

Package ‘scRepertoire’

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Title A toolkit for single-cell immune receptor profiling

Version 1.7.0

Description

scRepertoire was built to process data derived from the 10x Genomics Chromium Immune Profiling for both T-cell receptor (TCR) and immunoglobulin (Ig) enrichment workflows and subsequently interacts with the popular Seurat and SingleCellExperiment R packages. It also allows for general analysis of single-cell clonotype information without the use of expression information. The package functions as a wrapper for Startrac and powerTCR R packages.

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R topics documented:

| | |
|---------------------------------|-----------|
| abundanceContig | 2 |
| addVariable | 3 |
| alluvialClonotypes | 4 |
| clonalDiversity | 5 |
| clonalHomeostasis | 6 |
| clonalOverlap | 7 |
| clonalOverlay | 8 |
| clonalProportion | 9 |
| clonesizeDistribution | 10 |
| clusterTCR | 11 |
| combineBCR | 12 |
| combineExpression | 13 |
| combineTCR | 14 |
| compareClonotypes | 15 |
| contig_list | 16 |
| expression2List | 16 |
| getCirclize | 17 |
| highlightClonotypes | 18 |
| lengthContig | 19 |
| occupiedscRepertoire | 20 |
| quantContig | 21 |
| screp_example | 22 |
| Startrac | 22 |
| StartracDiversity | 23 |
| stripBarcode | 24 |
| subsetContig | 24 |
| Index | 26 |

| | |
|-----------------|---|
| abundanceContig | <i>Demonstrate the relative abundance of clonotypes by group or sample.</i> |
|-----------------|---|

Description

This function takes the output of `combineTCR()`, `combineBCR()`, or `expression2List()` and displays the number of clonotypes at specific frequencies by sample or group. Visualization can either be a line graph using calculated numbers or if `scale = TRUE`, the output will be a density plot. Multiple sequencing runs can be group together using the `group` parameter. If a matrix output for the data is preferred, set `exportTable = TRUE`.

Usage

```
abundanceContig(
  df,
  cloneCall = "gene+nt",
  scale = FALSE,
  group = NULL,
  exportTable = FALSE
)
```

Arguments

| | |
|-------------|---|
| df | The product of combineTCR(), combineBCR(), or expression2List(). |
| cloneCall | How to call the clonotype - CDR3 gene (gene), CDR3 nucleotide (nt), CDR3 amino acid (aa), or C DR3 gene+nucleotide (gene+nt). |
| scale | Converts the graphs into denisty plots in order to show relative distributions. |
| group | The column header for which you would like to analyze the data. |
| exportTable | Returns the data frame used for forming the graph to the visualization. |

Value

ggplot of the total or relative adundance of clonotypes across quanta

Examples

```
#Making combined contig data
x <- contig_list
combined <- combineTCR(x, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells ="T-AB")
abundanceContig(combined, cloneCall = "gene", scale = FALSE)
```

addVariable

Adding variables after the combination of contigs.

Description

This function adds variables to the product of combineTCR() combineBCR() or expression2List() to be used in later visualizations. For each element, the function will add a column (labeled by name) with the variable. The length of the variable paramater needs to match the length of the combined object.

Usage

```
addVariable(df, name = NULL, variables = NULL)
```

Arguments

df The product of combineTCR() combineBCR() or expression2List().
 name The column header to add.
 variables The exact values to add to each element of the list.

Value

list of contigs with a new column (name).

Examples

```
x <- contig_list
combined <- combineTCR(x, rep(c("PX", "PY", "PZ"), each=2),
  rep(c("P", "T"), 3), cells = "T-AB")
combined <- addVariable(combined, name = "batch", variables = c(1,1,1,1,2,2))
```

alluvialClonotypes *Exploring interaction of clonotypes by seurat or SCE dynamics*

Description

View the proportional contribution of clonotypes 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

```
alluvialClonotypes(
  sc,
  cloneCall = c("gene", "nt", "aa", "gene+nt"),
  y.axes = NULL,
  color = NULL,
  alpha = NULL,
  facet = NULL
)
```

Arguments

sc The seurat or SCE object to visualize after combineExpression(). For SCE objects, the cluster variable must be in the meta data under "cluster".
 cloneCall How to call the clonotype - CDR3 gene (gene), CDR3 nucleotide (nt) or CDR3 amino acid (aa), or CDR3 gene+nucleotide (gene+nt).
 y.axes The columns that will separate the proportional visualizations.
 color The column header or clonotype(s) to be highlighted.
 alpha The column header to have gradated opacity.
 facet The column label to separate.

Value

Alluvial ggplot comparing clonotype distribution across selected parameters.

Examples

```
#Getting the combined contigs
combined <- combineTCR(contig_list, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells="T-AB")

#Getting a sample of a Seurat object
screp_example <- get(data("screp_example"))
sce <- suppressMessages(Seurat::UpdateSeuratObject(screp_example))
sce <- Seurat::as.SingleCellExperiment(sce)

#Using combineExpression()
sce <- combineExpression(combined, sce)

#Using alluvialClonotypes()
alluvialClonotypes(sce, cloneCall = "gene",
y.axes = c("Patient", "cluster"), color = "cluster")
```

clonalDiversity

Examine the clonal diversity of samples

Description

This function calculates traditional measures of diversity - Shannon, inverse Simpson, Chao1 index, and abundance-based coverage estimators (ACE) by sample or group. The function automatically down samples the diversity metrics using 100 boot straps The group paramter can be used to condense the individual samples. If a matrix output for the data is preferred, set exportTable = TRUE.

Usage

```
clonalDiversity(
  df,
  cloneCall = "gene+nt",
  group = "samples",
  exportTable = FALSE,
  n.boots = 100
)
```

Arguments

| | |
|-----------|--|
| df | The product of combineTCR(), combineBCR(), or expression2List(). |
| cloneCall | How to call the clonotype - CDR3 gene (gene), CDR3 nucleotide (nt) or CDR3 amino acid (aa), or CDR3 gene+nucleotide (gene+nt). |
| group | The column header for which you would like to analyze the data. |

| | |
|-------------|--|
| exportTable | Exports a table of the data into the global environment in addition to the visualization |
| n.boots | number of bootstraps to downsample in order to get mean diversity |

Value

ggplot of the diversity of clonotype sequences across list

Author(s)

Andrew Malone, Nick Borcharding

Examples

```
#Making combined contig data
x <- contig_list
combined <- combineTCR(x, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells="T-AB")
clonalDiversity(combined, cloneCall = "gene")
```

clonalHomeostasis *Examining the clonal homeostasis*

Description

This function calculates the space occupied by clonotype proportions. The grouping of these clonotypes is based on the parameter cloneTypes, at default, cloneTypes will group the clonotypes 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 cloneTypes paramter. If a matrix output for the data is preferred, set exportTable = TRUE.

Usage

```
clonalHomeostasis(
  df,
  cloneTypes = c(Rare = 1e-04, Small = 0.001, Medium = 0.01, Large = 0.1, Hyperexpanded
    = 1),
  cloneCall = "gene+nt",
  exportTable = FALSE
)
```

Arguments

| | |
|------------|--|
| df | The product of CombineContig() or expression2List() |
| cloneTypes | The cutpoints of the proportions. |
| cloneCall | How to call the clonotype - CDR3 gene (gene), CDR3 nucleotide (nt) or CDR3 amino acid (aa), or CDR3 gene+nucleotide (gene+nt). |

`exportTable` Exports a table of the data into the global environment in addition to the visualization

Value

ggplot of the space occupied by the specific proportion of clonotypes

Examples

```
#Making combined contig data
x <- contig_list
combined <- combineTCR(x, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells = "T-AB")
clonalHomeostasis(combined, cloneCall = "gene")
```

clonalOverlap *Examining the clonal overlap between groups or samples*

Description

This functions allows for the calculation and visualizations of the overlap coefficient or morisita index for clonotypes using the product of `combineTCR()`, `combineBCR()` or `expression2list()`. The overlap coefficient is calculated using the intersection of clonotypes divided by the length of the smallest component. Morisita index is estimating the dispersion of a population, more information can be found [here](<https://en.wikipedia.org/wiki/Morisita>) If a matrix output for the data is preferred, set `exportTable = TRUE`.

Usage

```
clonalOverlap(
  df,
  cloneCall = c("gene", "nt", "aa", "gene+nt"),
  method = c("overlap", "morisita"),
  exportTable = FALSE
)
```

Arguments

`df` The product of `combineTCR()`, `combineBCR()`, or `expression2List()`.

`cloneCall` How to call the clonotype - CDR3 gene (gene), CDR3 nucleotide (nt) or CDR3 amino acid (aa), or CDR3 gene+nucleotide (gene+nt).

`method` The method to calculate the overlap, either the overlap coefficient or morisita index.

`exportTable` Exports a table of the data into the global environment in addition to the visualization

Value

ggplot of the clonotypic overlap between elements of a list

Examples

```
#Making combined contig data
x <- contig_list
combined <- combineTCR(x, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells ="T-AB")

clonalOverlap(combined, cloneCall = "gene", method = "overlap")
```

| | |
|---------------|---|
| clonalOverlay | <i>Visualize distribution of clonal frequency overlaid on dimensional reduction plots</i> |
|---------------|---|

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 Dr. Carmona and his work with [ProjectTIL](<https://github.com/carmonalab/ProjecTILs>).

Usage

```
clonalOverlay(
  sc,
  reduction = NULL,
  freq.cutpoint = 30,
  bins = 25,
  facet = NULL
)
```

Arguments

| | |
|---------------|---|
| sc | The seurat or SCE object to visualize after combineExpression(). |
| reduction | The dimensional reduction to visualize |
| freq.cutpoint | The overlay cutpoint to include, this corresponds to the Frequency variable in the single-cell objecter |
| bins | The number of contours to the overlay |
| facet | meta data variable to facet the comparison |

Value

ggplot object

Author(s)

Francesco Mazziotto, Nick Borcharding

Examples

```
#Getting the combined contigs
combined <- combineTCR(contig_list, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells="T-AB")

#Getting a sample of a Seurat object
screp_example <- get(data("screp_example"))
sce <- suppressMessages(Seurat::UpdateSeuratObject(screp_example))

#Using combineExpression()
sce <- combineExpression(combined, sce)

#Using clonalOverlay()
clonalOverlay(sce, freq.cutpoint = 0.3, bins = 5)
```

clonalProportion

Examining the clonal space occupied by specific clonotypes

Description

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

Usage

```
clonalProportion(
  df,
  split = c(10, 100, 1000, 10000, 30000, 1e+05),
  cloneCall = "gene+nt",
  exportTable = FALSE
)
```

Arguments

| | |
|--------------------------|--|
| <code>df</code> | The product of <code>combineTCR()</code> , <code>combineBCR()</code> , or <code>expression2List()</code> . |
| <code>split</code> | The cutpoints for the specific clonotypes. |
| <code>cloneCall</code> | How to call the clonotype - CDR3 gene (gene), CDR3 nucleotide (nt) or CDR3 amino acid (aa), or CDR3 gene+nucleotide (gene+nt). |
| <code>exportTable</code> | Exports a table of the data into the global environment in addition to the visualization |

Value

ggplot of the space occupied by the specific rank of clonotypes

Examples

```
#Making combined contig data
x <- contig_list
combined <- combineTCR(x, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells = "T-AB")
clonalProportion(combined, cloneCall = "gene")
```

clonesizeDistribution *Hierarchical clustering of clonotypes on clonotype size and Jensen-Shannon divergence*

Description

This function produces a hierarchical clustering of clonotypes by sample using the Jensen-Shannon distance and discrete gamma-GPD spliced threshold model in the [powerTCR R package] (<https://bioconductor.org/packages/>). Please read and cite PMID: 30485278 if using the function for analyses. If a matrix output for the data is preferred set `exportTable = TRUE`.

Usage

```
clonesizeDistribution(
  df,
  cloneCall = "gene+nt",
  method = "ward.D2",
  exportTable = FALSE
)
```

Arguments

| | |
|--------------------------|--|
| <code>df</code> | The product of <code>combineTCR()</code> , <code>combineBCR()</code> , or <code>expression2List()</code> . |
| <code>cloneCall</code> | How to call the clonotype - CDR3 gene (gene), CDR3 nucleotide (nt), CDR3 amino acid (aa), or CDR3 gene+nucleotide (gene+nt). |
| <code>method</code> | The clustering parameter for the dendrogram. |
| <code>exportTable</code> | Returns the data frame used for forming the graph. |

Value

ggplot dendrogram of the clone size distribution

Examples

```
#Making combined contig data
x <- contig_list
combined <- combineTCR(x, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells ="T-AB")
clonesizeDistribution(combined, cloneCall = "gene+nt", method="ward.D2")
```

clusterTCR

*Clustering T cell receptors***Description**

This function uses edit distances of either the nucleotide or amino acid sequences of the CDR3 to cluster similar TCRs together. The distance clustering will then be amended to the end of the list of combined contigs with the corresponding V gene. The cluster will appear as CHAIN.num if a unique sequence or CHAIN:LD.num if clustered together. This function will only two chains recovered, multiple chains will automatically be reduced. This function also underlies the combineBCR() function and therefore not needed for B cells. This may take some time to calculate the distances and cluster.

Usage

```
clusterTCR(df, chain = NULL, sequence = NULL, threshold = 0.85, group = NULL)
```

Arguments

| | |
|-----------|--|
| df | The product of CombineTCR() or CombineBCR(). |
| chain | The TCR to cluster |
| sequence | Clustering based on either "aa" or "nt" |
| threshold | The normalized edit distance to consider. The higher the number the more similarity of sequence will be used for clustering. |
| group | The column header used for to calculate the cluster by. |

Value

List of clonotypes for individual cell barcodes

Examples

```
combined <- combineTCR(contig_list, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells ="T-AB")

sub_combined <- clusterTCR(combined[[2]], chain = "TCRA", sequence = "aa")
```

`combineBCR`*Combining the list of B Cell Receptor contigs*

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,

Usage

```
combineBCR(  
  df,  
  samples = NULL,  
  ID = NULL,  
  removeNA = FALSE,  
  removeMulti = FALSE  
)
```

Arguments

| | |
|--------------------------|--|
| <code>df</code> | List of filtered contig annotations from 10x Genomics. |
| <code>samples</code> | The labels of samples. |
| <code>ID</code> | The additional sample labeling option. |
| <code>removeNA</code> | This will remove any chain without values. |
| <code>removeMulti</code> | This will remove barcodes with greater than 2 chains. |

Value

List of clonotypes for individual cell barcodes

See Also

[combineExpression](#). Unlike `combineTCR()`, `combineBCR` produces a column `CTstrict` of an index of nucleotide sequence and the corresponding `v-gene`. This index automatically calculates the Hamming distance between sequences of the same length and will index sequences with ≤ 0.15 normalized Levenshtein distance with the same ID for sequences with < 15 nucleotide difference in length. After which, clonotype clusters are called using the `igraph` `component()` function. Clonotype clusters will then be labeled with "LD" with the `CTstrict` header.

Examples

```
#Data derived from the 10x Genomics intratumoral NSCLC B cells  
BCR <- read.csv("https://ncborcherding.github.io/vignettes/b_contigs.csv",  
stringsAsFactors = FALSE)  
combined <- combineBCR(BCR, samples = "Patient1", ID = "Time1")
```

combineExpression *Adding clonotype information to a seurat or SCE 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 of the clonotypes by sequencing run (groupBy = "none"). To change how the frequencies are calculated, select a column header for the groupBy variable. Importantly, before using combineExpression() ensure the barcodes of the seurat or SCE object match the barcodes in the output of the combinedContig() call. Check changeNames() to change the prefix of the seurat object. If the dominant clonotypes have a greater frequency than 500, adjust the cloneTypes variable.

Usage

```
combineExpression(
  df,
  sc,
  cloneCall = "gene+nt",
  groupBy = "none",
  proportion = TRUE,
  cloneTypes = c(Rare = 1e-04, Small = 0.001, Medium = 0.01, Large = 0.1, Hyperexpanded
    = 1),
  filterNA = FALSE
)
```

Arguments

| | |
|------------|---|
| df | The product of CombineTCR() or CombineBCR(). |
| sc | The seurat or SingleCellExperiment (SCE) object to attach |
| cloneCall | How to call the clonotype - CDR3 gene (gene), CDR3 nucleotide (nt) CDR3 amino acid (aa), or CDR3 gene+nucleotide (gene+nt). |
| groupBy | The column label in the combined contig object in which clonotype frequency will be calculated. |
| proportion | Whether to use the total frequency (FALSE) or the proportion (TRUE) of the clonotype based on the groupBy variable. |
| cloneTypes | The bins for the grouping based on frequency |
| filterNA | Method to subset seurat object of barcodes without clonotype information |

Value

seurat or SingleCellExperiment object with attached clonotype information

Examples

```
#Getting the combined contigs
combined <- combineTCR(contig_list, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells ="T-AB")

#Getting a sample of a Seurat object
screp_example <- get(data("screp_example"))
sce <- suppressMessages(Seurat::UpdateSeuratObject(screp_example))
sce <- Seurat::as.SingleCellExperiment(sce)

#Using combineExpression()
sce <- combineExpression(combined, sce)
```

combineTCR

Combining the list of T Cell Receptor contigs

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, @seealso [combineExpression](#). Several levels of filtering exist - remove or filterMulti are parameters that control how the function deals with barcodes with multiple chains recovered.

Usage

```
combineTCR(
  df,
  samples = NULL,
  ID = NULL,
  cells = c("T-AB", "T-GD"),
  removeNA = FALSE,
  removeMulti = FALSE,
  filterMulti = FALSE
)
```

Arguments

| | |
|-------------|--|
| df | List of filtered contig annotations from 10x Genomics. |
| samples | The labels of samples. |
| ID | The additional sample labeling option. |
| cells | The type of T cell - T cell-AB or T cell-GD |
| removeNA | This will remove any chain without values. |
| removeMulti | This will remove barcodes with greater than 2 chains. |
| filterMulti | This option will allow for the selection of the 2 corresponding chains with the highest expression for a single barcode. |

Value

List of clonotypes for individual cell barcodes

Examples

```
combineTCR(contig_list, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells = "T-AB")
```

| | |
|-------------------|---|
| compareClonotypes | <i>Demonstrate the difference in clonal proportion between clonotypes</i> |
|-------------------|---|

Description

This function produces an alluvial or area graph of the proportion of the indicated clonotypes for all or selected samples. Clonotypes can be selected using the clonotypes parameter with the specific sequence of interest or using the number parameter with the top n clonotypes by proportion to be visualized. If multiple clonotypes have the same proportion and are within the selection by the number parameter, all the clonotypes will be visualized. In this instance, if less clonotypes are desired, reduce the number parameter.

Usage

```
compareClonotypes(
  df,
  cloneCall = "gene+nt",
  samples = NULL,
  clonotypes = NULL,
  numbers = NULL,
  graph = "alluvial",
  exportTable = FALSE
)
```

Arguments

| | |
|-------------|--|
| df | The product of combineTCR(), combineBCR(), or expression2List() |
| cloneCall | How to call the clonotype - CDR3 gene (gene), CDR3 nucleotide (nt), CDR3 amino acid (aa), or CDR3 gene+nucleotide (gene+nt). |
| samples | The specific samples to isolate for visualization. |
| clonotypes | The specific sequences of interest. |
| numbers | The top number clonotype sequences. |
| graph | The type of graph produced, either "alluvial" or "area". |
| exportTable | Returns the data frame used for forming the graph. |

Value

ggplot of the proportion of total sequencing read of selecting clonotypes

Examples

```
#Making combined contig data
x <- contig_list
combined <- combineTCR(x, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells ="T-AB")
compareClonotypes(combined, numbers = 10,
samples = c("PX_P", "PX_T"), cloneCall="aa")
```

| | |
|-------------|---|
| contig_list | <i>A data set of T cell contigs as a list outputed from the filter_contig_annotation files.</i> |
|-------------|---|

Description

A data set of T cell contigs as a list outputed from the filter_contig_annotation files.

| | |
|-----------------|--|
| expression2List | <i>Allows users to take the meta data in seurat/SCE and place it into a list that will work with all the functions</i> |
|-----------------|--|

Description

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.

Usage

```
expression2List(sc, group)
```

Arguments

| | |
|-------|--|
| sc | object after combineExpression(). |
| group | The column header to group the new list by |

Value

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

Examples

```
#Getting the combined contigs
combined <- combineTCR(contig_list, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells = "T-AB")

#Getting a sample of a Seurat object
screp_example <- get(data("screp_example"))
sce <- suppressMessages(Seurat::UpdateSeuratObject(screp_example))
sce <- Seurat::as.SingleCellExperiment(sce)

#Using expression2List
newList <- expression2List(sce, group = "seurat_clusters")
```

| | |
|-------------|---|
| getCirclize | <i>Generate data frame to be used with circlize R package to visualize clonotypes as a chord diagram.</i> |
|-------------|---|

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. The output is a measure of relative clonotype overlap between groups and does not reflect exact clonotype matches between groups.

Usage

```
getCirclize(sc, cloneCall = "gene+nt", groupBy = NULL, proportion = FALSE)
```

Arguments

| | |
|------------|---|
| sc | object after <code>combineExpression()</code> . |
| cloneCall | How to call the clonotype - CDR3 nucleotide (nt), CDR3 amino acid (aa). |
| groupBy | The group header for which you would like to analyze the data. |
| proportion | Binary will calculate relationship as unique clonotypes (<code>proportion = TRUE</code>) or proportion of unique clonotypes (<code>proportion = FALSE</code>) |

Value

data frame of shared clonotypes between groups

Author(s)

Dillon Corvino, Nick Borcharding

Examples

```
#Getting the combined contigs
combined <- combineTCR(contig_list, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells ="T-AB")

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

#Getting data frame output for Circilize
circles <- getCirclize(screp_example, groupBy = "seurat_clusters")
```

highlightClonotypes *Highlighting specific clonotypes in Seurat*

Description

Use a specific clonotype sequence to highlight on top of the dimensional reduction in seurat object.

Usage

```
highlightClonotypes(
  sc,
  cloneCall = c("gene", "nt", "aa", "gene+nt"),
  sequence = NULL
)
```

Arguments

| | |
|-----------|--|
| sc | The seurat object to attach |
| cloneCall | How to call the clonotype - CDR3 gene (gene), CDR3 nucleotide (nt), CDR3 amino acid (aa), or CDR3 gene+nucleotide (gene+nt). |
| sequence | The specific sequence or sequence to highlight |

Value

DimPlot with highlighted clonotypes

Examples

```
#' #Getting the combined contigs
combined <- combineTCR(contig_list, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells ="T-AB")

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

```
#Using combineExpression()
screp_example <- combineExpression(combined, screp_example )

#Using highlightClonotype()
screp_example <- highlightClonotypes(screp_example, cloneCall= "aa",
sequence = c("CAVNGGSQGNLIF_CSAEREDTDTQYF"))
```

lengthContig

Demonstrate the distribution of lengths filtered contigs.

Description

This function takes the output of `combineTCR()`, `combineBCR()`, or `expression2List()` and 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 clonotype (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` parameter. If a matrix output for the data is preferred, set `exportTable = TRUE`.

Usage

```
lengthContig(
  df,
  cloneCall = "aa",
  group = NULL,
  scale = FALSE,
  chains = "combined",
  exportTable = FALSE
)
```

Arguments

| | |
|--------------------------|--|
| <code>df</code> | The product of <code>combineTCR()</code> , <code>combineBCR()</code> , or <code>expression2List()</code> |
| <code>cloneCall</code> | How to call the clonotype - CDR3 nucleotide (nt), CDR3 amino acid (aa). |
| <code>group</code> | The group header for which you would like to analyze the data. |
| <code>scale</code> | Converts the graphs into denisty plots in order to show relative distributions. |
| <code>chains</code> | Whether to keep clonotypes "combined" or visualize by chain. |
| <code>exportTable</code> | Returns the data frame used for forming the graph. |

Value

ggplot of the discrete or relative length distributions of clonotype sequences

Examples

```
#Making combined contig data
x <- contig_list
combined <- combineTCR(x, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells ="T-AB")
lengthContig(combined, cloneCall="aa", chains = "combined")
```

occupiedscRepertoire *Visualize the number of single cells with clonotype frequencies by cluster*

Description

View the count of clonotypes frequency group in seurat or SCE object meta data after combineExpression(). The visualization will take the new meta data variable "cloneType" 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](<https://github.com/carmonalab/ProjectTILs>).

Usage

```
occupiedscRepertoire(sc, x.axis = "cluster", exportTable = FALSE)
```

Arguments

| | |
|-------------|--|
| sc | The seurat or SCE object to visualize after combineExpression(). For SCE objects, the cluster variable must be in the meta data under "cluster". |
| x.axis | The variable in the meta data to graph along the x.axis |
| exportTable | Exports a table of the data into the global environment in addition to the visualization |

Value

Stacked bar plot of counts of cells by clonotype frequency group

Examples

```
#Getting the combined contigs
combined <- combineTCR(contig_list, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells ="T-AB")

#Getting a sample of a Seurat object
screp_example <- get(data("screp_example"))
sce <- suppressMessages(Seurat::UpdateSeuratObject(screp_example))
sce <- Seurat::as.SingleCellExperiment(sce)

#Using combineExpression()
sce <- combineExpression(combined, sce)
```

```
#Using occupiedscRepertoire()
occupiedscRepertoire(sce, x.axis = "cluster")
table <- occupiedscRepertoire(sce, x.axis = "cluster", exportTable = TRUE)
```

quantContig

Quantify the unique clonotypes in the filtered contigs.

Description

This function takes the output from `combineTCR()`, `combineBCR()`, or `expression2List()` and quantifies unique clonotypes. The unique clonotypes can be either reported as a raw output or scaled to the total number of clonotypes recovered using the `scale` parameter. Multiple sequencing runs can be group together using the `group` parameter. If a matrix output for the data is preferred, set `exportTable = TRUE`.

Usage

```
quantContig(
  df,
  cloneCall = "gene+nt",
  scale = FALSE,
  group = NULL,
  exportTable = FALSE
)
```

Arguments

| | |
|--------------------------|--|
| <code>df</code> | The product of <code>combineTCR()</code> <code>combineBCR()</code> or <code>expression2List()</code> . |
| <code>cloneCall</code> | How to call the clonotype - CDR3 gene (gene), CDR3 nucleotide (nt), CDR3 amino acid (aa), or CDR3 gene+nucleotide (gene+nt). |
| <code>scale</code> | Converts the graphs into percentage of unique clonotypes. |
| <code>group</code> | The column header used for grouping. |
| <code>exportTable</code> | Returns the data frame used for forming the graph |

Value

ggplot of the total or relative unique clonotypes

Examples

```
#Making combined contig data
x <- contig_list
combined <- combineTCR(x, rep(c("PX", "PY", "PZ"), each=2),
  rep(c("P", "T"), 3), cells ="T-AB")
quantContig(combined, cloneCall="gene+nt", scale = TRUE)
```

| | |
|---------------|---|
| screp_example | <i>A seurat object of 1000 single T cells derived from 3 clear cell renal carcinoma patients.</i> |
|---------------|---|

Description

A seurat object of 1000 single T cells derived from 3 clear cell renal carcinoma patients.

| | |
|----------|---------------------------|
| Startrac | <i>The Startrac Class</i> |
|----------|---------------------------|

Description

The Startrac object store the data for tcr-based T cell dynamics analysis. The slots contained in Startrac object are listed below:

Slots

aid character. aid of the object, used for identification of the object. For example, patient id.
default: "AID"

cell.data data.frame. Each line for a cell, and these columns as required: 'Cell_Name', 'clone.id', 'patient', 'majorCluster', 'loc'

cell.perm.data object. list of 'Startrac' objects constructed from permuted cell data

clonotype.data data.frame. Each line for a clonotype; contain the clonotype level indexes information

cluster.data data.frame. Each line for a cluster; contain the cluster level indexes information

pIndex.migr data.frame. Each line for a cluster; pairwise migration index between the two locations indicated in the column name.

pIndex.tran data.frame. Each line for a cluster; pairwise transition index between the two major clusters indicated by the row name and column name.

cluster.sig.data data.frame. Each line for a cluster; contains the p values of cluster indices.

pIndex.sig.migr data.frame. Each line for a cluster; contains the p values of pairwise migration indices.

pIndex.sig.tran data.frame. Each line for a cluster; contains the p values of pairwise transition indices.

clonotype.dist.loc matrix. Each line for a clonotype and describe the cells distribution among the locations.

clonotype.dist.cluster matrix. Each line for a clonotype and describe the cells distribution among the clusters.

clust.size array. Number of cells of each major cluster.

patient.size array. Number of cells of each patient.

clone.size array. Number of cells of each clone.

clone2patient array. Mapping from patient id to clone id.

Description

This function utilizes the Startrac R package derived from [PMID: 30479382](<https://pubmed.ncbi.nlm.nih.gov/30479382/>) Required to run the function, the "type" variable needs to include the difference in where the cells were derived. The output of this function will produce 3 indices: expa (clonal expansion), migra (cross-tissue migration), and trans (state transition). In order to understand the underlying analyses of the outputs please read and cite the linked manuscript.

Usage

```
StartracDiversity(  
  sc,  
  type = "Type",  
  sample = NULL,  
  by = "overall",  
  exportTable = FALSE  
)
```

Arguments

| | |
|-------------|--|
| sc | The seurat or SCE object to visualize after combineExpression(). For SCE objects, the cluster variable must be in the meta data under "cluster". |
| type | The column header in the meta data that gives the where the cells were derived from, not the patient sample IDs |
| sample | The column header corresponding to individual samples or patients. |
| by | Method to subset the indices by either overall (across all samples) or by specific group |
| exportTable | Returns the data frame used for forming the graph |

Value

ggplot object of Startrac diversity metrics

Examples

```
#Getting the combined contigs  
combined <- combineTCR(contig_list, rep(c("PX", "PY", "PZ"), each=2),  
  rep(c("P", "T"), 3), cells = "T-AB")  
  
#Getting a sample of a Seurat object  
screp_example <- get(data("screp_example"))  
screp_example <- combineExpression(combined, screp_example)  
  
#Using occupiedscRepertoire()
```

```
StartracDiversity(screp_example, type = "Type", sample = "Patient", by = "overall")
```

| | |
|--------------|--|
| stripBarcode | <i>Removing any additional prefixes to the barcodes of filtered contigs.</i> |
|--------------|--|

Description

Removing any additional prefixes to the barcodes of filtered contigs.

Usage

```
stripBarcode(contigs, column = 1, connector = "_", num_connects = 3)
```

Arguments

| | |
|--------------|---|
| contigs | The raw loaded filtered_contig_annotation.csv |
| column | The column in which the barcodes are listed |
| connector | The type of character in which is attaching the default barcode with any other characters |
| num_connects | The number of strings combined with the connectors |

Value

list with the suffixes of the barcodes removed.

Examples

```
stripBarcode(contig_list[[1]], column = 1, connector = "_", num_connects = 1)
```

| | |
|--------------|---|
| subsetContig | <i>Subset the product of combineTCR() combineBCR() or expression2List()</i> |
|--------------|---|

Description

This function allows for the subsetting of the product of combineTCR() combineBCR() or expression2List() by the name of the individual list element. In general the names of are samples + _ + ID, allowing for users to subset the product of combineTCR(), combineBCR(), or expression2List() across a string or individual name.

Usage

```
subsetContig(df, name, variables = NULL)
```


Arguments

| | |
|------------------------|--|
| <code>df</code> | The product of <code>combineTCR()</code> , <code>combineBCR()</code> , or <code>expression2List()</code> . |
| <code>name</code> | The column header you'd like to use to subset. |
| <code>variables</code> | The values to subset by, must be in the <code>names(df)</code> . |

Value

list of contigs that have been filtered for the name parameter

Examples

```
x <- contig_list
combined <- combineTCR(x, rep(c("PX", "PY", "PZ"), each=2),
rep(c("P", "T"), 3), cells = "T-AB")
subset <- subsetContig(combined, name = "sample", variables = c("PX"))
```

Index

abundanceContig, [2](#)
addVariable, [3](#)
alluvialClonotypes, [4](#)

clonalDiversity, [5](#)
clonalHomeostasis, [6](#)
clonalOverlap, [7](#)
clonalOverlay, [8](#)
clonalProportion, [9](#)
clonesizeDistribution, [10](#)
clusterTCR, [11](#)
combineBCR, [12](#)
combineExpression, [12](#), [13](#), [14](#)
combineTCR, [14](#)
compareClonotypes, [15](#)
contig_list, [16](#)

expression2List, [16](#)

getCirclize, [17](#)

highlightClonotypes, [18](#)

lengthContig, [19](#)

occupiedscRepertoire, [20](#)

quantContig, [21](#)

screp_example, [22](#)
Startrac, [22](#)
Startrac-class (Startrac), [22](#)
StartracDiversity, [23](#)
stripBarcode, [24](#)
subsetContig, [24](#)