes the physicochemical properties of amino acids at each position along the CDR3 sequence. It calculates the mean property value and the 95% confidence interval for each position across one or more groups, visualizing the results as a line plot with a confidence ribbon.

Usage

```
positionalProperty(
  input.data,
  chain = "TRB",
  group.by = NULL,
  order.by = NULL,
  aa.length = 20,
  method = "atchleyFactors",
  export.table = NULL,
  palette = "inferno",
  exportTable = NULL,
  ...
)
```

Arguments

`input.data`	The product of `combineTCR()`, `combineBCR()`, or `combineExpression()`
`chain`	The TCR/BCR chain to use. Use `both` to include both chains (e.g., TRA/TRB). Accepted values: `TRA`, `TRB`, `TRG`, `TRD`, `IGH`, `IGL`, `IGK`, `Light` (for both light chains), or `both` (for TRA/B and Heavy/Light).

group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed by list element or active identity in the case of single-cell objects.
order.by	A character vector defining the desired order of elements of the group.by variable. Alternatively, use alphanumeric to sort groups automatically.
aa.length	The maximum length of the CDR3 amino acid sequence.
method	Character string (one of the supported names) Defaults to "atchleyFactors", but includes: "crucianiProperties", "FASGAI", "kideraFactors", "MSWHIM", "ProtFP", "stScales", "tScales", "VHSE", "zScales"
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Details

The function uses one of several established physicochemical property scales to convert amino acid sequences into numerical vectors. More information for the individual methods can be found at the following citations:

atchleyFactors: citation

crucianiProperties: citation

FASGAI: citation

kideraFactors: citation

MSWHIM: citation

ProtFP: citation

stScales: citation

tScales: citation

VHSE: citation

zScales: citation

Value

A ggplot object displaying property by amino acid position. If exportTable = TRUE, a matrix of the raw data is returned.

Author(s)

Florian Bach, Nick Borcherding

Examples

```
# Making combined contig data
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Using positionalProperty()
positionalProperty(combined,
                   chain = "TRB",
                   method = "atchleyFactors",
                   aa.length = 20)
```

`quietVDJgenes` *Remove TCR and BCR genes from variable gene results*

Description

Most single-cell workflows use highly-expressed and highly-variable genes for the initial calculation of PCA and subsequent dimensional reduction. This function will remove the TCR and/or BCR genes from the variable features in a Seurat object or from a vector (potentially generated by the Bioconductor scran workflow).

Usage

```
quietVDJgenes(input.data, ...)

quietTCRgenes(input.data, ...)

## Default S3 method:
quietTCRgenes(input.data, ...)

## S3 method for class 'Seurat'
quietTCRgenes(input.data, assay = NULL, ...)

quietBCRgenes(input.data, ...)

## Default S3 method:
quietBCRgenes(input.data, ...)

## S3 method for class 'Seurat'
quietBCRgenes(input.data, assay = NULL, ...)
```

Arguments

`input.data`	Single-cell object in Seurat format or vector of variable genes to use in reduction
`...`	Reserved for future arguments

assay The Seurat assay slot to use to remove immune receptor genes from, NULL value will default to the default assay

Value

Seurat object or vector list with TCR genes removed.

Author(s)

Nicky de Vrij, Nikolaj Pagh, Nick Borcherding, Qile Yang

Examples

```
example <- quietVDJgenes(scRep_example)
scRep <- quietTCRgenes(scRep_example)
ibex_example <- quietBCRgenes(scRep_example)
```

---

scRep_example *A Seurat Object of 500 Single T cells,*

Description

The object is compatible with `contig_list` and the TCR sequencing data can be added with `combineExpression`. The data is from 4 patients with acute respiratory distress, with samples taken from both the lung and peripheral blood. More information on the data can be found in the following manuscript.

StartracDiversity *Calculate Startrac-based Diversity Indices*

Description

This function utilizes the STARTRAC approach to calculate T cell diversity metrics based on the work of Zhang et al. (2018, Nature) PMID: 30479382. It can compute three distinct indices: clonal expansion (`expa`), cross-tissue migration (`migr`), and state transition (`tran`).

Usage

```
StartracDiversity(
  sc.data,
  clone.call = NULL,
  chain = "both",
  index = c("expa", "migr", "tran"),
  type = NULL,
  group.by = NULL,
```

```
  pairwise = NULL,
  export.table = NULL,
  palette = "inferno",
  cloneCall = NULL,
  exportTable = NULL,
  ...
)
```

Arguments

sc.data	The single-cell object after combineExpression(). For SCE objects, the cluster variable must be in the meta data under "cluster".
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
chain	The TCR/BCR chain to use. Use both to include both chains (e.g., TRA/TRB). Accepted values: TRA, TRB, TRG, TRD, IGH, IGL, IGK, Light (for both light chains), or both (for TRA/B and Heavy/Light).
index	A character vector specifying which indices to calculate. Options: "expa", "migr", "tran". Default is all three.
type	The metadata variable that specifies tissue type for migration analysis.
group.by	A column header in the metadata or lists to group the analysis by (e.g., "sample", "treatment"). If NULL, data will be analyzed as by list element or active identity in the case of single-cell objects.
pairwise	The metadata column to be used for pairwise comparisons. Set to the type variable for pairwise migration or "cluster" for pairwise transition.
export.table	If TRUE, returns a data frame or matrix of the results instead of a plot.
palette	Colors to use in visualization - input any hcl.pals.
cloneCall	**[Deprecated]** Use clone.call instead.
exportTable	**[Deprecated]** Use export.table instead.
...	Additional arguments passed to the ggplot theme

Details

The function requires a type variable in the metadata, which specifies the tissue origin or any other categorical variable for migration analysis.

Indices:

- **expa (Clonal Expansion):** Measures the extent of clonal proliferation within a T cell cluster. It is calculated as 1 - normalized Shannon entropy. A higher value indicates greater expansion of a few clones.
- **migr (Cross-Tissue Migration):** Quantifies the movement of clonal T cells across different tissues (as defined by the type parameter). It is based on the entropy of a clonotype's distribution across tissues.

- **tran (State Transition):** Measures the developmental transition of clonal T cells between different functional clusters. It is based on the entropy of a clonotype's distribution across clusters.

Pairwise Analysis: The `pairwise` parameter enables the calculation of migration or transition between specific pairs of tissues or clusters, respectively.

- For migration (`index = "migr"`), set `pairwise` to the `type` column (e.g., `pairwise = "Type"`).
- For transition (`index = "tran"`), set `pairwise` to `"cluster"`.

Value

A ggplot object visualizing STARTRAC diversity metrics or data.frame if `exportTable = TRUE`.

Author(s)

Liangtao Zheng

Examples

```
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Getting a sample of a Seurat object
scRep_example  <- get(data("scRep_example"))
scRep_example  <- combineExpression(combined, scRep_example)
scRep_example$Patient <- substring(scRep_example$orig.ident,1,3)
scRep_example$Type <- substring(scRep_example$orig.ident,4,4)

# Calculate a single index (expansion)
StartracDiversity(scRep_example,
                  type = "Type",
                  group.by = "Patient",
                  index = "expa")

# Calculate pairwise transition
StartracDiversity(scRep_example,
                  type = "Type",
                  group.by = "Patient",
                  index = "tran",
                  pairwise = "cluster")
```

subsetClones *Subset The Product of combineTCR() or combineBCR()*

Description

This function allows for the subsetting of the product of `combineTCR()` or `combineBCR()` by the name of the individual list element.

Usage

```
subsetClones(input.data, name, variables = NULL)
```

Arguments

input.data	The product of `combineTCR()` or `combineBCR()`.
name	The column header/name to use for subsetting.
variables	The values to subset by, must be in the in the variable.

Value

list of contigs that have been filtered for the name parameter

Examples

```
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))
subset <- subsetClones(combined, name = "sample", variables = c("P17B"))
```

vizCirclize *Visualize Clonal Relationships as a Chord Diagram*

Description

This function creates a chord diagram visualization of shared clones between groups using the circlize package. It provides a convenient wrapper around `getCirclize()` that handles the circlize plotting code automatically.

Usage

```
vizCirclize(
  sc.data,
  clone.call = NULL,
  group.by = NULL,
  method = c("unique", "jaccard", "overlap"),
  proportion = FALSE,
  directional = FALSE,
  self.link = 1,
  include.self = TRUE,
  transparency = 0.5,
  link.visible = TRUE,
  annotate.sectors = TRUE,
  min.shared = 0,
  palette = "inferno",
  sector.colors = NULL,
  export.table = FALSE
)
```

Arguments

sc.data	The single-cell object after combineExpression().
clone.call	Defines the clonal sequence grouping. Accepted values are: gene (VDJC genes), nt (CDR3 nucleotide sequence), aa (CDR3 amino acid sequence), or strict (VDJC + nt). A custom column header can also be used.
group.by	A column header (or vector of column headers for hierarchical grouping) in the metadata to group the analysis by.
method	The method for calculating link values: "unique" (default) counts unique shared clones, "jaccard" calculates Jaccard similarity, "overlap" calculates overlap coefficient.
proportion	Calculate the relationship by unique clones (FALSE, default) or normalized by proportion (TRUE).
directional	If TRUE, show directional arrows on chords. Default is FALSE.
self.link	How to handle self-links. 1 = show as loops, 2 = show as parallel lines. Default is 1.
include.self	Include self-links (clones within a single group). Default is TRUE.
transparency	Transparency of the chord links (0-1). Default is 0.5.
link.visible	If FALSE, hide the chord links and show only sectors.
annotate.sectors	If TRUE (default), display sector names.
min.shared	Minimum number of shared clones to include a link (default 0).
palette	Colors to use for sectors - input any hcl.pals.
sector.colors	Named vector of colors for specific sectors. Overrides palette.
export.table	If TRUE, returns the data instead of plotting.

Details

This function requires the circlize package to be installed. If circlize is not available, the function will return the data that would be used for plotting and provide instructions for manual plotting.

The chord diagram shows relationships between groups (sectors) where the width of each chord represents the number or proportion of shared clones between the connected groups.

Value

Invisibly returns the circlize data list. If circlize is not installed or `export.table = TRUE`, returns the data that would be used for plotting.

Author(s)

Nick Borcherding

See Also

`getCirclize()` for generating the underlying data

Examples

```
## Not run:
# Getting the combined contigs
combined <- combineTCR(contig_list,
                       samples = c("P17B", "P17L", "P18B", "P18L",
                                   "P19B","P19L", "P20B", "P20L"))

# Getting a sample of a Seurat object
scRep_example <- get(data("scRep_example"))
scRep_example <- combineExpression(combined,
                                   scRep_example)

# Simple chord diagram
vizCirclize(scRep_example, group.by = "seurat_clusters")

# Directional chord diagram with arrows
vizCirclize(scRep_example,
            group.by = "seurat_clusters",
            directional = TRUE)

# Multi-level grouping
scRep_example$Patient <- substring(scRep_example$orig.ident, 1, 3)
vizCirclize(scRep_example,
            group.by = c("Patient", "seurat_clusters"))

## End(Not run)
```

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