# Package 'transite'

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```
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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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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 permu-

tation 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 two 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

### See Also

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

### **Examples**

```
# define simple sequence sets for foreground and background
foreground_set1 <- c(</pre>
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU", "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA"
)
foreground_set2 <- c("UUAUUUA", "AUCCUUUACA", "UUUUUUU", "UUUCAUCAUU")</pre>
foreground_sets <- list(foreground_set1, foreground_set2)</pre>
background_set <- c(foreground_set1, foreground_set2,</pre>
                      "CCACACAC", "CUCAUUGGAG", "ACUUUGGGACA", "CAGGUCAGCA")
# single-threaded
kmer_enrichment_values_st <- calculate_kmer_enrichment(foreground_sets,</pre>
  background_set, 6, n_cores = 1)
## Not run:
# multi-threaded
kmer_enrichment_values_mt <- calculate_kmer_enrichment(foreground_sets,</pre>
  background_set, 6)
## End(Not run)
```

```
calculate_local_consistency
```

Local Consistency Score

# **Description**

C++ implementation of Local Consistency Score algorithm.

```
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

е

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)</pre>
```

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.

```
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

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

e

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
               unadjusted p-value from Monte Carlo test
    p_value
               number of Monte Carlo test permutations
  p_value_n
               adjusted p-value from Monte Carlo test (usually FDR)
adj_p_value
```

### See Also

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

### **Examples**

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

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minPermutations

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

е

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**

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

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### See Also

```
Other k-mer functions: calculate_kmer_enrichment(), compute_kmer_enrichment(), count_homopolymer_corrected draw_volcano_plot(), estimate_significance_core(), estimate_significance(), 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 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:

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```
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(</pre>
  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 \leftarrow rnorm(n = 40, mean = 0, sd = 0.5)
linear_sp <- score_spectrum(signal + noise, max_model_degree = 1,</pre>
  max_cs_permutations = 100000)
score <- classify_spectrum(</pre>
  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 \leftarrow rnorm(n = 40, mean = 0, sd = 0.2)
linear_sp <- score_spectrum(signal + noise, max_model_degree = 1,</pre>
  max_cs_permutations = 100000)
score <- classify_spectrum(</pre>
  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 \leftarrow rnorm(n = 40, mean = 0, sd = 0.2)
quadratic_sp <- score_spectrum(signal + noise, max_model_degree = 2,</pre>
  max_cs_permutations = 100000)
score <- classify_spectrum(</pre>
  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)</pre>
```

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

#### See Also

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

### **Examples**

```
# define simple sequence sets for foreground and background
foreground_set <- c(</pre>
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU",
  "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA"
)
background_set <- c(</pre>
  "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)</pre>
background_kmers <- generate_kmers(background_set, 6)</pre>
kmer_enrichment_values <- compute_kmer_enrichment(foreground_kmers,</pre>
 background_kmers)
```

 $\verb|count_homopolymer_corrected_kmers|$ 

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(), draw_volcano_plot(), estimate_significance_core(), estimate_significance(), 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.

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

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# 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"
)</pre>
```

# **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')

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### Value

```
object of class RBPMotif
```

# **Examples**

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

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 set of k-mers that are associated with a certain motif, will be highlighted in volcano plot 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(), estimate_significance_core(), estimate_significance(), generate_kmers(), generate_permuted_enrichments(), run_kmer_spma(), run_kmer_tsma()
```

### **Examples**

```
motif <- get_motif_by_id("951_12324455")</pre>
draw_volcano_plot(transite:::kmers_enrichment, get_hexamers(motif[[1]]),
  get_rbps(motif[[1]]))
## Not run:
foreground_set <- c("UGUGGG", "GUGGGG", "GUGUGG", "UGUGGU")</pre>
background_set <- unique(c(foreground_set, c(</pre>
  "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")</pre>
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.

```
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(), 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.

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

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### **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(), 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")</pre>
```

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 data frame that contains up-regulated genes after treatment 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:

Adenine Cytosine С Guanine U Uracil A or G C or U S G or C A or U Κ G or U A or C В C or G or U D A or G or U A or C or U Н A or C or G 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_meta_info(), get_motifs(), 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

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```
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_meta_info(), get_motifs(), 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(), draw_volcano_plot(), estimate_significance_core(), estimate_significance(), generate_permuted_enrichments(), run_kmer_spma(), run_kmer_tsma()
```

### **Examples**

```
# count hexamers in set of RNA sequences
rna_sequences <- c(</pre>
  "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)</pre>
# count heptamers in set of DNA sequences
dna_sequences <- c(</pre>
  "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)</pre>
```

generate\_kmers\_from\_iupac

Generates all k-mers for IUPAC string

### **Description**

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

```
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:

Adenine С Cytosine G Guanine U Uracil A or G R C or U S G or C W A or U G or U M A or C B C or G or U D A or G or U A or C or U A or C or G 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_meta_info(), get_motifs(), 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(), draw_volcano_plot(), estimate_significance_core(), estimate_significance(), generate_kmers(), run_kmer_spma(), run_kmer_tsma()
```

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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()</pre>
```

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```
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.

```
get_motif_by_id(id)
```

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# 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_meta_info(), get_motifs(), 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_meta_info(), get_motifs(), 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_meta_info(), get_motifs(), 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:

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- 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_meta_info(), get_motifs(), 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.

```
data(kmers_enrichment)
```

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# **Format**

A data frame with the following columns:

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kmer contains all hexamers (AAAAAA to UUUUUU)

foreground\_count absolute k-mer frequency in foreground set

absolute k-mer frequency in background set

enrichment enrichment of k-mer in foreground relative to background

associated p-value of enrichment

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 motifid

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 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

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### **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**

р	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.  $\Phi^{-1}$  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.

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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

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```
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)
```

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

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

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

# Arguments

object RBPMotif object
x RBPMotif object

#### Value

Object of type RBPMotif

#### **Slots**

### **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"
)</pre>
```

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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.

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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 consis-

ency score

min\_cs\_permutations

minimum number of permutations performed in Monte Carlo test for consis-

tency 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

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## 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(), draw_volcano_plot(), estimate_significance_core(),
estimate_significance(), 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)</pre>
# character vector of named and ranked (by signal-to-noise ratio) sequences
background_seqs <- gsub("T", "U", background_df$seq)</pre>
names(background_seqs) <- paste0(background_df$refseq, "|",</pre>
  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,</pre>
                          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,
```

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```
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)

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:

40 run\_kmer\_tsma

```
\begin{array}{ccc} & \text{enrichment\_df} & \text{the result of compute\_kmer\_enrichment} \\ & \text{motif\_df} \\ & \text{motif\_kmers\_dfs} \\ & \text{volcano\_plots} & \text{volcano plots for each motif (see draw\_volcano\_plot)} \\ & \text{perm\_test\_plots} & \text{plots of the empirical distribution of $k$-mer enrichment values for each motif} \\ & \text{enriched\_kmers\_combined\_p\_values} \end{array}
```

#### 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(), draw_volcano_plot(), estimate_significance_core(), estimate_significance(), generate_kmers(), generate_permuted_enrichments(), run_kmer_spma()
```

## **Examples**

```
# define simple sequence sets for foreground and background
foreground_set1 <- c(</pre>
  "CAACAGCCUUAAUU", "CAGUCAAGACUCC", "CUUUGGGGAAU", "UCAUUUUAUUAAA", "AAUUGGUGUCUGGAUACUUCCCUGUACAU",
  "AUCAAAUUA", "AGAU", "GACACUUAAAGAUCCU",
  "UAGCAUUAACUUAAUG", "AUGGA", "GAAGAGUGCUCA",
  "AUAGAC", "AGUUC", "CCAGUAA"
)
foreground_set2 <- c("UUAUUUA", "AUCCUUUACA", "UUUUUUU", "UUUCAUCAUU")</pre>
foreground_sets <- list(foreground_set1, foreground_set2)</pre>
background_set <- unique(c(foreground_set1, foreground_set2, c(</pre>
  "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)</pre>
# run TSMA with one motif:
motif_db <- get_motif_by_id("M178_0.6")</pre>
results <- run_kmer_tsma(foreground_sets, background_set, motifs = motif_db)
# define example sequence sets for foreground and background
foreground_set1 <- gsub("T", "U", transite:::ge$foreground1_df$seq)</pre>
foreground_set2 <- gsub("T", "U", transite:::ge$foreground2_df$seq)</pre>
foreground_sets <- list(foreground_set1, foreground_set2)</pre>
background_set <- gsub("T", "U", transite:::ge$background_df$seq)</pre>
# run TSMA with all Transite motifs
results <- run_kmer_tsma(foreground_sets, background_set)</pre>
# 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)</pre>
```

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 qual-

ifiers ("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  $\emptyset$ 

(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

integer-valued stop criterion for enrichment score Monte Carlo test: aborting permutation process after observing e random enrichment values with more ex-

treme values than the actual enrichment value

p\_adjust\_method

adjustment of p-values from Monte Carlo tests to avoid alpha error accumula-

tion, 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) the result of score_transcripts for the background set the result of score_transcripts for the background set a list of data frames, returned by calculate_motif_enrichment a data frame with the SPMA results a list of spectrum plots, as generated by score_spectrum 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_single_motif(),
```

```
score_transcripts()
```

#### **Examples**

```
# example data set
background_df <- transite:::ge$background_df</pre>
# sort sequences by signal-to-noise ratio
background_df <- dplyr::arrange(background_df, value)</pre>
# character vector of named and ranked (by signal-to-noise ratio) sequences
background_seqs <- gsub("T", "U", background_df$seq)</pre>
names(background_seqs) <- paste0(background_df$refseq, "|",</pre>
 background_df$seq_type)
results <- run_matrix_spma(background_seqs,
                            sorted_transcript_values = background_df$value,
                            transcript_values_label = "signal-to-noise ratio",
                            motifs = get_motif_by_id("M178_0.6"),
                            n_bins = 20,
                            max_fg_permutations = 10000)
## Not run:
results <- run_matrix_spma(background_seqs,</pre>
                            sorted_transcript_values = background_df$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<sup>6</sup>, 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

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

the number of cores that are used n\_cores

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**

e

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_single_motif(), score_transcripts()
```

# **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_9_DUMMY|3UTR", "NM_10_DUMMY|3UTR",
    "NM_8_DUMMY|3UTR", "NM_9_DUMMY|3UTR", "NM_10_DUMMY|3UTR",</pre>
```

```
"NM_11_DUMMY|3UTR",
  "NM_12_DUMMY|3UTR", "NM_13_DUMMY|3UTR", "NM_14_DUMMY|3UTR"
)
foreground_set2 <- c("UUAUUUA", "AUCCUUUACA", "UUUUUUU", "UUUCAUCAUU")</pre>
names(foreground_set2) <- c(</pre>
  "NM_15_DUMMY|3UTR", "NM_16_DUMMY|3UTR", "NM_17_DUMMY|3UTR",
  "NM_18_DUMMY|3UTR"
)
foreground_sets <- list(foreground_set1, foreground_set2)</pre>
background_set <- c(</pre>
  "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(</pre>
  "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)</pre>
# run uncached version with one motif:
motif_db <- get_motif_by_id("M178_0.6")</pre>
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$foreground1_df</pre>
foreground_set1 <- gsub("T", "U", foreground1_df$seq)</pre>
names(foreground_set1) <- paste0(foreground1_df$refseq, "|",</pre>
  foreground1_df$seq_type)
foreground2_df <- transite:::ge$foreground2_df</pre>
foreground_set2 <- gsub("T", "U", foreground2_df$seq)</pre>
names(foreground_set2) <- paste0(foreground2_df$refseq, "|",</pre>
  foreground2_df$seq_type)
```

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```
foreground_sets <- list(foreground_set1, foreground_set2)</pre>
background_df <- transite:::ge$background_df</pre>
background_set <- gsub("T", "U", background_df$seq)</pre>
names(background_set) <- paste0(background_df$refseq, "|",</pre>
 background_df$seq_type)
# run cached version of TSMA with all Transite motifs (recommended)
results <- run_matrix_tsma(foreground_sets, background_set)</pre>
# 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,</pre>
 motifs = get_motif_by_rbp("ELAVL1"))
# run TSMA with user-defined motif
toy_motif <- create_matrix_motif(</pre>
  "toy_motif", "example RBP", toy_motif_matrix,
  "example type", "example species", "user"
)
results <- run_matrix_tsma(foreground_sets, background_set,</pre>
 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**

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

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**

e

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

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

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

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

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

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

where m is the degree of the polynomial (usually  $m \leq 5$ ), and  $\epsilon_i$  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  $\beta$  are chosen in a way to minimize the deviance between the actual and the predicted values characterized by

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

$$M = argmin_{M}(\sum_{i=1}^{n} L(y_{i}, M(x_{i}))),$$

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

Ordinary least squares is used as estimation method for the model coefficients  $\beta$ . The loss function of ordinary least squares is the sum of squared residuals (SSR) and is defined as follows  $SSR(y, \hat{y}) = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2$ , 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
                               obtained by Monte Carlo sampling (randomly permuting the coordinates of the scores vecto
consistency_score_p_value
                               number of permutations
      consistency_score_n
                        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) /</pre>
                           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)
```

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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.

54 score\_transcripts

#### 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
the relative, i.e., absolute divided by total, frequency of binding sites per motif in all transcripts
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(</pre>
  "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(</pre>
  "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")</pre>
scores <- score_transcripts(foreground_set, motifs = motifs, cache = FALSE)</pre>
## Not run:
# all Transite motifs, cached (writes scores to disk)
scores <- score_transcripts(foreground_set)</pre>
# all Transite motifs, uncached
scores <- score_transcripts(foreground_set, cache = FALSE)</pre>
```

```
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)</pre>
```

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)

set\_motifs

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 total_sites total_sites

one_hit, two_hits, ... the motif identifier of the specified motif

the gene symbol of the RNA-binding protein(s)

the absolute frequency of binding sites per motif in all transcripts

the total number of potential binding sites

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_meta_info(), get_motifs(), get_ppm(), init_iupac_lookup_table()
```

SpectrumScore-class 57

## **Examples**

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

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'
```

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```
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

SpectrumScore object object SpectrumScore object Χ

#### 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")
```

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```
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.

meaningful meas

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

60 toy\_motif\_matrix

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

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 61

# 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

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