

Package ‘ISAnalytics’

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Title Analyze gene therapy vector insertion sites data identified from genomics next generation sequencing reads for clonal tracking studies

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Description In gene therapy, stem cells are modified using viral vectors to deliver the therapeutic transgene and replace functional properties since the genetic modification is stable and inherited in all cell progeny. The retrieval and mapping of the sequences flanking the virus-host DNA junctions allows the identification of insertion sites (IS), essential for monitoring the evolution of genetically modified cells in vivo. A comprehensive toolkit for the analysis of IS is required to foster clonal tracking studies and supporting the assessment of safety and long term efficacy in vivo. This package is aimed at (1) supporting automation of IS workflow, (2) performing base and advance analysis for IS tracking (clonal abundance, clonal expansions and statistics for insertional mutagenesis, etc.), (3) providing basic biology insights of transduced stem cells in vivo.

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URL <https://calabrialab.github.io/ISAnalytics>,
<https://github.com//calabrialab/isanalytics>

BugReports <https://github.com/calabrialab/ISAnalytics/issues>

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aggregate_metadata	<i>Performs aggregation on metadata contained in the association file.</i>
--------------------	--

Description

[Maturing] Groups metadata by the specified grouping keys and returns a summary of info for each group. For more details on how to use this function: `vignette("Working with aggregate functions", package = "ISAnalytics")`

Usage

```
aggregate_metadata(
  association_file,
  grouping_keys = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  aggregating_functions = default_meta_agg(),
  import_stats = lifecycle::deprecated()
)
```

Arguments

association_file	The imported association file (via <code>linkimport_association_file</code>)
grouping_keys	A character vector of column names to form a group
aggregating_functions	A data frame containing specifications of the functions to be applied to columns in the association file during aggregation. It defaults to <code>default_meta_agg</code> . The structure of this data frame should be maintained if the user wishes to change the defaults.
import_stats	[Deprecated] The import of VISPA2 stats has been moved to its dedicated function, see <code>import_Vispa2_stats</code> .

Value

An aggregated data frame

See Also

Other Aggregate functions: [aggregate_values_by_key\(\)](#), [default_meta_agg\(\)](#)

Examples

```
op <- options("ISAnalytics.widgets" = FALSE, "ISAnalytics.verbose" = FALSE)
path_AF <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_correct <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root_correct <- unzip_file_system(root_correct, "fs")
association_file <- import_association_file(path_AF, root_correct,
  dates_format = "dmy"
)
aggregated_meta <- aggregate_metadata(association_file)
options(op)
```

aggregate_values_by_key

Aggregates matrices values based on specified key.

Description

[Maturing] Performs aggregation on values contained in the integration matrices based on the key and the specified lambda. For more details on how to use this function: `vignette("Working with aggregate functions", package = "ISAnalytics")`

Usage

```
aggregate_values_by_key(
  x,
  association_file,
  value_cols = "Value",
  key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  lambda = list(sum = ~sum(.x, na.rm = TRUE)),
  group = c(mandatory_IS_vars(), annotation_IS_vars()),
  join_af_by = "CompleteAmplificationID"
)
```

Arguments

x	A single integration matrix (tibble) or a list of imported integration matrices (tibble)
association_file	The imported association file
value_cols	A character vector containing the names of the columns to apply the given lambdas. Must be numeric or integer columns.
key	A string or a character vector with column names of the association file to take as key
lambda	A named list of functions or purrr-style lambdas. See details section.
group	Other variables to include in the grouping besides key, can be set to NULL
join_af_by	A character vector representing the joining key between the matrix and the meta-data. Useful to re-aggregate already aggregated matrices.

Details**Setting the lambda parameter:**

The lambda parameter should always contain a named list of either functions or purrr-style lambdas. It is also possible to specify the namespace of the function in both ways, for example:

```
lambda = list(sum = sum, desc = psych::describe)
```

Using purrr-style lambdas allows to specify arguments for the functions, keeping in mind that the first parameter should always be `.x`:

```
lambda = list(sum = ~sum(.x, na.rm = TRUE))
```

It is also possible to use custom user-defined functions, keeping in mind that the symbol will be evaluated in the calling environment, for example if the function is called in the global environment and lambda contains "foo" as a function, "foo" will be evaluated in the global environment.

```
foo <- function(x) {
  sum(x)
}
```

```
lambda = list(sum = ~sum(.x, na.rm = TRUE), foo = foo)
```

```
# Or with lambda notation
```

```
lambda = list(sum = ~sum(.x, na.rm = TRUE), foo = ~foo(.x))
```

Constraints on aggregation functions:

Functions passed in the lambda parameters must respect a few constraints to properly work and it's the user responsibility to ensure this.

- Functions have to accept as input a numeric or integer vector
- Function should return a single value or a list/data frame: if a list or a data frame is returned as a result, all the columns will be added to the final data frame.

Value

A list of tibbles or a single tibble aggregated according to the specified arguments

See Also

Other Aggregate functions: [aggregate_metadata\(\)](#), [default_meta_agg\(\)](#)

Examples

```
op <- options("ISAnalytics.widgets" = FALSE, "ISAnalytics.verbose" = FALSE)
path_AF <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_correct <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root_correct <- unzip_file_system(root_correct, "fs")
association_file <- import_association_file(path_AF, root_correct,
  dates_format = "dmy"
)
matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = association_file, root = NULL,
  quantification_type = c("fragmentEstimate", "seqCount"),
  matrix_type = "annotated", workers = 2, matching_opt = "ANY"
)
agg <- aggregate_values_by_key(
  x = matrices,
  association_file = association_file,
  value_cols = c("fragmentEstimate", "seqCount")
)
options(op)
```

annotation_IS_vars *Names of the annotation variables for an integration matrix.*

Description

Contains the names of the columns that are present if the integration matrix is annotated.

Usage

```
annotation_IS_vars()
```

Value

A character vector

Examples

```
annotation_IS_vars()
```

`association_file_columns`*Names of the columns in the association file.*

Description

All the names of the columns present in the association file.

Usage

```
association_file_columns()
```

Value

A character vector

Examples

```
association_file_columns()
```

`as_sparse_matrix`*Converts tidy integration matrices in the original sparse matrix form.*

Description

[Maturing] This function is particularly useful when a sparse matrix structure is needed by a specific function (mainly from other packages).

Usage

```
as_sparse_matrix(  
  x,  
  fragmentEstimate = "fragmentEstimate",  
  seqCount = "seqCount",  
  barcodeCount = "barcodeCount",  
  cellCount = "cellCount",  
  ShsCount = "ShsCount"  
)
```

Arguments

x	A single tidy integration matrix or a list of integration matrices. Supports also multi-quantification matrices obtained via comparison_matrix
fragmentEstimate	For multi-quantification matrix support: the name of the fragment estimate values column
seqCount	For multi-quantification matrix support: the name of the sequence count values column
barcodeCount	For multi-quantification matrix support: the name of the barcode count values column
cellCount	For multi-quantification matrix support: the name of the cell count values column
ShsCount	For multi-quantification matrix support: the name of the Shs Count values column

Value

Depending on input, 2 possible outputs:

- A single sparse matrix (tibble) if input is a single quantification matrix
- A list of sparse matrices divided by quantification if input is a single multi-quantification matrix or a list of matrices

See Also

Other Utility functions: [generate_Vispa2_launch_AF\(\)](#), [generate_blank_association_file\(\)](#), [unzip_file_system\(\)](#)

Examples

```
path <- system.file("extdata", "ex_annotated_ISMatrix.tsv.xz",
  package = "ISAnalytics"
)
matrix <- import_single_Vispa2Matrix(path)
sparse <- as_sparse_matrix(matrix)
```

available_outlier_tests

A character vector containing all the names of the currently supported outliers tests that can be called in the function [outlier_filter](#).

Description

A character vector containing all the names of the currently supported outliers tests that can be called in the function [outlier_filter](#).

Usage

```
available_outlier_tests()
```

Value

A character vector

See Also

Other Outlier tests: [outliers_by_pool_fragments\(\)](#)

Examples

```
available_outlier_tests()
```

`blood_lineages_default`
Default blood lineages info

Description

A default table with info relative to different blood lineages associated with cell markers that can be supplied as a parameter to [HSC_population_size_estimate](#)

Usage

```
blood_lineages_default()
```

Value

A data frame

Examples

```
blood_lineages_default()
```

CIS_grubbs

*Grubbs test for Common Insertion Sites (CIS).***Description**

[Experimental] Statistical approach for the validation of common insertion sites significance based on the comparison of the integration frequency at the CIS gene with respect to other genes contained in the surrounding genomic regions. For more details please refer to this paper: <https://ashpublications.org/blood/article/117/20/5332/21206/Lentiviral-vector-common-integration-sites-in>

Usage

```
CIS_grubbs(
  x,
  genomic_annotation_file = system.file("extdata", "hg19.refGene.oracle.tsv.xz",
    package = "ISAnalytics"),
  grubbs_flanking_gene_bp = 1e+05,
  threshold_alpha = 0.05,
  add_standard_padjust = TRUE
)
```

Arguments

`x` An integration matrix, must include the mandatory `IS_vars()` columns and the `annotation_IS_vars()` columns

`genomic_annotation_file` Database file for gene annotation, see details

`grubbs_flanking_gene_bp` Number of base pairs flanking a gene

`threshold_alpha` Significance threshold

`add_standard_padjust` Compute the standard padjust?

Details**Genomic annotation file:**

This file is a data base, or more simply a .tsv file to import, with genes annotation for the specific genome. The annotations for the human genome (hg19) is already included in this package. If for any reason the user is performing an analysis on another genome, this file needs to be changed respecting the UCSC Genome Browser format, meaning the input file headers should be:

```
## name2, chrom, strand
## min_txStart, max_txEnd, minmax_TxLen
## average_TxLen, name, min_cdsStart
## max_cdsEnd, minmax_CdsLen, average_CdsLen
```

Value

A data frame

See Also

Other Analysis functions: [comparison_matrix\(\)](#), [compute_abundance\(\)](#), [cumulative_count_union\(\)](#), [sample_statistics\(\)](#), [separate_quant_matrices\(\)](#), [threshold_filter\(\)](#), [top_integrations\(\)](#)

Examples

```
op <- options(ISAnalytics.widgets = FALSE)

path_AF <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_correct <- system.file("extdata", "fs.zip",
  package = "ISAnalytics"
)
root_correct <- unzip_file_system(root_correct, "fs")

matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = path_AF, root = root_correct,
  quantification_type = c("seqCount", "fragmentEstimate"),
  matrix_type = "annotated", workers = 2, patterns = NULL,
  matching_opt = "ANY",
  dates_format = "dmy"
)

cis <- CIS_grubbs(matrices)

options(op)
```

CIS_volcano_plot *Trace volcano plot for computed CIS data.*

Description

[Experimental] Traces a volcano plot for IS frequency and CIS results.

Usage

```
CIS_volcano_plot(
  x,
  onco_db_file = system.file("extdata", "201806_uniprot-Proto-oncogene.tsv.xz", package
    = "ISAnalytics"),
  tumor_suppressors_db_file = system.file("extdata",
    "201806_uniprot-Tumor-suppressor.tsv.xz", package = "ISAnalytics"),
  species = "human",
  known_onco = known_clinical_oncogenes(),
```

```

suspicious_genes = clinical_relevant_suspicious_genes(),
significance_threshold = 0.05,
annotation_threshold_ontots = 0.1,
highlight_genes = NULL,
title_prefix = NULL,
return_df = FALSE
)

```

Arguments

x	Either a simple integration matrix or a data frame resulting from the call to CIS_grubbs with <code>add_standard_padjust = TRUE</code>
onco_db_file	Uniprot file for proto-oncogenes (see details)
tumor_suppressors_db_file	Uniprot file for tumor-suppressor genes
species	One between "human", "mouse" and "all"
known_onco	Data frame with known oncogenes. See details.
suspicious_genes	Data frame with clinical relevant suspicious genes. See details.
significance_threshold	The significance threshold
annotation_threshold_ontots	Value above which genes are annotated
highlight_genes	Either NULL or a character vector of genes to be highlighted in the plot even if they're not above the threshold
title_prefix	A string to be displayed in the title - usually the project name and other characterizing info
return_df	Return the data frame used to generate the plot? This can be useful if the user wants to manually modify the plot with <code>ggplot2</code> . If TRUE the function returns a list containing both the plot and the data frame.

Details

Input data frame:

Users can supply as x either a simple integration matrix or a data frame resulting from the call to [CIS_grubbs](#) with `add_standard_padjust = TRUE`. In the first case an internal call to the function `CIS_grubbs` is performed.

Oncogene and tumor suppressor genes files:

These files are included in the package for user convenience and are simply UniProt files with gene annotations for human and mouse. For more details on how this files were generated use the `help ?filename` function.

Known oncogenes:

The default values are contained in a data frame exported by this package, it can be accessed by doing:

```
head(known_clinical_oncogenes())

## # A tibble: 5 x 2
##   GeneName KnownClonalExpansion
##   <chr>    <lgl>
## 1 MECOM    TRUE
## 2 CCND2    TRUE
## 3 TAL1     TRUE
## 4 LMO2     TRUE
## 5 HMGA2    TRUE
```

If the user wants to change this parameter the input data frame must preserve the column structure. The same goes for the `suspicious_genes` parameter (DOIReference column is optional):

```
head(clinical_relevant_suspicious_genes())

## # A tibble: 6 x 3
##   GeneName ClinicalRelevance DOIReference
##   <chr>    <lgl>                <chr>
## 1 DNMT3A   TRUE                  https://doi.org/10.1182/blood-2018-01-829937
## 2 TET2     TRUE                  https://doi.org/10.1182/blood-2018-01-829937
## 3 ASXL1    TRUE                  https://doi.org/10.1182/blood-2018-01-829937
## 4 JAK2     TRUE                  https://doi.org/10.1182/blood-2018-01-829937
## 5 CBL      TRUE                  https://doi.org/10.1182/blood-2018-01-829937
## 6 TP53     TRUE                  https://doi.org/10.1182/blood-2018-01-829937
```

Value

A plot or a list containing a plot and a data frame

See Also

Other Plotting functions: [HSC_population_plot\(\)](#)

Examples

```
op <- options(ISAnalytics.widgets = FALSE)

path_AF <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_correct <- system.file("extdata", "fs.zip",
  package = "ISAnalytics"
)
root_correct <- unzip_file_system(root_correct, "fs")

matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = path_AF, root = root_correct,
  quantification_type = c("seqCount", "fragmentEstimate"),
  matrix_type = "annotated", workers = 2, patterns = NULL,
  matching_opt = "ANY",
  dates_format = "dmy"
```

```
)  
  
cis <- CIS_grubbs(matrices)  
plot <- CIS_volcano_plot(cis)  
options(op)
```

clinical_relevant_suspicious_genes

Clinical relevant suspicious genes (for mouse and human).

Description

Clinical relevant suspicious genes (for mouse and human).

Usage

```
clinical_relevant_suspicious_genes()
```

Value

A data frame

See Also

Other Plotting function helpers: [known_clinical_oncogenes\(\)](#)

Examples

```
clinical_relevant_suspicious_genes()
```

comparison_matrix

obtain a single integration matrix from individual quantification matrices.

Description

[Maturing] Takes a list of integration matrices referring to different quantification types and merges them in a single data frame that has multiple value columns, each renamed according to their quantification type of reference.

Usage

```
comparison_matrix(
  x,
  fragmentEstimate = "fragmentEstimate",
  seqCount = "seqCount",
  barcodeCount = "barcodeCount",
  cellCount = "cellCount",
  ShsCount = "ShsCount"
)
```

Arguments

x	A named list of integration matrices, ideally obtained via import_parallel_Vispa2Matrices_interactive or import_parallel_Vispa2Matrices_auto . Names must be quantification types.
fragmentEstimate	The name of the output column for fragment estimate values
seqCount	The name of the output column for sequence count values
barcodeCount	The name of the output column for barcode count values
cellCount	The name of the output column for cell count values
ShsCount	The name of the output column for Shs count values

Value

A tibble

See Also

[quantification_types](#)

Other Analysis functions: [CIS_grubbs\(\)](#), [compute_abundance\(\)](#), [cumulative_count_union\(\)](#), [sample_statistics\(\)](#), [separate_quant_matrices\(\)](#), [threshold_filter\(\)](#), [top_integrations\(\)](#)

Examples

```
op <- options("ISAnalytics.widgets" = FALSE)
path <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_pth <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root <- unzip_file_system(root_pth, "fs")
matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = path, root = root,
  quantification_type = c("fragmentEstimate", "seqCount"),
  matrix_type = "annotated", workers = 2, patterns = NULL,
  matching_opt = "ANY",
  dates_format = "dmy", multi_quant_matrix = FALSE
)
total_matrix <- comparison_matrix(matrices)
options(op)
```

compute_abundance	<i>Computes the abundance for every integration event in the input data frame.</i>
-------------------	--

Description

[Maturing] Abundance is obtained for every integration event by calculating the ratio between the single value and the total value for the given group.

Usage

```
compute_abundance(
  x,
  columns = "Value",
  percentage = TRUE,
  key = "CompleteAmplificationID",
  keep_totals = FALSE
)
```

Arguments

x	An integration matrix - aka a data frame that includes the mandatory_IS_vars() as columns. The matrix can either be aggregated (via aggregate_values_by_key()) or not.
columns	A character vector of column names to process, must be numeric or integer columns
percentage	Add abundance as percentage?
key	The key to group by when calculating totals
keep_totals	A value between TRUE, FALSE or df. If TRUE, the intermediate totals for each group will be kept in the output data frame as a dedicated column with a trailing "_tot". If FALSE, totals won't be included in the output data frame. If df, the totals are returned to the user as a separate data frame, together with the abundance data frame.

Details

Abundance will be computed upon the user selected columns in the columns parameter. For each column a corresponding relative abundance column (and optionally a percentage abundance column) will be produced.

Value

Either a single data frame with computed abundance values or a list of 2 data frames (abundance_df, quant_totals)

See Also

Other Analysis functions: [CIS_grubbs\(\)](#), [comparison_matrix\(\)](#), [cumulative_count_union\(\)](#), [sample_statistics\(\)](#), [separate_quant_matrices\(\)](#), [threshold_filter\(\)](#), [top_integrations\(\)](#)

Examples

```
path <- system.file("extdata", "ex_annotated_ISMatrix.tsv.xz",
  package = "ISAnalytics"
)
matrix <- import_single_Vispa2Matrix(path)

# Simple integration matrix - grouping by CompleteAmplificationID
abundance1 <- compute_abundance(matrix)
abundance1

# Keeping totals as a separate data frame
abundance2 <- compute_abundance(matrix, keep_totals = "df")
abundance2
```

compute_near_integrations

Scans input matrix to find and merge near integration sites.

Description

[Experimental] This function scans the input integration matrix to detect eventual integration sites that are too "near" to each other and merges them into single integration sites adjusting their values if needed.

Usage

```
compute_near_integrations(
  x,
  threshold = 4,
  keep_criteria = "max_value",
  strand_specific = TRUE,
  max_value_column = "seqCount",
  map_as_widget = TRUE,
  map_as_file = TRUE,
  file_path = ".",
  export_widget_path = NULL
)
```

Arguments

x A single integration matrix, either with a single "Value" column or multiple value columns corresponding to different quantification types (obtained via [comparison_matrix](#))

threshold	A single integer that represents an absolute number of bases for which two integrations are considered distinct
keep_criteria	While scanning, which integration should be kept? The 2 possible choices for this parameter are: <ul style="list-style-type: none"> • "max_value": keep the integration site which has the highest value (and collapse other values on that integration). • "keep_first": keeps the first integration
strand_specific	Should strand be considered? If yes, for example these two integration sites c(chr = "1", strand = "+", integration_locus = 14568) and c(chr = "1", strand = "-", integration_locus = 14568) are considered different and not grouped together.
max_value_column	The column that has to be considered for searching the maximum value
map_as_widget	Produce recalibration map as an HTML widget?
map_as_file	Produce recalibration map as a .tsv file?
file_path	String representing the path where the file will be saved. By default the function produces a folder in the current working directory and generates file names with time stamps.
export_widget_path	A path on disk to save produced widgets or NULL if the user doesn't wish to save the html file

Details

The whole matrix is scanned with a sliding window mechanism: for each row in the integration matrix an interval is calculated based on the threshold value, then a "look ahead" operation is performed to detect subsequent rows which integration locuses fall in the interval. If CompleteAmplificationIDs of the near integrations are different only the locus value (and optionally GeneName and GeneStrand if the matrix is annotated) is modified, otherwise rows with the same id are aggregated and values are summed. If one of the map parameters is set to true the function will also produce a re-calibration map: this data frame contains the reference of pre-recalibration values for chr, strand and integration locus and the value to which that integration was changed to after.

Value

An integration matrix with same or less number of rows

Note

We do recommend to use this function in combination with [comparison_matrix](#) to automatically perform re-calibration on all quantification matrices.

Examples

```
path <- system.file("extdata", "ex_annotated_ISMatrix.tsv.xz",
  package = "ISAnalytics"
)
```

```

matrix <- import_single_Vispa2Matrix(path)
near <- compute_near_integrations(matrix,
  map_as_widget = FALSE,
  map_as_file = FALSE
)

```

cumulative_count_union

Integrations cumulative count in time by sample

Description

[Experimental] This function computes the cumulative number of integrations observed in each sample at different time points by assuming that if an integration is observed at time point "t" then it is also observed in time point "t+1".

Usage

```

cumulative_count_union(
  x,
  association_file = NULL,
  timepoint_column = "TimePoint",
  key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  include_tp_zero = FALSE,
  zero = "0000",
  aggregate = FALSE,
  ...
)

```

Arguments

x	A simple integration matrix or an aggregated matrix (see details)
association_file	NULL or the association file for x if aggregate is set to TRUE
timepoint_column	What is the name of the time point column?
key	The aggregation key - must always contain the timepoint_column
include_tp_zero	Include timepoint 0?
zero	How is 0 coded in the data frame?
aggregate	Should x be aggregated?
...	Additional parameters to pass to aggregate_values_by_key

Details

Input data frame:

The user can provide as input for the `x` parameter both a simple integration matrix AND setting the `aggregate` parameter to `TRUE`, or provide an already aggregated matrix via [aggregate_values_by_key](#). If the user supplies a matrix to be aggregated the `association_file` parameter must not be `NULL`: aggregation will be done by an internal call to the aggregation function. If the user supplies an already aggregated matrix, the `key` parameter is the key used for aggregation - **NOTE: for this operation is mandatory that the time point column is included in the key.**

Assumptions on time point format:

By using the functions provided by this package, when imported, an association file will be correctly formatted for future usage. In the formatting process there is also a padding operation performed on time points: this means the functions expects the time point column to be of type character and to be correctly padded with 0s. If the chosen column for time point is detected as numeric the function will attempt the conversion to character and automatic padding. If you choose to import the association file not using the [import_association_file](#) function, be sure to check the format of the chosen column to avoid undesired results.

Value

A data frame

See Also

Other Analysis functions: [CIS_grubbs\(\)](#), [comparison_matrix\(\)](#), [compute_abundance\(\)](#), [sample_statistics\(\)](#), [separate_quant_matrices\(\)](#), [threshold_filter\(\)](#), [top_integrations\(\)](#)

Examples

```
op <- options(ISAnalytics.widgets = FALSE)

path_AF <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_correct <- system.file("extdata", "fs.zip",
  package = "ISAnalytics"
)
root_correct <- unzip_file_system(root_correct, "fs")

association_file <- import_association_file(path_AF, root_correct,
  dates_format = "dmy"
)
matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = association_file, root = NULL,
  quantification_type = c("seqCount", "fragmentEstimate"),
  matrix_type = "annotated", workers = 2, patterns = NULL,
  matching_opt = "ANY", multi_quant_matrix = FALSE
)

#### EXTERNAL AGGREGATION
```

```

aggregated <- aggregate_values_by_key(matrices$seqCount, association_file)
cumulative_count <- cumulative_count_union(aggregated)

#### INTERNAL AGGREGATION
cumulative_count_2 <- cumulative_count_union(matrices$seqCount,
  association_file,
  aggregate = TRUE
)

options(op)

```

date_columns_coll *Possible choices for date_col parameter.*

Description

Possible choices for date_col parameter.

Usage

```
date_columns_coll()
```

Value

A character vector of column names

See Also

[remove_collisions](#)

Examples

```
dates <- date_columns_coll()
```

date_formats	<i>Possible choices for the</i>	dates_format	<i>pa-</i>
	<i>parameter</i>	<i>in</i>	<i>import_association_file,</i>
		import_parallel_vispa2Matrices_interactive	<i>and</i>
		import_parallel_vispa2Matrices_auto.	

Description

All options correspond to lubridate functions:

- ymd: year, month, date
- ydm: year, day, month
- mdy: month, day, year
- myd: month, year, day
- dmy: day, month, year
- dym: day, year, month
- yq: year quantile

Usage

```
date_formats()
```

Details

NOTE: use the same date format across the association file.

Value

A character vector

See Also

[import_association_file](#), [import_parallel_Vispa2Matrices_auto](#)

Examples

```
date_formats()
```

default_iss_file_prefixes

Default regex prefixes for Vispa2 stats files.

Description

Note that each element is a regular expression.

Usage

```
default_iss_file_prefixes()
```

Value

A character vector of regexes

Examples

```
default_iss_file_prefixes()
```

default_meta_agg	<i>Default metadata aggregation function table</i>
------------------	--

Description

A default columns-function specifications for [aggregate_metadata](#)

Usage

```
default_meta_agg()
```

Details

This data frame contains four columns:

- **Column:** holds the name of the column in the association file that should be processed
- **Function:** contains either the name of a function (e.g. `mean`) or a purrr-style lambda (e.g. `~ mean(.x, na.rm = TRUE)`). This function will be applied to the corresponding column specified in **Column**
- **Args:** optional additional arguments to pass to the corresponding function. This is relevant **ONLY** if the corresponding **Function** is a simple function and not a purrr-style lambda.
- **Output_colname:** a glue specification that will be used to determine a unique output column name. See [glue](#) for more details.

Value

A data frame

See Also

Other Aggregate functions: [aggregate_metadata\(\)](#), [aggregate_values_by_key\(\)](#)

Examples

```
default_meta_agg()
```

default_stats	<i>A set of pre-defined functions for sample_statistics.</i>
---------------	--

Description

A set of pre-defined functions for sample_statistics.

Usage

```
default_stats()
```

Value

A named list of functions/purrr-style lambdas

Examples

```
default_stats()
```

generate_blank_association_file	<i>Creates a blank association file.</i>
---------------------------------	--

Description

This function is useful if you want a blank association file to start using both Vispa2 and this package or simply if you want a correct framework to fix a malformed association file you have already.

Usage

```
generate_blank_association_file(path)
```

Arguments

path	The path on disk where the file should be written
------	---

Value

returns NULL

See Also

Other Utility functions: [as_sparse_matrix\(\)](#), [generate_Vispa2_launch_AF\(\)](#), [unzip_file_system\(\)](#)

Examples

```
temp <- tempfile()
generate_blank_association_file(temp)
```

`generate_Vispa2_launch_AF`

Creates a reduced association file for Vispa2 run, given project and pool

Description

The function selects the appropriate columns and prepares a file for the launch of Vispa2 pipeline for each project/pool pair specified.

Usage

```
generate_Vispa2_launch_AF(association_file, project, pool, path)
```

Arguments

<code>association_file</code>	The imported association file (via <code>import_association_file</code>)
<code>project</code>	A vector of characters containing project names
<code>pool</code>	A vector of characters containing pool names. NOTE: the names should refer to the values contained in the PoolID column of the association file and NOT the concatenatePoolIDSeqRun column!
<code>path</code>	A single string representing the path to the folder where files should be written. If the folder doesn't exist it will be created.

Details

Note: the function is vectorized, meaning you can specify more than one project and more than one pool as vectors of characters, but you must ensure that:

- Both `project` and `pool` vectors have the same length
- You correctly type names in corresponding positions, for example `c("CLOEXP", "PROJECT1100", "PROJECT1100") - c("POOL6", "ABX-LR-PL5-POOL14-1", "ABX-LR-PL6-POOL15-1")`. If you type a pool in the position of a corresponding project that doesn't match no file will be produced since that pool doesn't exist in the corresponding project.

Value

returns NULL

See Also

Other Utility functions: [as_sparse_matrix\(\)](#), [generate_blank_association_file\(\)](#), [unzip_file_system\(\)](#)

Examples

```

op <- options("ISAnalytics.widgets" = FALSE)
temp <- tempdir()
path_af <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_pth <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root <- unzip_file_system(root_pth, "fs")
association_file <- import_association_file(path_af, root,
  dates_format = "dmy"
)
generate_Vispa2_launch_AF(association_file, "CLOEXP", "POOL6", temp)
options(op)

```

HSC_population_plot *Plot of the estimated HSC population size for each patient.*

Description

Plot of the estimated HSC population size for each patient.

Usage

```

HSC_population_plot(
  estimates,
  project_name,
  timepoints = "Consecutive",
  models = "Mth Chao (LB)"
)

```

Arguments

estimates	The estimates data frame, obtained via HSC_population_size_estimate
project_name	The project name, will be included in the plot title
timepoints	Which time points to plot? One between "All", "Stable" and "Consecutive"
models	Name of the models to plot (as they appear in the column of the estimates)

Value

A plot

See Also

Other Plotting functions: [CIS_volcano_plot\(\)](#)

Examples

```

op <- options("ISAnalytics.widgets" = FALSE, "ISAnalytics.verbose" = FALSE)
path_AF <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_correct <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root_correct <- unzip_file_system(root_correct, "fs")
association_file <- import_association_file(path_AF, root_correct,
  dates_format = "dmy"
)
aggregated_meta <- aggregate_metadata(association_file)
matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = association_file, root = NULL,
  quantification_type = c("fragmentEstimate", "seqCount"),
  matrix_type = "annotated", workers = 2, matching_opt = "ANY"
)
agg <- aggregate_values_by_key(
  x = matrices,
  association_file = association_file,
  value_cols = "seqCount"
)
estimate <- HSC_population_size_estimate(x = agg,
  metadata = aggregated_meta,
  stable_timepoints = NULL)
p <- HSC_population_plot(estimate, "PROJECT1")
options(op)

```

HSC_population_size_estimate

Hematopoietic stem cells population size estimate.

Description

[Experimental] Hematopoietic stem cells population size estimate with capture-recapture models.

Usage

```

HSC_population_size_estimate(
  x,
  metadata,
  stable_timepoints = NULL,
  aggregation_key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  blood_lineages = blood_lineages_default(),
  timepoint_column = "TimePoint",
  seqCount_column = "seqCount_sum",
  seqCount_threshold = 3,
  nIS_threshold = 5,
  cell_type = "MYELOID",
  tissue_type = "PB"
)

```

Arguments

x	An aggregated integration matrix. See details.
metadata	An aggregated association file. See details.
stable_timepoints	A numeric vector or NULL if there are no stable time points.
aggregation_key	A character vector indicating the key used for aggregating x and metadata. Note that x and metadata should always be aggregated with the same key.
blood_lineages	A data frame containing information on the blood lineages. Users can supply their own, provided the columns CellMarker and CellType are present.
timepoint_column	What is the name of the time point column to use? Note that this column must be present in the key.
seqCount_column	What is the name of the column in x holding the values of sequence count quantification?
seqCount_threshold	A single numeric value. After re-aggregating x, rows with a value greater or equal will be kept, the others will be discarded.
nIS_threshold	A single numeric value. If a group (row) in the metadata data frame has a count of distinct integration sites strictly greater than this number it will be kept, otherwise discarded.
cell_type	The cell types to include in the models. Note that the matching is case-insensitive.
tissue_type	The tissue types to include in the models. Note that the matching is case-insensitive.

Value

A data frame with the results of the estimates

Input formats

Both x and metadata should be supplied to the function in aggregated format (ideally through the use of [aggregate_metadata](#) and [aggregate_values_by_key](#)). Note that the aggregation_key, aka the vector of column names used for aggregation, must contain at least the columns SubjectID, CellMarker, Tissue and a time point column (the user can specify the name of the column in the argument timepoint_column).

On time points

If stable_timepoints is a vector with length > 1, the function will look for the first available stable time point and slice the data from that time point onward. If NULL is supplied instead, it means there are no stable time points available. Note that 0 time points are ALWAYS discarded. Also, to be included in the analysis, a group must have at least 2 distinct non-zero time points.

Examples

```

op <- options("ISAnalytics.widgets" = FALSE, "ISAnalytics.verbose" = FALSE)
path_AF <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_correct <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root_correct <- unzip_file_system(root_correct, "fs")
association_file <- import_association_file(path_AF, root_correct,
  dates_format = "dmy"
)
aggregated_meta <- aggregate_metadata(association_file)
matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = association_file, root = NULL,
  quantification_type = c("fragmentEstimate", "seqCount"),
  matrix_type = "annotated", workers = 2, matching_opt = "ANY"
)
agg <- aggregate_values_by_key(
  x = matrices,
  association_file = association_file,
  value_cols = "seqCount"
)
estimate <- HSC_population_size_estimate(x = agg,
  metadata = aggregated_meta,
  stable_timepoints = NULL)
options(op)

```

```
import_association_file
```

Import the association file from disk

Description

[Maturing] Imports the association file and immediately performs a check on the file system starting from the root to assess the alignment between the two.

Usage

```

import_association_file(
  path,
  root = NULL,
  tp_padding = 4,
  dates_format = "ymd",
  separator = "\t",
  filter_for = NULL,
  import_iss = FALSE,
  export_widget_path = NULL,
  convert_tp = TRUE,
  ...
)

```

Arguments

path	The path on disk to the association file.
root	The path on disk of the root folder of Vispa2 output or NULL. See details.
tp_padding	Timepoint padding, indicates the number of digits of the "Timepoint" column once imported. Fills the content with 0s up to the length specified (ex: 1 becomes 0001 with a tp_padding of 4)
dates_format	A single string indicating how dates should be parsed. Must be a value in: <code>date_formats()</code>
separator	The column separator used in the file
filter_for	A named list where names represent column names that must be filtered. For example: <code>list(ProjectID = c("PROJECT1", "PROJECT2"))</code> will filter the association file so that it contains only those rows for which the value of the column "ProjectID" is one of the specified values. If multiple columns are present in the list all filtering conditions are applied as a logical AND.
import_iss	Import Vispa2 stats and merge them with the association file?
export_widget_path	A path on disk to save produced widgets or NULL if the user doesn't wish to save the html file
convert_tp	Should be time points be converted into months and years?
...	Additional arguments to pass to <code>import_Vispa2_stats</code>

Details

The import series of functions is designed to work in combination with the use of Vispa2 pipeline, please refer to this article for more details: [VISPA2: A Scalable Pipeline for High-Throughput Identification and Annotation of Vector Integration Sites](#).

The pipeline automatically produces an hierarchical structure in the file system which follows this schema:

- /root_folder
 - Optional intermediate folders
 - * ProjectID
 - l_bam
 - l_bcmuxall
 - l_bed
 - l_iss
 - l_quality
 - l_report
 - l_quantification
 - *l___concatenatePoolIDSeqRun

For each ProjectID there may be several nested PoolIDs. The alignment function only looks for PoolIDs in the quantification folder, since it's the location of the matrices to import. For more details on how to properly use these functions, refer to the vignette - `vignette("how_to_import_functions")`. If 'NULL' the file system alignment step is skipped.

Value

A tibble with the contents of the association file plus columns containing the path in the file system for every project and pool if found.

See Also

[date_formats](#)

Other Import functions: [import_Vispa2_stats\(\)](#), [import_parallel_Vispa2Matrices_auto\(\)](#), [import_parallel_Vispa2Matrices_interactive\(\)](#), [import_single_Vispa2Matrix\(\)](#)

Examples

```
op <- options("ISAnalytics.widgets" = FALSE)
path <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_pth <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root <- unzip_file_system(root_pth, "fs")
association_file <- import_association_file(path, root, dates_format = "dmy")
options(op)
```

`import_parallel_Vispa2Matrices_auto`

Import integration matrices based on the association file.

Description

[Maturing] These functions are designed to import the appropriate integration matrix files given the association file and the root folder of the file system where Vispa2 matrices are generated.

Usage

```
import_parallel_Vispa2Matrices_auto(
  association_file,
  quantification_type,
  matrix_type = "annotated",
  workers = 2,
  multi_quant_matrix = TRUE,
  export_report_path = NULL,
  patterns = NULL,
  matching_opt = matching_options(),
  ...
)
```

Arguments

<code>association_file</code>	A single string containing the path to the association file on disk, or a data frame resulting from a previous call to <code>import_association_file</code>
<code>quantification_type</code>	A vector of requested <code>quantification_types</code> . Must be one in <code>quantification_types()</code>
<code>matrix_type</code>	A single string representing the type of matrices to be imported. Can only be one in "annotated" or "not_annotated"
<code>workers</code>	A single integer representing the number of parallel workers to use for the import
<code>multi_quant_matrix</code>	If set to TRUE will produce a multi-quantification matrix (data frame) through <code>comparison_matrix</code> instead of a list.
<code>export_report_path</code>	A path on disk to save produced import report or NULL if the user doesn't wish to save the html file
<code>patterns</code>	A character vector of additional patterns to match on file names. Please note that patterns must be regular expressions. Can be NULL if no patterns needs to be matched.
<code>matching_opt</code>	A single value between <code>matching_options</code>
<code>...</code>	<code><dynamic-dots></code> Additional named arguments to pass to <code>import_association_file</code> and <code>comparison_matrix</code>

Details

Import family functions are designed to work in combination with Vispa2, for more details on this take a look here: [VISPA2: A Scalable Pipeline for High-Throughput Identification and Annotation of Vector Integration Sites](#).

For more details on how to properly use these functions, refer to the vignette - `vignette("how_to_import_functions")`

Value

A named list of data frames containing data from all imported integration matrices, divided by quantification type or a multi-quantification matrix

Automatic version

The automatic version of `import_parallel_Vispa2Matrices` doesn't interact with the user directly, for this reason options in this modality are more limited compared to the interactive version. In automatic version you can't:

- Choose single projects or pools: to have a selection import the association file first and filter it according to your needs before calling the function (more details on this in the vignette)
- Choose duplicates: if, after filtering by the specified patterns, duplicates are found they are automatically ignored

Interactive version

The interactive version of `import_parallel_Vispa2Matrices` asks user for input and allows a more detailed choice of projects to import, pools to import and, if necessary, duplicate files. During the execution, a series of reports is shown in html format.

See Also

[matching_options](https://stringr.tidyverse.org/articles/regular-expressions.html), <https://stringr.tidyverse.org/articles/regular-expressions.html>

Other Import functions: `import_Vispa2_stats()`, `import_association_file()`, `import_parallel_Vispa2Matrices_i`
`import_single_Vispa2Matrix()`

Examples

```
op <- options("ISAnalytics.widgets" = FALSE)
path <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_pth <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root <- unzip_file_system(root_pth, "fs")
matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = path,
  quantification_type = c("fragmentEstimate", "seqCount"),
  patterns = NULL, matching_opt = "ANY",
  root = root,
  dates_format = "dmy",
  workers = 2
)
options(op)
```

```
import_parallel_Vispa2Matrices_interactive
```

Import integration matrices based on the association file.

Description

[Maturing] These functions are designed to import the appropriate integration matrix files given the association file and the root folder of the file system where Vispa2 matrices are generated.

Usage

```
import_parallel_Vispa2Matrices_interactive(
  association_file,
  quantification_type,
  matrix_type = "annotated",
  workers = 2,
  multi_quant_matrix = TRUE,
  export_report_path = NULL,
  ...
)
```

Arguments

<code>association_file</code>	A single string containing the path to the association file on disk, or a data frame resulting from a previous call to <code>import_association_file</code>
<code>quantification_type</code>	A vector of requested <code>quantification_types</code> . Must be one in <code>quantification_types()</code>
<code>matrix_type</code>	A single string representing the type of matrices to be imported. Can only be one in "annotated" or "not_annotated"
<code>workers</code>	A single integer representing the number of parallel workers to use for the import
<code>multi_quant_matrix</code>	If set to TRUE will produce a multi-quantification matrix (data frame) through <code>comparison_matrix</code> instead of a list.
<code>export_report_path</code>	A path on disk to save produced import report or NULL if the user doesn't wish to save the html file
<code>...</code>	<dynamic-dots> Additional named arguments to pass to <code>import_association_file</code> and <code>comparison_matrix</code>

Details

Import family functions are designed to work in combination with Vispa2, for more details on this take a look here: [VISPA2: A Scalable Pipeline for High-Throughput Identification and Annotation of Vector Integration Sites](#).

For more details on how to properly use these functions, refer to the vignette - `vignette("how_to_import_functions")`

Value

A named list of data frames containing data from all imported integration matrices, divided by quantification type or a multi-quantification matrix

Interactive version

The interactive version of `import_parallel_Vispa2Matrices` asks user for input and allows a more detailed choice of projects to import, pools to import and, if necessary, duplicate files. During the execution, a series of reports is shown in html format.

See Also

`comparison_matrix`, `import_association_file`

Other Import functions: `import_Vispa2_stats()`, `import_association_file()`, `import_parallel_Vispa2Matrices_a`
`import_single_Vispa2Matrix()`

Examples

```
## Not run:
# Can't run because it's interactive and requires user input
matrices <- import_parallel_Vispa2Matrices_interactive(
  association_file,
```

```
    quantification_type,  
    matrix_type = "annotated",  
    workers = 2,  
    multi_quant_matrix = FALSE,  
    export_report_path = NULL,  
  )  
  
## End(Not run)
```

```
import_single_Vispa2Matrix
```

Import a single integration matrix from file

Description

[Stable] This function allows to read and import an integration matrix produced as the output of Vispa2 pipeline and converts it to a tidy format.

Usage

```
import_single_Vispa2Matrix(path, to_exclude = NULL, separator = "\t")
```

Arguments

path	The path to the file on disk
to_exclude	Either NULL or a character vector of column names that should be ignored when importing
separator	The column delimiter used

Details

The import series of functions is designed to work in combination with the use of Vispa2 pipeline, please refer to this article for more details: [VISPA2: A Scalable Pipeline for High-Throughput Identification and Annotation of Vector Integration Sites](#). For more details on how to properly use these functions, refer to vignette("How to use import functions", package = "ISAnalytics")

Value

A data.table object in tidy format

See Also

Other Import functions: [import_Vispa2_stats\(\)](#), [import_association_file\(\)](#), [import_parallel_Vispa2Matrices_a](#), [import_parallel_Vispa2Matrices_interactive\(\)](#)

Examples

```
path_to_file <- system.file("extdata", "ex_annotated_ISMatrix.tsv.xz",
  package = "ISAnalytics"
)
isa_dataframe <- import_single_Vispa2Matrix(path_to_file)
```

```
import_Vispa2_stats Import Vispa2 stats given the aligned association file.
```

Description

[Experimental] Imports all the Vispa2 stats files for each pool provided the association file has been aligned with the file system (see [import_association_file](#)).

Usage

```
import_Vispa2_stats(
  association_file,
  file_prefixes = default_iss_file_prefixes(),
  join_with_af = TRUE,
  pool_col = "concatenatePoolIDSeqRun",
  export_widget_path = NULL
)
```

Arguments

association_file	The file system aligned association file (contains columns with absolute paths to the 'iss' folder)
file_prefixes	A character vector with known file prefixes to match on file names. NOTE: the elements represent regular expressions. For defaults see default_iss_file_prefixes .
join_with_af	Logical, if TRUE the imported stats files will be merged with the association file, if false a single data frame holding only the stats will be returned.
pool_col	A single string. What is the name of the pool column used in the Vispa2 run? This will be used as a key to perform a join operation with the stats files POOL column.
export_widget_path	Either NULL or the path on disk where the widget report should be saved.

Value

A data frame

See Also

Other Import functions: [import_association_file\(\)](#), [import_parallel_Vispa2Matrices_auto\(\)](#), [import_parallel_Vispa2Matrices_interactive\(\)](#), [import_single_Vispa2Matrix\(\)](#)

Examples

```
op <- options("ISAnalytics.widgets" = FALSE)
path <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_pth <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root <- unzip_file_system(root_pth, "fs")
association_file <- import_association_file(path, root, dates_format = "dmy")
af_with_stats <- import_Vispa2_stats(association_file)
options(op)
```

ISAnalytics

ISAnalytics: Analyze gene therapy vector insertion sites data identified from genomics next generation sequencing reads for clonal tracking studies

Description

[Maturing] In gene therapy, stem cells are modified using viral vectors to deliver the therapeutic transgene and replace functional properties since the genetic modification is stable and inherited in all cell progeny. The retrieval and mapping of the sequences flanking the virus-host DNA junctions allows the identification of insertion sites (IS), essential for monitoring the evolution of genetically modified cells in vivo. A comprehensive toolkit for the analysis of IS is required to foster clonal tracking studies and supporting the assessment of safety and long term efficacy in vivo. This package is aimed at (1) supporting automation of IS workflow, (2) performing base and advance analysis for IS tracking (clonal abundance, clonal expansions and statistics for insertional mutagenesis, etc.), (3) providing basic biology insights of transduced stem cells in vivo.

Useful resources

- [VISPA2: A Scalable Pipeline for High-Throughput Identification and Annotation of Vector Integration Sites](#)

ISAnalytics function families

- Import functions:
 - [import_single_Vispa2Matrix](#)
 - [import_association_file](#)
 - [import_Vispa2_stats](#)
 - [import_parallel_Vispa2Matrices_interactive](#)
 - [import_parallel_Vispa2Matrices_auto](#)
- Aggregation functions:
 - [aggregate_metadata](#)
 - [aggregate_values_by_key](#)
- Collision removal functions:

- `remove_collisions`
 - `realign_after_collisions`
- Removal of outliers from raw reads
 - `outlier_filter`
 - `outliers_by_pool_fragments`
- Recalibration functions:
 - `compute_near_integrations`
- Analysis functions:
 - `compute_abundance`
 - `comparison_matrix`
 - `separate_quant_matrices`
 - `threshold_filter`
 - `top_integrations`
 - `sample_statistics`
 - `CIS_grubbs`
 - `cumulative_count_union`
- HSC population size estimate:
 - `HSC_population_size_estimate`
- Plotting functions:
 - `CIS_volcano_plot`
 - `HSC_population_plot`
- Utility functions:
 - `generate_blank_association_file`
 - `generate_Vispa2_launch_AF`
 - `unzip_file_system`
 - `as_sparse_matrix`

Vignettes

- `vignette("How to use import functions", package = "ISAnalytics")`
- `vignette("Collision removal functionality", package = "ISAnalytics")`
- `vignette("Working with aggregate functions", package = "ISAnalytics")`

`known_clinical_oncogenes`*Known clinical oncogenes (for mouse and human).*

Description

Known clinical oncogenes (for mouse and human).

Usage

```
known_clinical_oncogenes()
```

Value

A data frame

See Also

Other Plotting function helpers: [clinical_relevant_suspicious_genes\(\)](#)

Examples

```
known_clinical_oncogenes()
```

`mandatory_IS_vars`*Names of mandatory variables for an integration matrix.*

Description

Contains the names of the columns that need to be present in order for a tibble to be considered an integration matrix.

Usage

```
mandatory_IS_vars()
```

Value

A character vector

Examples

```
mandatory_IS_vars()
```

matching_options *Possible choices for the matching_opt parameter.*

Description

These are all the possible values for the matching_opt parameter in import_parallel_vispa2Matrices_auto.

Usage

```
matching_options()
```

Details

The values "ANY", "ALL" and "OPTIONAL", represent how the patterns should be matched, more specifically

- ANY = look only for files that match AT LEAST one of the patterns specified
- ALL = look only for files that match ALL of the patterns specified
- OPTIONAL = look preferentially for files that match, in order, all patterns or any pattern and if no match is found return what is found (keep in mind that duplicates are discarded in automatic mode)

Value

A vector of characters for matching_opt

See Also

[import_parallel_Vispa2Matrices_auto](#)

Other Import functions helpers: [quantification_types\(\)](#)

Examples

```
opts <- matching_options()
```

outliers_by_pool_fragments
Identify and flag outliers based on pool fragments.

Description

[Experimental] Identify and flag outliers

Usage

```

outliers_by_pool_fragments(
  metadata,
  key = "BARCODE_MUX",
  outlier_p_value_threshold = 0.05,
  normality_test = FALSE,
  normality_p_value_threshold = 0.05,
  transform_log2 = TRUE,
  per_pool_test = TRUE,
  pool_col = "PoolID",
  min_samples_per_pool = 5,
  flag_logic = "AND",
  keep_calc_cols = TRUE,
  save_widget_path = NULL
)

```

Arguments

metadata	The metadata data frame
key	A character vector of numeric column names
outlier_p_value_threshold	The p value threshold for a read to be considered an outlier
normality_test	Perform normality test? Normality is assessed for each column in the key using Shapiro-Wilk test and if the values do not follow a normal distribution, other calculations are skipped
normality_p_value_threshold	Normality threshold
transform_log2	Perform a log2 transformation on values prior the actual calculations?
per_pool_test	Perform the test for each pool?
pool_col	A character vector of the names of the columns that uniquely identify a pool
min_samples_per_pool	The minimum number of samples that a pool needs to contain in order to be processed - relevant only if per_pool_test = TRUE
flag_logic	A character vector of logic operators to obtain a global flag formula - only relevant if the key is longer than one. All operators must be chosen between: AND, OR, XOR, NAND, NOR, XNOR
keep_calc_cols	Keep the calculation columns in the output data frame?
save_widget_path	Either null or a string containing the path on disk where the report should be saved

Details

This particular test calculates for each column in the key

- The zscore of the values

- The tstudent of the values
- The the distribution of the tstudent values

Optionally the test can be performed for each pool and a normality test can be run prior the actual calculations. Samples are flagged if this condition is respected:

- $\text{tdist} < \text{outlier_p_value_threshold} \ \& \ \text{zscore} < 0$

If the key contains more than one column an additional flag logic can be specified for combining the results. Example: let's suppose the key contains the names of two columns, X and Y $\text{key} = \text{c}("X", "Y")$ if we specify the the argument `flag_logic = "AND"` then the reads will be flagged based on this global condition: $(\text{tdist}_X < \text{outlier_p_value_threshold} \ \& \ \text{zscore}_X < 0) \ \text{AND} \ (\text{tdist}_Y < \text{outlier_p_value_threshold} \ \& \ \text{zscore}_Y < 0)$

The user can specify one or more logical operators that will be applied in sequence.

Value

A data frame of metadata with the column `to_remove`

See Also

Other Outlier tests: [available_outlier_tests\(\)](#)

Examples

```
op <- options(ISAnalytics.widgets = FALSE)

path_AF <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
association_file <- import_association_file(path_AF, root = NULL,
  dates_format = "dmy"
)
filtered_af <- outliers_by_pool_fragments(association_file, key = "VCN")
options(op)
```

outlier_filter

Filter out outliers in metadata, identified by the chosen outlier test.

Description

[Experimental] Filter out outliers in metadata.

Usage

```
outlier_filter(
  metadata,
  outlier_test = "outliers_by_pool_fragments",
  negate = FALSE,
  ...
)
```

Arguments

metadata	The metadata data frame
outlier_test	A string representing a function name. The name must be one of the available outlier tests, see available_outlier_tests .
negate	If TRUE will return only the metadata that was flagged to be removed. If FALSE will return only the metadata that wasn't flagged to be removed.
...	Additional named arguments passed to outliers_test

Value

A data frame of metadata which has less or the same amount of rows

Examples

```
op <- options(ISAnalytics.widgets = FALSE)

path_AF <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
association_file <- import_association_file(path_AF, root = NULL,
  dates_format = "dmy"
)
filtered_af <- outlier_filter(association_file, key = "VCN")
options(op)
```

quantification_types *Possible choices for the quantification_type parameter.*

Description

These are all the possible values for the quantification_type parameter in import_parallel_vispa2Matrices_interac and import_parallel_vispa2Matrices_auto.

Usage

```
quantification_types()
```

Details

The possible values are:

- fragmentEstimate
- seqCount
- barcodeCount
- cellCount
- ShsCount

Value

A vector of characters for quantification types

See Also

[import_parallel_Vispa2Matrices_interactive](#), [import_parallel_Vispa2Matrices_auto](#)

Other Import functions helpers: [matching_options\(\)](#)

Examples

```
quant_types <- quantification_types()
```

```
realign_after_collisions
```

Re-aligns matrices of other quantification types based on the processed sequence count matrix.

Description

[Experimental] This function should be used to keep data consistent among the same analysis: if for some reason you removed the collisions by passing only the sequence count matrix to the `remove_collisions` function, you should call this function afterwards, providing a list of other quantification matrices. NOTE: if you provided a list of several quantification types to `remove_collisions` before, there is no need to call this function.

Usage

```
realign_after_collisions(sc_matrix, other_matrices)
```

Arguments

`sc_matrix` The sequence count matrix already processed for collisions via `remove_collisions`
`other_matrices` A named list of matrices to re-align. Names in the list must be quantification types (`quantification_types()`) except "seqCount".

Details

For more details on how to use collision removal functionality: `vignette("Collision removal functionality", package = "ISAnalytics")`

Value

A named list with re-aligned matrices

See Also

[remove_collisions](#)

Other Collision removal: [remove_collisions\(\)](#)

Examples

```
op <- options("ISAnalytics.widgets" = FALSE)
path <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_pth <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root <- unzip_file_system(root_pth, "fs")
association_file <- import_association_file(path, root,
  dates_format = "dmy"
)
matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = association_file, root = NULL,
  quantification_type = c("fragmentEstimate", "seqCount"),
  matrix_type = "annotated", workers = 2,
  patterns = NULL, matching_opt = "ANY",
  multi_quant_matrix = FALSE
)
sc_matrix <- remove_collisions(matrices$seqCount, association_file)
others <- matrices[!names(matrices) %in% "seqCount"]
aligned_matrices <- realign_after_collisions(sc_matrix, others)
options(op)
```

reduced_AF_columns	<i>Names of the columns of the association file to consider for Vispa2 launch.</i>
--------------------	--

Description

Selection of column names from the association file to be considered for Vispa2 launch. NOTE: the TagID column appears only once but needs to be repeated twice for generating the launch file. Use the appropriate function to generate the file automatically.

Usage

```
reduced_AF_columns()
```

Value

A character vector

Examples

```
reduced_AF_columns()
```

remove_collisions *Identifies and removes collisions based on the sequence count matrix.*

Description

[Experimental] A collision is an integration (aka a unique combination of chr, integration_locus and strand) which is observed in more than one independent sample (a unique pair of ProjectID and SubjectID). The function tries to decide to which subject an integration should be assigned and if no decision can be taken, the integration is completely removed from the data frame.

Usage

```
remove_collisions(  
  x,  
  association_file,  
  date_col = "SequencingDate",  
  reads_ratio = 10,  
  seq_count_col = "seqCount",  
  max_rows_reports = 50,  
  save_widget_path = NULL  
)
```

Arguments

x	A named list of matrices (names must be quantification types), a single integration matrix representing the sequence count matrix of interest or a multi-quantification matrix obtained via comparison_matrix
association_file	The association file imported via <code>import_association_file</code>
date_col	The date column that should be considered for the analysis. Must be one value in <code>date_columns_coll()</code>
reads_ratio	A single numeric value that represents the ratio that has to be considered when deciding between seqCount value.
seq_count_col	For support of multi-quantification matrix - the name of the sequence count values column
max_rows_reports	A numeric value, represents the maximum number of rows of the reports data frames that can be printed on console if the option <code>ISAnalytics.verbose</code> is active. If the data frames are too large they won't be printed on console - we recommend using widgets for detailed and more accessible info.
save_widget_path	Either NULL or a path where the html report file should be saved. If NULL the report is visualized via browser ONLY (not saved on disk).

Details

If you don't want the function to show details and messages do: `options(ISAnalytics.verbose = FALSE)`. To restore to the original value: `options(ISAnalytics.verbose = TRUE)`. For more details on how to use collision removal functionality: `vignette("Collision removal functionality", package = "ISAnalytics")`

Value

A list of tibbles with removed collisions

See Also

[date_columns_coll](#)

Other Collision removal: [realign_after_collisions\(\)](#)

Examples

```
op <- options("ISAnalytics.widgets" = FALSE)
path <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_pth <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root <- unzip_file_system(root_pth, "fs")
association_file <- import_association_file(path, root,
  dates_format = "dmy"
)
matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = association_file, root = NULL,
  quantification_type = c("fragmentEstimate", "seqCount"),
  matrix_type = "annotated", workers = 2,
  patterns = NULL, matching_opt = "ANY",
  multi_quant_matrix = FALSE
)
matrices <- remove_collisions(matrices, association_file)
options(op)
```

sample_statistics	<i>Computes user specified functions on numerical columns and updates the metadata data frame accordingly.</i>
-------------------	--

Description

[Experimental] The function operates on a data frame by grouping the content by the sample key and computing every function specified on every column in the `value_columns` parameter. After that the metadata data frame is updated by including the computed results as columns for the corresponding key. For this reason it's required that both `x` and `metadata` have the same sample key, and it's particularly important if the user is working with previously aggregated data. For example:

```

### Importing association file and matrices
path_AF <- system.file("extdata", "ex_association_file.tsv",
package = "ISAnalytics")
root_correct <- system.file("extdata", "fs.zip",
package = "ISAnalytics")
root_correct <- unzip_file_system(root_correct, "fs")

association_file <- import_association_file(path_AF, root_correct)
matrices <- import_parallel_Vispa2Matrices_auto(
association_file = association_file , root = NULL,
quantification_type = c("seqCount","fragmentEstimate"),
matrix_type = "annotated", workers = 2, patterns = NULL,
matching_opt = "ANY", dates_format = "dmy")

### Aggregating data (both by same key)
aggregated_x <- aggregate_values_by_key(matrices$seqCount,
association_file)
aggregated_meta <- aggregate_metadata(association_file)

### Sample statistics
sample_stats <- sample_statistics(x = aggregated_x,
metadata = aggregated_meta,
sample_key = c("SubjectID", "CellMarker","Tissue", "TimePoint"))

```

Usage

```

sample_statistics(
  x,
  metadata,
  sample_key = "CompleteAmplificationID",
  value_columns = "Value",
  functions = default_stats()
)

```

Arguments

x	A data frame
metadata	The metadata data frame
sample_key	Character vector representing the key for identifying a sample
value_columns	THE name of the columns to be computed, must be numeric or integer
functions	A named list of function or purrr-style lambdas

Value

A list with modified x and metadata data frames

See Also

Other Analysis functions: [CIS_grubbs\(\)](#), [comparison_matrix\(\)](#), [compute_abundance\(\)](#), [cumulative_count_union\(\)](#), [separate_quant_matrices\(\)](#), [threshold_filter\(\)](#), [top_integrations\(\)](#)

Examples

```
op <- options(ISAnalytics.widgets = FALSE)

path_AF <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_correct <- system.file("extdata", "fs.zip",
  package = "ISAnalytics"
)
root_correct <- unzip_file_system(root_correct, "fs")

association_file <- import_association_file(path_AF, root_correct,
  dates_format = "dmy"
)
matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = association_file, root = NULL,
  quantification_type = c("seqCount", "fragmentEstimate"),
  matrix_type = "annotated", workers = 2, patterns = NULL,
  matching_opt = "ANY", multi_quant_matrix = FALSE
)

stats <- sample_statistics(matrices$seqCount, association_file)
options(op)
```

```
separate_quant_matrices
```

Separate a multiple-quantification matrix into single quantification matrices.

Description

[Maturing] The function separates a single multi-quantification integration matrix, obtained via [comparison_matrix](#), into single quantification matrices as a named list of tibbles.

Usage

```
separate_quant_matrices(
  x,
  fragmentEstimate = "fragmentEstimate",
  seqCount = "seqCount",
  barcodeCount = "barcodeCount",
  cellCount = "cellCount",
  ShsCount = "ShsCount",
  key = c(mandatory_IS_vars(), annotation_IS_vars(), "CompleteAmplificationID")
)
```

Arguments

x	Single integration matrix with multiple quantification value columns, likely obtained via comparison_matrix .
fragmentEstimate	Name of the fragment estimate values column in input
seqCount	Name of the sequence count values column in input
barcodeCount	Name of the barcode count values column in input
cellCount	Name of the cell count values column in input
ShsCount	Name of the shs count values column in input
key	Key columns to perform the joining operation

Value

A named list of tibbles, where names are quantification types

See Also

[quantification_types](#)

Other Analysis functions: [CIS_grubbs\(\)](#), [comparison_matrix\(\)](#), [compute_abundance\(\)](#), [cumulative_count_union\(\)](#), [sample_statistics\(\)](#), [threshold_filter\(\)](#), [top_integrations\(\)](#)

Examples

```
op <- options("ISAnalytics.widgets" = FALSE)
path <- system.file("extdata", "ex_association_file.tsv",
  package = "ISAnalytics"
)
root_pth <- system.file("extdata", "fs.zip", package = "ISAnalytics")
root <- unzip_file_system(root_pth, "fs")
association_file <- import_association_file(
  path = path, root = root,
  dates_format = "dmy"
)
matrices <- import_parallel_Vispa2Matrices_auto(
  association_file = association_file,
  quantification_type = c("seqCount", "fragmentEstimate"),
  matrix_type = "annotated", workers = 2, patterns = NULL,
  matching_opt = "ANY"
)
separated_matrix <- separate_quant_matrices(matrices)
options(op)
```

threshold_filter	<i>Filter data frames with custom predicates</i>
------------------	--

Description

[Experimental] Filter a single data frame or a list of data frames with custom predicates assembled from the function parameters.

Usage

```
threshold_filter(x, threshold, cols_to_compare = "Value", comparators = ">")
```

Arguments

x	A data frame or a list of data frames
threshold	A numeric/integer vector or a named list of numeric/integer vectors
cols_to_compare	A character vector or a named list of character vectors
comparators	A character vector or a named list of character vectors. Must be one of the allowed values between c("<", ">", "==", "!=", ">=", "<=")

Details

A single data frame as input:

If the user chooses to operate on a single data frame, the other parameters should only be vectors: numeric vector for `threshold` and character vectors for both `cols_to_compare` and `comparators`. A filtering condition is obtained by combining element by element `cols_to_compare + comparators + threshold` (similarly to the `paste` function). For example:

```
threshold = c(20, 35, 50)
cols_to_compare = c("a", "b", "c")
comparators = "<"
```

given these vectors, the input data frame will be filtered by checking which values in column "a" are less than 20 **AND** which values in column "b" are less than 35 **AND** which values in column "c" are less than 50. Things the user should keep in mind are:

- The vectors of length 1 are going to be recycled if one or more parameters are longer (in the example, the `comparators` value)
- If vectors are not of length 1 they must have the same length
- Columns to compare, of course, need to be included in the input data frame and need to be numeric/integer
- The filtering will perform a logical "AND" on all the conditions, only rows that satisfy ALL the conditions are preserved

A list of data frames as input:

The input for the function may also be a list of data frames, either named or unnamed.


```

print(filtered)
## $first
## # A tibble: 1 x 4
##   a     b c     d
##   <dbl> <dbl> <chr> <int>
## 1    30    50 b     4
##
## $second
## # A tibble: 3 x 4
##   a     b c     d
##   <dbl> <dbl> <chr> <int>
## 1    20    40 a     3
## 2    30    50 b     4
## 3    40    60 c     5
##
## $third
## # A tibble: 2 x 4
##   a     b c     d
##   <dbl> <dbl> <chr> <int>
## 1    30    50 b     4
## 2    40    60 c     5

```

The above signature will roughly be translated as:

- Filter the element "first" in the list by checking that values in column "a" are bigger than 20 AND values in column "b" are less than 60
- Don't apply any filter to the element "second" (returns the data frame as is)
- Filter the element "third" by checking that values in column "a" are equal or bigger than 25.

It is also possible to use some parameters as vectors and some as lists: vectors will be recycled for every element filtered.

```

filtered <- threshold_filter(example_list,
                             threshold = list(first = c(20, 60),
                                                third = c(25, 65)),
                             cols_to_compare = c("a", "b"),
                             comparators = list(first = c(">", "<"),
                                                  third = c(">=", "<=")))

```

In this example, different threshold and comparators will be applied to the same columns in all data frames.

Things the user should keep in mind are:

- Names for the list parameters must be the same names in the input list
- Only elements explicited in list parameters as names will be filtered
- Lengths of both vectors and lists must be consistent

Value

A data frame or a list of data frames

See Also

Other Analysis functions: [CIS_grubbs\(\)](#), [comparison_matrix\(\)](#), [compute_abundance\(\)](#), [cumulative_count_union\(\)](#), [sample_statistics\(\)](#), [separate_quant_matrices\(\)](#), [top_integrations\(\)](#)

Examples

```
example_df <- tibble::tibble(
  a = c(20, 30, 40),
  b = c(40, 50, 60),
  c = c("a", "b", "c"),
  d = c(3L, 4L, 5L)
)
example_list <- list(
  first = example_df,
  second = example_df,
  third = example_df
)

filtered <- threshold_filter(example_list,
  threshold = list(
    first = c(20, 60),
    third = c(25)
  ),
  cols_to_compare = list(
    first = c("a", "b"),
    third = c("a")
  ),
  comparators = list(
    first = c(">", "<"),
    third = c(">=")
  )
)
```

top_integrations	<i>Sorts and keeps the top n integration sites based on the values in a given column.</i>
------------------	---

Description

[Experimental] The input data frame will be sorted by the highest values in the columns specified and the top n rows will be returned as output. The user can choose to keep additional columns in the output by passing a vector of column names or passing 2 "shortcuts":

- keep = "everything" keeps all columns in the original data frame
- keep = "nothing" only keeps the mandatory columns (mandatory_IS_vars()) plus the columns in the columns parameter.

Usage

```
top_integrations(
  x,
  n = 50,
  columns = "fragmentEstimate_sum_RelAbundance",
  keep = "everything",
  key = NULL
)
```

Arguments

x	An integration matrix (data frame containing mandatory_IS_vars())
n	How many integrations should be sliced (in total or for each group)? Must be numeric or integer and greater than 0
columns	Columns to use for the sorting. If more than a column is supplied primary ordering is done on the first column, secondary ordering on all other columns
keep	Names of the columns to keep besides mandatory_IS_vars() and columns
key	Either NULL or a character vector of column names to group by. If not NULL the input will be grouped and the top fraction will be extracted from each group.

Value

Either a data frame with at most n rows or a data frames with at most n*(number of groups) rows.

See Also

Other Analysis functions: [CIS_grubbs\(\)](#), [comparison_matrix\(\)](#), [compute_abundance\(\)](#), [cumulative_count_union\(\)](#), [sample_statistics\(\)](#), [separate_quant_matrices\(\)](#), [threshold_filter\(\)](#)

Examples

```
smpl <- tibble::tibble(
  chr = c("1", "2", "3", "4", "5", "6"),
  integration_locus = c(14536, 14544, 14512, 14236, 14522, 14566),
  strand = c("+", "+", "-", "+", "-", "+"),
  CompleteAmplificationID = c("ID1", "ID2", "ID1", "ID1", "ID3", "ID2"),
  Value = c(3, 10, 40, 2, 15, 150),
  Value2 = c(456, 87, 87, 9, 64, 96),
  Value3 = c("a", "b", "c", "d", "e", "f")
)
top <- top_integrations(smpl,
  n = 3,
  columns = c("Value", "Value2"),
  keep = "nothing"
)
top_key <- top_integrations(smpl,
  n = 3,
  columns = "Value",
  keep = "Value2",
```

```
    key = "CompleteAmplificationID"  
  )
```

unzip_file_system	<i>A utility function to unzip and use example file systems included in the package</i>
-------------------	---

Description

This utility function is a simple shortcut to create a temporary directory, unzip and reference the examples file systems included in the package for testing purposes.

Usage

```
unzip_file_system(zipfile, name)
```

Arguments

zipfile	The zipped file to decompress
name	The name of the folder in the zipped archive ("fs" or "fserr")

Value

A path to reference

See Also

Other Utility functions: [as_sparse_matrix\(\)](#), [generate_Vispa2_launch_AF\(\)](#), [generate_blank_association_file\(\)](#)

Examples

```
root_pth <- system.file("extdata", "fs.zip", package = "ISAnalytics")  
root <- unzip_file_system(root_pth, "fs")
```


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