Package 'MutSeqR'

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Title Analysis of Error-Corrected Sequencing Data for Mutation Detection

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Description Standard methods for analysis of mutation data following error-corrected sequencing (ECS) for the purpose of mutagencity assessment. Functions include importing the mutation lists provided by a variant caller, and a set of analytical tools for statistical testing and visualization of mutation data; comparison to COSMIC and/or germline signatures; etc.

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Depends R (>= 4.5.0)

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Suggests binom, BiocManager, BiocStyle, bs4Dash, BSgenome.Hsapiens.UCSC.hg38, BSgenome.Mmusculus.UCSC.mm10, car, colorspace, dendsort, doBy, DT, ExperimentHub, fmsb, fs, ggrepel, gtools, htmltools, httr, knitr, lme4, magick, MutSeqRData, openxlsx, packcircles, patchwork, RColorBrewer, reticulate, rmarkdown, scales, shiny, testthat (>= 3.0.0), trackViewer, withr, yaml, xml2

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`annotate_cpg_sites` *Annotate CpG sites*

Description

A simple method to test whether your trinucleotide context contains a CpG site. Vectorized version of Biostrings::vcountPattern is used.

Usage

```
annotate_cpg_sites(mutation_data, motif = "CG", column_query = "context", ...)
```

Arguments

`mutation_data`	A dataframe or GRanges object containing the genomic regions of interest in which to look for CpG sites.
`motif`	Default "CG", which returns CpG sites. You could in theory use an arbitrary string to look at different motifs. Use with caution. In this case the pattern being searched must be a column in the mutation data.
`column_query`	Default "context" but can be any column in the mutation data that you wish to look for a motif in.
`...`	Additional arguments to vcountPattern()

Value

A data frame with the same number of rows as there were ranges in the input, but with an additional metadata column indicating CpG sites in the target sequence of the mutation.

bmd_proast *BMD modeling using PROAST*

Description

Calculate the benchmark dose (BMD) of continuous, individual-level data with optional model averaging. This function is intended to model the dose-response of mutation frequency. This function is an extension of the PROAST software (copyright RIVM National Institute for Public Health and the Environment).

Usage

```
bmd_proast(
  mf_data,
  dose_col = "dose",
  response_col = "mf_min",
  covariate_col = NULL,
  bmr = 0.5,
  adjust_bmr_to_group_sd = FALSE,
  model_averaging = TRUE,
  num_bootstraps = 200,
  plot_results = FALSE,
  output_path = NULL,
  raw_results = FALSE,
  seed = 125
)
```

Arguments

mf_data	A data frame containing the data to be analyzed. Data should be individual for each sample. Required columns are the column containing the dose dose_col the column(s) containing the mutation frequency response_col, and the column containing the covariate covariate_col, if applicable.
dose_col	The column in mf_data containing the dose data. Values must be numeric. Default is "dose".
response_col	The column(s) in mf_data containing the mutation frequency. Multiple response_cols can be provided. Default is "mf_min".
covariate_col	The column in mf_data containing the covariate. If no covariate is present, set to NULL (default).
bmr	The Benchmark Response value. The BMR is defined as a bmr-percent change in mean response relative to the controls. Default is 0.5 (50% change).
adjust_bmr_to_group_sd	A logical value indicating whether the group standard deviation should be used as the BMR. If TRUE, the BMR will be bet set to one standard deviation above the control group mean. Default is FALSE.

`model_averaging`
: A logical value indicating whether confidence intervals should be calculated using model averaging. Default is TRUE (recommended).

`num_bootstraps`
: The number of bootstrap resamples to be used in the model averaging. Default is 200 (recommended).

`plot_results`
: A logical value indicating whether to plot the BMD models and/or the Cleveland plots. Default is FALSE. Plots may be exported directly to an output_path, or returned within a list to the user.

`output_path`
: The file path indicating where to save the plots. If NULL, the plots will automatically be displayed to the graphics window and then returned as a list alongside the bmd results.

`raw_results`
: A logical value indicating whether to return the raw results from the PROAST analysis. If FALSE, data is returned as a summary table.

`seed`
: An integer value to set the random seed for reproducibility when using model averaging. Default is 125. Use 0 for a random seed each time.

Details

This function is a modified version of the original interactive PROAST software (https://www.rivm.nl/en/proast that allows for batch processing of data. The function is designed to be used with the output of `calculate_mf` for the purpose of calculating the Benchmark Dose of mutation frequency data. As such, some functionality of the original PROAST software has been removed.

This function will accept continuous data, with an observation for each individual subject. It is assumed that data are lognormally distributed. The response data is log-transformed, then back-transformed after the statistical analysis. The function will fit model 3 or 5 from various families of models (Exponential, Hill, Inverse Exponential, LogNormal). It will then compare the fits of models 3 and 5 for each model family and select the model with the lowest AIC. The BMD 90% confidence intervals will be calculated based on the selected model (3 or 5) for each model family using the profile likelihood method. The BMD 90% confidence interval may also be calculated using the bootstrap method if `model_averaging = TRUE`. It is recommended to use 200 bootstraps for model averaging.

To replicate these results in the PROAST interactive software, select the following menu options:

1. f.proast(mf_data)
2. What type of response data do you want to consider? *1: continuous, individual data*
3. Do you want to fit a single model or fit various nested families of models? *3: select model 3 or 5 from various families of models*
4. Q1: Which variable do you want to consider as the independent variable? *# : dose_col*
5. Give number(s) of the response(s) you want to analyse. *# : response_col*
6. Give number of factor serving as potential covariate (e.g.sex) type 0 if none. *# : covariate_col*
7. Do you want to adjust CES to within group SD? *1: no, 2: yes | adjust_bmr_to_group_sd: FALSE/TRUE*
8. Give value for CES (always positive) type 0 to avoid calculation of CIs. *bmr*
9. Do you want to calculate the BMD confidence interval by model averaging? *1: no 2: yes | model_averaging: FALSE/TRUE*

10. give number of bootstrap runs for calculating BMD confidence interval based on MA (e.g. 200) *num_bootstraps*
11. Which models do you want to be fitted? *4 : previous option with lognormal DR model added*

Value

A summmary data frame of final results. If plots or raw results are selected, all data will be returned within a list.

The summary will include the following for each response variable and covariate subgroup (if applicable):

- `Model`: The m3 or m5 model selected for each model family (Exponential, Hill, Inverse Exponential, LogNormal).
- `Response`: The response variable.
- `Covariate`: The covariate subgroup, if applicable.
- `bmr`: The specified Benchmark Response.
- `BMD`: The Benchmark Dose, in original dose units, estimated for the given model.
- `BMDL`: The lower bound of the 90% confidence interval for the BMD, calculated by the profile likelihood method.
- `BMDU`: The upper bound of the 90% confidence interval for the BMD, calculated by the profile likelihood method.
- `AIC`: The Akaike Information Criterion for the selected model. Lower values indicate a better fit. It is advised to choose the BMD value from the model with the lowest AIC.
- `weights`: The weight of the model in the model averaging process, if applicable.
- `Model averaging`: The BMDL and BMDU calculated by the bootstrap method if `model_averaging = TRUE`.

If there is no significant response in the data, the function will return an empty data frame.

If `plot_results = TRUE` the function will create the following plots for each response variable. The plots will be saved to the output_path. If no output_path is provided, then they will be returned within a list alongside the summary data frame.

- Model Plots. The following plot will be created for each model family (Exponential, Hill, Inverse Exponential, LogNormal): The fitted curve of the selected (3 or 5) model. Data is log-transformed. Individual data points are plotted using small triangles. The geometric mean (median) at each dose is plotted as a large triangle. The BMD is indicated by the dotted line. If applicable, the covariate subgroup is indicated by color. When output_path = NULL, plots are returned alongside results as recordedPlot objects. View plots with replayPlot().
- bootstrap_curves If `model_averaging = TRUE`, the bootstrap curves based on model averaging. The geometric mean (median) at each dose is plotted as a large triangle. Data is log-transformed. When output_path = NULL, plots are returned alongside results as recordedPlot objects. View plots with replayPlot().
- cleveland plot if `model_averaging = TRUE` The BMD estimate for each model is plotted as a red point alongside the 90% confidence intervals. The size of the BMD point represents the model weight assigned during model averaging, based on the AIC. When output_path = NULL, plots are returned alongside results as ggplots. View plots by calling object name.

If raw_results = TRUE, the function will return the raw results of the PROAST analysis alongside the summary data frame. PROAST raw_results is a list of variables and data that is continuously modified as it is passed through the proast functions. It can be given to f.proast() to resume analysis.

Examples

```
# Example data  consists of 24 mouse bone marrow
# samples exposed to three doses of BaP alongside vehicle controls.
# Libraries were sequenced with Duplex Sequencing using
# the TwinStrand Mouse Mutagenesis Panel which consists of 20 2.4kb
# targets = 48kb of sequence. Example data can be retrieved from
# MutSeqRData, an ExperimentHub data package:
## library(ExperimentHub)
## eh <- ExperimentHub()
## query(eh, "MutSeqRData")
# Mutation frequency data was precalculated using
## mf_data_global <- calculate_mf(mutation_data = eh[["EH9861"]],
##   cols_to_group = "sample",
##   retain_metadata_cols = c("dose_group", "dose"))
mf_example <- readRDS(system.file("extdata/Example_files/mf_data_global.rds",
  package = "MutSeqR"
))
# We will calculate the BMD for a 50% increase in mutation frequency from
# control with Model averaging.
# For the purpose of this example, num_bootstraps is set to 3 to reduce
# run time. 200 bootstraps is recommended.
bmd <- bmd_proast(
  mf_data = mf_example,
  dose_col = "dose",
  response_col = "mf_min",
  bmr = 0.5,
  model_averaging = TRUE,
  num_bootstraps = 3
)
```

BS_org_map *BS genome organism dictionary*

Description

A dictionary to map common organism names to their corresponding BSgenome organism names.

Usage

```
BS_org_map
```

Format

A list with organism names as keys and corresponding BSgenome organism names as values.

Examples

```
BS_org_map["Hsapiens"]
```

`calculate_mf` *Calculate mutation frequency*

Description

Calculates mutation frequencies for arbitrary groupings and creates a new dataframe with the results. Mutation frequency is calculated by dividing the sum of mutations by the sum of the total_depth for a given group (mutations/bp). The operation is run using both the minimum and maximum independent mutation counting methods.

Usage

```
calculate_mf(
  mutation_data,
  cols_to_group = "sample",
  subtype_resolution = "none",
 variant_types = c("snv", "deletion", "insertion", "complex", "mnv", "sv", "ambiguous",
    "uncategorized"),
  calculate_depth = TRUE,
  correct_depth = TRUE,
  correct_depth_by_indel_priority = FALSE,
  precalc_depth_data = NULL,
  d_sep = "\t",
  summary = TRUE,
  retain_metadata_cols = NULL
)
```

Arguments

`mutation_data`	The data frame (or GRanges) to be processed containing mutation data. Required columns are listed in details.
`cols_to_group`	A vector of grouping variables. This should be the groups of interest that you want to calculate a frequency for. For instance, getting the frequency by `"sample"`. Other options might include an experimental group Ex. `"dose"` or a locus Ex. `c("sample", "locus")`. All listed variables must be a column in the mutation_data. Do not include mutation subtype columns in this field. Please refer to subtype_resolution to group by subtype as the calculation will differ.
`subtype_resolution`	The degree at which to resolve the mutation subtypes when calculating frequencies. Mutation frequency will be calculated across all col_to_groups for each mutation subtype given the desired resolution. Subtype proportions will also be calculated. Options are "none", "type", "base_6", "base_12", "base_96", and "base_192". See details for definitions.

variant_types Use this parameter to choose which variation types to include in the mutation counts. Provide a character vector of the variation types that you want to include. Alternatively, provide a character vector of the variation types that you want to exclude preceded by "-". Options are: "snv", "complex", "deletion", "insertion", "mnv", "sv", "ambiguous", "uncategorized". Ex. inclusion: "snv", exclusion: "-snv". Default includes all variants. For `calculate_depth = TRUE`: Regardless of whether or not a variant is included in the mutation counts, the total_depth for that position will be counted.

calculate_depth
A logical variable, whether to calculate the per-group total_depth from the mutation data. If set to TRUE, the mutation data must contain a total_depth value for every sequenced base (including variants AND no-variant calls). If set to FALSE, pre-calculated per-group total_depth values may be supplied at the desired subtype_resolution using the precalc_depth_data parameter. Alternatively, if no per-group total_depth is available, per-group mutation counts will be calculated, but mutation frequency will not. In such cases, mutation subtype proportions will not be normalized to the total_depth.

correct_depth A logical value. If TRUE, the function will correct the `total_depth` column in `mutation_data` in order to prevent double-counting the `total_depth` values for the same genomic position. For rows with the same sample, contig, and start values, the `total_depth` will be retained for only one row. All other rows in the group will have their `total_depth` set to 0. The default is TRUE.

correct_depth_by_indel_priority
A logical value. If TRUE, during depth correction, should there be different `total_depth` values within a group of rows with the same sample, contig, and start values, the `total_depth` value for the row with the highest priority `variation_type` will be retained, while the other rows will have their `total_depth` set to 0. `variation_type` priority order is: deletion, complex, insertion, snv, mnv, sv, uncategorised, ambiguous, no_variant. If FALSE, the `total_depth` value for the first row in the group will be retained, while the other rows will have their `total_depth` set to 0. The default is FALSE.

precalc_depth_data
A data frame or a file path to a text file containing pre-calculated per-group total_depth values. This data frame should contain the columns for the desired grouping variable(s) and the reference context at the desired subtype resolution (if applicable). The precalculated total_depth column(s) should be called one of `group_depth` and `subtype_depth`. `group_depth` is used for subtype resolutions of "none", "type", and all non-snv mutations in "base_6", "base_12", "base_96", and "base_192". `subtype_depth` is used for snv mutations in "base_6", "base_12", "base_96", and "base_192". You can access a list of context values for each subtype resolution using `MutSeqR::context_list$your_subtype_resolution`.

d_sep The delimiter used in the precalc_depth_data, if applicable. Default is tab-delimited.

summary A logical variable, whether to return a summary table (i.e., where only relevant columns for frequencies and groupings are returned). Setting this to false returns all columns in the original mutation_data, which might make plotting more difficult, but may provide additional flexibility to power users.

`retain_metadata_cols`
: a character vector that contains the names of the metadata columns that you would like to retain in the summary table. This may be useful for plotting your summary data. Ex. retain the "dose" column when summarising by "sample".

Details

Required columns:

- `contig`: (or `seqnames`) The reference sequence name.
- `start`: 1-based start position of the feature.
- `alt_depth`: The read depth supporting the alternate allele.
- `variation_type`: The category to which this variant is assigned.
- subtype_col: The column containing the mutation subtype. This column depends on the `subtype_resolution` parameter.
- reference context: The column containing the referene base(s) for the mutation. This column depends on the `subtype_resolution` parameter.
- cols to group: all columns across which you want to calculate the mutation frequency. Ex. `c("tissue", "dose")`. These columns should be listed in cols_to_group.

It is also required to include the total_depth column if you are calculating depth from the mutation data. If you are using precalculated depth data, the total_depth column is not required.

Subtype Resolutions:

- "none" calculates mutation frequencies across all selected grouping columns.
- "type" calculates mutation frequencies across all selected grouping columns for each `variation_type` seperately; snv, mnv, deletion, insertion, complex, sv, ambiguous, uncategorized.
- "base_6" calculates mutation frequencies across all selected grouping columns for each variation_type with snv mutations separated by `normalized_subtype`; C>A, C>G, C>T, T>A, T>C, T>G. The reference context is `normalized_ref`.
- "base_12" calculates mutation frequencies across all selected grouping columns for each variation_type with snv mutations separated by `subtype`; A>C, A>G, A>T, C>A, C>G, C>T, G>A, G>C, G>T, T>A, T>C, T>G. The reference context is `short_ref`.
- "base_96" calculates mutation frequencies across all selected grouping columns for each variation_type with snv mutations separated by `normalized_context_with_mutation`, i.e. the 96-base trinucleotide context. Ex. A[C>T]A. The reference context is `normalized_context`.
- "base_192" calculates mutation frequencies across all selected grouping columns for each variation_type with snv mutations separated by `context_with_mutation`, i.e. the 192-base trinucleotide context. Ex A[G>A]A. The reference context is `context`.

Subtype depth: For SNV subtypes, the total_depth is summed based on the sequence context in which the SNV subtype occurs. Ex. for base_6, the two possible reference bases are C or T; hence, the total_depth is summed seperately for C:G positions and T:A positions. The MF for C>T mutations is calculated as total # C>T mutations / total_depth for C>G positions (sum / subtype_depth). Non-SNV mutation types will be caluclated as their sum / group_depth, since they can occur in the context of any nucleotide.

retain_metadata_cols at subtype_resolution: The summary table uses a pre-defined list of possible subtypes for each resolution. If a particular subtype within a given group is not recorded in the mutation data, the summary table will have no frame of reference for populating the metadata_cols. Thus, for subtypes that do not occur in the mutation data for a given group, the corresponding metadata_col will be NA.

Variant filtering: Variants flagged as TRUE in the `filter_mut` column will be excluded from the mutation counts. However, the total_depth of these variants will be included in the group/subtype depths if calculating depth.

Depth correction is important for preventing double-counting of reads in mutation data when summing the total_depth across samples or other groups. Generally, when several mutations have been detected at the same genomic position, within a sample, the total_depth value will be the same for all of them. However, in some datasets, whenever a deletion is detected, the data may contain an additional row with the same genomic position calling a "no_variant". The total_depth will differ between the deletion and the no_variant. In these cases, correct_depth_by_indel_priority == TRUE will ensure that the total_depth value for the deletion is retained, while the total_depth value for the no_variant is removed.

Value

A data frame with the mutation frequency calculated. If summary is set to TRUE, the data frame will be a summary table with the mutation frequency calculated for each group. If summary is set to FALSE, the mutation frequency will be appended to each row of the original mutation_data.

- `sum_min`: The sum of all mutations within the group, calculated using the "min" method for mutation counting. All identical mutations within a samples are assumed to be the result of clonal expansion and are thus only counted once.
- `sum_max`: The sum of all mutations within the group, calculated using the "max" method for mutaiton counting. All identical mutations within a sample are assumed to be idenpendant mutational evens and are included in the mutation frequency calculation.
- `group_depth`: The total_depth summed across groups.
- `subtype_depth`: The total_depth summed across groups for a given sequence context. Used for calculating subtype frequencies.
- `mf_min`: The mutation frequency calculated using the "min" method for mutation counting. mf_min = sum_min / depth.
- `mf_max`: The mutation frequency calculated using the "max" method for mutation counting. mf_max = sum_max / depth.
- `proportion_min`: The proportion of each mutation subtype within the group, normalized to the depth. Calculated using the "min" method. This is only calculated if `subtype_resolution` is not "none". If no depth is calculated or provided, proportion is calculated without normalization to the depth.
- `proportion_max`: The proportion of each mutation subtype within the group, normalized to its read depth. Calculated using the "max" method. This is only calculated if `subtype_resolution` is not "none". If no depth is calculated or provided, proportion is calculated without normalization to the depth.

Examples

```
# Mutation data is just for example purposes. It does not reflect real data
mutation_data <- readRDS(system.file("extdata", "Example_files",
                                     "filtered_simple_mutation_data.rds",
                                     package = "MutSeqR"))
# Calculate mutation frequency by sample.
# Calculate depth from the mutation data (default)
# Correct the Depth (default) with indel priority (set)
mf_example <- calculate_mf(
  mutation_data = mutation_data,
  cols_to_group = "sample",
  correct_depth_by_indel_priority = TRUE
)
```

---

characterize_variants *Characterize Variants*

Description

This function generates additional columns for the mutation data, including a breakdown of the mutation subtypes at various resolutions.

Usage

```
characterize_variants(mutation_data)
```

Arguments

mutation_data	A data frame containing mutation data.

Value

A data frame with additional columns for variant characterization.

---

check_required_columns *Check that all required columns are present before proceeding with the function*

Description

A utility function that will check that all required columns are present.

Usage

```
check_required_columns(data, required_columns)
```

Arguments

data	mutation data
required_columns	a list of required column names.

Value

an error

Examples

```
df <- data.frame(
  contig = c("chr1", "chr2", "chr3"),
  start = c(100, 200, 300),
  end = c(100, 200, 300),
  sample = c("S1", "S2", "S3"),
  ref = c("G", "C", "T"),
  alt = c("A", "T", "G")
)
check_required_columns(df, required_columns = op$base_required_mut_cols)
```

---

classify_variation *classify_variation*

Description

Classify the variation type of a mutation based on its ref and alt values.

Usage

```
classify_variation(ref, alt)
```

Arguments

ref	The reference allele.
alt	The alternate allele.

Value

A character indicating the type of variation.

Examples

```
df <- data.frame(
  ref = c("A", "CAGT", "GCC", "T", "ACG", "C", "G", "T", "A"),
  alt = c("R", "TGA", "G", "TC", "TAC", "C", "<DEL>", "G", "???")
)
df$variation_type <- mapply(classify_variation, df$ref, df$alt)
df
```

cleveland_plot *Cleveland Plot*

Description

Make a Cleveland plot for the PROAST results.

Usage

```
cleveland_plot(results, covariate_col = NULL, output_path = NULL)
```

Arguments

results	PROAST results object.
covariate_col	Covariate column name.
output_path	Output path for the plot. If the output_path doesn't exist, it will be created. If NULL, the plots will not be exported.

Value

A single ggplot object with facets for each response.

cluster_spectra *Hierarchical Clustering*

Description

perform hierarchical clustering of samples based on the mutation spectra.

Usage

```
cluster_spectra(
  mf_data,
  group_col = "sample",
  response_col = "proportion_min",
  subtype_col = "normalized_subtype",
  dist = "cosine",
  cluster_method = "ward.D"
)
```

Arguments

mf_data	A data frame containing the mutation data. This data must include a column containing the mutation subtypes, a column containing the sample/cohort names, and a column containing the response variable.
group_col	The name of the column in data that contains the sample/cohort names.
response_col	The name of the column in data that contains the response variable. Typical response variables can be the subtype mf, proportion, or count.
subtype_col	The name of the column in data that contains the mutation subtypes.
dist	the distance measure to be used. This must be one of "cosine", "euclidean", "maximum", "manhattan","canberra", "binary" or "minkowski". See dist for details.
cluster_method	The agglomeration method to be used. See hclust for details.

Details

The cosine distance measure represents the inverted cosine similarity between samples:

Cosine Dissimilarity $= 1 - \frac{\mathbf{A} \cdot \mathbf{B}}{\|\mathbf{A}\| \cdot \|\mathbf{B}\|}$

This equation calculates the cosine dissimilarity between two vectors A and B.

Leaves are sorted using dendsort, if installed, otherwise leaves are unsorted.

Value

A dendrogram object representing the hierarchical clustering of the samples.

---

context_list *A list of reference contexts at different resolutions*

Description

A list of reference contexts at different resolutions

Usage

```
context_list
```

Format

A list with corresponding values

Examples

```
context_list[["base_6"]]
context_list[["base_12"]]
```

denominator_dict *Values used for denominators in frequency calculations*

Description

These values are used to cross reference base substitution types to their appropriate denominators for calculations. That is", "for example, the 6 base substitution frequency should be subsetted based on the normalized_ref column which would contain only T or C (i.e., the pyrimidine context for base substitutions).

Usage

```
denominator_dict
```

Format

A vector with corresponding values

Examples

```
denominator_dict["type"]
```

dm_loglik_ridge_stable *dm_loglik_ridge_stable*

Description

Computes the robust, ridge-penalized log-likelihood for a Dirichlet-Multinomial distribution. It safely handles massive counts utilizing lgamma components and matrix operations. The ridge penalty shrinks probability vectors toward a uniform distribution to stabilize optimization for sparse or heavily overdispersed data.

Usage

```
dm_loglik_ridge_stable(x, p, theta, lambda = 1)
```

Arguments

x	A numeric matrix of mutational counts (subtypes as rows, samples as columns).
p	A numeric vector representing the expected probability of each subtype.
theta	Numeric scalar. The overdispersion parameter (concentration parameter). Larger values of theta indicate lower overdispersion (approaching multinomial).
lambda	Numeric scalar. The strength of the ridge penalty. Default is 1.0. Set to 0 for unpenalized (raw) likelihood calculation.

Value

A numeric scalar representing the computed log-likelihood value, adjusted by the penalty term.

`f.plot.gui` *Manages plotting for PROAST*

Description

Runs through the plotting functions depending on data type and plot type.

Usage

```
f.plot.gui(
  ans.all,
  HTML = FALSE,
  model.summ = TRUE,
  display_plots = TRUE,
  .proast_env = NULL,
  output_type = NULL,
  filename = NULL,
  interactive_mode = TRUE,
  knitting = FALSE,
  return_plots = FALSE
)
```

Arguments

`ans.all`	The proast object that gets passed to all functions.
`HTML`	Keep FALSE
`model.summ`	Keep TRUE
`display_plots`	A logical variable - whether we want to display the plots or not.
`.proast_env`	environment
`output_type`	The format that you wish to export the plots as.
`filename`	The name of the file to be read.
`interactive_mode`	A TRUE/FALSE value specifying whether you want to run interactively (i.e., TRUE, the default) or using command-line mode (i.e., FALSE, non-interactive). If FALSE, you must provide all other parameters.
`knitting`	A TRUE/FALSE value specifying whether you are knitting a document. If TRUE, the function will adjust the plot display accordingly.
`return_plots`	A logical variable indicating whether you want to return the plots as a list (TRUE) or not (FALSE, the default). If TRUE, the function will return a list of recorded plots.

Value

Either the proast object (default) or a list of recorded plots if return_plots = TRUE.

`f.plot.result` *Plot the PROAST results*

Description

Independently generate the model plots from the raw results.

Usage

```
f.plot.result(
  proast_results_list,
  output_path = NULL,
  output_type = "svg",
  prefix = NULL,
  model_averaging = FALSE
)
```

Arguments

`proast_results_list`	The raw results list. This is the output of `f.proast`
`output_path`	Where to save the exported plots. A file path. If the output_path is NULL, plots will be saved to the working directory. If the output_path doesn't exist, it will be created.
`output_type`	How do you want to output the plots. If "none", the plots will be displayed to the graphics window and returned as a list. Plots can be replayed using replayPlot(). Alternatively, the plots may be saved to file. Options are 'svg', 'jpeg', 'pdf', 'png', or 'tiff'. Plots will be save to the output_path.
`prefix`	A custom prefix to append to the file names of exported plots. Plot names are PROAST_modeltype.
`model_averaging`	A logical variable indicating whether you want to generate the model averaging figure (TRUE) or the plots of the individual models (FALSE). You plot one or the other, not both. Plotting the model averaging figure will require the function to re-run the bootstrapping so it might take a while. You may think this seems rather inefficient. Well, it is, but I'm too tired to fix it, so we all just have to deal with it for now.

Value

Generates plots. Either saves them to an output path or records them and returns them as a list.

`f.proast` *Run dose-response modeling using PROAST.*

Description

Run dose-response modeling using PROAST.

Usage

```
f.proast(
  odt = list(),
  ans.all = 0,
  er = FALSE,
  resize = FALSE,
  scale.ans = FALSE,
  const.var = FALSE,
  show.warnings = FALSE,
  interactive_mode = TRUE,
  datatype = NULL,
  model_choice = NULL,
  setting_choice = NULL,
  nested_model_choice = NULL,
  indep_var_choice = NULL,
  Vyans_input = NULL,
  covariates = NULL,
  custom_CES = 0.05,
  model_selection = NULL,
  lower_dd = NULL,
  upper_dd = NULL,
  selected_model = NULL,
  adjust_CES_to_group_SD = NULL,
  model_averaging = NULL,
  num_bootstraps = NULL,
  display_plots = TRUE,
  add_nonzero_val_to_dat = FALSE,
  nonzero_val = NULL,
  detection_limit = NULL,
  seed = 125
)
```

Arguments

`odt`	List. Internal state object/list passed between PROAST functions. Usually, users do not need to set this.
`ans.all`	Output from a previous fit, or internal results object. Used to resume or adjust analyses. Usually 0 (default) to start a new session.

`er` Logical. If TRUE, attempt to resume analysis from previously stored state. Used internally/recoverably. Defaults to FALSE.

`resize` Logical. If TRUE, resize the graphics window during execution; passed to graphics helper functions. Defaults to FALSE.

`scale.ans` Logical. If TRUE, applies scaling to the answers/results (advanced use only). Defaults to FALSE.

`const.var` Logical. If TRUE, constrains variance during model fitting (advanced option for troubleshooting). Defaults to FALSE.

`show.warnings` Logical. If TRUE, print extra warning messages during model fitting (for debugging or detailed output). Defaults to FALSE.

`interactive_mode` A TRUE/FALSE value specifying whether you want to run interactively (i.e., TRUE, the default) or using command-line mode (i.e., FALSE, non-interactive). If FALSE, you must provide all other parameters.

`datatype` Non-interactive mode parameter. What type of response data do you want to consider? Currently only 'continuous, individual data'is supported.

`model_choice` Non-interactive mode parameter. Do you want to fit a single model or fit various nested families of models? Options are 'single model', 'select model 3 or 5 from various families of models', 'select model 3 from various nested families of models', 'select model 5 from various nested families of models', 'select model 15 in terms of RPF'. Recommended: 'select model 3 or 5 from various families of models'.

`setting_choice` Non-interactive mode parameter. Do you want to fit a set of models, or choose a single model? Options are 'single model', 'set of models'. Recommended: 'set of models'.

`nested_model_choice` Non-interactive mode parameter. Which subset of nested models to fit, if changing model settings non-interactively. Options match those provided in interactive menus. See details in documentation.

`indep_var_choice` Non-interactive mode parameter. The column name for the independent variable to use.

`Vyans_input` Non-interactive mode parameter. The column name(s) for the response variable(s) to use. If multiple, provide as a vector.

`covariates` Non-interactive mode parameter. The column name for the covariate to use. If none, enter 0.

`custom_CES` Non-interactive mode parameter. The critical effect size (BMR) to use, when adjust_CES_to_group_SD = 1 (FALSE).

`model_selection` Non-interactive mode parameter. The model selection to use. Options are "Exponential model only", "Exponential and Hill model", "previous option with inverse exponential model added" (run Expon, Hill, and Inv-Expon), "previous option with lognormal DR model added" (run Expon, Hill, Inv-Expon, and LN). Recommended: "previous option with lognormal DR model added".

`lower_dd` Non-interactive mode parameter. The lower constraint on d parameter. If NULL, existing defaults are used.

`upper_dd` Non-interactive mode parameter. The upper constraint on d parameter. If NULL, existing defaults are used.

`selected_model` Non-interactive mode parameter. Which model do you want to continue with? Options are "exponential", "Hill", "inverse exponential", "lognormal DR". The function will output results for all models regardless of this choice. Really just to bypass the menu option. Recommended: "exponential".

`adjust_CES_to_group_SD` Non-interactive mode parameter. Set the BMR to the group standard deviation. Options are 1 (FALSE) or 2 (TRUE).

`model_averaging` Non-interactive mode parameter. Whether to perform model averaging to calculate 90% confidence intervals. TRUE/FALSE.

`num_bootstraps` Non-interactive mode parameter. The number of bootstraps to perform for model averaging. Recommended: 200.

`display_plots` Non-interactive mode parameter. Whether to display plots. TRUE/FALSE.

`add_nonzero_val_to_dat` Non-interactive mode parameter. When the response data contains 0s, whether to add a non-zero value to each observation. TRUE/FALSE. If TRUE, set the nonzero_val parameter with your desired (positive) number. If FALSE, a detection limit will used. Provide the detection limit in the detection_limit parameter. If no detection_limit is given, the function will use the minimum non-zero value in the data. Values below the detection limit will be plotted as half the detection limit.

`nonzero_val` Non-interactive mode parameter. The non-zero value to add to each observation when add_nonzero_val_to_dat = TRUE. Must be a positive number.

`detection_limit` Non-interactive mode parameter. The detection limit to use when add_nonzero_val_to_dat = FALSE. If NULL, the minimum non-zero value in the data will be used. This paramater accepts a numeric value, which will be applied to all response values, or a column name in the data, which will be used to apply different detection limits to different observations.

`seed` Integer. Random seed for reproducibility. Defaults to 125. Use 0 to get a random seed each time.

Value

Results from PROAST.

`filter_mut` *Filter your mutation data*

Description

This function creates a `filter_mut`` column that will be read by the \code{calculate_mf} function and other d
ter_mut == TRUE“ will be excluded from group mutation counts. This function may also remove records upon on user specification. Running this function again on the same data will not overide the previous filters. To reset previous filters, set the filter_mut column values to FALSE.

Usage

```
filter_mut(
  mutation_data,
  vaf_cutoff = 1,
  snv_in_germ_mnv = FALSE,
  rm_abnormal_vaf = FALSE,
  custom_filter_col = NULL,
  custom_filter_val = NULL,
  custom_filter_rm = FALSE,
  regions = NULL,
  regions_filter,
  allow_half_overlap = FALSE,
  rg_sep = "\t",
  is_0_based_rg = TRUE,
  rm_filtered_mut_from_depth = FALSE,
  return_filtered_rows = FALSE
)
```

Arguments

`mutation_data`	Your mutation data. This can be a data frame or a GRanges object.
`vaf_cutoff`	Filter out ostensibly germline variants using a cutoff for variant allele fraction (VAF). Any variant with a `vaf` larger than the cutoff will be filtered. The default is 1 (no filtering). It is recommended to use a value of 0.01 (i.e. 1%) as a conservative approach to retain only somatic variants.
`snv_in_germ_mnv`	Filter out snv variants that overlap with germline mnv variants within the same samples IF they show the same variation at the same position. mnv variants will be considered germline if their vaf > vaf_cutoff. Default is FALSE. Ex. Position 101-103 MNV is CAG > TGG. SNV at position 101 C>T will be filtered out but SNV at position 101 C>A will not be filtered out. Helps identify sequencing artifacts generated by N-calls in MNVs.
`rm_abnormal_vaf`	A logical value. If TRUE, rows in `mutation_data` with a variant allele fraction (VAF) between 0.05 and 0.45 or between 0.55 and 0.95 will be removed.

We expect variants to have a VAF ~0. 0.5, or 1, reflecting rare somatic mutations, heterozygous germline mutations, and homozygous germline mutations, respectively. Default is FALSE.

`custom_filter_col`
: The name of the column in mutation_data to apply a custom filter to. This column will be checked for specific values, as defined by `custom_filter_val`. If any row in this column contains one of the specified values, that row will either be flagged in the `filter_mut` column or, if specified by `custom_filter_rm`, removed from mutation_data.

`custom_filter_val`
: A set of values used to filter rows in `mutation_data` based on `custom_filter_col`. If a row in `custom_filter_col` matches any value in `custom_filter_val`, it will either be set to TRUE in the `filter_mut` column or removed, depending on `custom_filter_rm`.

`custom_filter_rm`
: A logical value. If TRUE, rows in custom_filter_col that match any value in custom_filter_val will be removed from the mutation_data. If FALSE, `filter_mut` will be set to TRUE for those rows.

`regions`
: Remove rows that are within/outside of specified regions. `regions` can be either a file path, a data frame, or a GRanges object containing the genomic ranges by which to filter. File paths will be read using the rg_sep. Users can also choose from the built-in TwinStrand's Mutagenesis Panels by inputting "TSpanel_human", "TSpanel_mouse", or "TSpanel_rat". Required columns for the regions file are "contig", "start", and "end". In a GRanges object, the required columns are "seqnames", "start", and "end".

`regions_filter`
: Specifies how the provided `regions` should be applied to `mutation_data`. Acceptable values are "remove_within" or "keep_within". If set to "remove_within", records that fall within the specified regions wil be removed from mutation_data. If set to "keep_within", only records within the specified regions will be kept in mutation_data, and all other records will be removed.

`allow_half_overlap`
: A logical value. If TRUE, records that start or end in your `regions`, but extend outside of them in either direction will be included in the filter. If FALSE, only records that start and end within the `regions` will be included in the filter. Default is FALSE.

`rg_sep`
: The delimiter for importing the custom_regions. The default is tab-delimited "\t".

`is_0_based_rg`
: A logical variable. Indicates whether the position coordinates in `regions` are 0 based (TRUE) or 1 based (FALSE). If TRUE, positions will be converted to 1-based (start + 1). Need not be supplied for TSpanels. Default is TRUE.

`rm_filtered_mut_from_depth`
: A logical value. If TRUE, the function will subtract the `alt_depth` of records that were flagged by the `filter_mut` column from their `total_depth`. This will treat flagged variants as No-calls. This will not apply to variants flagged as germline by the `vaf_cutoff`. However, if the germline variant has additional filters applied, then the subtraction will still occur. If FALSE, the `alt_depth` will be retained in the `total_depth` for all variants. Default is FALSE.

return_filtered_rows
: A logical value. If TRUE, the function will return both the filtered mutation data and the records that were removed/flagged in a seperate data frame. The two dataframes will be returned inside a list, with names mutation_data and filtered_rows. Default is FALSE.

Value

A data frame or a list of two data frames, depending on the value of return_filtered_rows. If return_filtered_rows is FALSE (default), a data frame of the same structure as mutation_data is returned, with an additional column, filter_mut, indicating whether each record has been flagged for filtering (TRUE) or not (FALSE). If return_filtered_rows is TRUE, a list containing two data frames is returned. The first data frame, named mutation_data, is the filtered mutation data as described above. The second data frame, named filtered_rows, contains all records that were either removed from mutation_data or flagged with filter_mut == TRUE.

Examples

```
# Mutation data is just for example purposes. It does not reflect real data.
mutation_data <- readRDS(system.file("extdata", "Example_files",
                                     "simple_mutation_data.rds",
                                     package = "MutSeqR"))
  # In this example, we will apply the following filters:
  # 1) Filter out putative germline variants using a VAF cutoff of 0.01
  # 2) Flag snv variants that overlap with germline mnv variants and
  # 3) Subtract the alt_depth of these variants from their total_depth
  #    (treat them as No-calls).
  filter_example <- filter_mut(
    mutation_data = mutation_data,
    vaf_cutoff = 0.01,
    snv_in_germ_mnv = TRUE,
    rm_filtered_mut_from_depth = TRUE,
    return_filtered_rows = FALSE
  )
 # Flagging germline mutations...
 # Found 15 germline mutations.
 # Flagging SNVs overlapping with germline MNVs...
 # Found 1 SNVs overlapping with germline MNVs.
 # Removing filtered mutations from the total_depth...
 # Filtering complete.
```

---

find_BS_genome *Find the appropriate BS genome for the specified organism and genome.*

Description

This function will browse available BSgenomes, indicating which one should be installed for the specified organism and genome assembly version. If you cannot specify both organism and genome, the function can return a list of available genomes for a specified species.

Usage

```
find_BS_genome(organism, genome, masked = FALSE)
```

Arguments

organism	the name of the organism for which to install the reference genome. This can be the scientific name or a common name. For example Homo Sapiens, H. sapiens, or human
genome	The reference genome assembly version. Ex. hg18, mm10, rn6.
masked	Logical value. Whether to search for the 'masked' BSgenome. Default is FALSE.

Value

a BSgenome package name or a dataframe of possibilities

Examples

```
# Find the reference genome for Mouse, mm10 assembly:
mouse_mm10 <- find_BS_genome("mouse", "mm10")
```

---

get_binom_ci *Add binomial confidence intervals to mutation frequencies.*

Description

Uses the binomial distribution to create confidence intervals for mutation frequencies calculated from a single point estimate. Calculating binomial confidence intervals for mutation frequencies is not part of MutSeqR's recommended workflow, but is provided here for users who wish to use it.

Usage

```
get_binom_ci(
  mf_data,
  sum_col = "sum_min",
  depth_col = "group_depth",
  conf_level = 0.95,
  method = "wilson"
)
```

Arguments

mf_data	The data frame containing the mutation frequencies per sample. Obtained as an output from `calculate_mf`.
sum_col	Column name that specifies the mutation count (e.g., sum_min)
depth_col	Column name that specifies the sequencing depth (e.g., total_depth)

conf_level	Confidence interval to calculate, default 95% (0.95)
method	The method used by binom::binom.confint to calculate intervals. Default is "wilson" (recommended).

Value

A mf data frame with added columns indicating the confidence intervals.

Examples

```
# Example data  consists of 24 mouse bone marrow
# samples exposed to three doses of BaP alongside vehicle controls.
# Libraries were sequenced with Duplex Sequencing using
# the TwinStrand Mouse Mutagenesis Panel which consists of 20 2.4kb
# targets = 48kb of sequence. Example data can be retrieved from
# MutSeqRData, an ExperimentHub data package:
## library(ExperimentHub)
## eh <- ExperimentHub()
## query(eh, "MutSeqRData")
# Mutation frequency data was precalculated using
## mf_data_global <- calculate_mf(mutation_data = eh[["EH9861"]],
##   cols_to_group = "sample",
##   retain_metadata_cols = c("dose_group", "dose"))

 mf <- readRDS(system.file("extdata", "Example_files",
                           "mf_data_global.rds",
                           package = "MutSeqR"))
 confint <- get_binom_ci(
   mf_data = mf,
    sum_col = "sum_min",
    depth_col = "group_depth"
  )
```

---

get_cpg_mutations *Get mutations at CpG sites.*

Description

Needs to be reworked for variants >1bp. Subset the mutation data and return only mutations that are found at positions with a specific motif. The default is CpG sites, but can be customizable.

Usage

```
get_cpg_mutations(
  mutation_data,
  regions,
  variant_types = c("-no_variant"),
  motif = "CG",
  filter_mut = TRUE
)
```

Arguments

mutation_data	A dataframe or GRanges object containing the mutation data to be interrogated. If supplying a data frame, the genomic coordinates must be 1-based (true for mutation data imported using import_mut_data or import_vcf_data).
regions	A GRanges object containing the genomic regions of interest in which to look for CpG sites. Must have the metadata column "sequence" populated with the raw nucleotide sequence to search for CpGs. This object can be obtained using the get_seq.R function.
variant_types	Use this parameter to choose which variation_types to include in the output. Provide a character vector of the variation _types that you want to include. Options are "ambiguous", "complex", "deletion", "insertion", "mnv", "no_variant", "snv", "sv", "uncategorized". Alternatively, provide a character vector of the variation_types that you want to exclude preceded by "-". All variation_types except those excluded will be returned. Ex. inclusion: variant_types = "snv", will return only rows with variation_type == "snv". Ex. exclusion: variant_types = "-no_variant" will return all rows, except those with variation_type == "no_variant" (default).
motif	Default "CG", which returns CpG sites. You could in theory use an arbitrary string to look at different motifs. Use with caution.
filter_mut	A logical value indicating whether the function should exclude rows flagged in the filter_mut column from the output. Default is TRUE.

Value

A GRanges object where each range is a mutation at a CpG site (a subset of mutations from the larger object provided to the function).

---

get_cpg_regions *Get the coordinates of the CpG sites within your genomic regions*

Description

Filters the ranges of your genomic regions to find all positions with a specific motif. The default is CpG sites, but can be customizable.

Usage

```
get_cpg_regions(regions, motif = "CG")
```

Arguments

regions	A GRanges object containing the genomic regions of interest in which to look for CpG sites. Must have the metadata column "sequence" populated with the raw nucleotide sequence to search for CpGs. This object can be obtained using the get_seq() function.
motif	Default "CG", which returns CpG sites. You could in theory use an arbitrary string to look at different motifs. Use with caution.

Value

A GRanges object where each range is a CpG site (a subset of ranges from the larger object provided to the function).

`get_mutation_palette` *Get Mutation Palette*

Description

Internal helper function to determine the color palette based on resolution. Supports types, base_6, base_12, base_96, base_192

Usage

```
get_mutation_palette(custom_palette = NULL, subtype_resolution, num_colours)
```

`get_ref_of_mut` *A utility function that will return the reference context of a mutation*

Description

A utility function that will return the reference context of a mutation

Usage

```
get_ref_of_mut(mut_string)
```

Arguments

`mut_string`	the mutation. Ex. T>C, `A[G>T]C`

Value

the reference context of the mutation

get_seq *Get sequence of genomic target regions*

Description

Create a GRanges object from the genomic target ranges and import raw nucleotide sequences.

Usage

```
get_seq(
  regions,
  rg_sep = "\t",
  is_0_based_rg = TRUE,
  padding = 0,
  BS_genome = NULL,
  ucsc = FALSE,
  species = NULL,
  genome = NULL
)
```

Arguments

regions	The regions metadata file to import. Can be either a file path, a data frame, or a GRanges object. File paths will be read using the rg_sep. Users can also choose from the built-in TwinStrand's Mutagenesis Panels by inputting "TSpanel_human", "TSpanel_mouse", or "TSpanel_rat". Required columns for the regions file are "contig", "start", and "end". In a GRanges object, the required columns are "seqnames", "start", and "end".
rg_sep	The delimiter for importing the regions file. The default is tab-delimited ("\t").
is_0_based_rg	A logical variable. Indicates whether the position coordinates in regions are 0 based (TRUE) or 1 based (FALSE). If TRUE, positions will be converted to 1-based (start + 1). Need not be supplied for TSpanels. Default is TRUE.
padding	An integer value by which the function will extend the range of the target sequence on both sides. Start and end coordinates will be adjusted accordingly. Default is 0.
BS_genome	The name of the appropriate BSgenome package to use for sequence retrieval. Ex. "BSgenome.Hsapiens.UCSC.hg38", "BSgenome.Mmusculus.UCSC.mm10", "BSgenome.Rnorvegicus.UCSC.rn6". Use the function find_BS_genome() to help identify the appropriate BSgenome package if needed. Need not be supplied for TSpanels. BS_genome must be installed if using this method.
ucsc	A logical value. If TRUE, the function will retrieve the sequences from the UCSC genome browser using an API. If FALSE, the function will retrieve sequences using the appropriate BSgenome package, which will be installed as needed. Default is FALSE.

species — The species for which to retrieve the sequences. Only required if using the UCSC method. Species may be given as the scientific name or the common name. Ex. "Human", "Homo sapien". Used to choose the appropriate BS genome. Need not be supplied for TSpanels.

genome — The genome assembly version for which to retrieve the sequences. Only required if using the UCSC method. Ex. hg38, hg19, mm10, mm39, rn6, rn7. Need not be supplied for TSpanels.

Details

Consult `available.genomes(splitNameParts=FALSE, type=getOption("pkgType"))` for a full list of the available BS genomes and their associated species/genome/masked values. The BSgenome package will be installed if not already available. If using the UCSC API, the function will retrieve the sequences from the UCSC genome browser using the DAS API. See the UCSC website for available genomes: https://genome.ucsc.edu.

Value

a GRanges object with sequences of targeted regions.

Examples

```
#  Retrieve the sequences for custom regions
# We will load the TSpanel_human regions file as an example
# and supply it to the function as a GRanges object.
human <- load_regions_file("TSpanel_human")
regions_seq <- get_seq(
  regions = human,
  is_0_based_rg = FALSE,
  BS_genome = "BSgenome.Hsapiens.UCSC.hg38",
  padding = 0
)
```

---

get_vcf_end_positions *Derive end coordinates from a VCF object*

Description

`VariantAnnotation::rowRanges()` defines the range width from `REF`, which is appropriate for small variants but does not honor structural-variant span annotations such as `INFO/END`. This helper prefers explicit `END` values from the INFO field and falls back to `SVLEN` for symbolic non-insertion alleles when `END` is absent.

Usage

```
get_vcf_end_positions(vcf)
```

Arguments

vcf	A VCF object from VariantAnnotation::readVcf().

Value

An integer vector of end coordinates aligned to the rows of vcf.

---

import_mut_data *Import tabular mutation data*

Description

Imports tabular mutation file into the local R environment.

Usage

```
import_mut_data(
  mut_file,
  mut_sep = "\t",
  is_0_based_mut = TRUE,
  sample_data = NULL,
  sd_sep = "\t",
  regions = NULL,
  rg_sep = "\t",
  is_0_based_rg = TRUE,
  padding = 0,
  BS_genome = NULL,
  custom_column_names = NULL,
  output_granges = FALSE,
  add_chr = FALSE
)
```

Arguments

mut_file	The mutation data file(s) to be imported. This can be either a data frame object or a filepath to a file or directory. If you specify a directory, the function will attempt to read all files in the directory and combine them into a single data frame. Mutation data should consist of a row for each variant. Required columns are listed in details.
mut_sep	The delimiter for importing the mutation file. Default is tab-delimited.
is_0_based_mut	A logical variable. Indicates whether the position coordinates in the mutation data are 0 based (TRUE) or 1 based (FALSE). If TRUE, positions will be converted to 1-based.
sample_data	An optional file containing additional sample metadata (dose, timepoint, etc.). This can be a data frame or a file path. Metadata will be joined with the mutation data based on the sample column. Required columns are sample and any additional columns you wish to include.

sd_sep	The delimiter for importing sample data. Default is tab-delimited.
regions	An optional file containing metadata of genomic regions. Region metadata will be joined with mutation data and variants will be checked for overlap with the regions. regions can be either a file path, a data frame, or a GRanges object. File paths will be read using the rg_sep. Users can also choose from the built-in TwinStrand's Mutagenesis Panels by inputting "TSpanel_human", "TSpanel_mouse", or "TSpanel_rat". Required columns for the regions file are "contig", "start", and "end". For a GRanges object, the required columns are "seqnames", "start", and "end". Default is NULL.
rg_sep	The delimiter for importing the custom_regions. The default is tab-delimited "\t".
is_0_based_rg	A logical variable. Indicates whether the position coordinates in regions are 0 based (TRUE) or 1 based (FALSE). If TRUE, positions will be converted to 1-based (start + 1). Need not be supplied for TSpanels. Default is TRUE.
padding	An integer >= 0. Extend the range of your regions in both directions by the given amount. Ex. Structural variants and indels may start outside of the regions. Adjust the padding to include these variants in your region's ranges.
BS_genome	The pkgname of a BS genome. A BS genome must be installed prior to import to populate the context column (trinucleotide context for each position). Only required if data does not already include a context column. Please install the appropriate BS genome using BiocManager::install("pkgname") where pkgname is the name of the BSgenome package. The pkgname can be found using the find_BS_genome() function, which requires the species and assembly version. Ex."BSgenome.Hsapiens.UCSC.hg38" | "BSgenome.Hsapiens.UCSC.hg19" | "BSgenome.Mmusculus.UC | "BSgenome.Mmusculus.UCSC.mm39" | "BSgenome.Rnorvegicus.UCSC.rn6".
custom_column_names	A list of names to specify the meaning of column headers. Since column names can vary with data, this might be necessary to digest the mutation data properly. Typical defaults are set, but can be substituted in the form of list(my_custom_contig_name = "contig", my_custom_sample_column_name = "sample"). You can change one or more of these. Set column synonyms are defined in MutSeqR::op$column and will automatically be changed to their default value.
output_granges	A logical variable; whether you want the mutation data to output as a GRanges object. Default output (FALSE) is as a dataframe.
add_chr	A logical variable. If TRUE, prepends "chr" to contig names missing it (e.g., "1" becomes "chr1") and changes "MT" to "chrM" to ensure compatibility with BSgenome packages. Default is FALSE.

Details

Required columns for mut files are:

- contig: The name of the reference sequence.
- start: The start position of the feature.
- end: The half-open end position of the feature.
- sample: The sample name.

- `ref`: The reference allele at this position
- `alt`: The left-aligned, normalized, alternate allele at this position. Multiple alt alleles called for a single position should be represented as separate rows in the table.

The following columns are not required, but are recommended for full package functionality:

- `alt_depth`: The read depth supporting the alternate allele. If not included, the function will add this column, assuming a value of 1.
- `total_depth`: The total read depth at this position, excluding no-calls (N calls). If not present, the function will attempt to calculate the `total_depth` as `depth - no_calls`. If no_calls is not present, the function will use `depth` as the `total_depth`.
- `depth`: The total read depth at this position, including no-calls.
- `no_calls`: The number of no-calls (N-calls) at this position.

We recommend that files include a record for every sequenced position, regardless of whether a variant was called, along with the `total_depth` for each record. This enables site-specific depth calculations required for some downstream analyses.

Value

A table where each row is a mutation, and columns indicate the location, type, and other data. If `output_granges` is set to TRUE, the mutation data will be returned as a GRanges object, otherwise mutation data is returned as a dataframe.

Output Column Definitions:

- `short_ref`: The reference base at the start position.
- `normalized_ref`: The short_ref in C/T-base notation for this position (e.g. A -> T, G -> C).
- `context` The trinucleotide context at this position. Consists of the reference base and the two flanking bases (e.g. TAC).
- `normalized_context`: The trinucleotide context in C/T base notation for this position (e.g. TAG -> CTA).
- `variation_type` The type of variant (snv, mnv, insertion, deletion, complex, sv, no_variant, ambiguous, uncategorized).
- `subtype` The substitution type for the snv variant (12-base spectrum; e.g. A>C).
- `normalized_subtype` The C/T-based substitution type for the snv variant (6-base spectrum; e.g. A>C -> T>G).
- `context_with_mutation`: The substitution type for the snv variant including the two flanking nucleotides (192-trinucleotide spectrum; e.g. `T[A>C]G`)
- `normalized_context_with_mutation`: The C/T-based substitution type for the snv variant including the two flanking nucleotides (96-base spectrum e.g. `T[A>C]G` -> `C[T>G]A`).
- `nchar_ref`: The length (in bp) of the reference allele.
- `nchar_alt`: The length (in bp) of the alternate allele.
- `varlen`: The length (in bp) of the variant.
- `ref_depth`: The depth of the reference allele. Calculated as `total_depth - alt_depth`, if applicable.

- vaf : The variant allele fraction. Calculated as alt_depth/total_depth.
- gc_content: % GC of the trinucleotide context at this position.
- is_known: TRUE or FALSE. Flags known variants (ID != ".").
- row_has_duplicate: TRUE or FALSE. Flags rows whose position is the same as that of at least one other row for the same sample.
- filter_mut : A logical value, initially set to FALSE that indicates to calculte_mf() if the variant should be excluded from mutation counts. See the filter_mut function for more detail.

Examples

```
# Mutation data is just for example purposes. It does not reflect real data
file <- system.file("extdata", "Example_files",
                    "simple_mut_import.txt", package = "MutSeqR")
# Import the data
imported_example_data <- import_mut_data(mut_file = file)
```

---

import_regions_metadata

Join Regions Metadata

Description

This function imports the regions metadata and joins it with the mutation data.

Usage

```
import_regions_metadata(
  mutation_granges,
  regions,
  rg_sep,
  is_0_based_rg,
  padding
)
```

Arguments

mutation_granges	A data frame containing mutation data.
regions	The path to the file containing the regions metadata. Alternatively, a data frame can be provided directly.
rg_sep	The separator used in the regions metadata file. Default is tab (\t).
is_0_based_rg	A logical value indicating whether the regions file is 0-based (TRUE) or 1-based (FALSE). Default is FALSE.
padding	An integer value indicating the number of base pairs to pad the regions on either side. Default is 0.

Value

A GRanges object that combines the mutation data with the regions metadata.

`import_sample_data` *Import Sample Metadata*

Description

This function imports sample metadata from a file or accepts a data frame directly, and performs basic validation checks.

Usage

```
import_sample_data(sample_data, sd_sep = "\t")
```

Arguments

`sample_data`	The path to the file containing the sample metadata, or a data frame provided directly.
`sd_sep`	The separator used in the sample metadata file. Default is tab (`\t`).

Value

A validated data frame containing sample metadata, including a required column named `sample`.

`import_vcf_data` *Import a VCF file*

Description

The function reads VCF file(s) and extracts the data into a dataframe.

Usage

```
import_vcf_data(
  vcf_file,
  sample_data = NULL,
  sd_sep = "\t",
  regions = NULL,
  rg_sep = "\t",
  is_0_based_rg = FALSE,
  padding = 0,
  BS_genome = NULL,
  output_granges = FALSE,
  remove_sample_suffix = NULL,
  add_chr = FALSE
)
```

Arguments

vcf_file	The path to the .vcf (.gvcf, gzip, bgzip) to be imported. If you specify a directory, the function will attempt to read all files in the directory and combine them into a single table. VCF files should follow the VCF specifications, version 4.5. Multisample VCF files are not supported; VCF files must contain one sample each. Required fields are listed in details.
sample_data	An optional file containing additional sample metadata (dose, timepoint, etc.). This can be a data frame or a file path. Metadata will be joined with the mutation data based on the sample column. Required columns are `sample` and any additional columns you wish to include.
sd_sep	The delimiter for importing sample metadata tables. Default is tab-delimited.
regions	An optional file containing metadata of genomic regions. Region metadata will be joined with mutation data and variants will be checked for overlap with the regions. `regions` can be either a file path, a data frame, or a GRanges object. File paths will be read using the rg_sep. Users can also choose from the built-in TwinStrand's Mutagenesis Panels by inputting "TSpanel_human", "TSpanel_mouse", or "TSpanel_rat". Required columns for the regions file are "contig", "start", and "end". For a GRanges object, the required columns are "seqnames", "start", and "end". Default is NULL.
rg_sep	The delimiter for importing the custom_regions. The default is tab-delimited "\t".
is_0_based_rg	A logical variable. Indicates whether the position coordinates in `regions` are 0 based (TRUE) or 1 based (FALSE). If TRUE, positions will be converted to 1-based (start + 1). Need not be supplied for TSpanels. Default is TRUE.
padding	Extend the range of your regions in both directions by the given amount. Ex. Structural variants and indels may start outside of the regions. Adjust the `padding` to include these variants in your region's ranges.
BS_genome	The pkgname of a BS genome. A BS genome must be installed prior to import to populate the context column (trinucleotide context for each position). Only required if data does not already include a context column. Please install the appropriate BS genome using BiocManager::install("pkgname") where pkgname is the name of the BSgenome package. The pkgname can be found using the find_BS_genome() function, which requires the species and assembly version. Ex. "BSgenome.Hsapiens.UCSC.hg38" | "BSgenome.Hsapiens.UCSC.hg19" | "BSgenome.Mmusculus.UCSC.mm10" | "BSgenome.Mmusculus.UCSC.mm39" | "BSgenome.Rnorvegicus.UCSC.rn6"
output_granges	`TRUE` or `FALSE`; whether you want the mutation data to output as a GRanges object. Default output is as a dataframe.
remove_sample_suffix	An optional character string representing a regular expression to remove unwanted suffixes from VCF sample names prior to joining with metadata. For example, if your VCF sample is "Sample1.cons.filtered" but your metadata sheet just says "Sample1", you can use `remove_sample_suffix = "\\.cons\\.filtered$"`. Default is NULL.

add_chr — A logical variable. If TRUE, prepends "chr" to contig names missing it (e.g., "1" becomes "chr1") and changes "MT" to "chrM" to ensure compatibility with BSgenome packages. Default is FALSE.

Details

The required fields are:

FIXED FIELDS

- CHROM: The name of the reference sequence. Equivalent to contig.
- POS: The 1-based start position of the feature. Equivalent to start.
- REF: The reference allele at this position.
- ALT: The left-aligned, normalized, alternate allele at this position. Multiple alt alleles called for a single position should be represented as separate rows in the table.

INFO FIELDS

- sample: An identifying field for your samples; either in the INFO field or as the header to the FORMAT field.

SUGGESTED FIELDS

The following **FORMAT** fields are not required, but are recommended for full package functionality:

- AD: The allelic depths for the reference and alternate allele in the order listed. The sum of AD is equivalent to the total_depth (read depth at this position excluding N-calls).
- DP: The read depth at this position (including N-calls). Equivalent to depth. Note that in many VCF files, the DP field is defined as total_depth. However, in most cases, the DP field includes N-calls.
- VD: The read depth supporting the alternate allele. If not included, the function will add this column, assuming a value of 1. Equivalent to alt_depth.

We recommend that files include a record for every sequenced position, regardless of whether a variant was called, along with the AD for each record. This enables site-specific depth calculations required for some downstream analyses. AD is used to calculate the total_depth (the read depth excluding No-calls). If AD is not available, the DP field will be used as the total_depth.

Value

A table where each row is a mutation, and columns indicate the location, type, and other data. If output_granges is set to TRUE, the mutation data will be returned as a GRanges object, otherwise mutation data is returned as a dataframe.

Output Column Definitions:

- 'end': The half-open end position of the feature. This is calculated as the 1-based start position + the length of the reference allele - 1. For structural variants, if the INFO field contains SVLEN, end is calculated as the 1-based start position + SVLEN - 1. End is only calculated if not already present in the VCF INFO fields.
- short_ref: The reference base at the start position.

- normalized_ref: The short_ref in C/T-base notation for this position (e.g. A -> T, G -> C).
- context The trinucleotide context at this position. Consists of the reference base and the two flanking bases (e.g. TAC).
- normalized_context: The trinucleotide context in C/T base notation for this position (e.g. TAG -> CTA).
- variation_type The type of variant (snv, mnv, insertion, deletion, complex, sv, no_variant, ambiguous, uncategorized).
- subtype The substitution type for the snv variant (12-base spectrum; e.g. A>C).
- normalized_subtype The C/T-based substitution type for the snv variant (6-base spectrum; e.g. A>C -> T>G).
- context_with_mutation: The substitution type for the snv variant including the two flanking nucleotides (192-trinucleotide spectrum; e.g. T[A>C]G)
- normalized_context_with_mutation: The C/T-based substitution type for the snv variant including the two flanking nucleotides (96-base spectrum e.g. T[A>C]G -> C[T>G]A).
- nchar_ref: The length (in bp) of the reference allele.
- nchar_alt: The length (in bp) of the alternate allele.
- varlen: The length (in bp) of the variant.
- ref_depth: The depth of the reference allele. Calculated as total_depth - alt_depth, if applicable.
- vaf : The variant allele fraction. Calculated as alt_depth/total_depth.
- gc_content: % GC of the trinucleotide context at this position.
- is_known: TRUE or FALSE. Flags known variants (ID != ".").
- row_has_duplicate: TRUE or FALSE. Flags rows whose position is the same as that of at least one other row for the same sample.
- filter_mut : A logical value, initially set to FALSE that indicates to calculte_mf() if the variant should be excluded from mutation counts. See the filter_mut function for more detail.

Examples

```
# Mutation data is just for example purposes. It does not reflect real data
file <- system.file("extdata", "Example_files",
                    "simple_vcf_data.vcf", package = "MutSeqR")
# Import the data
imported_example_data <- import_vcf_data(
 vcf_file = file,
BS_genome = find_BS_genome("mouse", "mm10"))
```

load_regions_file *Imports the regions file*

Description

A helper function to import the regions metadata file and return a GRanges object.

Usage

```
load_regions_file(regions, rg_sep = "\t", is_0_based_rg = TRUE)
```

Arguments

regions	The regions metadata file to import. Can be either a file path, a data frame, or a GRanges object. File paths will be read using the rg_sep. Users can also choose from the built-in TwinStrand's Mutagenesis Panels by inputting "TSpanel_human", "TSpanel_mouse", or "TSpanel_rat". Required columns for the regions file are "contig", "start", and "end". In a GRanges object, the required columns are "seqnames", "start", and "end".
rg_sep	The delimiter for importing the custom_regions. The default is tab-delimited "\t".
is_0_based_rg	A logical variable. Indicates whether the position coordinates in regions are 0 based (TRUE) or 1 based (FALSE). If TRUE, positions will be converted to 1-based (start + 1). Need not be supplied for TSpanels. Default is TRUE.

Value

a GRanges object of the imported regions metadata file.

Examples

```
#' # Example 1: Load built-in TwinStrand's Human Mutagenesis
human_rg <- load_regions_file(regions = "TSpanel_human")
human_rg
# Load a custom regions file from an interval list
# We will use the human TSpanel system file for this example,
# but any file can be imported.
file <- system.file("extdata",
  "inputs",
  "metadata",
  "human_mutagenesis_panel_hg38.txt",
  package = "MutSeqR"
)
custom_rg <- load_regions_file(regions = file, rg_sep = "\t", is_0_based_rg = TRUE)
custom_rg
```

`model_mf` *Perform linear modelling on mutation frequency for given fixed and random effects*

Description

`model_mf` will fit a linear model to analyse the effect(s) of given factor(s) on mutation frequency and perform specified pairwise comparisons. This function will fit either a generalized linear model (glm) or, if supplied random effects, a generalized linear mixed-effects model (glmer). Pairwise comparisons are conducted using the doBy library (esticon) and estimates are then back-transformed. The delta method is employed to approximate the back-transformed standard-errors. A Sidak correction is applied to adjust p-values for multiple comparisons.

Usage

```
model_mf(
  mf_data,
  fixed_effects,
  test_interaction = TRUE,
  random_effects = NULL,
  reference_level,
  muts = "sum_min",
  total_count = "group_depth",
  contrasts = NULL,
  cont_sep = "\t",
  ...
)
```

Arguments

`mf_data`	The data frame containing the mutation frequency data. Mutation counts and total sequencing depth should be summarized per sample alongside columns for your fixed effects. This data can be obtained using `calculate_mf(summary=TRUE)`.
`fixed_effects`	The name(s) of the column(s) that will act as the fixed_effects (factor/independent variable) for modelling mutation frequency.
`test_interaction`	a logical value. Whether or not your model should include the interaction between the `fixed_effects`.
`random_effects`	The name of the column(s) to be analysed as a random effect in the model. Providing this effect will cause the function to fit a generalized linear mixed-effects model.
`reference_level`	Refers to one of the levels within each of your fixed_effects. The coefficient for the reference level will represent the baseline effect. The coefficients of the other levels will be interpreted in relation to the reference_level as deviations from the baseline effect.

muts	The column containing the mutation count per sample.
total_count	The column containing the sequencing depth per sample.
contrasts	a data frame or a filepath to a file that will provide the information necessary to make pairwise comparisons between groups. The table must consist of two columns. The first column will be a group within your fixed_effects and the second column must be the group that it will be compared to. The values must correspond to entries in your mf_data column for each fixed effect. Put the group that you expect to have the higher mutation frequency in the 1st column and the group that you expect to have a lower mutation frequency in the second column. For multiple fixed effects, separate the levels of each fixed_effect of a group with a colon. Ensure that all fixed_effects are represented in each entry for the table. See details for examples.
cont_sep	The delimiter for importing the contrast table file. Default is tab-delimited.
...	Extra arguments for glm or glmer. The glmer function is used when a random_effect is supplied, otherwise, the model uses the glm function.

Details

fixed_effects are variables that have a direct and constant effect on the dependent variable (ie mutation frequency).They are typically the experimental factors or covariates of interest for their impact on the dependent variable. One or more fixed_effect may be provided. If you are providing more than one fixed effect, avoid using correlated variables; each fixed effect must independently predict the dependent variable. Ex. fixed_effects = c("dose", "genomic_target", "tissue", "age", etc).

Interaction terms enable you to examine whether the relationship between the dependent and independent variable changes based on the value of another independent variable. In other words, if an interaction is significant, then the relationship between the fixed effects is not constant across all levels of each variable. Ex. Consider investigating the effect of dose group and tissue on mutation frequency. An interaction between dose and tissue would capture whether the dose response differs between tissues.

random_effects account for the unmeasured sources of statistical variance that affect certain groups in the data. They help account for unobserved heterogeneity or correlation within groups. Ex. If your model uses repeated measures within a sample, random_effects = "sample".

Setting a reference_level for your fixed effects enhances the interpretability of the model. Ex. Consider a fixed_effect "dose" with levels 0, 25, 50, and 100 mg/kg. Intuitively, the reference_level would refer to the negative control dose, "0" since we are interested in testing how the treatment might change mutation frequency relative to the control.

Examples of contrasts:

If you have a fixed_effect "dose" with dose groups 0, 25, 50, 100, then the first column would contain the treated groups (25, 50, 100), while the second column would be 0, thus comparing each treated group to the control group.

25 0

50 0

100 0

Alternatively, if you would like to compare mutation frequency between treated dose groups, then the contrast table would look as follows, with the lower dose always in the second column, as we expect it to have a lower mutation frequency. Keeping this format aids in interpretability of the estimates for the pairwise comparisons. Should the columns be reversed, with the higher group in the second column, then the model will compute the fold-decrease instead of the fold-increase.

100 25

100 50

50 25

Ex. Consider the scenario where the `fixed_effects` are "dose" (0, 25, 50, 100) and "genomic_target" ("chr1", "chr2"). To compare the three treated dose groups to the control for each genomic target, the contrast table would look like:

25:chr1 0:chr1

50:chr1 0:chr1

100:chr1 0:chr1

25:chr2 0:chr2

50:chr2 0:chr2

100:chr2 0:chr2

Troubleshooting: If you are having issues with convergence for your generalized linear mixed-effects model, it may be advisable to increase the tolerance level for convergence checking during model fitting. This is done through the `control` argument for the `lme4::glmer` function. The default tolerance is tol = 0.002. Add this argument as an extra argument in the `model_mf` function. Ex. `control = lme4::glmerControl(check.conv.grad = lme4::.makeCC("warning", tol = 3e-3, relTol = NULL))` Alternate approach: `control = lme4::glmerControl(optimizer = "bobyqa", optCtrl = list(maxfun = 2e5))` Similar approaches may be taken for glm models.

Value

Model results are output as a list. Included are:

- model_data: the supplied mf_data with added column for the Pearson's residuals of the model.
- summary: the summary of the model.
- anova: the analysis of variance for models with two or more effects. Anova`(model)`
- residuals_histogram: the Pearson's residuals plotted as a histogram. This is used to check whether the variance is normally distributed. A symmetric bell-shaped histogram, evenly distributed around zero indicates that the normality assumption is likely to be true.
- residuals_qq_plot: the Pearson's residuals plotted in a quantile-quantile plot. For a normal distribution, we expect points to roughly follow the y=x line.
- point_estimates_matrix: the contrast matrix used to generate point-estimates for the fixed effects.
- point_estimates: the point estimates for the fixed effects.
- pairwise_comparisons_matrix: the contrast matrix used to conduct the pairwise comparisons specified in the `contrasts`.
- pairwise_comparisons: the results of pairwise comparisons specified in the `contrasts`.

Examples

```
# Example data  consists of 24 mouse bone marrow
# samples exposed to three doses of BaP alongside vehicle controls.
# Libraries were sequenced with Duplex Sequencing using
# the TwinStrand Mouse Mutagenesis Panel which consists of 20 2.4kb
# targets = 48kb of sequence. Example data can be retrieved from
# MutSeqRData, an ExperimentHub data package:
## library(ExperimentHub)
## eh <- ExperimentHub()
## query(eh, "MutSeqRData")
# Mutation frequency data was precalculated using
## mf_data_global <- calculate_mf(mutation_data = eh[["EH9861"]],
##   cols_to_group = "sample",
##   retain_metadata_cols = c("dose_group", "dose"))

# We will model the effect of dose on mutation frequency min.
mf_example <- readRDS(system.file("extdata/Example_files/mf_data_global.rds",
  package = "MutSeqR"
))
# We will compare all BaP dose groups to the control group
# using pairwise comparisons.
contrasts <- data.frame(
  col1 = c("12.5", "25", "50"),
  col2 = c("0", "0", "0")
)
# Fit the model
model <- model_mf(
  mf_data = mf_example,
  fixed_effects = "dose",
  reference_level = "0",
  muts = "sum_min",
  total_count = "group_depth",
  contrasts = contrasts
)
```

---

op *Dictionary of column name synonyms for required mutation data columns*

Description

A list of column specifications

Usage

```
op
```

Format

A list with potential variable column names

Examples

```
# examples of "alt" synonyms
op$column$alternate
```

`plot_bubbles` *Generate Bubble Plots*

Description

Produces a ggplot object of bubble plots from given mutation data. Optionally, bubble plots can be facetted and coloured by a specified column.

Usage

```
plot_bubbles(
  mutation_data,
  size_by = "alt_depth",
  facet_col = NULL,
  color_by = "normalized_subtype",
  circle_spacing = 1,
  circle_outline = "none",
  circle_resolution = 50,
  custom_palette = NULL
)
```

Arguments

`mutation_data`	Data frame containing the mutation data.
`size_by`	The column name by which to size the circles. Recommended values are "alt_depth" or "vaf".
`facet_col`	The column name by which to facet . If NULL, no facetting will be done. Default is NULL.
`color_by`	The column name by which to colour the mutations. Default is "normalized_subtype".
`circle_spacing`	Numerical value to adjust the spacing between circles. Default is 1.
`circle_outline`	Colour for the circle outline. Default is "none", resulting in no outline colour. Other accepted values are colours in the R language.
`circle_resolution`	Number of points to use for the circle resolution. Default is 50.
`custom_palette`	A named vector of colors to be used for the mutation subtypes. The names of the vector should correspond to the levels in color_by. Alternatively, you can specify a color palette from the RColorBrewer package. See `brewer.pal` for palette options. You may visualize the palettes at the ColorBrewer website: `https://colorbrewer2.org/`. Default is `NULL`.

Details

The function will plot a circle for each mutation in `mutation_data`. Mutations flagged by the `filter_mut` column will be excluded from the plot. The size of the circle is determined by the `size_by` parameter. Sizing by the "alt_depth" or the "vaf" will give users the ability to visualize the the distribution of recurrent mutations within their data with large multiplets having a large circle.

Value

A ggplot object with the bubble plot, facetted if specified.

Examples

```
# The example data is a subset of variants from the target chr1
# from samples of the high dose group (50mg).
example_data <- readRDS(system.file("extdata", "Example_files",
                                    "variants_subset_d50_chr1.rds",
                                     package = "MutSeqR")
)
 plot <- plot_bubbles(
    mutation_data = example_data
  )
```

`plot_ci` *plot_ci*

Description

Plot confidence intervals

Usage

```
plot_ci(
  data,
  order = "none",
  custom_order = NULL,
  nudge = 0.3,
  log_scale = FALSE,
  x_lab = NULL,
  y_lab = NULL,
  title = NULL
)
```

Arguments

data	A data frame with the results of the BMD analysis. Data must contain columns "Response", "BMD", "BMDL", and "BMDU". BMD values can be NA.
order	Indicates how the responses should be ordered. Options are "none" (default), "asc" for ascending BMD values, "desc" for descending BMD values, or a custom order.
custom_order	A character vector with the custom order of the Responses.
nudge	A numeric value to nudge the text labels away from points. Default is 0.3.
log_scale	A logical value indicating if the x-axis should be in log10 scale. Default is false.
x_lab	A character string with the x-axis label. Default is "BMD" or "log10(BMD)" if log_scale is TRUE.
y_lab	A character string with the y-axis label. Default is "Response".
title	A character string with the plot title. Default is "BMD with 90% Confidence Intervals".

Value

a ggplot object

Examples

```
if (requireNamespace("MutSeqRData", quietly = TRUE)) {
  # Plot results from PROAST
  dat <- data.frame(
    Response = c("PROAST MF Min", "PROAST MF Max"),
    BMD = c(NA, NA),
    BMDL = c(7.38, 2.98),
    BMDU = c(10.9, 7.68)
  )
  plot <- plot_ci(dat)
}
```

---

plot_lollipop *Plot recurrent mutations in a lollipop plot using ggplot2*

Description

Plot recurrent mutations in a lollipop plot using ggplot2

Usage

```
plot_lollipop(
  mutation_data,
  min_recurrence = 2,
  group_col = "contig",
  subtype_resolution = "base_6",
  custom_palette = NULL
)
```

Arguments

mutation_data	A data frame containing mutation data.
min_recurrence	An integer specifying the minimum number of times a mutation must be observed at the same position to be plotted. Default is 2.
group_col	A character vector specifying the column name(s) to group mutation_data by. Default is "contig".
subtype_resolution	The subtype resolution by which to group and colour the mutations. Options are "none", "type", "base_6", "base_12", "base_96", and "base_192".
custom_palette	A named vector of colors to be used for the mutation subtypes. The names of the vector should correspond to the mutation subtypes in the data. Alternatively, you can specify a color palette from the RColorBrewer package. See brewer.pal for palette options. You may visualize the palettes at the ColorBrewer website: https://colorbrewer2.org/. Default is NULL.

Value

A list of ggplot objects.

Examples

```
# For this example, we will use a subset of the example mutation data.
# The subset contains mutations from target chr1 in samples from the high
# dose group (50mg).
 example_data <- readRDS(system.file("extdata", "Example_files",
     "variants_subset_d50_chr1.rds", package = "MutSeqR"))
 # We will plot mutations that recoccur in at least two samples, grouped
 # by the "label" column, which signifies the target region (chr1).
 # Mutations will be grouped and coloured by their base 6 subtype (default)
 plot <- plot_lollipop(
     mutation_data = example_data,
     min_recurrence = 2,
     group_col = "label"
 )
```

---

plot_mean_mf *Plot the Mean Mutatation Frequency*

Description

This function calculates the mean mutation frequency across samples for given groups and plots the results.

Usage

```
plot_mean_mf(
  mf_data,
  group_col = "dose",
  fill_col = NULL,
  mf_type = "both",
  plot_type = "line",
  plot_error_bars = TRUE,
  plot_indiv_vals = TRUE,
  group_order = "none",
  group_order_input = NULL,
  add_labels = "mean_count",
  scale_y_axis = "linear",
  x_lab = NULL,
  y_lab = NULL,
  plot_title = NULL,
  custom_palette = NULL,
  plot_legend = TRUE,
  rotate_labels = FALSE,
  label_size = 3
)
```

Arguments

mf_data	A data frame containing the mutation frequency data. This is obtained from the calculate_mf function with SUMMARY = TRUE.
group_col	The column(s) in mf_data by which to calculate the mean. When supplying more than one column, the values of all group columns will be concatenated into a single value by which to calculate the mean. Values will be displayed along the x-axis. Ex. "dose" or c("dose", "tissue").
fill_col	An optional column name in the data used to define the fill aesthetic in the plot. If fill_col has multiple levels within each group_col level, the mean will be calculated for each level of fill_col (recommend plot_type = "line" for this use case). Default is NULL.
mf_type	The type of mutation frequency to plot. Options are "min", "max", "both", or "stacked". If "both", the min and max mutation frequencies are plotted side by side. "stacked" can be chosen for bar plot_type only. If "stacked", the difference between the min and max MF is stacked on top of the min MF such that the total height of both bars represent the max MF. Default is "min".
plot_type	The type of plot to create. Options are "bar" or "line". Default is "bar".
plot_error_bars	Whether to plot the error bars. Default is TRUE. Error bars are standard error of the mean.
plot_indiv_vals	Whether to plot the individual values as data points. Default is FALSE.
group_order	The order of the groups along the x-axis. ' Options include:

- `none`: No ordering is performed. Default.
- `smart`: Groups are ordered based on the sample names.
- `arranged`: Groups are ordered based on one or more factor column(s) in mf_data. Factor column names are passed to the function using the `group_order_input`.
- `custom`: Groups are ordered based on a custom vector of group names. The custom vector is passed to the function using the `group_order_input`.

`group_order_input`
: The order of the groups if group_order is "custom". The column name by which to arrange groups if group_order is "arranged". If "custom", and using more than one group_col, values are concatenated in the order listed, separated by a "_".

`add_labels`
: The data labels to display on the plot. Either "indiv_count", "indiv_MF", "mean_count", "mean_MF", or "none". Count labels display the number of mutations, MF labels display the mutation frequency. Mean plots the mean value. Indiv plots the labels for individual data points (only if plot_indiv_vals = TRUE). Default is "none".

`scale_y_axis`
: The scale of the y axis. Either "linear" or "log". Default is "linear".

`x_lab`
: The x-axis label. Default is the value of group_col.

`y_lab`
: The y-axis label. Default is "Mutation Frequency (mutations/bp)".

`plot_title`
: The title of the plot. Default is "Mean Mutation Frequency".

`custom_palette`
: A custom color palette to use for the plot. Input a character vector of colours. Input a named character vector to specify olours to specific groups. Fill labels will be constructed by the following components

1. "Mean/Individual" if plot_indiv_vals = TRUE, fill labels will specify Mean/Individual values.
2. "min/max" if mf_type = "both" or "stacked", fill labels will specify min/max values.
3. fill_col value. Name colours to match the fill labels. Default is NULL. If no custom_palette, a rainbow palette is generated. Min/Max values and Mean/Individual values will be the same colour, different shades.

`plot_legend`
: Logical. Whether to show the fill (and color) legend. Default is TRUE.

`rotate_labels`
: A logical value indicating whether data labels should be rotated 90 degrees. Default is FALSE.

`label_size`
: A numeric value that controls the size of the data labels.

Value

a ggplot object

Examples

```
# Example data  consists of 24 mouse bone marrow
# samples exposed to three doses of BaP alongside vehicle controls.
# Libraries were sequenced with Duplex Sequencing using
```

```
# the TwinStrand Mouse Mutagenesis Panel which consists of 20 2.4kb
# targets = 48kb of sequence. Example data can be retrieved from
# MutSeqRData, an ExperimentHub data package:
## library(ExperimentHub)
## eh <- ExperimentHub()
## query(eh, "MutSeqRData")
# Mutation frequency data was precalculated using
## mf_data_global <- calculate_mf(mutation_data = eh[["EH9861"]],
##   cols_to_group = "sample",
##   retain_metadata_cols = c("dose_group", "dose"))

mf_example <- readRDS(system.file("extdata/Example_files/mf_data_global.rds",
  package = "MutSeqR"
))
# Specify the order of the dose groups along the x-axis
mf_example$dose_group <- factor(mf_example$dose_group,
  levels = c(
    "Control", "Low",
    "Medium", "High"
  )
)
# Plot the mean min MF per dose group as a bar plot with error bars
plot <- plot_mean_mf(
  mf_data = mf_example,
  group_col = "dose_group",
  mf_type = "min",
  plot_type = "line",
  fill_col = "dose_group",
  plot_error_bars = TRUE,
  plot_indiv_vals = TRUE,
  add_labels = "none"
)
```

---

plot_mf *Plot the Mutation Frequency*

Description

This function creates a plot of the mutation frequency.

Usage

```
plot_mf(
  mf_data,
  group_col,
  plot_type = "bar",
  mf_type = "min",
  fill_col = NULL,
  custom_palette = NULL,
  group_order = "none",
```

```
  group_order_input = NULL,
  labels = "count",
  scale_y_axis = "linear",
  x_lab = NULL,
  y_lab = NULL,
  title = NULL,
  rotate_labels = FALSE,
  label_size = 3
)
```

Arguments

mf_data	A data frame containing the mutation frequency data. This is obtained from the calculate_mf function with SUMMARY = TRUE.
group_col	The name of the column containing the sample/group names for the x-axis.
plot_type	The type of plot to create. Options are "bar" or "point".
mf_type	The type of mutation frequency to plot. Options are "min", "max", "both", or "stacked". If "both", the min and max mutation frequencies are plotted side by side. "stacked" can be chosen for bar plot_type only. If "stacked", the difference between the min and max MF is stacked on top of the min MF such that the total height of both bars represent the max MF.
fill_col	The name of the column containing the fill variable.
custom_palette	A character vector of colour codes to use for the plot. If NULL, a default palette is used
group_order	The order of the samples/groups along the x-axis. ' Options include: • none: No ordering is performed. Default. • smart: Samples are ordered based on the sample names. • arranged: Samples are ordered based on one or more factor column(s) in mf_data. Factor column names are passed to the function using the group_order_input. • custom: Samples are ordered based on a custom vector of sample names. The custom vector is passed to the function using the group_order_input.
group_order_input	The order of the samples/groups if group_order is "custom". The column name by which to arrange samples/groups if group_order is "arranged"
labels	The data labels to display on the plot. Either "count", "MF", or "none". Count labels display the number of mutations, MF labels display the mutation frequency.
scale_y_axis	The scale of the y axis. Either "linear" or "log".
x_lab	The label for the x axis.
y_lab	The label for the y axis.
title	The title of the plot.
rotate_labels	A logical value aplied when labels is not "none". Indicates whether the labels should be rotated 90 degrees. Default is FALSE.
label_size	A numeric value that adjusts the size of the labels. Default is 3.

Value

A ggplot object

Examples

```
# Example data  consists of 24 mouse bone marrow
# samples exposed to three doses of BaP alongside vehicle controls.
# Libraries were sequenced with Duplex Sequencing using
# the TwinStrand Mouse Mutagenesis Panel which consists of 20 2.4kb
# targets = 48kb of sequence. Example data can be retrieved from
# MutSeqRData, an ExperimentHub data package:
## library(ExperimentHub)
## eh <- ExperimentHub()
## query(eh, "MutSeqRData")
# Mutation frequency data was precalculated using
## mf_data_global <- calculate_mf(mutation_data = eh[["EH9861"]],
##   cols_to_group = "sample",
##   retain_metadata_cols = c("dose_group", "dose"))
# Load Example MF data
mf_example <- readRDS(system.file("extdata/Example_files/mf_data_global.rds",
  package = "MutSeqR"
))
# Specify the order of the dose groups along the x-axis
mf_example$dose_group <- factor(mf_example$dose_group,
  levels = c(
    "Control", "Low",
    "Medium", "High"
  )
)
# Plot the min MF per sample as a bar plot with count labels
plot <- plot_mf(
  mf_data = mf_example,
  group_col = "sample",
  plot_type = "bar",
  mf_type = "min",
  fill_col = "dose_group",
  group_order = "arranged",
  group_order_input = "dose_group",
  labels = "count",
  title = "Mutation Frequency per Sample"
)
```

`plot_model_mf` *Plot your mf model*

Description

Provide a visualization of the point estimates derived using model_mf()

Usage

```
plot_model_mf(
  model,
  plot_type = "point",
  x_effect = NULL,
  plot_error_bars = TRUE,
  plot_signif = TRUE,
  ref_effect = NULL,
  x_order = NULL,
  fill_order = NULL,
  x_label = NULL,
  y_label = NULL,
  plot_title = NULL,
  fill_label = NULL,
  custom_palette = NULL
)
```

Arguments

model	A model object created using model_mf()
plot_type	The type of plot to create. Options are "bar" or "point".
x_effect	If there are multiple fixed effects in the model, specify the fixed effect to plot on the x-axis. The other will be used in the fill aesthetic. Currently, only 2 fixed effects are supported.
plot_error_bars	Logical. If TRUE, the estimated standard error will be added to the plot.
plot_signif	Logical. If TRUE, will add significance labels based on the pairwise_comparisons data frame in the model object. This is only valid if you supplied a contrasts table to model_mf(). Symbols will be applied to plotted values that are significantly different from the reference. Your contrasts table is structured as a data frame with two columns, each containing levels of the fixed effects to be contrasted. When adding significance labels, symbols will be added to the values defined in the first column, while the second column will represent the reference. A different symbol will be used for each unique reference level. If a single plotted value has been contrasted against multiple references, then it will gain multiple symbols for each significance difference.
ref_effect	The fixed effect to use as the reference level when adding significance labels. Only applicable if using two fixed effects.
x_order	A character vector indicating the order of the levels for the x_effect.
fill_order	A character vector indicating the order of the levels for the fill aesthetic, if applicable.
x_label	The label for the x-axis.
y_label	The label for the y-axis.
plot_title	The title of the plot.
fill_label	The label for the fill aesthetic, if applicable.

custom_palette A vector of colors to use for the fill and color aesthetics. If not provided, a default palette will be used. When plotting a model that has a single fixed effect, you can specify colors for "fill" and "color" using a named vector. Likewise, when plotting a model with two fixed effects, you can specify colors for the levels within your fill variable.

Details

See model_mf() for examples.

Value

A ggplot object.

Examples

```
# Example data consists of 24 mouse bone marrow DNA samples imported
# using import_mut_data() and filtered with filter_mut.
# Data was summarized per sample using calculate_mf() and modeled using
# model_mf() (see example).
file <- system.file("extdata/Example_files/mf_model_global.rds",
     package = "MutSeqR")
model <- readRDS(file)
# Plot the results using plot_model_mf()
plot <- plot_model_mf(model,
  plot_type = "bar",
  x_effect = "dose",
  plot_error_bars = TRUE,
  plot_signif = TRUE,
  x_order = c("0", "12.5", "25", "50"),
  x_label = "Dose (mg/kg-bw/d)",
  y_label = "Estimated Mean MF (mutations/bp)",
  plot_title = ""
)
```

---

plot_radar *Create a radar plot*

Description

Create a radar plot

Usage

```
plot_radar(mf_data, response_col, label_col, facet_col, indiv_y = TRUE)
```

Arguments

mf_data	A data frame with the data to plot
response_col	The column with the response values
label_col	The column with the labels for the radar plot.
facet_col	The column with the group to facet the radar plots.
indiv_y	A logical indicating whether to use individual y-axis scales for each plot.

Value

A radar plot

Examples

```
# Plot the MFmin for each variation type, including the 6 SNV subtypes
# Facet the plots by dose group
mf_ex <- readRDS(system.file("extdata", "Example_files", "mf_data_6.rds",
     package = "MutSeqR")
)
plot <- plot_radar(
     mf_data = mf_ex,
     response_col = "mf_min",
     label_col = "normalized_subtype",
     facet_col = "dose_group",
     indiv_y = TRUE
)
```

---

plot_spectra *Plot spectra*

Description

Given mf data, construct a plot displaying the mutation subtypes observed in a cohort.

Usage

```
plot_spectra(
  mf_data,
  group_col = "sample",
  subtype_resolution = "base_6",
  response = "proportion",
  mf_type = "min",
  group_order = "none",
  group_order_input = NULL,
  dist = "cosine",
  cluster_method = "ward.D",
  custom_palette = NULL,
  x_lab = NULL,
```

```
  y_lab = NULL,
  rotate_xlabs = FALSE
)
```

Arguments

mf_data	A data frame containing the mutation frequency data at the desired subtype resolution. This is obtained using the 'calculate_mf' function with subtype_resolution set to the desired resolution. Data must include a column containing the group_col, a column containing the mutation subtypes, a column containing the desired response variable (mf, proportion, sum) for the desired mf_type (min or max), and if applicable, a column containing the variable by which to order the samples/groups.
group_col	The name of the column(s) in the mf data that contains the sample/group names. This will generally be the same values used for the cols_to_group argument in the calculate_mf function. However, you may also use groups that are at a higher level of the aggregation in mf_data.
subtype_resolution	The subtype resolution of the mf data. Options are `base_6`, `base_12`, `base_96`, `base_192`, or `type`. Default is `base_6`.
response	The desired response variable to be plotted. Options are mf, proportion, or sum. Default is `proportion`. Your mf_data must contain columns with the name of your desired response: `mf_min`, `mf_max`, `proportion_min`, `proportion_max`, `sum_min`, and `sum_max`.
mf_type	The mutation counting method to use. Options are min or max. Default is `min`.
group_order	The method for ordering the samples within the plot. Options include: • `none`: No ordering is performed. Default. • `smart`: Groups are automatically ordered based on the group names (alphabetical, numerical) • `arranged`: Groups are ordered based on one or more factor column(s) in mf_data. Column names are passed to the function using the `group_order_input`. • `custom`: Groups are ordered based on a custom vector of group names. The custom vector is passed to the function using the `group_order_input`. • `clustered`: Groups are ordered based on hierarchical clustering. The dissimilarity matrix can be specified using the `dist` argument. The agglomeration method can be specified using the `cluster_method` argument.
group_order_input	A character vector specifying details for the group order method. If `group_order` is `arranged`, `group_order_input` should contain the column name(s) to be used for ordering the samples. If `group_order` is `custom`, `group_order_input` should contain the custom vector of group names.
dist	The dissimilarity matrix for hierarchical clustering. Options are `cosine`, `euclidean`, `maximum`, `manhattan`, `canberra`, `binary` or `minkowski`. The default is `cosine`. See dist for details.
cluster_method	The agglomeration method for hierarchical clustering. Options are `ward.D`, `ward.D2`, `single`, `complete`, `average` (= UPGMA), `mcquitty` (= WPGMA),

	median (= WPGMC) or centroid (= UPGMC). The default is Ward.D. See hclust for details.
custom_palette	A named vector of colors to be used for the mutation subtypes. The names of the vector should correspond to the mutation subtypes in the data. Alternatively, you can specify a color palette from the RColorBrewer package. See brewer.pal for palette options. You may visualize the palettes at the ColorBrewer website: https://colorbrewer2.org/. Default is NULL.
x_lab	The label for the x-axis. Default is the value of group_col.
y_lab	The label for the y-axis. Default is the value of response_col.
rotate_xlabs	A logical value indicating whether the x-axis labels should be rotated 90 degrees. Default is FALSE.

Value

A ggplot object representing the mutation spectra plot.

Examples

```
# Example data consists of 24 mouse bone marrow DNA samples imported
# using import_mut_data() and filtered with filter_mut. Filtered
# mutation data is available in the MutSeqRData ExperimentHub package:
# eh <- ExperimentHub::ExperimentHub()
# Example 1: Visualized the 6-base mutation proportions per dose group.
# Data was summarized per dose_group using:
# calculate_mf(mutation_data = eh[["EH9861"]],
#              cols_to_group = "dose_group",
#              subtype_resolution = "base_6")
# Load the example data
mf_example <- readRDS(system.file("extdata", "Example_files", "mf_data_6.rds",
  package = "MutSeqR"
))
# Convert dose_group to a factor with the desired order.
mf_example$dose_group <- factor(mf_example$dose_group,
  levels = c("Control", "Low", "Medium", "High")
)
# Plot the mutation spectra
plot <- plot_spectra(
  mf_data = mf_example,
  group_col = "dose_group",
  subtype_resolution = "base_6",
  response = "proportion",
  group_order = "arranged",
  group_order_input = "dose_group"
)

# Example 2: plot the proportion of 6-based mutation subtypes
# for each sample, ordered by hierarchical clustering:
# Data was summarized per dose_group using:
# calculate_mf(mutation_data = eh[["EH9861"]],
#              cols_to_group = "sample",
```

```
#              subtype_resolution = "base_6")
# Load the example data
mf_example2 <- readRDS(system.file("extdata", "Example_files", "mf_data_6_sample.rds",
  package = "MutSeqR"
))
plot <- plot_spectra(
  mf_data = mf_example2,
  group_col = "sample",
  subtype_resolution = "base_6",
  response = "proportion",
  group_order = "clustered"
)
```

`plot_trinucleotide` *Plot the trinucleotide spectrum*

Description

Creates barplots of the trinucleotide spectrum for all levels of a given group.

Usage

```
plot_trinucleotide(
  mf_96,
  response = "proportion",
  mf_type = "min",
  group_col = "dose",
  indiv_y = FALSE,
  sum_totals = TRUE,
  output_path = NULL,
  output_type = "svg"
)
```

Arguments

`mf_96`	A data frame containing the mutation frequency data at the 96-base resolution. This should be obtained using the 'calculate_mf' with subtype_resolution set to 'base_96'. Generally, cols_to_group should be the same as 'group_col'.
`response`	A character string specifying the type of response to plot. Must be one of 'frequency', 'proportion', or 'sum'.
`mf_type`	A character string specifying the mutation count method to plot. Must be one of 'min' or 'max'. Default is 'min'.
`group_col`	A character string specifying the column(s) in 'mf_96' to group the data by. Default is 'sample'. The sum, proportion, or frequency will be plotted for all unique levels of this group. You can specify more than one column to group by. Generally the same as the 'cols_to_group' parameter in 'calculate_mf' when generating mf_96.

indiv_y	A logical value specifying whether the the max response value for the y-axis should be scaled independently for each group (TRUE) or scaled the same for all groups (FALSE). Default is FALSE.
sum_totals	A logical value specifying whether to display the total sum of mutations in the mutation labels. Default is TRUE.
output_path	An optional file path to an output directory. If provided, the plots will be automatically exported using the graphics device specified in output_type. The function will create the output directory if it doesn't already exist. If NULL, plots will not be exported. Default is NULL.
output_type	A character string specifying the type of output file. Options are 'eps', 'ps', 'tex', 'pdf', or 'jpeg', 'tiff', 'png', 'bmp', 'svg', or 'wmf' (windows only). Default is 'svg'.

Details

The function plots the trinucleotide spectrum for all levels of a given group from the provided mf_96 data; the output of calculate_mf with subtype_resolution = "base_96".

Value

A named list containing ggplots.

Examples

```
 # Calculate the mutation frequency data at the 96-base resolution
 mf_96 <- readRDS(system.file("extdata", "Example_files", "mf_data_96.rds",
    package = "MutSeqR"))
# Plot the trinucleotide proportions for the control and high dose groups
mf_96 <- dplyr::filter(mf_96, dose_group %in% c("Control", "High"))

# Scale y-axis the same for all groups
  plots <- plot_trinucleotide(
    mf_96 = mf_96,
    response = "proportion",
    mf_type = "min",
    group_col = "dose_group",
    indiv_y = FALSE,
    output_path = NULL
  )
```

---

plot_trinucleotide_heatmap

Create a heatmap plot of mutation subtype proportions.

Description

This function creates a heatmap plot of subtype proportions for a given grouping variable. The groups may be facetted by a second variable. Mutation sums for each facet group and normalized subtype are calculated and displayed.

Usage

```
plot_trinucleotide_heatmap(
  mf_data,
  group_col = "sample",
  facet_col = "dose",
  mf_type = "min",
  mut_proportion_scale = "turbo",
  max = 0.2,
  rescale_data = FALSE,
  condensed = FALSE
)
```

Arguments

mf_data	A data frame containing the mutation frequency data at the desired base resolution. This is obtained using the 'calculate_mf' with subtype_resolution set to the desired resolution. cols_to_group should be the same as 'group_col'.
group_col	The variable to group by.
facet_col	The variable to facet by.
mf_type	The type of mutation frequency to plot. Options are "min" or "max". (Default: "min")
mut_proportion_scale	The scale option for the mutation proportion. Options are passed to viridis::scale_fill_viridis_c. One of # inferno, magma, plasma, viridis, cividis, turbo, mako, or rocket. We highly reccomend the default for its ability to disciminate hard to see patterns. (Default: "turbo")
max	Maximum value used for plotting the proportions. Proportions that are higher will have the maximum colour. (Default: 0.2)
rescale_data	Logical value indicating whether to rescale the mutation proportions to increase the dynamic range of colors shown on the plot. (Default: TRUE)
condensed	More condensed plotting format. Default = FALSE.

Value

A ggplot object representing the heatmap plot.

Examples

```
mf_96 <- readRDS(system.file("extdata/Example_files/mf_data_96_sample.rds",
package = "MutSeqR"))
# define dose_group order
mf_96$dose_group <- factor(mf_96$dose_group,
     levels = c("Control", "Low","Medium", "High")
)
plot <- plot_trinucleotide_heatmap(mf_96,
  group_col = "sample",
  facet_col = "dose_group"
)
```

populate_sequence_context *Populate Sequence context*

Description

This function populates the trinucleotide context for each mutation in the mutation data.

Usage

```
populate_sequence_context(mutation_granges, BS_genome, n = 1)
```

Arguments

mutation_granges	A GRanges object containing mutation data.
BS_genome	The name of the Bioconductor BSgenome package to use for retrieving the reference genome sequence.
n	An integer value indicating the number of base pairs to include on either side of the mutation for context. Default is 1 (trinucleotide context including the mutation).

Value

A GRanges object with an additional column for the trinucleotide context.

print_ascii_art *This function prints ASCII art when the package is loaded*

Description

This function prints ASCII art when the package is loaded

Usage

```
print_ascii_art()
```

Value

None

rdm_fast *rdm_fast*

Description

Rapidly simulates count matrices drawn from a Dirichlet-Multinomial distribution. This function is highly optimized for parametric bootstrapping, utilizing a Gamma-Multinomial approximation for speed.

Usage

```
rdm_fast(depths, p, theta)
```

Arguments

depths	A numeric vector representing the total sequencing depth (total mutation counts) for each sample to be simulated.
p	A numeric vector representing the expected probability of each subtype.
theta	Numeric scalar. The overdispersion parameter (concentration parameter).

Value

A numeric matrix of simulated mutation counts, with subtypes as rows and simulated samples as columns.

rename_columns *Map column names of mutation data to default column names. A utility function that renames columns of mutation data to default columns names.*

Description

Map column names of mutation data to default column names. A utility function that renames columns of mutation data to default columns names.

Usage

```
rename_columns(data, column_map = op$column)
```

Arguments

data	mutation data
column_map	a list that maps synonymous column names to their default.

Value

the mutation data with column names changed to match default.

Examples

```
df <- data.frame(
  chromosome = c("chr1", "chr2", "chr3"),
  pos = c(100, 200, 300),
  end = c(100, 200, 300),
  sample_id = c("S1", "S2", "S3"),
  reference = c("G", "C", "T"),
  alternate = c("A", "T", "G")
)
renamed_data <- rename_columns(df, column_map = op$column)
```

---

render_report *Read configuration file and render R Markdown document*

Description

This function reads a configuration file in YAML format, extracts the parameters, and renders an R Markdown document using the specified parameters.

Usage

```
render_report(
  config_filepath,
  output_file = "./MutSeqR_Summary_Report.html",
  output_format = "html_document"
)
```

Arguments

config_filepath	The path to the configuration file.
output_file	The name of the output file. Will be saved to the outputdir in config params.
output_format	The format of the output file. Options are "html_document" (default), "pdf_document", or "all".

Value

A rendered R Markdown document.

Examples

```
# Step 1: Copy the example configuration file to your working directory
## config <- system.file("extdata", "inputs", "summary_config.yaml", package = "MutSeqR")
## file.copy(from = config, to = "your/working/directory/summary_config.yaml")
# Step 2: Edit the configuration file with your inputs
# Step 3: Render the report
## render_report(config_filepath = "your/working/directory/summary_config.yaml",
##  output_file = "MutSeqR_Summary_Report.html",
##  output_format = "html_document")
```

`reverseComplement` *Get the reverse complement of a DNA or RNA sequence.*

Description

Get the reverse complement of a DNA or RNA sequence.

Usage

```
reverseComplement(
  x,
  content = c("dna", "rna"),
  case = c("lower", "upper", "as is")
)
```

Arguments

`x`	A character vector of DNA or RNA sequences.
`content`	c("dna", "rna") The type of sequence to be reversed.
`case`	c("lower", "upper", "as is") The case of the output sequence.

Details

This file is part of the source code for SPGS: an R package for identifying statistical patterns in genomic sequences. Copyright (C) 2015 Universidad de Chile and INRIA-Chile A copy of Version 2 of the GNU Public License is available in the share/licenses/gpl-2 file in the R installation directory or from http://www.R-project.org/Licenses/GPL-2. reverseComplement.R

Value

A character vector of the reverse complement sequences.

run_penalized_comparison *run_penalized_comparison*

Description

Core statistical engine for the RP-DMM test. Evaluates whether two count matrices represent significantly different mutational spectra. It utilizes a "hybrid logic": robust parameters (thetas) are estimated utilizing a ridge penalty (QA phase), while the test statistic (LRT) is evaluated using the unpenalized likelihood. Significance is evaluated via parametric bootstrap.

Usage

```
run_penalized_comparison(g1, g2, lambda = 1, n_boot = 500)
```

Arguments

g1	A numeric count matrix for Group 1 (subtypes as rows, samples as columns).
g2	A numeric count matrix for Group 2 (subtypes as rows, samples as columns).
lambda	Numeric. The ridge penalty strength used during parameter estimation.
n_boot	Integer. The number of parametric bootstrap iterations to perform.

Value

A list containing 5 items:

- p_value: Numeric. The bootstrapped p-value.
- lrt: Numeric. The observed unpenalized Likelihood Ratio Test statistic.
- theta_g1: Numeric. The optimized robust theta (overdispersion) for Group 1.
- theta_g2: Numeric. The optimized robust theta for Group 2.
- theta_shared: Numeric. The optimized robust theta under the Null hypothesis.

select_optimal_lambda *select_optimal_lambda*

Description

Performs leave-one-out cross-validation (LOOCV) to empirically select the optimal ridge penalty (lambda) for a given count matrix. Models are fit on $N-1$ samples using a specified lambda, and unpenalized likelihood is evaluated on the held-out sample.

Usage

```
select_optimal_lambda(count_matrix, lambdas = c(0.1, 0.5, 1, 5, 10, 50, 100))
```

Arguments

count_matrix	A numeric matrix of mutation counts with subtypes as rows and samples as columns.
lambdas	A numeric vector of lambda penalty values to evaluate. Default is c(0.1, 0.5, 1, 5, 10, 50, 100).

Value

A list containing two items:

- best_lambda: Numeric scalar. The lambda value from the provided vector that yielded the highest overall cross-validation log-likelihood.
- scores: A named numeric vector detailing the cumulative log-likelihood score achieved by each tested lambda.

---

setup_mutseqr_python *Set up Python environment for MutSeqR*

Description

This function initializes the Python environment used by MutSeqR. It is not run automatically to avoid issues during installation and checks.

Usage

```
setup_mutseqr_python(force = FALSE)
```

Arguments

force	Logical. Whether to force reconfiguration even if an environment already exists.

Value

None

sidak *Correct p-values for multiple comparisons*

Description

Correct p-values for multiple comparisons

Usage

```
sidak(vecP)
```

Arguments

vecP	vector of p-values

Details

This function corrects a vector of probabilities for multiple testing using the Bonferroni (1935) and Sidak (1967) corrections. References: Bonferroni (1935), Sidak (1967), Wright (1992). Bonferroni, C. E. 1935. Il calcolo delle assicurazioni su gruppi di teste. Pp. 13-60 in: Studi in onore del Professore Salvatore Ortu Carboni. Roma. Sidak, Z. 1967. Rectangular confidence regions for the means of multivariate normal distributions. Journal of the American Statistical Association 62:626-633. Wright, S. P. 1992. Adjusted P-values for simultaneous inference. Biometrics 48: 1005-1013. Pierre Legendre, May 2007

Value

adjusted p-values

Examples

```
p_values <- c(0.01, 0.04, 0.03, 0.08, 0.05)
adjusted_p <- sidak(p_values)
adjusted_p$SidakP
```

signature_fitting *Run COSMIC signatures comparison using SigProfilerAssignment*

Description

Run COSMIC signatures comparison using SigProfilerAssignment

Usage

```
signature_fitting(
  mutation_data,
  project_name = "Default",
  project_genome = "GRCh38",
  env_name = "MutSeqR",
  group = "sample",
  output_path = NULL,
  python_version
)
```

Arguments

mutation_data	A data frame containing mutation data.
project_name	The name of the project. This is used to format the data into required .txt format for SigProfiler tools.
project_genome	The reference genome to use. On first use, the function will install the genome using SigProfilerMatrixGenerator::install. e.x. GRCh37, GRCH38, mm10, mm9, rn6
env_name	The name of the virtual environment. This will be created on first use.
group	The column in the mutation data used to aggregate groups. Signature assignment will be performed on each group separately.
output_path	The filepath to the directory in which the output folder will be created to store results. Default is NULL. This will store results in the current working directory.
python_version	The version of python installed on the user's computer.

Details

Assign COSMIC SBS signatures to mutation data using SigProfilerAssignment. Data is cleaned and formatted for input into SigProfiler tools. This function will create a virtual environment using reticulate to run python, as this is a requirement for the SigProfiler suite of tools. Note that it will also install several python dependencies using a conda virtual environment on first use. Please be aware of the implications of this. For advanced use, it is suggested to use the SigProfiler python tools directly in python as described in their respective documentation. Users must have python installed on their computer to use this function.

Mutation data will be filtered to only include SNVs. Variants flagged by the filter_mut column will be excluded.

Value

Creates a subfolder "SigProfiler" in the output directory with SigProfiler tools results. For a complete breakdown of the results, see the Readme file for MutSeqR. Most relevant results are stored in SigProfiler > ggplot2::group > matrices > output > Assignment_Solution > Activities > SampleReconstruction > WebPNGs. These plots show a summary of the signature assignment results for each group. In each plot, the top left panel represents the base_96 mutation count for the group. The bottom left panel represents the reconstructed profile. Below the reconstruction are the solution statistics that indicate the goodness of fit of the reconstructed profile to the observed profile.

(Recommended cosine similarity > 0.9). The panels on the right represent the SBS signatures that contribute to the reconstructed profile. The signature name and its contribution % are shown in the panel. A high contribution means a high association of the signature with the group's mutation spectra.

Examples

```
if (requireNamespace("MutSeqRData", quietly = TRUE)) {
  # Example data consists of 24 mouse bone marrow DNA samples imported
  # using import_mut_data() and filtered with filter_mut as in Example 4.
  # Sequenced on TS Mouse Mutagenesis Panel. Example data is
  # retrieved from MutSeqRData, an ExperimentHub data package.
  library(ExperimentHub)
  eh <- ExperimentHub()
  example_data <- eh[["EH9861"]]
  output_path <- tempdir()

  signature_fitting(
    mutation_data = example_data,
    project_name = "Example",
    project_genome = "mm10",
    env_name = "MutSeqR",
    group = "dose",
    python_version = "3.11",
    output_path = output_path
  )
}
```

---

spectra_comparison *Compare the overall mutation spectra between groups*

Description

spectra_comparison compares the mutation spectra of groups using a modified contingency table approach.

Usage

```
spectra_comparison(
  mf_data,
  exp_variable,
  mf_type = "min",
  contrasts,
  cont_sep = "\t"
)
```

Arguments

mf_data	A data frame containing the MF data. This is the output from calculate_mf(). MF data should be at the desired subtype resolution. Required columns are the exp_variable column(s), the subtype column, and sum_min or sum_max.
exp_variable	The column names of the experimental variable(s) to be compared.
mf_type	The type of mutation frequency to use. Default is "min" (recommended).
contrasts	a filepath to a file OR a dataframe that specifies the comparisons to be made between levels of the exp_variable(s) The table must consist of two columns, each containing a level of the exp_variable. The level in the first column will compared to the level in the second column for each row in contrasts. When using more than one exp_variable, separate the levels of each variable with a colon. Ensure that all variables listed in exp_variable are represented in each entry for the table. See details for examples.
cont_sep	The delimiter used to import the contrasts table. Default is tab.

Details

This function creates an R * 2 contigency table of the subtype counts, where R is the number of subtypes for the 2 groups being compared. The G2 likelihood ratio statistic is used to evaluate whether the proportion (count/group total) of each mutation subtype equals that of the other group.

The G2 statistic refers to a chi-squared distribution to compute the p-value for large sample sizes. When N / (R-1) < 20, where N is the total mutation counts across both groups, the function will use a F-distribution to compute the p-value in order to reduce false positive rates.

The comparison assumes independance among the observations, as such, it is highly recommended to use mf_type = "min".

Examples of contrasts: For 'exp_variable = "dose"' with dose groups 0, 12.5, 25, 50, compare each treated dose to the control:

12.5 0

25 0

50 0

Ex. Consider two 'exp_variables = c("dose", "tissue")'; with levels dose (0, 12.5, 25, 50) and tissue("bone_marrow", "liver"). To compare the mutation spectra between tissues for each dose group, the contrast table would look like:

0:bone_marrow 0:liver

12.5:bone_marrow 12.5:liver

25:bone_marrow 25:liver

50:bone_marrow 50:liver

Value

the log-likelihood statistic G2 for the specified comparisons with the p-value adjusted for multiple-comparisons.

Examples

```
# Example data consists of 24 mouse bone marrow DNA samples imported
# using import_mut_data() and filtered with filter_mut. Filtered
# mutation data is available in the MutSeqRData ExperimentHub package:
# eh <- ExperimentHub::ExperimentHub()
# Data was summarized per sample using:
# calculate_mf(mutation_data = eh[["EH9861"]],
#              cols_to_group = "dose_group",
#              subtype_resolution = "base_6")

# Example: compare 6-base mutation spectra between dose groups
# Load the example data
mf_example <- readRDS(
     system.file("extdata", "Example_files", "mf_data_6.rds",
         package = "MutSeqR"
     )
)
# Create the contrasts table
contrasts <- data.frame(
  col1 = c("Low", "Medium", "High"),
  col2 = rep("Control", 3)
)
# Run the comparison
spectra_comparison(
  mf_data = mf_example,
  exp_variable = "dose_group",
  mf_type = "min",
  contrasts = contrasts
)
```

`spectra_comparison_rpdmm`

spectra_comparison_rpdmm

Description

Ridge-penalized Dirichlet-Multinomial test for comparing mutation spectra between two groups. Robust for large mutation counts.

Usage

```
spectra_comparison_rpdmm(
  mf_data,
  exp_variable,
  contrasts,
  cont_sep = "\t",
  mf_type = "min",
  lambda = 1,
  n_boot = 500
)
```

Arguments

mf_data	A data frame containing the MF data. This is the output from calculate_mf(). MF data should be calculate at the *sample level* and at the desired subtype resolution. Required columns are sample, the exp_variable column(s), the subtype column, and sum_min or sum_max.
exp_variable	The column names of the experimental variable(s) to be compared.
contrasts	A filepath (character) OR a data frame specifying the comparisons to be made. Must consist of exactly two columns. The level in the first column will be compared to the level in the second column for each row. If using multiple exp_variables, separate levels with a colon (e.g., "Drug:High").
cont_sep	Character. The delimiter used to import the contrasts table if a filepath is provided. Default is tab.
mf_type	Character. The type of mutation frequency count to use. Choices are "min" or "max". Default is "min" (recommended).
lambda	Numeric. The strength of the ridge penalty. Default is 1.0. Higher values increase shrinkage towards a uniform distribution, stabilizing estimation in sparse datasets.
n_boot	Integer. The number of bootstrap iterations to perform for p-value calculation. Default is 500.

Value

A data frame containing one row per specified contrast. Columns include the group names, comparison string, bootstrap p-value, observed Likelihood Ratio Test (LRT) statistic, and robust overdispersion metrics (theta) for group 1, group 2, and the shared null model.

---

subtype_dict *Subtype Resolution Dictionary*

Description

Associates the subtype resolution names with their corresponding column names in the mutation data.

Usage

```
subtype_dict
```

Format

A vector with corresponding values

Examples

```
subtype_dict["base_96"]
subtype_dict["type"]
```

subtype_list *A comprehensive list of mutation subtypes at different resolutions*

Description

A comprehensive list of mutation subtypes at different resolutions

Usage

```
subtype_list
```

Format

A list with corresponding values

Examples

```
subtype_list[["type"]]
```

validate_BS_genome *Validate BSgenome Input*

Description

Internal utility function to validate the BS_genome argument prior to sequence context extraction. Ensures that the provided genome is a valid **BSgenome** package name and that it is installed locally.

Usage

```
validate_BS_genome(BS_genome)
```

Arguments

BS_genome	A character string specifying the package name of a **BSgenome** object (e.g., "BSgenome.Hsapiens.UCSC.hg38"), or NULL.

Details

This function performs three checks:

1. If BS_genome is NULL, an error is thrown indicating that a genome must be provided when sequence context is required.
2. If BS_genome is not among the available **BSgenome** packages, an error is thrown.
3. If BS_genome is valid but not installed locally, an error is thrown with instructions to install it via BiocManager::install().

This function is intended to be called only when sequence context needs to be populated (i.e., when a context column is absent or incomplete).

Value

Invisibly returns TRUE if validation passes; otherwise, an error is raised.

---

vcf_sample_fix *Retrieve the sample column from VCF files*

Description

Checks to find the sample name of the vcf in the INFO field or in the FORMAT header. Can also handle sample name synonyms.

Usage

```
vcf_sample_fix(vcf)
```

Arguments

vcf	The imported VCF

Value

The vcf with sample column name corrected

---

write_excel *Write results to Excel tables*

Description

Writes data to an Excel file.

Usage

```
write_excel(data, output_path = NULL, workbook_name, model_results = FALSE)
```

Arguments

data	A data frame, a list of data frames, or model_mf output.
output_path	Directory to write to. Defaults to current working directory.
workbook_name	Filename (without extension).
model_results	Logical. Set to TRUE if data is output from model_mf.

Value

A saved Excel workbook.

Examples

```
# Example data consists of 24 mouse bone marrow DNA samples imported
# using import_mut_data(), filtered with filter_mut, and summarized
# using calculate_mf().
outputpath <- tempdir()
mf_example <- readRDS(system.file("extdata/Example_files/mf_data_global.rds",
  package = "MutSeqR"
))
mf_example2 <- readRDS(system.file("extdata/Example_files/mf_data_6.rds",
  package = "MutSeqR"
))
mf_example3 <- readRDS(
 system.file("extdata/Example_files/mf_data_6_sample.rds",
     package = "MutSeqR"
 )
)
list <- list(mf_example, mf_example2, mf_example3)
names(list) <- c("Global MF", "Base 6 Spectra", "Base 6 Sample Spectra")

# save a single data frame to an Excel file
write_excel(
 mf_example,
 output_path = outputpath,
 workbook_name = "test_single"
)

# save a list of data frames to an Excel file
write_excel(list, output_path = outputpath, workbook_name = "test_list")
```

`write_mutational_matrix` *Write a Mutational Matrix to input into the sigprofiler web application*

Description

Creates a .txt file from mutation data that can be used for mutational signatures analysis using the SigProfiler web application. Can handle group analyses (ex dose, tissue, etc). Currently only supports SBS matrices i.e. snvs.

Usage

```
write_mutational_matrix(
  mutation_data,
  group = "dose",
  subtype_resolution = "base_96",
  mf_type = "min",
  output_path = NULL
)
```

Arguments

mutation_data	The object containing the mutation data. The output of import_mut_data() or import_vcf_data().
group	The column in the mutation data used to aggregate groups (e.g., sample, tissue, dose).
subtype_resolution	The resolution of the mutation subtypes. Options are "base_6" or "base_96". Default is "base_96".
mf_type	The mutation counting method to use. Options are "min" or "max". Default is "min".
output_path	The path to save the output file. If not provided, the file will be saved in the current working directory. Default is NULL.

Details

Mutations will be be filtered for SNVs. Mutations flagged in `filter_mut` will be excluded from the output. Mutations will be summed across the groups specified in the `group` argument.

Value

a .txt file that can be uploaded to the SigProfiler Assignment web application (https://cancer.sanger.ac.uk/signatures/assignmen as a "Mutational Matrix".

Examples

```
# Example data  consists of 24 mouse bone marrow
# samples exposed to three doses of BaP alongside vehicle controls.
# Libraries were sequenced with Duplex Sequencing using
# the TwinStrand Mouse Mutagenesis Panel which consists of 20 2.4kb
# targets = 48kb of sequence. Example data can be retrieved from
# MutSeqRData, an ExperimentHub data package:
## library(ExperimentHub)
## eh <- ExperimentHub()
## query(eh, "MutSeqRData")
# The data is a subset of variants from the target chr1
# from samples of the high dose group (50mg).
example_data <- readRDS(system.file("extdata", "Example_files",
                                    "variants_subset_d50_chr1.rds",
                                     package = "MutSeqR")
)

  write_mutational_matrix(
    mutation_data = example_data,
    group = "sample",
    subtype_resolution = "base_96",
    mf_type = "min",
    output_path = tempdir()
  )
  list.files(tempdir())
  # The file is saved in the temporary directory
```

```
# To view the file, use the following code:
## output_file <- file.path(tempdir(), "sample_base_96_mutational_matrix.txt")
## file.show(output_file)
```

`write_mutation_calling_file` *Write the mutation calling file to input into the SigProfiler Assignment web application.*

Description

Creates a .txt file from mutation data that can be used for mutational signatures analysis using the SigProfiler Assignment web application. Currently only supports SBS analysis i.e. snvs.

Usage

```
write_mutation_calling_file(
  mutation_data,
  project_name = "Example",
  project_genome = "GRCm38",
  output_path = NULL
)
```

Arguments

`mutation_data`	The object containing the mutation data. The output of import_mut_data() or import_vcf_data().
`project_name`	The name of the project. Default is "Example".
`project_genome`	The reference genome to use. (e.g., Human: GRCh38, Mouse mm10: GRCm38)
`output_path`	The path to save the output file. If NULL, files will be saved in the current working directory. Default is NULL.

Details

Mutations will be be filtered for SNVs. Mutations flagged in `filter_mut` will be excluded from the output.

Value

a .txt file that can be uploaded to the SigProfiler Assignment web application (https://cancer.sanger.ac.uk/signatures/assignmen as a "Mutational calling file".

Examples

```
# Example data  consists of 24 mouse bone marrow
# samples exposed to three doses of BaP alongside vehicle controls.
# Libraries were sequenced with Duplex Sequencing using
# the TwinStrand Mouse Mutagenesis Panel which consists of 20 2.4kb
# targets = 48kb of sequence. Example data can be retrieved from
# MutSeqRData, an ExperimentHub data package:
## library(ExperimentHub)
## eh <- ExperimentHub()
## query(eh, "MutSeqRData")
# The data is a subset of variants from the target chr1
# from samples of the high dose group (50mg).
example_data <- readRDS(system.file("extdata", "Example_files",
                                    "variants_subset_d50_chr1.rds",
                                     package = "MutSeqR")
)
 write_mutation_calling_file(
    mutation_data = example_data,
    project_name = "Example",
    project_genome = "GRCm38",
    output_path = tempdir()
  )
  list.files(tempdir())
  # The file is saved in the temporary directory
  # To view the file, use the following code:
  ## output_file <- file.path(tempdir(), "mutation_calling_file.txt")
  ## file.show(output_file)
```

`write_reference_fasta` *Write FASTA file of reference sequences.*

Description

Write FASTA file of reference sequences.

Usage

```
write_reference_fasta(regions_gr, output_path = NULL)
```

Arguments

`regions_gr`	A GRanges object including the sequences of the reference regions included for the data. This can be generated from the `get_seq` function.
`output_path`	The directory where the FASTA file should be written. Default is NULL, which will write the file to the current working directory.

Details

Generate an arbitrary multi-sequence FASTA file from GRanges including the reference sequences.

Value

Writes a FASTA reference file "reference_output.fasta". If multiple ranges are included in the GRanges object, the sequences will be written to a single FASTA file. Sequences names will be the seqnames (contig) of the range.

Examples

```
# Write FASTA files for the 20 genomic target sequences
# of TwinStrand's Mouse Mutagenesis Panel.
output_path <- tempdir()
rg <- get_seq("TSpanel_mouse")
write_reference_fasta(rg, output_path = output_path)
```

---

write_vcf_from_mut *Write mutation_data to a VCF file*

Description

Export your mutation_data to a VCF file for downstream applications.

Usage

```
write_vcf_from_mut(mutation_data, output_path = NULL)
```

Arguments

mutation_data	A data frame of a GRanges object containing your mutation data. This can be the output of import_mut_data, import_vcf_data, or filter_mut. Coordinates must be 1-based. Required columns are "contig", "start", "end", "ref", "alt", "sample", "alt_depth", "total_depth", and "ref_depth". Additional columns are allowed.
output_path	The directory where the VCF file should be written. Default is NULL, which will write the file to the current working directory.

Value

Writes a VCF file of mutations "mutation_output.vcf".

Examples

```
# Example data  consists of 24 mouse bone marrow
# samples exposed to three doses of BaP alongside vehicle controls.
# Libraries were sequenced with Duplex Sequencing using
# the TwinStrand Mouse Mutagenesis Panel which consists of 20 2.4kb
# targets = 48kb of sequence. Example data can be retrieved from
# MutSeqRData, an ExperimentHub data package:
## library(ExperimentHub)
## eh <- ExperimentHub()
```

```
## query(eh, "MutSeqRData")
# The data is a subset of variants from the target chr1
# from samples of the high dose group (50mg).
example_data <- readRDS(system.file("extdata", "Example_files",
                                    "variants_subset_d50_chr1.rds",
                                     package = "MutSeqR")
)
# Export mutation data of the four samples to a multi-sample VCF file.
write_vcf_from_mut(mutation_data = example_data, output_path = tempdir())
```

`%>%` *Pipe operator*

Description

See `magrittr::%>%` for details.

Usage

```
lhs %>% rhs
```

Arguments

`lhs`	A value or the magrittr placeholder.
`rhs`	A function call using the magrittr semantics.

Value

The result of calling `rhs(lhs)`.

Examples

```
df <- data.frame(x = 1:5, y = rnorm(5))
df %>% dplyr::mutate(z = x + y)
df %>% head(3) %>% summary()
```

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