

Package ‘spatzie’

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Type Package

Title Identification of enriched motif pairs from chromatin interaction data

Version 1.3.0

Description Identifies motifs that are significantly co-enriched from enhancer-promoter interaction data. While enhancer-promoter annotation is commonly used to define groups of interaction anchors, spatzie also supports co-enrichment analysis between preprocessed interaction anchors. Supports BEDPE interaction data derived from genome-wide assays such as HiC, ChIA-PET, and HiChIP. Can also be used to look for differentially enriched motif pairs between two interaction experiments.

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URL <https://spatzie.mit.edu>

Depends R (>= 4.1)

Imports BiocGenerics, BSgenome, GenomeInfoDb, GenomicFeatures, GenomicInteractions, GenomicRanges, ggplot2, IRanges, matrixStats, motifmatchr, S4Vectors, stats, SummarizedExperiment, TFBSTools, utils

Suggests BiocManager, Biostrings, knitr, pheatmap, rmarkdown, testthat, TxDb.Hsapiens.UCSC.hg19.knownGene, TxDb.Hsapiens.UCSC.hg38.knownGene, TxDb.Mmusculus.UCSC.mm10.knownGene, TxDb.Mmusculus.UCSC.mm9.knownGene

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R topics documented:

anchor_pair_enrich	2
anchor_pair_example_count	5
anchor_pair_example_match	5
anchor_pair_example_score	6
compare_motif_pairs	6
compare_pairs_example	7
filter_motifs	7
filter_pairs_example	8
filter_pair_motifs	9
find_ep_coenrichment	10
get_specific_interactions	12
interactions_yy1	13
interactions_yy1_enhancer	14
interactions_yy1_ep	14
interactions_yy1_promoter	15
int_data_k562	15
int_data_mslcl	16
int_data_yy1	16
plot_motif_occurrence	17
scan_interactions_example	18
scan_interactions_example_filtered	18
scan_motifs	19
spatzie	20

Index	21
--------------	-----------

anchor_pair_enrich	<i>Determine enriched motifs in anchors</i>
--------------------	---

Description

Determine whether motifs between paired bed regions have a statistically significant relationship. Options for significance are motif score correlation, motif count correlation, or hypergeometric motif co-occurrence.

Usage

```
anchor_pair_enrich(interaction_data, method = c("count", "score", "match"))
```

Arguments

`interaction_data` an interactionData object of paired genomic regions

`method` method for co-occurrence, valid options include:

`count`: correlation between counts (for each anchor, tally positions where motif score $> 5 * 10^{-5}$)

`score`: correlation between motif scores (for each anchor, use the maximum score over all positions)

`match`: association between motif matches (for each anchor, a match is defined if there is at least one position with a motif score

Value

an interactionData object where `obj$pair_motif_enrich` contains the p-values for significance of seeing a higher co-occurrence than what we get by chance.

Score-based correlation

We assume motif scores follow a normal distribution and are independent between enhancers and promoters. We can therefore compute how correlated scores of any two transcription factor motifs are between enhancer and promoter regions using Pearson's product-moment correlation coefficient:

$$r = \frac{\sum(x'_i - \bar{x}')(y'_i - \bar{y}')}{\sqrt{\sum(x'_i - \bar{x}')^2 \sum(y'_i - \bar{y}')^2}}$$

, where the input vectors \mathbf{x} and \mathbf{y} from above are transformed to vectors \mathbf{x}' and \mathbf{y}' by replacing the set of scores with the maximum score for each region:

$$x'_i = \max x_i$$

x'_i is then the maximum motif score of motif a in the promoter region of interaction i , y'_i is the maximum motif score of motif b in the enhancer region of interaction i , and \bar{x}' and \bar{y}' are the sample means.

Significance is then computed by transforming the correlation coefficient r to test statistic t , which is Student t -distributed with $n - 2$ degrees of freedom.

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

All p-values are calculated as one-tailed p-values of the probability that scores are greater than or equal to r .

Count-based correlation

Instead of calculating the correlation of motif scores directly, the count-based correlation metric first tallies the number of instances of a given motif within an enhancer or a promoter region, which are defined as all positions in those regions with motif score p-values of less than $5 * 10^{-5}$. Formally,

the input vectors \mathbf{x} and \mathbf{y} are transformed to vectors \mathbf{x}'' and \mathbf{y}'' by replacing the set of scores with the cardinality of the set:

$$x_i'' = |x_i|$$

And analogous for y_i'' . Finally, the correlation coefficient r between \mathbf{x}'' and \mathbf{y}'' and its associated significance are calculated as described above.

Match-based association

Instance co-occurrence uses the presence or absence of a motif within an enhancer or promoter to determine a statistically significant association, thus \mathbf{x}''' and \mathbf{y}''' are defined by:

$$x_i''' = \mathbf{1}_{x_i'' > 0}$$

Instance co-occurrence is computed using the hypergeometric test:

$$p = \sum_{k=I_{ab}}^{P_a} \frac{\text{binom}(P_a, k) \text{binom}(n - P_a, E_b - k)}{\text{binom}(n, E_b)},$$

where I_{ab} is the number of interactions that contain a match for motif a in the promoter and motif b in the enhancer, P_a is the number of promoters that contain motif a ($P_a = \sum_i^n x_i'''$), E_b is the number of enhancers that contain motif b ($E_b = \sum_i^n y_i'''$), and n is the total number of interactions, which is equal to the number of promoters and to the number of enhancers.

Author(s)

Jennifer Hammelman
Konstantin Krismer

Examples

```
## Not run:
genome_id <- "BSgenome.Mmusculus.UCSC.mm9"
if (!(genome_id %in% rownames(utils::installed.packages()))) {
  BiocManager::install(genome_id, update = FALSE, ask = FALSE)
}
genome <- BSgenome::getBSgenome(genome_id)

motifs_file <- system.file("extdata/motifs_subset.txt.gz",
                           package = "spatzie")
motifs <- TFBSTools::readJASPARMatrix(motifs_file, matrixClass = "PFM")

yy1_pd_interaction <- scan_motifs(spatzie::interactions_yy1, motifs, genome)
yy1_pd_interaction <- filter_motifs(yy1_pd_interaction, 0.4)
yy1_pd_count_corr <- anchor_pair_enrich(yy1_pd_interaction, method = "count")

## End(Not run)

res <- anchor_pair_enrich(spatzie::scan_interactions_example_filtered,
                         method = "score")
```

anchor_pair_example_count
spatzie count correlation data set

Description

This object contains genomic interactions obtained by mouse YY1 ChIA-PET scanned for mouse transcription factor motifs, filtered for motifs present in at least 10 interactions with count correlation. It serves as unit test data.

Usage

```
data(anchor_pair_example_count)
```

Format

An interactionData object

anchor_pair_example_match
spatzie match association data set

Description

This object contains genomic interactions obtained by mouse YY1 ChIA-PET scanned for mouse transcription factor motifs, filtered for motifs present in at least 10 interactions with using the hypergeometric test. It serves as unit test data.

Usage

```
data(anchor_pair_example_match)
```

Format

A interactionData object

anchor_pair_example_score
spatzie score correlation data set

Description

This object contains genomic interactions obtained by mouse YY1 ChIA-PET scanned for mouse transcription factor motifs, filtered for motifs present in at least 10 interactions with score correlation. It serves as unit test data.

Usage

```
data(anchor_pair_example_score)
```

Format

An interactionData object

compare_motif_pairs *Compare pairs of motifs between two interaction datasets*

Description

Compute the log-likelihood ratio that a motif pair is differential between two interaction datasets. Note that motif pair significance should have been computed using the same method for both datasets.

Usage

```
compare_motif_pairs(
  interaction_data1,
  interaction_data2,
  differential_p = 0.05
)
```

Arguments

interaction_data1
an interactionData object of paired genomic regions that has been scanned for significant motif:motif interactions

interaction_data2
an interactionData object of paired genomic regions that has been scanned for significant motif:motif interactions

differential_p threshold for significance of differential p-value

Value

a matrix of the log likelihood ratio of motif pairs that are significantly differential between two interactionData sets

Author(s)

Jennifer Hammelman

Examples

```
pheatmap::pheatmap(compare_motif_pairs(spatzie::int_data_k562,
                                       spatzie::int_data_mslcl, 5e-06),
                    fontsize = 6)
```

compare_pairs_example *compare_motif_pairs example*

Description

This is a matrix containing example result from compare_motif_pairs. It serves as unit test data.

Usage

```
data(compare_pairs_example)
```

Format

A matrix

filter_motifs *Filter motifs based on occurrence within interaction data*

Description

Select a subset of motifs that are in at least a threshold fraction of regions. Motif subsets are selected separately for anchor one and anchor two regions.

Usage

```
filter_motifs(interaction_data, threshold)
```

Arguments

interaction_data
 an interactionData object of paired genomic regions

threshold
 fraction of interactions that should contain a motif for a motif to be considered

Value

an interactionData object where obj\$anchor1_motif_indices and obj\$anchor2_motif_indices have been filtered to motifs that are present in a threshold fraction of interactions

Author(s)

Jennifer Hammelman

Examples

```
## Not run:
genome_id <- "BSgenome.Mmusculus.UCSC.mm9"
if (!(genome_id %in% rownames(utils::installed.packages()))) {
  BiocManager::install(genome_id, update = FALSE, ask = FALSE)
}
genome <- BSgenome::getBSgenome(genome_id)

motifs_file <- system.file("extdata/motifs_subset.txt.gz",
                           package = "spatzie")
motifs <- TFBSTools::readJASPARMatrix(motifs_file, matrixClass = "PFM")

yy1_pd_interaction <- scan_motifs(spatzie::interactions_yy1, motifs, genome)
yy1_pd_interaction <- filter_motifs(yy1_pd_interaction, 0.4)

## End(Not run)

res <- filter_motifs(spatzie::scan_interactions_example, threshold = 0.1)
```

filter_pairs_example *spatzie score correlation filtered data set*

Description

This object contains genomic interactions obtained by mouse YY1 ChIA-PET scanned for mouse transcription factor motifs, filtered for motifs present in at least 10 interactions with score correlation, and filtered for pairs with $p < 0.5$. It serves as unit test data.

Usage

```
data(filter_pairs_example)
```

Format

An interactionData object

filter_pair_motifs	<i>Filter significant motif interactions</i>
--------------------	--

Description

Multiple hypothesis correction applied to filter for significant motif interactions.

Usage

```
filter_pair_motifs(interaction_data, method = "fdr", threshold = 0.05)
```

Arguments

interaction_data	an interactionData object of paired genomic regions
method	statistical method for multiple hypothesis correction, defaults to Benjamini-Hochberg ("fdr") (see p.adjust for options)
threshold	p-value threshold for significance cut-off

Value

an interactionData object where `obj$pair_motif_enrich` contains multiple hypothesis corrected p-values for significance of seeing a higher co-occurrence than what we get by chance and `obj$pair_motif_enrich_sig` contains only motifs that have at least one significant interaction.

Author(s)

Jennifer Hammelman

Examples

```
## Not run:
genome_id <- "BSgenome.Mmusculus.UCSC.mm9"
if (!(genome_id %in% rownames(utils::installed.packages()))) {
  BiocManager::install(genome_id, update = FALSE, ask = FALSE)
}
genome <- BSgenome::getBSgenome(genome_id)

motifs_file <- system.file("extdata/motifs_subset.txt.gz",
                           package = "spatzie")
motifs <- TFBSTools::readJASPARMatrix(motifs_file, matrixClass = "PFM")

yy1_pd_interaction <- scan_motifs(spatzie::interactions_yy1, motifs, genome)
yy1_pd_interaction <- filter_motifs(yy1_pd_interaction, 0.4)
yy1_pd_score_corr <- anchor_pair_enrich(yy1_pd_interaction, method = "score")
yy1_pd_score_corr_adj <- filter_pair_motifs(yy1_pd_score_corr)

## End(Not run)
```

```
res <- filter_pair_motifs(spatzie::anchor_pair_example_count,
                        threshold = 0.5)
```

find_ep_coenrichment *Find co-enriched motif pairs in enhancer-promoter interactions*

Description

Identifies co-enriched pairs of motifs in enhancer-promoter interactions selected from a data frame of general genomic interactions.

If `identify_ep`: Promoters and enhancers are identified using genomic annotations, where anchors close to promoter annotations (within 2500 base pairs) are considered promoters and all other anchors are considered gene-distal enhancers. Only interactions in `int_raw_data` between promoters and enhancers are used for motif co-enrichment analysis.

If `!identify_ep`: Instead of automatically identifying promoters and enhancers based on genomic annotations, all interactions in `int_raw_data` must be preprocessed in a way that anchor 1 contains promoters and anchor 2 contains enhancers. Motif co-enrichment analysis is performed under this assumption.

Calls functions `scan_motifs`, `filter_motifs`, and `anchor_pair_enrich` internally.

Usage

```
find_ep_coenrichment(
  int_raw_data,
  motifs_file,
  motifs_file_matrix_format = c("pfm", "ppm", "pwm"),
  genome_id = c("hg38", "hg19", "mm9", "mm10"),
  identify_ep = TRUE,
  cooccurrence_method = c("count", "score", "match"),
  filter_threshold = 0.4
)
```

Arguments

`int_raw_data` a `GenomicInteractions` object or a data frame with at least six columns:

- column 1: character; genomic location of interaction anchor 1 - chromosome (e.g., "chr3")
- column 2: integer; genomic location of interaction anchor 1 - start coordinate
- column 3: integer; genomic location of interaction anchor 1 - end coordinate
- column 4: character; genomic location of interaction anchor 2 - chromosome (e.g., "chr3")
- column 5: integer; genomic location of interaction anchor 2 - start coordinate
- column 6: integer; genomic location of interaction anchor 2 - end coordinate

motifs_file **JASPAR format** matrix file containing multiple motifs to scan for, gz-zipped files allowed
motifs_file_matrix_format
 type of position-specific scoring matrices in `motifs_file`, valid options include:

pfm: position frequency matrix, elements are absolute frequencies, i.e., counts (default)
ppm: position probability matrix, elements are probabilities, i.e., Laplace smoothing corrected relative frequencies
pwm: position weight matrix, elements are log likelihoods

genome_id ID of genome assembly interactions in `int_raw_data` were aligned to, valid options include hg19, hg38, mm9, and mm10, defaults to hg38
identify_ep logical, set FALSE if enhancers and promoters should not be identified based on genomic annotations, but instead assumes anchor 1 contains promoters and anchor 2 contains enhancers, for all interactions in `int_raw_data`, defaults to TRUE, i.e., do identify enhancers and promoters of interactions in `int_raw_data` based on genomic interactions and filter all interactions which are not between promoters and enhancers
cooccurrence_method
 method for co-occurrence, valid options include:

count: correlation between counts (for each anchor, tally positions where motif score $> 5 * 10^{-5}$)
score: correlation between motif scores (for each anchor, use the maximum score over all positions)
match: association between motif matches (for each anchor, a match is defined if there is at least one position with a motif score)

 See [anchor_pair_enrich](#) for details.

filter_threshold
 fraction of interactions that should contain a motif for a motif to be considered, see [filter_motifs](#), defaults to 0.4

Value

a list with the following items:

int_data [GenomicInteractions](#) object; promoter-enhancer interactions
int_data_motifs: interactionData object; return value of [scan_motifs](#)
filtered_int_data_motifs: interactionData object; return value of [filter_motifs](#)
annotation_pie_chart: ggplot2 plot; return value of [plotInteractionAnnotations](#)
motif_cooccurrence: interactionData object; return value of [anchor_pair_enrich](#)

Author(s)

Jennifer Hammelman
 Konstantin Krismer

Examples

```
## Not run:
```

```
interactions_file <- system.file("extdata/yy1_interactions.bedpe.gz",
                                package = "spatzie")
motifs_file <- system.file("extdata/motifs_subset.txt.gz",
                           package = "spatzie")

df <- read.table(gzfile(interactions_file), header = TRUE, sep = "\t")
res <- find_ep_coenrichment(df, motifs_file,
                           motifs_file_matrix_format = "pfm",
                           genome_id = "mm10")

## End(Not run)
```

get_specific_interactions

Get interactions that contain a specific motif pair

Description

Select interactions that contain anchor1_motif within anchor 1 and anchor2_motif within anchor 2.

Usage

```
get_specific_interactions(
  interaction_data,
  anchor1_motif = NULL,
  anchor2_motif = NULL
)
```

Arguments

interaction_data
an interactionData object of paired genomic regions

anchor1_motif Motif name from interactionData\$anchor1_motifs

anchor2_motif Motif name from interactionData\$anchor2_motifs

Value

a [GenomicInteractions](#) object containing a subset subset of interactions that contain an instance of anchor1_motif in anchor 1 and anchor2_motif in anchor 2

Author(s)

Jennifer Hammelman

Examples

```
## Not run:
genome_id <- "BSgenome.Mmusculus.UCSC.mm9"
if (!(genome_id %in% rownames(utils::installed.packages()))) {
  BiocManager::install(genome_id, update = FALSE, ask = FALSE)
}
genome <- BSgenome::getBSgenome(genome_id)

motifs_file <- system.file("extdata/motifs_subset.txt.gz",
                           package = "spatzie")
motifs <- TFBSTools::readJASPARMatrix(motifs_file, matrixClass = "PFM")

yy1_pd_interaction <- scan_motifs(spatzie::interactions_yy1, motifs, genome)
yy1_pd_interaction <- filter_motifs(yy1_pd_interaction, 0.4)
yy1_pd_count_corr <- anchor_pair_enrich(yy1_pd_interaction,
                                       method = "score")
yy1_yy1_interactions <- get_specific_interactions(
  yy1_pd_interaction,
  anchor1_motif = "YY1",
  anchor2_motif = "YY1")

## End(Not run)

res <- get_specific_interactions(spatzie::int_data_yy1,
                                anchor1_motif = "YY1",
                                anchor2_motif = "YY1")
```

interactions_yy1

Mouse YY1 Enhancer - Promoter Interactions Data Set

Description

This object contains genomic interactions obtained by mouse YY1 ChIA-PET and serves as example and unit test data. The same data set is used in the vignette.

Usage

```
data(interactions_yy1)
```

Format

A [GenomicInteractions](#) object

interactions_yy1_enhancer

Mouse YY1 Enhancer - Promoter Interactions Data Set - YY1 enhancers

Description

This is a [GenomicInteractions](#) object containing processed results from YY1 ChIA-PET of interactions that contain a YY1 motif in the enhancer (anchor 2) region. It serves as unit test data.

Usage

```
data(interactions_yy1_enhancer)
```

Format

A [GenomicInteractions](#) object

interactions_yy1_ep

Mouse YY1 Enhancer - Promoter Interactions Data Set - YY1 enhancers/promoters

Description

This is a [GenomicInteractions](#) object containing processed results from YY1 ChIA-PET of interactions that contain a YY1 motif in the promoter (anchor 1) region and a YY1 motif in the enhancer (anchor 2) region. It serves as unit test data.

Usage

```
data(interactions_yy1_ep)
```

Format

A [GenomicInteractions](#) object

`interactions_yy1_promoter`*Mouse YY1 Enhancer - Promoter Interactions Data Set - YY1 promoters*

Description

This is a [GenomicInteractions](#) object containing processed results from YY1 ChIA-PET of interactions that contain a YY1 motif in the promoter (anchor 1) region. It serves as unit test data.

Usage

```
data(interactions_yy1_promoter)
```

Format

A [GenomicInteractions](#) object

`int_data_k562`*K562 Enhancer - Promoter Interactions Data Set*

Description

This object contains genomic interactions obtained by human RAD21 ChIA-PET from K562 cells and serves as unit test data.

Usage

```
data(int_data_k562)
```

Format

An `interactionData` object

int_data_mslcl	<i>MSLCL Enhancer - Promoter Interactions Data Set</i>
----------------	--

Description

This object contains genomic interactions obtained by human RAD21 ChIA-PET from MSLCL cells and serves as unit test data.

Usage

```
data(int_data_mslcl)
```

Format

An interactionData object

int_data_yy1	<i>Mouse YY1 Enhancer - Promoter Interactions Data Set</i>
--------------	--

Description

This object contains genomic interactions obtained by mouse YY1 ChIA-PET and serves as example and unit test data.

Usage

```
data(int_data_yy1)
```

Format

An interactionData object

plot_motif_occurrence *Plot motif occurrence*

Description

Plots a histogram of motif values (either counts, instances, or scores) for anchor 1 and anchor 2 regions.

Usage

```
plot_motif_occurrence(  
  interaction_data,  
  method = c("counts", "instances", "scores")  
)
```

Arguments

interaction_data	an interactionData object of paired genomic regions
method	way to interpret motif matching for each anchor region as "counts" number of motifs per region, "instances" motif present or absent each region, or "scores" maximum motif PWM match score for each region

Value

plot containing histogram for each anchor

Author(s)

Jennifer Hammelman

Examples

```
## Not run:  
genome_id <- "BSgenome.Mmusculus.UCSC.mm9"  
if (!(genome_id %in% rownames(utils::installed.packages()))) {  
  BiocManager::install(genome_id, update = FALSE, ask = FALSE)  
}  
genome <- BSgenome::getBSgenome(genome_id)  
  
motifs_file <- system.file("extdata/motifs_subset.txt.gz",  
  package = "spatzie")  
motifs <- TFBSTools::readJASPARMatrix(motifs_file, matrixClass = "PFM")  
  
yy1_pd_interaction <- scan_motifs(spatzie::interactions_yy1, motifs, genome)  
yy1_pd_interaction <- filter_motifs(yy1_pd_interaction, 0.4)  
plot_motif_occurrence(yy1_pd_interaction, "counts")  
  
## End(Not run)
```

```
plot_motif_occurrence(spatzie::anchor_pair_example_score)
```

```
scan_interactions_example
```

Interactions scanned for motifs - interactionData object

Description

This object contains genomic interactions obtained by mouse YY1 ChIA-PET scanned for mouse transcription factor motifs and serves as unit test data.

Usage

```
data(scan_interactions_example)
```

Format

An interactionData object

```
scan_interactions_example_filtered
```

Interactions with motifs filtered for significance - interactionData object

Description

This object contains genomic interactions obtained by mouse YY1 ChIA-PET scanned for mouse transcription factor motifs and filtered for motifs present in at least 10

Usage

```
data(scan_interactions_example_filtered)
```

Format

An interactionData object

scan_motifs	<i>Scans interaction file for motif instances</i>
-------------	---

Description

Uses motifmatchR to scan interaction regions for given motifs.

Usage

```
scan_motifs(int_data, motifs, genome)
```

Arguments

int_data	a GenomicInteractions object of paired genomic regions
motifs	a TFBS tools matrix of DNA binding motifs
genome	BSgenome object or DNASTringSet object, must match chromosomes from interaction data file

Value

an interaction data object where obj\$anchor1_motifs and obj\$anchor2_motifs contain information about the scores and matches to motifs from anchor one and anchor two of interaction data genomic regions

Author(s)

Jennifer Hammelman

Examples

```
## Not run:
genome_id <- "BSgenome.Mmusculus.UCSC.mm9"
if (!(genome_id %in% rownames(utils::installed.packages()))) {
  BiocManager::install(genome_id, update = FALSE, ask = FALSE)
}
genome <- BSgenome::getBSgenome(genome_id)

motifs_file <- system.file("extdata/motifs_subset.txt.gz",
  package = "spatzie")
motifs <- TFBSTools::readJASPARMatrix(motifs_file, matrixClass = "PFM")

yy1_pd_interaction <- scan_motifs(spatzie::interactions_yy1, motifs, genome)

## End(Not run)

motifs_file <- system.file("extdata/motifs_subset.txt.gz",
  package = "spatzie")
motifs <- TFBSTools::readJASPARMatrix(motifs_file, matrixClass = "PFM")
```

```
left <- GenomicRanges::GRanges(  
  seqnames = c("chr1", "chr1", "chr1"),  
  ranges = IRanges::IRanges(start = c(1, 15, 20),  
                             end = c(10, 35, 31))  
right <- GenomicRanges::GRanges(  
  seqnames = c("chr1", "chr2", "chr2"),  
  ranges = IRanges::IRanges(start = c(17, 47, 41),  
                             end = c(28, 54, 53))  
test_interactions <- GenomicInteractions::GenomicInteractions(left, right)  
  
# toy DNASTringSet to replace BSgenome object  
seqs <- c("chr1" = "CCACTAGCCACGCGTCACTGGTTAGCGTGATTGAACTAAATCGTATGAAAATCC",  
          "chr2" = "CTACAAACTAGGAATTTAGGCAAACCTGTGTTAAAATCTTAGCTCATTCAAT")  
toy_genome <- Biostrings::DNASTringSet(seqs, use.names = TRUE)  
  
res <- scan_motifs(test_interactions, motifs, toy_genome)
```

spatzie

spatzie

Description

Looks for motifs which are significantly co-enriched from enhancer-promoter interaction data, derived from assays such as as HiC, ChIA-PET, etc. It can also look for differentially enriched motif pairs between to interaction experiments.

Author(s)

Jennifer Hammelman

Konstantin Krismer

Index

* datasets

- anchor_pair_example_count, 5
 - anchor_pair_example_match, 5
 - anchor_pair_example_score, 6
 - compare_pairs_example, 7
 - filter_pairs_example, 8
 - int_data_k562, 15
 - int_data_mslcl, 16
 - int_data_yy1, 16
 - interactions_yy1, 13
 - interactions_yy1_enhancer, 14
 - interactions_yy1_ep, 14
 - interactions_yy1_promoter, 15
 - scan_interactions_example, 18
 - scan_interactions_example_filtered, 18
- anchor_pair_enrich, 2, 10, 11
- anchor_pair_example_count, 5
- anchor_pair_example_match, 5
- anchor_pair_example_score, 6
- compare_motif_pairs, 6
- compare_pairs_example, 7
- filter_motifs, 7, 10, 11
- filter_pair_motifs, 9
- filter_pairs_example, 8
- find_ep_coenrichment, 10
- GenomicInteractions, 10–15, 19
- get_specific_interactions, 12
- int_data_k562, 15
- int_data_mslcl, 16
- int_data_yy1, 16
- interactions_yy1, 13
- interactions_yy1_enhancer, 14
- interactions_yy1_ep, 14
- interactions_yy1_promoter, 15
- p.adjust, 9
- plot_motif_occurrence, 17
- plotInteractionAnnotations, 11
- scan_interactions_example, 18
- scan_interactions_example_filtered, 18
- scan_motifs, 10, 11, 19
- spatzie, 20