Package 'transite'

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Description transite is a computational method that allows comprehensive analysis of the regulatory role of RNA-binding proteins in various cellular processes by leveraging preexisting gene expression data and current knowledge of binding preferences of RNA-binding proteins.

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URL https://transite.mit.edu

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`calculate_kmer_enrichment` k-*mer Enrichment between Foreground and Background Sets*

Description

Calls `compute_kmer_enrichment` to compute *k*-mer enrichment values for multiple foregrounds. Calculates enrichment for foreground sets in parallel.

Usage

```
calculate_kmer_enrichment(
  foreground_sets,
  background_set,
  k,
  permutation = FALSE,
  chisq_p_value_threshold = 0.05,
  p_adjust_method = "BH",
  n_cores = 4
)
```

Arguments

`foreground_sets`	list of foreground sets; a foreground set is a character vector of DNA or RNA sequences (not both) and a strict subset of the `background_set`
`background_set`	character vector of DNA or RNA sequences that constitute the background set
`k`	length of *k*-mer, either `6` for hexamers or `7` for heptamers
`permutation`	if `TRUE`, only the enrichment value is returned (efficiency mode used for permutation testing)
`chisq_p_value_threshold`	threshold below which Fisher's exact test is used instead of Pearson's chi-squared test
`p_adjust_method`	see `p.adjust`
`n_cores`	number of computing cores to use

Value

A list with three entries:

dfs	a list of data frames with results from compute_kmer_enrichment for each of the foreground sets
kmers	a character vector of all k-mers
kmer_origins	a list of k-mers and their original sequence(s)

See Also

Other *k*-mer functions: check_kmers(), compute_kmer_enrichment(), count_homopolymer_corrected_kmers(), create_kmer_origin_list(), draw_volcano_plot(), estimate_significance(), estimate_significance_core(), generate_kmers(), generate_permuted_enrichments(), run_kmer_spma(), run_kmer_tsma()

Examples

```
# define simple sequence sets for foreground and background
foreground_set1 <- c(
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
  "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA"
)
foreground_set2 <- c("UUAUUUA", "AUCCUUUACA", "UUUUUUU", "UUUCAUCAUU")
foreground_sets <- list(foreground_set1, foreground_set2)
background_set <- c(foreground_set1, foreground_set2,
                    "CCACACAC", "CUCAUUGGAG", "ACUUUGGGACA", "CAGGUCAGCA")

# single-threaded
kmer_enrichment_values_st <- calculate_kmer_enrichment(foreground_sets,
  background_set, 6, n_cores = 1)
## Not run:
# multi-threaded
kmer_enrichment_values_mt <- calculate_kmer_enrichment(foreground_sets,
  background_set, 6)
## End(Not run)
```

---

calculate_local_consistency

Local Consistency Score

Description

C++ implementation of Local Consistency Score algorithm.

Usage

```
calculate_local_consistency(x, numPermutations, minPermutations, e)
```

Arguments

x	numeric vector that contains values for shuffling
numPermutations	maximum number of permutations performed in Monte Carlo test for consistency score
minPermutations	minimum number of permutations performed in Monte Carlo test for consistency score
e	stop criterion for consistency score Monte Carlo test: aborting permutation process after observing e random consistency values with more extreme values than the actual consistency value

Value

list with score, p_value, and n components, where score is the raw local consistency score (usually not used), p_value is the associated p-value for that score, obtained by Monte Carlo testing, and n is the number of permutations performed in the Monte Carlo test (the higher, the more significant)

Examples

```
poor_enrichment_spectrum <- c(0.1, 0.5, 0.6, 0.4,
  0.7, 0.6, 1.2, 1.1, 1.8, 1.6)
local_consistency <- calculate_local_consistency(poor_enrichment_spectrum,
  1000000, 1000, 5)

enrichment_spectrum <- c(0.1, 0.3, 0.6, 0.7, 0.8,
  0.9, 1.2, 1.4, 1.6, 1.4)
local_consistency <- calculate_local_consistency(enrichment_spectrum,
  1000000, 1000, 5)
```

---

calculate_motif_enrichment

Binding Site Enrichment Value Calculation

Description

This function is used to calculate binding site enrichment / depletion scores between predefined foreground and background sequence sets. Significance levels of enrichment values are obtained by Monte Carlo tests.

Usage

```
calculate_motif_enrichment(
  foreground_scores_df,
  background_scores_df,
  background_total_sites,
  background_absolute_hits,
```

```
  n_transcripts_foreground,
  max_fg_permutations = 1e+06,
  min_fg_permutations = 1000,
  e = 5,
  p_adjust_method = "BH"
)
```

Arguments

foreground_scores_df	result of score_transcripts on foreground sequence set (foreground sequence sets must be a subset of the background sequence set)
background_scores_df	result of score_transcripts on background sequence set
background_total_sites	number of potential binding sites per sequence (returned by score_transcripts)
background_absolute_hits	number of putative binding sites per sequence (returned by score_transcripts)
n_transcripts_foreground	number of sequences in the foreground set
max_fg_permutations	maximum number of foreground permutations performed in Monte Carlo test for enrichment score
min_fg_permutations	minimum number of foreground permutations performed in Monte Carlo test for enrichment score
e	integer-valued stop criterion for enrichment score Monte Carlo test: aborting permutation process after observing e random enrichment values with more extreme values than the actual enrichment value
p_adjust_method	adjustment of p-values from Monte Carlo tests to avoid alpha error accumulation, see p.adjust

Value

A data frame with the following columns:

motif_id	the motif identifier that is used in the original motif library
motif_rbps	the gene symbol of the RNA-binding protein(s)
enrichment	binding site enrichment between foreground and background sequences
p_value	unadjusted p-value from Monte Carlo test
p_value_n	number of Monte Carlo test permutations
adj_p_value	adjusted p-value from Monte Carlo test (usually FDR)

See Also

Other matrix functions: run_matrix_spma(), run_matrix_tsma(), score_transcripts(), score_transcripts_single_

Examples

```
foreground_seqs <- c("CAGUCAAGACUCC", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AGAU", "CCAGUAA")
background_seqs <- c(foreground_seqs, "CAACAGCCUUAAUU", "CUUUGGGGAAU",
                     "UCAUUUUAUUAAA", "AUCAAAUUA", "GACACUUAAAGAUCCU",
                     "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
                     "AUAGAC", "AGUUC")
foreground_scores <- score_transcripts(foreground_seqs, cache = FALSE)
background_scores <- score_transcripts(background_seqs, cache = FALSE)
enrichments_df <- calculate_motif_enrichment(foreground_scores$df,
  background_scores$df,
  background_scores$total_sites, background_scores$absolute_hits,
  length(foreground_seqs),
  max_fg_permutations = 1000
)
```

`calculate_transcript_mc` *Motif Enrichment calculation*

Description

C++ implementation of Motif Enrichment calculation

Usage

```
calculate_transcript_mc(
  absoluteHits,
  totalSites,
  relHitsForeground,
  n,
  maxPermutations,
  minPermutations,
  e
)
```

Arguments

`absoluteHits`	number of putative binding sites per sequence (returned by `score_transcripts`)
`totalSites`	number of potential binding sites per sequence (returned by `score_transcripts`)
`relHitsForeground`	relative number of hits in foreground set
`n`	number of sequences in the foreground set
`maxPermutations`	maximum number of foreground permutations performed in Monte Carlo test for enrichment score

minPermutations
: minimum number of foreground permutations performed in Monte Carlo test for enrichment score

e
: stop criterion for enrichment score Monte Carlo test: aborting permutation process after observing e random enrichment values with more extreme values than the actual enrichment value

Value

list with p-value and number of iterations of Monte Carlo sampling for foreground enrichment

Examples

```
foreground_seqs <- c("CAGUCAAGACUCC", "AAUUGGUUGUGGGGCUUCCCUGUACAU",
                     "AGAU", "CCAGUAA", "UGUGGGG")
background_seqs <- c(foreground_seqs, "CAACAGCCUUAAUU", "CUUUGGGGAAU",
                     "UCAUUUUAUUAAA", "AUCAAAUUA", "GACACUUAAAGAUCCU",
                     "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
                     "AUAGAC", "AGUUC")
motif_db <- get_motif_by_id("M178_0.6")
fg <- score_transcripts(foreground_seqs, cache = FALSE,
  motifs = motif_db)
bg <- score_transcripts(background_seqs, cache = FALSE,
  motifs = motif_db)

mc_result <- calculate_transcript_mc(unlist(bg$absolute_hits),
 unlist(bg$total_sites),
 fg$df$absolute_hits / fg$df$total_sites,
 length(foreground_seqs), 1000, 500, 5)
```

---

check_kmers *Check Validity of Set of* k*-mers*

Description

Checks if the provided set of *k*-mers is valid. A valid set of *k*-mers is (1) non-empty, (2) contains either only hexamers or only heptamers, and (3) contains only characters from the RNA alphabet (A, C, G, U)

Usage

```
check_kmers(kmers)
```

Arguments

kmers
: set of *k*-mers

Value

TRUE if set of *k*-mers is valid

See Also

Other *k*-mer functions: calculate_kmer_enrichment(), compute_kmer_enrichment(), count_homopolymer_corrected(), create_kmer_origin_list(), draw_volcano_plot(), estimate_significance(), estimate_significance_core(), generate_kmers(), generate_permuted_enrichments(), run_kmer_spma(), run_kmer_tsma()

Examples

```
# valid set
check_kmers(c("ACGCUC", "AAACCC", "UUUACA"))

# invalid set (contains hexamers and heptamers)
check_kmers(c("ACGCUC", "AAACCC", "UUUACAA"))
```

---

classify_spectrum *Simple spectrum classifier based on empirical thresholds*

Description

Spectra can be classified based on the aggregate spectrum classifier score. If `sum(score) == 3` spectrum considered non-random, random otherwise.

Usage

```
classify_spectrum(
  adj_r_squared,
  degree,
  slope,
  consistency_score_n,
  n_significant,
  n_bins
)
```

Arguments

adj_r_squared	adjusted R^2 of polynomial model, returned by score_spectrum
degree	degree of polynomial, returned by score_spectrum
slope	coefficient of the linear term of the polynomial model (spectrum "direction"), returned by score_spectrum
consistency_score_n	number of performed permutations before early stopping, returned by score_spectrum
n_significant	number of bins with statistically significant enrichment
n_bins	number of bins

Value

a three-dimensional binary vector with the following components:

coordinate 1	adj_r_squared >= 0.4
coordinate 2	consistency_score_n > 1000000
coordinate 3	n_significant >= floor(n_bins / 10)

See Also

Other SPMA functions: run_kmer_spma(), run_matrix_spma(), score_spectrum(), subdivide_data()

Examples

```
n_bins <- 40

# random spectrum
random_sp <- score_spectrum(runif(n = n_bins, min = -1, max = 1),
  max_model_degree = 1)
score <- classify_spectrum(
  get_adj_r_squared(random_sp), get_model_degree(random_sp),
  get_model_slope(random_sp), get_consistency_score_n(random_sp), 0, n_bins
)
sum(score)

# non-random linear spectrum with strong noise component
signal <- seq(-1, 0.99, 2 / 40)
noise <- rnorm(n = 40, mean = 0, sd = 0.5)
linear_sp <- score_spectrum(signal + noise, max_model_degree = 1,
  max_cs_permutations = 100000)
score <- classify_spectrum(
  get_adj_r_squared(linear_sp), get_model_degree(linear_sp),
  get_model_slope(linear_sp), get_consistency_score_n(linear_sp), 10, n_bins
)
sum(score)
## Not run:
# non-random linear spectrum with weak noise component
signal <- seq(-1, 0.99, 2 / 40)
noise <- rnorm(n = 40, mean = 0, sd = 0.2)
linear_sp <- score_spectrum(signal + noise, max_model_degree = 1,
  max_cs_permutations = 100000)
score <- classify_spectrum(
  get_adj_r_squared(linear_sp), get_model_degree(linear_sp),
  get_model_slope(linear_sp), get_consistency_score_n(linear_sp), 10, n_bins
)
sum(score)

## End(Not run)

# non-random quadratic spectrum with strong noise component
signal <- seq(-1, 0.99, 2 / 40)^2 - 0.5
noise <- rnorm(n = 40, mean = 0, sd = 0.2)
quadratic_sp <- score_spectrum(signal + noise, max_model_degree = 2,
```

```
  max_cs_permutations = 100000)
score <- classify_spectrum(
  get_adj_r_squared(quadratic_sp), get_model_degree(quadratic_sp),
  get_model_slope(quadratic_sp),
  get_consistency_score_n(quadratic_sp), 10, n_bins
)
sum(score)
## Not run:
# non-random quadratic spectrum with weak noise component
signal <- seq(-1, 0.99, 2 / 40)^2 - 0.5
noise <- rnorm(n = 40, mean = 0, sd = 0.1)
quadratic_sp <- score_spectrum(signal + noise, max_model_degree = 2)
score <- classify_spectrum(
  get_adj_r_squared(quadratic_sp), get_model_degree(quadratic_sp),
  get_model_slope(quadratic_sp),
  get_consistency_score_n(quadratic_sp), 10, n_bins
)
sum(score)

## End(Not run)
```

`compute_kmer_enrichment` k-*mer Enrichment between Foreground and Background Sets*

Description

Compares foreground sequence set to background sequence set and computes enrichment values for each possible *k*-mer.

Usage

```
compute_kmer_enrichment(
  foreground_kmers,
  background_kmers,
  permutation = FALSE,
  chisq_p_value_threshold = 0.05,
  p_adjust_method = "BH"
)
```

Arguments

`foreground_kmers`	*k*-mer counts of the foreground set (generated by `generate_kmers`)
`background_kmers`	*k*-mer counts of the background set (generated by `generate_kmers`)
`permutation`	if `TRUE`, only the enrichment value is returned (efficiency mode used for permutation testing)

`chisq_p_value_threshold`
: threshold below which Fisher's exact test is used instead of Pearson's chi-squared test

`p_adjust_method`
: see `p.adjust`

Details

Usually uses Pearson's chi-squared test, but recalculates p-values with Fisher's exact test for Pearson's chi-squared test p-values <= `chisq_p_value_threshold`. The reason this is done is computational efficiency. Fisher's exact tests are computationally demanding and are only performed in situations, where exact p-values are preferred, e.g., if expected < 5 or significant p-values.

Value

enrichment of *k*-mers in specified foreground sequences. A data frame with the following columns is returned:

`foreground_count`	foreground counts for each *k*-mer
`background_count`	background counts for each *k*-mer
`enrichment`	*k*-mer enrichment
`p_value`	p-value of *k*-mer enrichment (either from Fisher's exact test or Pearson's chi-squared test)
`adj_p_value`	multiple testing corrected p-value

See Also

Other *k*-mer functions: `calculate_kmer_enrichment()`, `check_kmers()`, `count_homopolymer_corrected_kmers()`, `create_kmer_origin_list()`, `draw_volcano_plot()`, `estimate_significance()`, `estimate_significance_core()`, `generate_kmers()`, `generate_permuted_enrichments()`, `run_kmer_spma()`, `run_kmer_tsma()`

Examples

```
# define simple sequence sets for foreground and background
foreground_set <- c(
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
  "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA"
)
background_set <- c(
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
  "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA",
  "UUAUUUA", "AUCCUUUACA", "UUUUUUU", "UUUCAUCAUU",
  "CCACACAC", "CUCAUUGGAG", "ACUUUGGGACA", "CAGGUCAGCA"
)
foreground_kmers <- generate_kmers(foreground_set, 6)
```

```
background_kmers <- generate_kmers(background_set, 6)


kmer_enrichment_values <- compute_kmer_enrichment(foreground_kmers,
  background_kmers)
```

`count_homopolymer_corrected_kmers`
Correction for Homopolymeric Stretches

Description

Counts all non-overlapping instances of *k*-mers in a given set of sequences.

Usage

```
count_homopolymer_corrected_kmers(sequences, k, kmers, is_rna = FALSE)
```

Arguments

`sequences`	character vector of DNA or RNA sequences
`k`	length of *k*-mer, either `6` for hexamers or `7` for heptamers
`kmers`	column sums of return value of `Biostrings::oligonucleotideFrequency(sequences)`
`is_rna`	if `sequences` are RNA sequences, this flag needs to be set

Value

Returns a named numeric vector, where the elements are *k*-mer counts and the names are *k*-mers.

See Also

Other *k*-mer functions: `calculate_kmer_enrichment()`, `check_kmers()`, `compute_kmer_enrichment()`, `create_kmer_origin_list()`, `draw_volcano_plot()`, `estimate_significance()`, `estimate_significance_core()`, `generate_kmers()`, `generate_permuted_enrichments()`, `run_kmer_spma()`, `run_kmer_tsma()`

create_kmer_motif *Creates Transite motif object from character vector of* k*-mers*

Description

Takes a position weight matrix (PWM) and meta info and returns an object of class `RBPMotif`.

Usage

```
create_kmer_motif(id, rbps, kmers, type, species, src)
```

Arguments

`id`	motif id (character vector of length 1)
`rbps`	character vector of names of RNA-binding proteins associated with this motif
`kmers`	character vector of *k*-mers that are associated with the motif, set of *k*-mers is valid if (1) all *k*-mers must have the same length, (2) only hexamers or heptamers allowed, (3) allowed characters are A, C, G, U
`type`	type of motif (e.g., `'HITS-CLIP'`, `'EMSA'`, `'SELEX'`, etc.)
`species`	species where motif was discovered (e.g., `'Homo sapiens'`)
`src`	source of motif (e.g., `'RBPDB v1.3.1'`)

Value

object of class `RBPMotif`

Examples

```
custom_motif <- create_kmer_motif(
  "custom_motif", "RBP1",
  c("AAAAAAA", "CAAAAAA"), "HITS-CLIP",
  "Homo sapiens", "user"
)
```

create_kmer_origin_list *Create* k*-mer lists with their original sequences*

Description

Counts occurrences of *k*-mers of length k in the given set of sequences. A list of the sequences the *k*-mer originated from is created and returned. If the *k*-mer does not exist in any of the sequences, the list is empty for that *k*-mer.

Usage

```
create_kmer_origin_list(sequences, k)
```

Arguments

sequences	character vector of DNA or RNA sequences that constitute the whole set of sequences being analyzed
k	number of characters in *k*-mer

Value

A list with *k*-mers as the identifier and a vector of sequences for each *k*-mer that the *k*-mer is found in

See Also

Other *k*-mer functions: `calculate_kmer_enrichment()`, `check_kmers()`, `compute_kmer_enrichment()`, `count_homopolymer_corrected_kmers()`, `draw_volcano_plot()`, `estimate_significance()`, `estimate_significance_core()`, `generate_kmers()`, `generate_permuted_enrichments()`, `run_kmer_spma()`, `run_kmer_tsma()`

Examples

```
# define simple sequence sets for foreground and background
sequence_set <- c(
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
  "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA"
)
kmer_origin_list <- create_kmer_origin_list(sequence_set, k = 6)
```

---

create_matrix_motif *Creates Transite motif object from position weight matrix*

Description

Takes a position weight matrix (PWM) and meta info and returns an object of class `RBPMotif`.

Usage

```
create_matrix_motif(id, rbps, matrix, type, species, src)
```

Arguments

id	motif id (character vector of length 1)
rbps	character vector of names of RNA-binding proteins associated with this motif
matrix	data frame with four columns (A, C, G, U) and 6 - 15 rows (positions), where cell (i, j) contains weight of nucleotide j on position i
type	type of motif (e.g., 'HITS-CLIP', 'EMSA', 'SELEX', etc.)
species	species where motif was discovered (e.g., 'Homo sapiens')
src	source of motif (e.g., 'RBPDB v1.3.1')

Value

object of class RBPMotif

Examples

```
custom_motif <- create_matrix_motif(
  "custom_motif", "RBP1",
  transite:::toy_motif_matrix, "HITS-CLIP",
  "Homo sapiens", "user"
)
```

---

draw_volcano_plot k-*mer Enrichment Volcano Plot*

Description

Uses a volcano plot to visualize *k*-mer enrichment. X-axis is $\log_2$ enrichment value, y-axis is $\log_1 0$ significance, i.e., multiple testing corrected p-value from Fisher's exact test or Pearson's chi-squared test.

Usage

```
draw_volcano_plot(
  kmers,
  motif_kmers,
  motif_rbps,
  significance_threshold = 0.01,
  show_legend = TRUE
)
```

Arguments

kmers	data frame with the following columns: kmer, adj_p_value, enrichment
motif_kmers	set of *k*-mers that are associated with a certain motif, will be highlighted in volcano plot
motif_rbps	name of RNA-binding proteins associated with highlighted *k*-mers (character vector of length 1)
significance_threshold	p-value threshold for significance, e.g., 0.05 or 0.01
show_legend	whether or not a legend should be shown

Value

volcano plot

See Also

Other TSMA functions: run_kmer_tsma(), run_matrix_tsma()

Other *k*-mer functions: calculate_kmer_enrichment(), check_kmers(), compute_kmer_enrichment(), count_homopolymer_corrected_kmers(), create_kmer_origin_list(), estimate_significance(), estimate_significance_core(), generate_kmers(), generate_permuted_enrichments(), run_kmer_spma(), run_kmer_tsma()

Examples

```
motif <- get_motif_by_id("951_12324455")
draw_volcano_plot(transite:::kmers_enrichment, get_hexamers(motif[[1]]),
  get_rbps(motif[[1]]))

## Not run:
foreground_set <- c("UGUGGG", "GUGGGG", "GUGUGG", "UGUGGU")
background_set <- unique(c(foreground_set, c(
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
  "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA",
  "CCACACAC", "CUCAUUGGAG", "ACUUUCCCACA", "CAGGUCAGCA",
  "CCACACCAG", "CCACACAUCAGU", "CACACACUCC", "CAGCCCCCCACAGGCA"
)))

motif <- get_motif_by_id("M178_0.6")
results <- run_kmer_tsma(list(foreground_set), background_set,
                         motifs = motif)
draw_volcano_plot(results[[1]]$motif_kmers_dfs[[1]],
    get_hexamers(motif[[1]]), "test RBP")
## End(Not run)
```

`estimate_significance` *Permutation Test Based Significance of Observed Mean*

Description

`estimate_significance` returns an estimate of the significance of the observed mean, given a set of random permutations of the data.

Usage

```
estimate_significance(
  actual_mean,
  motif_kmers,
  random_permutations,
  alternative = c("two_sided", "less", "greater"),
  conf_level = 0.95,
  produce_plot = TRUE
)
```

Arguments

`actual_mean`	observed mean
`motif_kmers`	set of *k*-mers that were used to compute the `actual_mean`
`random_permutations`	a set of random permutations of the original data, used to generate an empirical null distribution.
`alternative`	side of the test, one of the following: `"two_sided"`, `"less"`, `"greater"`
`conf_level`	confidence level for the returned confidence interval
`produce_plot`	if distribution plot should be part of the returned list

Value

A list with the following components:

`p_value_estimate`	the estimated p-value of the observed mean
`conf_int`	the confidence interval around that estimate
`plot`	plot of the empirical distribution of geometric means of the enrichment values

See Also

Other *k*-mer functions: `calculate_kmer_enrichment()`, `check_kmers()`, `compute_kmer_enrichment()`, `count_homopolymer_corrected_kmers()`, `create_kmer_origin_list()`, `draw_volcano_plot()`, `estimate_significance_core()`, `generate_kmers()`, `generate_permuted_enrichments()`, `run_kmer_spma()`, `run_kmer_tsma()`

`estimate_significance_core` *Significance of Observed Mean*

Description

`estimate_significance_core` returns an estimate of the significance of the observed mean, given a vector of means based on random permutations of the data.

Usage

```
estimate_significance_core(
  random_means,
  actual_mean,
  alternative = c("two_sided", "less", "greater"),
  conf_level = 0.95
)
```

Arguments

`random_means`	numeric vector of means based on random permutations of the data (empirical null distribution)
`actual_mean`	observed mean
`alternative`	side of the test, one of the following: `"two_sided"`, `"less"`, `"greater"`
`conf_level`	confidence level for the returned confidence interval

Value

A list with the following components:

`p_value_estimate`	the estimated p-value of the observed mean
`conf_int`	the confidence interval around that estimate

See Also

Other *k*-mer functions: `calculate_kmer_enrichment()`, `check_kmers()`, `compute_kmer_enrichment()`, `count_homopolymer_corrected_kmers()`, `create_kmer_origin_list()`, `draw_volcano_plot()`, `estimate_significance()`, `generate_kmers()`, `generate_permuted_enrichments()`, `run_kmer_spma()`, `run_kmer_tsma()`

Examples

```
test_sd <- 1.0
test_null_distribution <- rnorm(n = 10000, mean = 1.0, sd = test_sd)

estimate_significance_core(test_null_distribution, test_sd * 2, "greater")
```

ge *Toy Gene Expression Data Set*

Description

This object contains a toy data set based on gene expression measurements and 3'-UTR sequences of 1000 genes. It comprises three data frames with RefSeq identifiers, log fold change values, and 3'-UTR sequences of genes, which are either upregulated or downregulated after some hypothetical treatment, as well as all measured genes. The actual values are not important. This data set merely serves as an example input for various functions.

Usage

```
data(ge)
```

Format

A list with the following components:

`foreground1_df`	data frame that contains down-regulated genes after treatment
`foreground2_df`	data frame that contains up-regulated genes after treatment
`background_df`	data frame that contains all genes measured

generate_iupac_by_kmers *Generates IUPAC code for a character vector of* k*-mers*

Description

Generates a compact logo of a motif based on IUPAC codes given by a character vector of *k*-mers

Usage

```
generate_iupac_by_kmers(kmers, code = NULL)
```

Arguments

`kmers`	character vector of *k*-mers
`code`	if IUPAC code table has already been initialized by `init_iupac_lookup_table`, it can be specified here

Details

IUPAC RNA nucleotide code:

A	Adenine
C	Cytosine
G	Guanine
U	Uracil
R	A or G
Y	C or U
S	G or C
W	A or U
K	G or U
M	A or C
B	C or G or U
D	A or G or U
H	A or C or U
V	A or C or G
N	any base

Value

the IUPAC string of the binding site

References

http://www.chem.qmul.ac.uk/iubmb/misc/naseq.html

See Also

Other motif functions: generate_iupac_by_matrix(), generate_kmers_from_iupac(), get_motif_by_id(), get_motif_by_rbp(), get_motifs(), get_motifs_meta_info(), get_ppm(), init_iupac_lookup_table(), set_motifs()

Examples

```
generate_iupac_by_kmers(c("AACCAA", "AACCGG", "CACCGA"))
```

---

generate_iupac_by_matrix

Generates IUPAC code for motif matrix

Description

Generates a compact logo of a motif based on IUPAC codes given by a position weight matrix

Usage

```
generate_iupac_by_matrix(matrix, threshold = 0.215, code = NULL)
```

Arguments

matrix	the position probability matrix of an RNA-binding protein
threshold	the threshold probability (nucleotides with lower probabilities are ignored)
code	if IUPAC code table has already been initialized by init_iupac_lookup_table, it can be specified here

Details

IUPAC RNA nucleotide code:

A	Adenine
C	Cytosine
G	Guanine
U	Uracil
R	A or G
Y	C or U
S	G or C
W	A or U
K	G or U
M	A or C
B	C or G or U
D	A or G or U
H	A or C or U
V	A or C or G
N	any base

Value

the IUPAC string of the binding site

References

http://www.chem.qmul.ac.uk/iubmb/misc/naseq.html

See Also

Other motif functions: generate_iupac_by_kmers(), generate_kmers_from_iupac(), get_motif_by_id(), get_motif_by_rbp(), get_motifs(), get_motifs_meta_info(), get_ppm(), init_iupac_lookup_table(), set_motifs()

Examples

```
generate_iupac_by_matrix(get_motif_matrix(get_motif_by_id("M178_0.6")[[1]]))
```

generate_kmers k-*mer Counts for Sequence Set*

Description

Counts occurrences of *k*-mers of length k in the given set of sequences. Corrects for homopolymeric stretches.

Usage

```
generate_kmers(sequences, k)
```

Arguments

sequences	character vector of DNA or RNA sequences
k	length of *k*-mer, either 6 for hexamers or 7 for heptamers

Value

Returns a named numeric vector, where the elements are *k*-mer counts and the names are DNA *k*-mers.

Warning

generate_kmers always returns DNA *k*-mers, even if sequences contains RNA sequences. RNA sequences are internally converted to DNA sequences. It is not allowed to mix DNA and RNA sequences.

See Also

Other *k*-mer functions: calculate_kmer_enrichment(), check_kmers(), compute_kmer_enrichment(), count_homopolymer_corrected_kmers(), create_kmer_origin_list(), draw_volcano_plot(), estimate_significance(), estimate_significance_core(), generate_permuted_enrichments(), run_kmer_spma(), run_kmer_tsma()

Examples

```
# count hexamers in set of RNA sequences
rna_sequences <- c(
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
  "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA",
  "UUAUUUA", "AUCCUUUACA", "UUUUUUU", "UUUCAUCAUU",
  "CCACACAC", "CUCAUUGGAG", "ACUUUGGGACA", "CAGGUCAGCA"
)
hexamer_counts <- generate_kmers(rna_sequences, 6)
```

```
# count heptamers in set of DNA sequences
dna_sequences <- c(
  "CAACAGCCTTAATT", "CAGTCAAGACTCC", "CTTTGGGGAAT",
  "TCATTTTATTAAA", "AATTGGTGTCTGGATACTTCCCTGTACAT",
  "ATCAAATTA", "AGAT", "GACACTTAAAGATCCT",
  "TAGCATTAACTTAATG", "ATGGA", "GAAGAGTGCTCA",
  "ATAGAC", "AGTTC", "CCAGTAA",
  "TTATTTA", "ATCCTTTACA", "TTTTTTT", "TTTCATCATT",
  "CCACACAC", "CTCATTGGAG", "ACTTTGGGACA", "CAGGTCAGCA"
)
hexamer_counts <- generate_kmers(dna_sequences, 7)
```

`generate_kmers_from_iupac` *Generates all* k*-mers for IUPAC string*

Description

Generates all possible *k*-mers for a given IUPAC string.

Usage

```
generate_kmers_from_iupac(iupac, k)
```

Arguments

`iupac`	IUPAC string
`k`	length of *k*-mer, `6` (hexamers) or `7` (heptamers)

Details

IUPAC RNA nucleotide code:

`A`	Adenine
`C`	Cytosine
`G`	Guanine
`U`	Uracil
`R`	A or G
`Y`	C or U
`S`	G or C
`W`	A or U
`K`	G or U
`M`	A or C
`B`	C or G or U
`D`	A or G or U
`H`	A or C or U

V	A or C or G
N	any base

Value

list of *k*-mers

References

http://www.chem.qmul.ac.uk/iubmb/misc/naseq.html

See Also

Other motif functions: generate_iupac_by_kmers(), generate_iupac_by_matrix(), get_motif_by_id(), get_motif_by_rbp(), get_motifs(), get_motifs_meta_info(), get_ppm(), init_iupac_lookup_table(), set_motifs()

Examples

```
generate_kmers_from_iupac(get_iupac(get_motif_by_id("M178_0.6")[[1]]), k = 6)
```

---

generate_permuted_enrichments

Generate Random Permutations of the Enrichment Data

Description

Calculates *k*-mer enrichment values for randomly sampled (without replacement) foreground sets.

Usage

```
generate_permuted_enrichments(
  n_transcripts_foreground,
  background_set,
  k,
  n_permutations = 1000,
  n_cores = 4
)
```

Arguments

n_transcripts_foreground	number of transcripts in the original foreground set
background_set	character vector of DNA or RNA sequences that constitute the background set
k	length of *k*-mer, either 6 for hexamers or 7 for heptamers
n_permutations	number of permutations to perform
n_cores	number of computing cores to use

Value

The result of calculate_kmer_enrichment for the random foreground sets.

See Also

Other *k*-mer functions: calculate_kmer_enrichment(), check_kmers(), compute_kmer_enrichment(), count_homopolymer_corrected_kmers(), create_kmer_origin_list(), draw_volcano_plot(), estimate_significance(), estimate_significance_core(), generate_kmers(), run_kmer_spma(), run_kmer_tsma()

---

geometric_mean *Geometric Mean*

Description

Calculates the geometric mean of the specified values.

Usage

```
geometric_mean(x, na_rm = TRUE)
```

Arguments

x	numeric vector of values for which the geometric mean will be computed
na_rm	logical. Should missing values (including NaN) be removed?

Value

Geometric mean of x or 1 if length of x is 0

Examples

```
geometric_mean(c(0.123, 0.441, 0.83))
```

get_motifs *Retrieve list of all motifs*

Description

Retrieves all Transite motifs

Usage

```
get_motifs()
```

Value

A list of objects of class Motif

See Also

Other motif functions: generate_iupac_by_kmers(), generate_iupac_by_matrix(), generate_kmers_from_iupac(), get_motif_by_id(), get_motif_by_rbp(), get_motifs_meta_info(), get_ppm(), init_iupac_lookup_table(), set_motifs()

Examples

```
transite_motifs <- get_motifs()
```

get_motifs_meta_info *Displays motif meta information.*

Description

Generates a data frame with meta information about all Transite motifs.

Usage

```
get_motifs_meta_info()
```

Value

A data frame containing meta information for all Transite motifs, with the following columns:

- id
- rbps
- length
- iupac
- type
- species
- src

See Also

Other motif functions: generate_iupac_by_kmers(), generate_iupac_by_matrix(), generate_kmers_from_iupac(), get_motif_by_id(), get_motif_by_rbp(), get_motifs(), get_ppm(), init_iupac_lookup_table(), set_motifs()

Examples

```
get_motifs_meta_info()
```

---

get_motif_by_id *Retrieve motif objects by id*

Description

Retrieves one or more motif objects identified by motif id.

Usage

```
get_motif_by_id(id)
```

Arguments

id	character vector of motif identifiers

Value

A list of objects of class RBPMotif

See Also

Other motif functions: generate_iupac_by_kmers(), generate_iupac_by_matrix(), generate_kmers_from_iupac(), get_motif_by_rbp(), get_motifs(), get_motifs_meta_info(), get_ppm(), init_iupac_lookup_table(), set_motifs()

Examples

```
get_motif_by_id("M178_0.6")

get_motif_by_id(c("M178_0.6", "M188_0.6"))
```

get_motif_by_rbp *Retrieve motif objects by gene symbol*

Description

Retrieves one or more motif objects identified by gene symbol.

Usage

```
get_motif_by_rbp(rbp)
```

Arguments

rbp	character vector of gene symbols of RNA-binding proteins

Value

A list of objects of class RBPMotif

See Also

Other motif functions: generate_iupac_by_kmers(), generate_iupac_by_matrix(), generate_kmers_from_iupac(), get_motif_by_id(), get_motifs(), get_motifs_meta_info(), get_ppm(), init_iupac_lookup_table(), set_motifs()

Examples

```
get_motif_by_rbp("ELAVL1")

get_motif_by_rbp(c("ELAVL1", "ELAVL2"))
```

get_ppm *Get Position Probability Matrix (PPM) from motif object*

Description

Return the position probability matrix of the specified motif.

Usage

```
get_ppm(motif)
```

Arguments

motif	object of class RBPMotif

Value

The position probability matrix of the specified motif

See Also

Other motif functions: generate_iupac_by_kmers(), generate_iupac_by_matrix(), generate_kmers_from_iupac(), get_motif_by_id(), get_motif_by_rbp(), get_motifs(), get_motifs_meta_info(), init_iupac_lookup_table(), set_motifs()

Examples

```
get_ppm(get_motif_by_id("M178_0.6")[[1]])
```

---

init_iupac_lookup_table

Initializes the IUPAC lookup table

Description

Initializes a hash table that serves as a IUPAC lookup table for the generate_iupac_by_matrix function.

Usage

```
init_iupac_lookup_table()
```

Details

IUPAC RNA nucleotide code:

A	Adenine
C	Cytosine
G	Guanine
U	Uracil
R	A or G
Y	C or U
S	G or C
W	A or U
K	G or U
M	A or C
B	C or G or U
D	A or G or U
H	A or C or U
V	A or C or G
N	any base

Value

an environment, the IUPAC lookup hash table

References

http://www.chem.qmul.ac.uk/iubmb/misc/naseq.html

See Also

Other motif functions: generate_iupac_by_kmers(), generate_iupac_by_matrix(), generate_kmers_from_iupac(), get_motif_by_id(), get_motif_by_rbp(), get_motifs(), get_motifs_meta_info(), get_ppm(), set_motifs()

Examples

```
generate_iupac_by_matrix(get_motif_matrix(get_motif_by_id("M178_0.6")[[1]]),
  code = init_iupac_lookup_table())
```

---

kmers_enrichment *Example* k-*mer Enrichment Data*

Description

This data frame with *k*-mer enrichment data (as produced by run_kmer_tsma) is used in a code example for k-mer volcano plot function draw_volcano_plot.

Usage

```
data(kmers_enrichment)
```

Format

A data frame with the following columns:

kmer	contains all hexamers (AAAAAA to UUUUUU)
foreground_count	absolute *k*-mer frequency in foreground set
background_count	absolute *k*-mer frequency in background set
enrichment	enrichment of *k*-mer in foreground relative to background
p_value	associated p-value of enrichment
adj_p_value	multiple testing corrected p-value

motifs *Transite Motif Database*

Description

The Transite motif database contains sequence motifs and associated *k*-mers of more than 100 different RNA-binding proteins, obtained from publicly available motif databases.

Usage

```
data(motifs)
```

Format

A list of lists with the following components:

id	motif id
rbps	gene symbols of RNA-binding proteins associated with motif
matrix	data frame of sequence motif (position weight matrix)
hexamers	all motif-associated hexamers
heptamers	all motif-associated heptamers
length	length of motif in nucleotides
iupac	IUPAC string of sequence motif
type	type of motif, e.g., RNAcompete
species	usually human
src	source of motif, e.g., RNA Zoo

References

http://cisbp-rna.ccbr.utoronto.ca/

http://rbpdb.ccbr.utoronto.ca/

p_combine *P-value aggregation*

Description

p_combine is used to combine the p-values of independent significance tests.

Usage

```
p_combine(p, method = c("fisher", "SL", "MG", "tippett"), w = NULL)
```

Arguments

p	vector of p-values
method	one of the following: Fisher (1932) (`'fisher'`), Stouffer (1949), Liptak (1958) (`'SL'`), Mudholkar and George (1979) (`'MG'`), and Tippett (1931) (`'tippett'`)
w	weights, only used in combination with Stouffer-Liptak. If `is.null(w)` then weights are set in an unbiased way

Details

The problem can be specified as follows: Given a vector of n p-values $p_1, ..., p_n$, find p_c, the combined p-value of the n significance tests. Most of the methods introduced here combine the p-values in order to obtain a test statistic, which follows a known probability distribution. The general procedure can be stated as:

$$T(h, C) = \sum_{i=1}^{n} h(p_i) * C$$

The function T, which returns the test statistic t, takes two arguments. h is a function defined on the interval $[0, 1]$ that transforms the individual p-values, and C is a correction term.

Fisher's method (1932), also known as the inverse chi-square method is probably the most widely used method for combining p-values. Fisher used the fact that if p_i is uniformly distributed (which p-values are under the null hypothesis), then $-2 \log p_i$ follows a chi-square distribution with two degrees of freedom. Therefore, if p-values are transformed as follows,

$$h(p) = -2 \log p,$$

and the correction term C is neutral, i.e., equals 1, the following statement can be made about the sampling distribution of the test statistic T_f under the null hypothesis: t_f is distributed as chi-square with $2n$ degrees of freedom, where n is the number of p-values.

Stouffer's method, or the inverse normal method, uses a p-value transformation function h that leads to a test statistic that follows the standard normal distribution by transforming each p-value to its corresponding normal score. The correction term scales the sum of the normal scores by the root of the number of p-values.

$$h(p) = \Phi^{-1}(1 - p)$$

$$C = \frac{1}{\sqrt{n}}$$

Under the null hypothesis, t_s is distributed as standard normal. Φ^{-1} is the inverse of the cumulative standard normal distribution function.

An extension of Stouffer's method with weighted p-values is called Liptak's method.

The logit method by Mudholkar and George uses the following transformation:

$$h(p) = -\ln(p/(1 - p))$$

When the sum of the transformed p-values is corrected in the following way:

$$C = \sqrt{\frac{3(5n + 4)}{\pi^2 n(5n + 2)}},$$

the test statistic t_m is approximately t-distributed with $5n + 4$ degrees of freedom.

In Tippett's method the smallest p-value is used as the test statistic t_t and the combined significance is calculated as follows:

$$Pr(t_t) = 1 - (1 - t_t)^n$$

Value

A list with the following components:

`statistic`	the test statistic
`p_value`	the corresponding p-value
`method`	the method used
`statistic_name`	the name of the test statistic

Examples

```
p_combine(c(0.01, 0.05, 0.5))

p_combine(c(0.01, 0.05, 0.5), method = "tippett")
```

`RBPMotif-class` *An S4 class to represent a RBPMotif*

Description

An S4 class to represent a RBPMotif

Getter Method get_id

Getter Method get_rbps

Getter Method get_motif_matrix

Getter Method get_hexamers

Getter Method get_heptamers

Getter Method get_width

Getter Method get_iupac

Getter Method get_type

Getter Method get_species

Getter Method get_source

Usage

```
get_id(object)

## S4 method for signature 'RBPMotif'
get_id(object)

get_rbps(object)

## S4 method for signature 'RBPMotif'
get_rbps(object)

get_motif_matrix(object)

## S4 method for signature 'RBPMotif'
get_motif_matrix(object)

get_hexamers(object)

## S4 method for signature 'RBPMotif'
get_hexamers(object)

get_heptamers(object)

## S4 method for signature 'RBPMotif'
get_heptamers(object)

get_width(object)

## S4 method for signature 'RBPMotif'
get_width(object)

get_iupac(object)

## S4 method for signature 'RBPMotif'
get_iupac(object)

get_type(object)

## S4 method for signature 'RBPMotif'
get_type(object)

get_species(object)

## S4 method for signature 'RBPMotif'
get_species(object)

get_source(object)
```

```
## S4 method for signature 'RBPMotif'
get_source(object)

## S4 method for signature 'RBPMotif'
show(object)
```

Arguments

`object`	RBPMotif object

Value

Object of type RBPMotif

Slots

`id` motif id (character vector of length 1)

`rbps` character vector of names of RNA-binding proteins associated with this motif

`matrix` data frame with four columns (A, C, G, U) and 6 - 15 rows (positions), where cell (i, j) contains weight of nucleotide j on position i

`hexamers` character vector of hexamers associated with this motif

`heptamers` character vector of heptamers associated with this motif

`length` length of the motif (i.e., `nrow(matrix)`)

`iupac` IUPAC code for motif matrix (see `generate_iupac_by_matrix`)

`type` type of motif (e.g., `'HITS-CLIP'`, `'EMSA'`, `'SELEX'`, etc.)

`species` species where motif was discovered (e.g., `'Homo sapiens'`)

`src` source of motif (e.g., `'RBPDB v1.3.1'`)

Examples

```
kmers <- c("AAAAAAA", "CAAAAAA")
iupac <- generate_iupac_by_kmers(kmers,
  code = init_iupac_lookup_table())
hexamers <- generate_kmers_from_iupac(iupac, 6)
heptamers <- generate_kmers_from_iupac(iupac, 7)
new("RBPMotif", id = "custom_motif", rbps = "RBP1",
  matrix = NULL, hexamers = hexamers, heptamers = heptamers, length = 7L,
  iupac = iupac, type = "HITS-CLIP", species = "Homo sapiens", src = "user"
)
```

run_kmer_spma k-*mer-based Spectrum Motif Analysis*

Description

SPMA helps to illuminate the relationship between RBP binding evidence and the transcript sorting criterion, e.g., fold change between treatment and control samples.

Usage

```
run_kmer_spma(
  sorted_transcript_sequences,
  sorted_transcript_values = NULL,
  transcript_values_label = "transcript value",
  motifs = NULL,
  k = 6,
  n_bins = 40,
  midpoint = 0,
  x_value_limits = NULL,
  max_model_degree = 1,
  max_cs_permutations = 1e+07,
  min_cs_permutations = 5000,
  fg_permutations = 5000,
  p_adjust_method = "BH",
  p_combining_method = "fisher",
  n_cores = 1
)
```

Arguments

sorted_transcript_sequences	character vector of ranked sequences, either DNA (only containing upper case characters A, C, G, T) or RNA (A, C, G, U). The sequences in sorted_transcript_sequences must be ranked (i.e., sorted). Commonly used sorting criteria are measures of differential expression, such as fold change or signal-to-noise ratio (e.g., between treatment and control samples in gene expression profiling experiments).
sorted_transcript_values	vector of sorted transcript values, i.e., the fold change or signal-to-noise ratio or any other quantity that was used to sort the transcripts that were passed to run_matrix_spma or run_kmer_spma (default value is NULL). These values are displayed as a semi-transparent area over the enrichment value heatmaps of spectrum plots.
transcript_values_label	label of transcript sorting criterion (e.g., "log fold change", default value is "transcript value"), only shown if !is.null(sorted_transcript_values)
motifs	a list of motifs that is used to score the specified sequences. If is.null(motifs) then all Transite motifs are used.

k	length of *k*-mer, either 6 for hexamers or 7 for heptamers
n_bins	specifies the number of bins in which the sequences will be divided, valid values are between 7 and 100
midpoint	for enrichment values the midpoint should be 1, for log enrichment values 0 (defaults to 0)
x_value_limits	sets limits of the x-value color scale (used to harmonize color scales of different spectrum plots), see limits argument of continuous_scale (defaults to NULL, i.e., the data-dependent default scale range)
max_model_degree	maximum degree of polynomial
max_cs_permutations	maximum number of permutations performed in Monte Carlo test for consistency score
min_cs_permutations	minimum number of permutations performed in Monte Carlo test for consistency score
fg_permutations	numer of foreground permutations
p_adjust_method	see p.adjust
p_combining_method	one of the following: Fisher (1932) ("fisher"), Stouffer (1949), Liptak (1958) ("SL"), Mudholkar and George (1979) ("MG"), and Tippett (1931) ("tippett") (see p_combine)
n_cores	number of computing cores to use

Details

In order to investigate how motif targets are distributed across a spectrum of transcripts (e.g., all transcripts of a platform, ordered by fold change), Spectrum Motif Analysis visualizes the gradient of RBP binding evidence across all transcripts.

The *k*-mer-based approach differs from the matrix-based approach by how the sequences are scored. Here, sequences are broken into *k*-mers, i.e., oligonucleotide sequences of *k* bases. And only statistically significantly enriched or depleted *k*-mers are then used to calculate a score for each RNA-binding protein, which quantifies its target overrepresentation.

Value

A list with the following components:

foreground_scores	the result of run_kmer_tsma for the binned data
spectrum_info_df	a data frame with the SPMA results
spectrum_plots	a list of spectrum plots, as generated by score_spectrum
classifier_scores	a list of classifier scores, as returned by classify_spectrum

See Also

Other SPMA functions: classify_spectrum(), run_matrix_spma(), score_spectrum(), subdivide_data()

Other *k*-mer functions: calculate_kmer_enrichment(), check_kmers(), compute_kmer_enrichment(), count_homopolymer_corrected_kmers(), create_kmer_origin_list(), draw_volcano_plot(), estimate_significance(), estimate_significance_core(), generate_kmers(), generate_permuted_enrichments(run_kmer_tsma()

Examples

```
# example data set
background_df <- transite:::ge$background_df
# sort sequences by signal-to-noise ratio
background_df <- dplyr::arrange(background_df, value)
# character vector of named and ranked (by signal-to-noise ratio) sequences
background_seqs <- gsub("T", "U", background_df$seq)
names(background_seqs) <- paste0(background_df$refseq, "|",
  background_df$seq_type)

results <- run_kmer_spma(background_seqs,
                         sorted_transcript_values = background_df$value,
                         transcript_values_label = "signal-to-noise ratio",
                         motifs = get_motif_by_id("M178_0.6"),
                         n_bins = 20,
                         fg_permutations = 10)

## Not run:
results <- run_kmer_spma(background_seqs,
                         sorted_transcript_values = background_df$value,
                         transcript_values_label = "signal-to-noise ratio")
## End(Not run)
```

---

run_kmer_tsma k-*mer-based Transcript Set Motif Analysis*

Description

Calculates the enrichment of putative binding sites in foreground sets versus a background set using *k*-mers to identify putative binding sites

Usage

```
run_kmer_tsma(
  foreground_sets,
  background_set,
  motifs = NULL,
  k = 6,
  fg_permutations = 5000,
```

```
  kmer_significance_threshold = 0.01,
  produce_plot = TRUE,
  p_adjust_method = "BH",
  p_combining_method = "fisher",
  n_cores = 1
)
```

Arguments

foreground_sets	list of foreground sets; a foreground set is a character vector of DNA or RNA sequences (not both) and a strict subset of the background_set
background_set	character vector of DNA or RNA sequences that constitute the background set
motifs	a list of motifs that is used to score the specified sequences. If is.null(motifs) then all Transite motifs are used.
k	length of *k*-mer, either 6 for hexamers or 7 for heptamers
fg_permutations	numer of foreground permutations
kmer_significance_threshold	p-value threshold for significance, e.g., 0.05 or 0.01 (used for volcano plots)
produce_plot	if TRUE volcano plots and distribution plots are created
p_adjust_method	see p.adjust
p_combining_method	one of the following: Fisher (1932) ("fisher"), Stouffer (1949), Liptak (1958) ("SL"), Mudholkar and George (1979) ("MG"), and Tippett (1931) ("tippett") (see p_combine)
n_cores	number of computing cores to use

Details

Motif transcript set analysis can be used to identify RNA binding proteins, whose targets are significantly overrepresented or underrepresented in certain sets of transcripts.

The aim of Transcript Set Motif Analysis (TSMA) is to identify the overrepresentation and underrepresentation of potential RBP targets (binding sites) in a set (or sets) of sequences, i.e., the foreground set, relative to the entire population of sequences. The latter is called background set, which can be composed of all sequences of the genes of a microarray platform or all sequences of an organism or any other meaningful superset of the foreground sets.

The *k*-mer-based approach breaks the sequences of foreground and background sets into *k*-mers and calculates the enrichment on a *k*-mer level. In this case, motifs are not represented as position weight matrices, but as lists of *k*-mers.

Statistically significantly enriched or depleted *k*-mers are then used to calculate a score for each RNA-binding protein, which quantifies its target overrepresentation.

Value

A list of lists (one for each transcript set) with the following components:

enrichment_df	the result of compute_kmer_enrichment
motif_df	
motif_kmers_dfs	
volcano_plots	volcano plots for each motif (see draw_volcano_plot)
perm_test_plots	plots of the empirical distribution of *k*-mer enrichment values for each motif
enriched_kmers_combined_p_values	
depleted_kmers_combined_p_values	

See Also

Other TSMA functions: draw_volcano_plot(), run_matrix_tsma()

Other *k*-mer functions: calculate_kmer_enrichment(), check_kmers(), compute_kmer_enrichment(), count_homopolymer_corrected_kmers(), create_kmer_origin_list(), draw_volcano_plot(), estimate_significance(), estimate_significance_core(), generate_kmers(), generate_permuted_enrichments(run_kmer_spma()

Examples

```
# define simple sequence sets for foreground and background
foreground_set1 <- c(
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
  "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA"
)
foreground_set2 <- c("UUAUUUA", "AUCCUUUACA", "UUUUUUU", "UUUCAUCAUU")
foreground_sets <- list(foreground_set1, foreground_set2)
background_set <- unique(c(foreground_set1, foreground_set2, c(
  "CCACACAC", "CUCAUUGGAG", "ACUUUGGGACA", "CAGGUCAGCA",
  "CCACACCGG", "GUCAUCAGU", "GUCAGUCC", "CAGGUCAGGGGCA"
)))

# run k-mer based TSMA with all Transite motifs (recommended):
# results <- run_kmer_tsma(foreground_sets, background_set)

# run TSMA with one motif:
motif_db <- get_motif_by_id("M178_0.6")
results <- run_kmer_tsma(foreground_sets, background_set, motifs = motif_db)
## Not run:
# define example sequence sets for foreground and background
foreground_set1 <- gsub("T", "U", transite:::ge$foreground1_df$seq)
foreground_set2 <- gsub("T", "U", transite:::ge$foreground2_df$seq)
foreground_sets <- list(foreground_set1, foreground_set2)
background_set <- gsub("T", "U", transite:::ge$background_df$seq)

# run TSMA with all Transite motifs
results <- run_kmer_tsma(foreground_sets, background_set)
```

```
# run TSMA with a subset of Transite motifs
results <- run_kmer_tsma(foreground_sets, background_set,
  motifs = get_motif_by_rbp("ELAVL1"))

# run TSMA with user-defined motif
toy_motif <- create_kmer_motif(
  "toy_motif", "example RBP",
  c("AACCGG", "AAAACG", "AACACG"), "example type", "example species", "user"
)
results <- run_matrix_tsma(foreground_sets, background_set,
  motifs = list(toy_motif))

## End(Not run)
```

`run_matrix_spma` *Matrix-based Spectrum Motif Analysis*

Description

SPMA helps to illuminate the relationship between RBP binding evidence and the transcript sorting criterion, e.g., fold change between treatment and control samples.

Usage

```
run_matrix_spma(
  sorted_transcript_sequences,
  sorted_transcript_values = NULL,
  transcript_values_label = "transcript value",
  motifs = NULL,
  n_bins = 40,
  midpoint = 0,
  x_value_limits = NULL,
  max_model_degree = 1,
  max_cs_permutations = 1e+07,
  min_cs_permutations = 5000,
  max_hits = 5,
  threshold_method = "p_value",
  threshold_value = 0.25^6,
  max_fg_permutations = 1e+06,
  min_fg_permutations = 1000,
  e = 5,
  p_adjust_method = "BH",
  n_cores = 1,
  cache = paste0(tempdir(), "/sc/")
)
```

Arguments

`sorted_transcript_sequences`
: named character vector of ranked sequences (only containing upper case characters A, C, G, T), where the names are RefSeq identifiers and sequence type qualifiers (`"3UTR"`, `"5UTR"` or `"mRNA"`), separated by `"|"`, e.g. `"NM_010356|3UTR"`. Names are only used to cache results. The sequences in `sorted_transcript_sequences` must be ranked (i.e., sorted). Commonly used sorting criteria are measures of differential expression, such as fold change or signal-to-noise ratio (e.g., between treatment and control samples in gene expression profiling experiments).

`sorted_transcript_values`
: vector of sorted transcript values, i.e., the fold change or signal-to-noise ratio or any other quantity that was used to sort the transcripts that were passed to `run_matrix_spma` or `run_kmer_spma` (default value is `NULL`). These values are displayed as a semi-transparent area over the enrichment value heatmaps of spectrum plots.

`transcript_values_label`
: label of transcript sorting criterion (e.g., `"log fold change"`, default value is `"transcript value"`), only shown if `!is.null(sorted_transcript_values)`

`motifs`
: a list of motifs that is used to score the specified sequences. If `is.null(motifs)` then all Transite motifs are used.

`n_bins`
: specifies the number of bins in which the sequences will be divided, valid values are between 7 and 100

`midpoint`
: for enrichment values the midpoint should be `1`, for log enrichment values `0` (defaults to `0`)

`x_value_limits`
: sets limits of the x-value color scale (used to harmonize color scales of different spectrum plots), see `limits` argument of `continuous_scale` (defaults to `NULL`, i.e., the data-dependent default scale range)

`max_model_degree`
: maximum degree of polynomial

`max_cs_permutations`
: maximum number of permutations performed in Monte Carlo test for consistency score

`min_cs_permutations`
: minimum number of permutations performed in Monte Carlo test for consistency score

`max_hits`
: maximum number of putative binding sites per mRNA that are counted

`threshold_method`
: either `"p_value"` (default) or `"relative"`. If `threshold_method` equals `"p_value"`, the default `threshold_value` is `0.25^6`, which is lowest p-value that can be achieved by hexamer motifs, the shortest supported motifs. If `threshold_method` equals `"relative"`, the default `threshold_value` is `0.9`, which is 90% of the maximum PWM score.

`threshold_value`
: semantics of the `threshold_value` depend on `threshold_method` (default is 0.25^6)

max_fg_permutations
: maximum number of foreground permutations performed in Monte Carlo test for enrichment score

min_fg_permutations
: minimum number of foreground permutations performed in Monte Carlo test for enrichment score

e
: integer-valued stop criterion for enrichment score Monte Carlo test: aborting permutation process after observing e random enrichment values with more extreme values than the actual enrichment value

p_adjust_method
: adjustment of p-values from Monte Carlo tests to avoid alpha error accumulation, see p.adjust

n_cores
: the number of cores that are used

cache
: either logical or path to a directory where scores are cached. The scores of each motif are stored in a separate file that contains a hash table with RefSeq identifiers and sequence type qualifiers as keys and the number of putative binding sites as values. If cache is FALSE, scores will not be cached.

Details

In order to investigate how motif targets are distributed across a spectrum of transcripts (e.g., all transcripts of a platform, ordered by fold change), Spectrum Motif Analysis visualizes the gradient of RBP binding evidence across all transcripts.

The matrix-based approach skips the *k*-merization step of the *k*-mer-based approach and instead scores the transcript sequence as a whole with a position specific scoring matrix.

For each sequence in foreground and background sets and each sequence motif, the scoring algorithm evaluates the score for each sequence position. Positions with a relative score greater than a certain threshold are considered hits, i.e., putative binding sites.

By scoring all sequences in foreground and background sets, a hit count for each motif and each set is obtained, which is used to calculate enrichment values and associated p-values in the same way in which motif-compatible hexamer enrichment values are calculated in the *k*-mer-based approach. P-values are adjusted with one of the available adjustment methods.

An advantage of the matrix-based approach is the possibility of detecting clusters of binding sites. This can be done by counting regions with many hits using positional hit information or by simply applying a hit count threshold per sequence, e.g., only sequences with more than some number of hits are considered. Homotypic clusters of RBP binding sites may play a similar role as clusters of transcription factors.

Value

A list with the following components:

foreground_scores
: the result of score_transcripts for the foreground sets (the bins)

background_scores
: the result of score_transcripts for the background set

enrichment_dfs
: a list of data frames, returned by calculate_motif_enrichment

spectrum_info_df
: a data frame with the SPMA results

spectrum_plots
: a list of spectrum plots, as generated by score_spectrum

classifier_scores a list of classifier scores, as returned by classify_spectrum

See Also

Other SPMA functions: classify_spectrum(), run_kmer_spma(), score_spectrum(), subdivide_data()

Other matrix functions: calculate_motif_enrichment(), run_matrix_tsma(), score_transcripts(), score_transcripts_single_motif()

Examples

```
# example data set
background_df <- transite:::ge$background_df
# sort sequences by signal-to-noise ratio
background_df <- dplyr::arrange(background_df, value)
# character vector of named and ranked (by signal-to-noise ratio) sequences
background_seqs <- gsub("T", "U", background_df$seq)
names(background_seqs) <- paste0(background_df$refseq, "|",
  background_df$seq_type)

results <- run_matrix_spma(background_seqs,
                           sorted_transcript_values = background_df$value,
                           transcript_values_label = "signal-to-noise ratio",
                           motifs = get_motif_by_id("M178_0.6"),
                           n_bins = 20,
                           max_fg_permutations = 10000)

## Not run:
results <- run_matrix_spma(background_seqs,
                           sorted_transcript_values = background_df$value,
                           transcript_values_label = "SNR")
## End(Not run)
```

---

run_matrix_tsma *Matrix-based Transcript Set Motif Analysis*

Description

Calculates motif enrichment in foreground sets versus a background set using position weight matrices to identify putative binding sites

Usage

```
run_matrix_tsma(
  foreground_sets,
  background_set,
  motifs = NULL,
  max_hits = 5,
  threshold_method = "p_value",
```

```
  threshold_value = 0.25^6,
  max_fg_permutations = 1e+06,
  min_fg_permutations = 1000,
  e = 5,
  p_adjust_method = "BH",
  n_cores = 1,
  cache = paste0(tempdir(), "/sc/")
)
```

Arguments

foreground_sets	a list of named character vectors of foreground sequences (only containing upper case characters A, C, G, T), where the names are RefSeq identifiers and sequence type qualifiers ("3UTR", "5UTR", "mRNA"), e.g. "NM_010356|3UTR". Names are only used to cache results.
background_set	a named character vector of background sequences (naming follows same rules as foreground set sequences)
motifs	a list of motifs that is used to score the specified sequences. If is.null(motifs) then all Transite motifs are used.
max_hits	maximum number of putative binding sites per mRNA that are counted
threshold_method	either "p_value" (default) or "relative". If threshold_method equals "p_value", the default threshold_value is 0.25^6, which is lowest p-value that can be achieved by hexamer motifs, the shortest supported motifs. If threshold_method equals "relative", the default threshold_value is 0.9, which is 90% of the maximum PWM score.
threshold_value	semantics of the threshold_value depend on threshold_method (default is 0.25^6)
max_fg_permutations	maximum number of foreground permutations performed in Monte Carlo test for enrichment score
min_fg_permutations	minimum number of foreground permutations performed in Monte Carlo test for enrichment score
e	integer-valued stop criterion for enrichment score Monte Carlo test: aborting permutation process after observing e random enrichment values with more extreme values than the actual enrichment value
p_adjust_method	adjustment of p-values from Monte Carlo tests to avoid alpha error accumulation, see p.adjust
n_cores	the number of cores that are used
cache	either logical or path to a directory where scores are cached. The scores of each motif are stored in a separate file that contains a hash table with RefSeq identifiers and sequence type qualifiers as keys and the number of putative binding sites as values. If cache is FALSE, scores will not be cached.

Details

Motif transcript set analysis can be used to identify RNA binding proteins, whose targets are significantly overrepresented or underrepresented in certain sets of transcripts.

The aim of Transcript Set Motif Analysis (TSMA) is to identify the overrepresentation and underrepresentation of potential RBP targets (binding sites) in a set (or sets) of sequences, i.e., the foreground set, relative to the entire population of sequences. The latter is called background set, which can be composed of all sequences of the genes of a microarray platform or all sequences of an organism or any other meaningful superset of the foreground sets.

The matrix-based approach skips the *k*-merization step of the *k*-mer-based approach and instead scores the transcript sequence as a whole with a position specific scoring matrix.

For each sequence in foreground and background sets and each sequence motif, the scoring algorithm evaluates the score for each sequence position. Positions with a relative score greater than a certain threshold are considered hits, i.e., putative binding sites.

By scoring all sequences in foreground and background sets, a hit count for each motif and each set is obtained, which is used to calculate enrichment values and associated p-values in the same way in which motif-compatible hexamer enrichment values are calculated in the k -mer-based approach. P-values are adjusted with one of the available adjustment methods.

An advantage of the matrix-based approach is the possibility of detecting clusters of binding sites. This can be done by counting regions with many hits using positional hit information or by simply applying a hit count threshold per sequence, e.g., only sequences with more than some number of hits are considered. Homotypic clusters of RBP binding sites may play a similar role as clusters of transcription factors.

Value

A list with the following components:

`foreground_scores`	the result of `score_transcripts` for the foreground sets
`background_scores`	the result of `score_transcripts` for the background set
`enrichment_dfs`	a list of data frames, returned by `calculate_motif_enrichment`

See Also

Other TSMA functions: `draw_volcano_plot()`, `run_kmer_tsma()`

Other matrix functions: `calculate_motif_enrichment()`, `run_matrix_spma()`, `score_transcripts()`, `score_transcripts_single_motif()`

Examples

```
# define simple sequence sets for foreground and background
foreground_set1 <- c(
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
  "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA"
)
```

```
names(foreground_set1) <- c(
  "NM_1_DUMMY|3UTR", "NM_2_DUMMY|3UTR", "NM_3_DUMMY|3UTR",
  "NM_4_DUMMY|3UTR", "NM_5_DUMMY|3UTR", "NM_6_DUMMY|3UTR",
  "NM_7_DUMMY|3UTR",
  "NM_8_DUMMY|3UTR", "NM_9_DUMMY|3UTR", "NM_10_DUMMY|3UTR",
  "NM_11_DUMMY|3UTR",
  "NM_12_DUMMY|3UTR", "NM_13_DUMMY|3UTR", "NM_14_DUMMY|3UTR"
)

foreground_set2 <- c("UUAUUUA", "AUCCUUUACA", "UUUUUUU", "UUUCAUCAUU")
names(foreground_set2) <- c(
  "NM_15_DUMMY|3UTR", "NM_16_DUMMY|3UTR", "NM_17_DUMMY|3UTR",
  "NM_18_DUMMY|3UTR"
)

foreground_sets <- list(foreground_set1, foreground_set2)

background_set <- c(
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
  "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA",
  "UUAUUUA", "AUCCUUUACA", "UUUUUUU", "UUUCAUCAUU",
  "CCACACAC", "CUCAUUGGAG", "ACUUUGGGACA", "CAGGUCAGCA"
)
names(background_set) <- c(
  "NM_1_DUMMY|3UTR", "NM_2_DUMMY|3UTR", "NM_3_DUMMY|3UTR",
  "NM_4_DUMMY|3UTR", "NM_5_DUMMY|3UTR", "NM_6_DUMMY|3UTR",
  "NM_7_DUMMY|3UTR",
  "NM_8_DUMMY|3UTR", "NM_9_DUMMY|3UTR", "NM_10_DUMMY|3UTR",
  "NM_11_DUMMY|3UTR",
  "NM_12_DUMMY|3UTR", "NM_13_DUMMY|3UTR", "NM_14_DUMMY|3UTR",
  "NM_15_DUMMY|3UTR",
  "NM_16_DUMMY|3UTR", "NM_17_DUMMY|3UTR", "NM_18_DUMMY|3UTR",
  "NM_19_DUMMY|3UTR",
  "NM_20_DUMMY|3UTR", "NM_21_DUMMY|3UTR", "NM_22_DUMMY|3UTR"
)

# run cached version of TSMA with all Transite motifs (recommended):
# results <- run_matrix_tsma(foreground_sets, background_set)

# run uncached version with one motif:
motif_db <- get_motif_by_id("M178_0.6")
results <- run_matrix_tsma(foreground_sets, background_set, motifs = motif_db,
cache = FALSE)

## Not run:
# define example sequence sets for foreground and background
foreground1_df <- transite:::ge$foreground1_df
foreground_set1 <- gsub("T", "U", foreground1_df$seq)
names(foreground_set1) <- paste0(foreground1_df$refseq, "|",
  foreground1_df$seq_type)
```

```
foreground2_df <- transite:::ge$foreground2_df
foreground_set2 <- gsub("T", "U", foreground2_df$seq)
names(foreground_set2) <- paste0(foreground2_df$refseq, "|",
  foreground2_df$seq_type)

foreground_sets <- list(foreground_set1, foreground_set2)

background_df <- transite:::ge$background_df
background_set <- gsub("T", "U", background_df$seq)
names(background_set) <- paste0(background_df$refseq, "|",
  background_df$seq_type)

# run cached version of TSMA with all Transite motifs (recommended)
results <- run_matrix_tsma(foreground_sets, background_set)

# run uncached version of TSMA with all Transite motifs
results <- run_matrix_tsma(foreground_sets, background_set, cache = FALSE)

# run TSMA with a subset of Transite motifs
results <- run_matrix_tsma(foreground_sets, background_set,
  motifs = get_motif_by_rbp("ELAVL1"))

# run TSMA with user-defined motif
toy_motif <- create_matrix_motif(
  "toy_motif", "example RBP", toy_motif_matrix,
  "example type", "example species", "user"
)
results <- run_matrix_tsma(foreground_sets, background_set,
  motifs = list(toy_motif))

## End(Not run)
```

`score_sequences` *Score Sequences with PWM*

Description

C++ implementation of PWM scoring algorithm

Usage

```
score_sequences(sequences, pwm)
```

Arguments

`sequences`	list of sequences
`pwm`	position weight matrix

Value

list of PWM scores for each sequence

Examples

```
motif <- get_motif_by_id("M178_0.6")[[1]]
sequences <- c("CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
               "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
               "AUCAAAUUA", "UGUGGGG", "GACACUUAAAGAUCCU",
               "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA", "AUAGAC",
               "AGUUC", "CCAGUAA")
seq_char_vectors <- lapply(sequences, function(seq) {
  unlist(strsplit(seq, ""))
})
score_sequences(seq_char_vectors, as.matrix(get_motif_matrix(motif)))
```

---

score_spectrum *Calculates spectrum scores and creates spectrum plots*

Description

Spectrum scores are a means to evaluate if a spectrum has a meaningful (i.e., biologically relevant) or a random pattern.

Usage

```
score_spectrum(
  x,
  p_values = array(1, length(x)),
  x_label = "log enrichment",
  sorted_transcript_values = NULL,
  transcript_values_label = "transcript value",
  midpoint = 0,
  x_value_limits = NULL,
  max_model_degree = 3,
  max_cs_permutations = 1e+07,
  min_cs_permutations = 5000,
  e = 5
)
```

Arguments

x	vector of values (e.g., enrichment values, normalized RBP scores) per bin
p_values	vector of p-values (e.g., significance of enrichment values) per bin
x_label	label of values (e.g., "enrichment value")

`sorted_transcript_values`
: vector of sorted transcript values, i.e., the fold change or signal-to-noise ratio or any other quantity that was used to sort the transcripts that were passed to `run_matrix_spma` or `run_kmer_spma` (default value is `NULL`). These values are displayed as a semi-transparent area over the enrichment value heatmaps of spectrum plots.

`transcript_values_label`
: label of transcript sorting criterion (e.g., `"log fold change"`, default value is `"transcript value"`), only shown if `!is.null(sorted_transcript_values)`

`midpoint`
: for enrichment values the midpoint should be `1`, for log enrichment values `0` (defaults to `0`)

`x_value_limits`
: sets limits of the x-value color scale (used to harmonize color scales of different spectrum plots), see `limits` argument of `continuous_scale` (defaults to `NULL`, i.e., the data-dependent default scale range)

`max_model_degree`
: maximum degree of polynomial

`max_cs_permutations`
: maximum number of permutations performed in Monte Carlo test for consistency score

`min_cs_permutations`
: minimum number of permutations performed in Monte Carlo test for consistency score

`e`
: integer-valued stop criterion for consistency score Monte Carlo test: aborting permutation process after observing `e` random consistency values with more extreme values than the actual consistency value

Details

One way to quantify the meaningfulness of a spectrum is to calculate the deviance between the linear interpolation of the scores of two adjoining bins and the score of the middle bin, for each position in the spectrum. The lower the score, the more consistent the trend in the spectrum plot. Formally, the local consistency score x_c is defined as

$$x_c = \frac{1}{n}\sum_{i=1}^{n-2} |\frac{s_i + s_{i+2}}{2} - s_{i+1}|.$$

In order to obtain an estimate of the significance of a particular score x_c', Monte Carlo sampling is performed by randomly permuting the coordinates of the scores vector s and recomputing x_c. The probability estimate $\hat{p}$ is given by the lower tail version of the cumulative distribution function

$$\hat{Pr}(T(x)) = \frac{\sum_{i=1}^{n} 1(T(y_i) \leq T(x)) + 1}{n+1},$$

where 1 is the indicator function, n is the sample size, i.e., the number of performed permutations, and T equals x_c in the above equation.

An alternative approach to assess the consistency of a spectrum plot is via polynomial regression. In a first step, polynomial regression models of various degrees are fitted to the data, i.e., the dependent

variable s (vector of scores), and orthogonal polynomials of the independent variable b (vector of bin numbers). Secondly, the model that reflects best the true nature of the data is selected by means of the F-test. And lastly, the adjusted R^2 and the sum of squared residuals are calculated to indicate how well the model fits the data. These statistics are used as scores to rank the spectrum plots. In general, the polynomial regression equation is

$$y_i = \beta_0 + \beta_1 x_i + \beta_2 x_i^2 + \cdots + \beta_m x_i^m + \epsilon_i,$$

where m is the degree of the polynomial (usually $m \leq 5$), and ϵ_i is the error term. The dependent variable y is the vector of scores s and x to x^m are the orthogonal polynomials of the vector of bin numbers b. Orthogonal polynomials are used in order to reduce the correlation between the different powers of b and therefore avoid multicollinearity in the model. This is important, because correlated predictors lead to unstable coefficients, i.e., the coefficients of a polynomial regression model of degree m can be greatly different from a model of degree $m + 1$.

The orthogonal polynomials of vector b are obtained by centering (subtracting the mean), QR decomposition, and subsequent normalization. Given the dependent variable y and the orthogonal polynomials of b x to x^m, the model coefficients β are chosen in a way to minimize the deviance between the actual and the predicted values characterized by

$$M(x) = \beta_0 + \beta_1 x + \beta_2 x^2 + \cdots + \beta_m x^m$$

$$M = argmin_M(\sum_{i=1}^{n} L(y_i, M(x_i))),$$

where L(actual value, predicted value) denotes the loss function.

Ordinary least squares is used as estimation method for the model coefficients β. The loss function of ordinary least squares is the sum of squared residuals (SSR) and is defined as follows $\text{SSR}(y, \hat{y}) = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2$, where y are the observed data and $\hat{y}$ the model predictions.

Thus the ordinary least squares estimate of the coefficients $\hat{\beta}$ (including the intercept $\hat{\beta}_0$) of the model M is defined by

$$\hat{\beta} = argmin_\beta(\sum_{i=1}^{n} (y_i - \beta_0 - \sum_{j=1}^{m} \beta_j x_i^j)^2).$$

After polynomial models of various degrees have been fitted to the data, the F-test is used to select the model that best fits the data. Since the SSR monotonically decreases with increasing model degree (model complexity), the relative decrease of the SSR between the simpler model and the more complex model must outweigh the increase in model complexity between the two models. The F-test gives the probability that a relative decrease of the SSR between the simpler and the more complex model given their respective degrees of freedom is due to chance. A low p-value indicates that the additional degrees of freedom of the more complex model lead to a better fit of the data than would be expected after a mere increase of degrees of freedom.

The F-statistic is calculated as follows

$$F = \frac{(SSR_1 - SSR_2)/(p_2 - p_1)}{SSR_2/(n - p_2)},$$

where SSR_i is the sum of squared residuals and p_i is the number of parameters of model i. The number of data points, i.e., bins, is denoted as n. F is distributed according to the F-distribution with $df_1 = p_2 - p_1$ and $df_2 = n - p_2$.

Value

A list object of class `SpectrumScore` with the following components:

`adj_r_squared`	adjusted R^2 of polynomial model
`degree`	maximum degree of polynomial
`residuals`	residuals of polynomial model
`slope`	coefficient of the linear term of the polynomial model (spectrum "direction")
`f_statistic`	statistic of the F-test
`f_statistic_p_value`	p-value of F-test
`consistency_score`	normalized sum of deviance between the linear interpolation of the scores of two adjoining b
`consistency_score_p_value`	obtained by Monte Carlo sampling (randomly permuting the coordinates of the scores vecto
`consistency_score_n`	number of permutations
`plot`	

See Also

Other SPMA functions: `classify_spectrum()`, `run_kmer_spma()`, `run_matrix_spma()`, `subdivide_data()`

Examples

```
# random spectrum
score_spectrum(runif(n = 40, min = -1, max = 1), max_model_degree = 1)

# two random spectrums with harmonized color scales
plot(score_spectrum(runif(n = 40, min = -1, max = 1), max_model_degree = 1,
     x_value_limits = c(-2.0, 2.0)))
plot(score_spectrum(runif(n = 40, min = -2, max = 2), max_model_degree = 1,
     x_value_limits = c(-2.0, 2.0)))

# random spectrum with p-values
score_spectrum(runif(n = 40, min = -1, max = 1),
               p_values = runif(n = 40, min = 0, max = 1),
               max_model_degree = 1)

# random spectrum with sorted transcript values
log_fold_change <- log(runif(n = 1000, min = 0, max = 1) /
                         runif(n = 1000, min = 0, max = 1))
score_spectrum(runif(n = 40, min = -1, max = 1),
               sorted_transcript_values = sort(log_fold_change),
               max_model_degree = 1)

# non-random linear spectrum
signal <- seq(-1, 0.99, 2 / 40)
noise <- rnorm(n = 40, mean = 0, sd = 0.5)
score_spectrum(signal + noise, max_model_degree = 1,
               max_cs_permutations = 100000)

# non-random quadratic spectrum
signal <- seq(-1, 0.99, 2 / 40)^2 - 0.5
noise <- rnorm(n = 40, mean = 0, sd = 0.2)
score_spectrum(signal + noise, max_model_degree = 2,
```

```
                max_cs_permutations = 100000)
```

`score_transcripts` *Scores transcripts with position weight matrices*

Description

This function is used to count the binding sites in a set of sequences for all or a subset of RNA-binding protein sequence motifs and returns the result in a data frame, which is subsequently used by `calculate_motif_enrichment` to obtain binding site enrichment scores.

Usage

```
score_transcripts(
  sequences,
  motifs = NULL,
  max_hits = 5,
  threshold_method = c("p_value", "relative"),
  threshold_value = 0.25^6,
  n_cores = 1,
  cache = paste0(tempdir(), "/sc/")
)
```

Arguments

`sequences`	character vector of named sequences (only containing upper case characters A, C, G, T), where the names are RefSeq identifiers and sequence type qualifiers (`"3UTR"`, `"5UTR"`, `"mRNA"`), e.g. `"NM_010356|3UTR"`
`motifs`	a list of motifs that is used to score the specified sequences. If `is.null(motifs)` then all Transite motifs are used.
`max_hits`	maximum number of putative binding sites per mRNA that are counted
`threshold_method`	either `"p_value"` (default) or `"relative"`. If `threshold_method` equals `"p_value"`, the default `threshold_value` is `0.25^6`, which is lowest p-value that can be achieved by hexamer motifs, the shortest supported motifs. If `threshold_method` equals `"relative"`, the default `threshold_value` is `0.9`, which is 90% of the maximum PWM score.
`threshold_value`	semantics of the `threshold_value` depend on `threshold_method` (default is 0.25^6)
`n_cores`	the number of cores that are used
`cache`	either logical or path to a directory where scores are cached. The scores of each motif are stored in a separate file that contains a hash table with RefSeq identifiers and sequence type qualifiers as keys and the number of putative binding sites as values. If `cache` is `FALSE`, scores will not be cached.

Value

A list with three entries:

(1) df: a data frame with the following columns:

motif_id	the motif identifier that is used in the original motif library
motif_rbps	the gene symbol of the RNA-binding protein(s)
absolute_hits	the absolute frequency of putative binding sites per motif in all transcripts
relative_hits	the relative, i.e., absolute divided by total, frequency of binding sites per motif in all transcripts
total_sites	the total number of potential binding sites
one_hit, two_hits, ...	number of transcripts with one, two, three, ... putative binding sites

(2) total_sites: a numeric vector with the total number of potential binding sites per transcript

(3) absolute_hits: a numeric vector with the absolute (not relative) number of putative binding sites per transcript

See Also

Other matrix functions: calculate_motif_enrichment(), run_matrix_spma(), run_matrix_tsma(), score_transcripts_single_motif()

Examples

```
foreground_set <- c(
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
  "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA"
)
# names are used as keys in the hash table (cached version only)
# ideally sequence identifiers (e.g., RefSeq ids) and region labels
# (e.g., 3UTR for 3'-UTR)
names(foreground_set) <- c(
  "NM_1_DUMMY|3UTR", "NM_2_DUMMY|3UTR", "NM_3_DUMMY|3UTR",
  "NM_4_DUMMY|3UTR", "NM_5_DUMMY|3UTR", "NM_6_DUMMY|3UTR",
  "NM_7_DUMMY|3UTR", "NM_8_DUMMY|3UTR", "NM_9_DUMMY|3UTR",
  "NM_10_DUMMY|3UTR", "NM_11_DUMMY|3UTR", "NM_12_DUMMY|3UTR",
  "NM_13_DUMMY|3UTR", "NM_14_DUMMY|3UTR"
)

# specific motifs, uncached
motifs <- get_motif_by_rbp("ELAVL1")
scores <- score_transcripts(foreground_set, motifs = motifs, cache = FALSE)
## Not run:
# all Transite motifs, cached (writes scores to disk)
scores <- score_transcripts(foreground_set)

# all Transite motifs, uncached
scores <- score_transcripts(foreground_set, cache = FALSE)
```

```
foreground_df <- transite:::ge$foreground1_df
foreground_set <- foreground_df$seq
names(foreground_set) <- paste0(foreground_df$refseq, "|",
   foreground_df$seq_type)
scores <- score_transcripts(foreground_set)

## End(Not run)
```

`score_transcripts_single_motif` *Scores transadsadscripts with position weight matrices*

Description

This function is used to count the putative binding sites (i.e., motifs) in a set of sequences for the specified RNA-binding protein sequence motifs and returns the result in a data frame, which is aggregated by `score_transcripts` and subsequently used by `calculate_motif_enrichment` to obtain binding site enrichment scores.

Usage

```
score_transcripts_single_motif(
  motif,
  sequences,
  max_hits = 5,
  threshold_method = c("p_value", "relative"),
  threshold_value = 0.25^6,
  cache_path = paste0(tempdir(), "/sc/")
)
```

Arguments

`motif`	a Transite motif that is used to score the specified sequences
`sequences`	character vector of named sequences (only containing upper case characters A, C, G, T), where the names are RefSeq identifiers and sequence type qualifiers (`"3UTR"`, `"5UTR"`, `"mRNA"`), e.g. `"NM_010356|3UTR"`
`max_hits`	maximum number of putative binding sites per mRNA that are counted
`threshold_method`	either `"p_value"` (default) or `"relative"`. If `threshold_method` equals `"p_value"`, the default `threshold_value` is `0.25^6`, which is lowest p-value that can be achieved by hexamer motifs, the shortest supported motifs. If `threshold_method` equals `"relative"`, the default `threshold_value` is `0.9`, which is 90% of the maximum PWM score.
`threshold_value`	semantics of the `threshold_value` depend on `threshold_method` (default is 0.25^6)

cache_path	the path to a directory where scores are cached. The scores of each motif are stored in a separate file that contains a hash table with RefSeq identifiers and sequence type qualifiers as keys and the number of binding sites as values. If is.null(cache_path), scores will not be cached.

Value

A list with the following items:

motif_id	the motif identifier of the specified motif
motif_rbps	the gene symbol of the RNA-binding protein(s)
absolute_hits	the absolute frequency of binding sites per motif in all transcripts
relative_hits	the relative, i.e., absolute divided by total, frequency of binding sites per motif in all transcripts
total_sites	the total number of potential binding sites
one_hit, two_hits, ...	number of transcripts with one, two, three, ... binding sites

See Also

Other matrix functions: calculate_motif_enrichment(), run_matrix_spma(), run_matrix_tsma(), score_transcripts()

---

set_motifs *Set Transite motif database*

Description

Globally sets Transite motif database, use with care.

Usage

```
set_motifs(value)
```

Arguments

value	list of Motif objects

Value

void

See Also

Other motif functions: generate_iupac_by_kmers(), generate_iupac_by_matrix(), generate_kmers_from_iupac(), get_motif_by_id(), get_motif_by_rbp(), get_motifs(), get_motifs_meta_info(), get_ppm(), init_iupac_lookup_table()

Examples

```
custom_motif <- create_kmer_motif(
  "custom_motif", "RBP1",
  c("AAAAAAA", "CAAAAAA"), "HITS-CLIP",
  "Homo sapiens", "user"
)
set_motifs(list(custom_motif))
```

`SpectrumScore-class` *An S4 class to represent a scored spectrum*

Description

An S4 class to represent a scored spectrum

Getter Method get_adj_r_squared

Getter Method get_model_degree

Getter Method get_model_residuals

Getter Method get_model_slope

Getter Method get_model_f_statistic

Getter Method get_model_f_statistic_p_value

Getter Method get_consistency_score

Getter Method get_consistency_score_p_value

Getter Method get_consistency_score_n

Usage

```
get_adj_r_squared(object)

## S4 method for signature 'SpectrumScore'
get_adj_r_squared(object)

get_model_degree(object)

## S4 method for signature 'SpectrumScore'
get_model_degree(object)

get_model_residuals(object)

## S4 method for signature 'SpectrumScore'
get_model_residuals(object)

get_model_slope(object)

## S4 method for signature 'SpectrumScore'
```

```
get_model_slope(object)

get_model_f_statistic(object)

## S4 method for signature 'SpectrumScore'
get_model_f_statistic(object)

get_model_f_statistic_p_value(object)

## S4 method for signature 'SpectrumScore'
get_model_f_statistic_p_value(object)

get_consistency_score(object)

## S4 method for signature 'SpectrumScore'
get_consistency_score(object)

get_consistency_score_p_value(object)

## S4 method for signature 'SpectrumScore'
get_consistency_score_p_value(object)

get_consistency_score_n(object)

## S4 method for signature 'SpectrumScore'
get_consistency_score_n(object)

## S4 method for signature 'SpectrumScore'
show(object)

## S4 method for signature 'SpectrumScore,ANY'
plot(x)
```

Arguments

object	SpectrumScore object
x	SpectrumScore object

Value

Object of type SpectrumScore

Slots

`adj_r_squared` adjusted R^2 of polynomial model

`degree` degree of polynomial (integer between 0 and 5)

`residuals` residuals of the polynomial model

`slope` coefficient of the linear term of the polynomial model (spectrum "direction")

`f_statistic` F statistic from the F test used to determine the degree of the polynomial model

`f_statistic_p_value` p-value associated with the F statistic

`consistency_score` raw local consistency score of the spectrum

`consistency_score_p_value` p-value associated with the local consistency score

`consistency_score_n` number of permutations performed to calculate p-value of local consistency score (permutations performed before early stopping criterion reached)

`plot` spectrum plot

Examples

```
new("SpectrumScore",
    adj_r_squared = 0,
    degree = 0L,
    residuals = 0,
    slope = 0,
    f_statistic = 0,
    f_statistic_p_value = 1,
    consistency_score = 1,
    consistency_score_p_value = 1,
    consistency_score_n = 1000L,
    plot = NULL
)
```

`subdivide_data` *Subdivides Sequences into* n *Bins*

Description

Preprocessing function for SPMA, divides transcript sequences into *n* bins.

Usage

```
subdivide_data(sorted_transcript_sequences, n_bins = 40)
```

Arguments

`sorted_transcript_sequences`
: character vector of named sequences (names are usually RefSeq identifiers and sequence region labels, e.g., "NM_1_DUMMY|3UTR"). It is important that the sequences are already sorted by fold change, signal-to-noise ratio or any other meaningful measure.

`n_bins`
: specifies the number of bins in which the sequences will be divided, valid values are between 7 and 100

Value

An array of `n_bins` length, containing the binned sequences

See Also

Other SPMA functions: classify_spectrum(), run_kmer_spma(), run_matrix_spma(), score_spectrum()

Examples

```
# toy example
toy_seqs <- c(
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU", "UCAUUUUAUUAAA",
  "AAUUGGUGUCUGGAUACUUCCCUGUACAU", "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA", "AUAGAC", "AGUUC", "CCAGUAA"
)
# names are used as keys in the hash table (cached version only)
# ideally sequence identifiers (e.g., RefSeq ids) and
# sequence region labels (e.g., 3UTR for 3'-UTR)
names(toy_seqs) <- c(
  "NM_1_DUMMY|3UTR", "NM_2_DUMMY|3UTR", "NM_3_DUMMY|3UTR",
  "NM_4_DUMMY|3UTR", "NM_5_DUMMY|3UTR", "NM_6_DUMMY|3UTR",
  "NM_7_DUMMY|3UTR",
  "NM_8_DUMMY|3UTR", "NM_9_DUMMY|3UTR", "NM_10_DUMMY|3UTR",
  "NM_11_DUMMY|3UTR",
  "NM_12_DUMMY|3UTR", "NM_13_DUMMY|3UTR", "NM_14_DUMMY|3UTR"
)

foreground_sets <- subdivide_data(toy_seqs, n_bins = 7)

# example data set
background_df <- transite:::ge$background_df
# sort sequences by signal-to-noise ratio
background_df <- dplyr::arrange(background_df, value)
# character vector of named sequences
background_seqs <- background_df$seq
names(background_seqs) <- paste0(background_df$refseq, "|",
  background_df$seq_type)

foreground_sets <- subdivide_data(background_seqs)
```

---

toy_motif_matrix *Toy Motif Matrix*

Description

This toy motif matrix is used in code examples for various functions.

Usage

```
data(toy_motif_matrix)
```

Format

A data frame with four columns (A, C, G, U) and seven rows (position 1 - 7)

transite *transite*

Description

transite is a computational method that allows comprehensive analysis of the regulatory role of RNA-binding proteins in various cellular processes by leveraging preexisting gene expression data and current knowledge of binding preferences of

Author(s)

Konstantin Krismer

See Also

Useful links:

- https://transite.mit.edu

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