

# Package ‘motifTestR’

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**Title** Perform key tests for binding motifs in sequence data

**Version** 1.0.3

**Description** Taking a set of sequence motifs as PWMs, test a set of sequences for over-representation of these motifs, as well as any positional features within the set of motifs. Enrichment analysis can be undertaken using multiple statistical approaches. The package also contains core functions to prepare data for analysis, and to visualise results.

**License** GPL-3

**Encoding** UTF-8

**URL** <https://github.com/smped/motifTestR>

**BugReports** <https://github.com/smped/motifTestR/issues>

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**Imports** GenomeInfoDb, harmonicmeanp, IRanges, matrixStats, methods, parallel, patchwork, rlang, S4Vectors, stats, universalmotif

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## Contents

motifTestR-package . . . . .	2
ar_er_peaks . . . . .	3
ar_er_seq . . . . .	4
countPwmMatches . . . . .	4
ex_pwm . . . . .	5
getPwmMatches . . . . .	6
hg19_mask . . . . .	7
makeRMRanges . . . . .	8
plotMatchPos . . . . .	10
testMotifEnrich . . . . .	12
testMotifPos . . . . .	14
zr75_enh . . . . .	16

**Index** **18**

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motifTestR-package	<i>motifTestR: Perform Key Analyses on Transcription Factor Binding Motifs</i>
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## Description

The package motifTestR has been designed for two primary analyses of TFBMs, testing for positional bias and overall enrichment.

## Details

The package motifTestR provides two primary functions for testing TFBMs within a set of sequences

- `testMotifPos()` for detecting positional bias within a set of test sequences
- `testMotifEnrich()` for testing overall enrichment of a TFBM within a set of test sequences

The main functions rely on lower-level functions such as:

- `countPwmMatches()` simply counts the number of matches within an XStringSet
- `getPwmMatches()` returns the position of matches within an XStringSet
- `makeRMRanges()` which produces a set of random, matching ranges based on key characteristics of the set of test sequences/ranges

A simple utility function is provided to enable visualisation of results

- `plotMatchPos()` enables visualisation of the matches within a set of sequences using multiple strategies

**Author(s)**

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**See Also**

Useful links:

- <https://github.com/smped/motifTestR>
- Report bugs at <https://github.com/smped/motifTestR/issues>

---

ar\_er\_peaks

*A set of peaks with AR and ER detected*

---

**Description**

A set of ChIP-Seq peaks where AR and ER were both detected

**Usage**

```
data("ar_er_peaks")
```

**Format**

An object of class GRanges of length 229.

**Details**

The subset of peaks found on chr1, and which contained signal from AR and ER, along with H3K27ac signal were taken from GSE123767. Peaks were resized to a uniform width of 400bp after downloading

Generation of these ranges is documented in `system.file("scripts/ar_er_peaks.R", package = "motifTestR")`

**Source**

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE123767>

**Examples**

```
data("ar_er_peaks")
ar_er_peaks
```

---

ar_er_seq	<i>Sequences from peaks with AR and ER detected</i>
-----------	---

---

**Description**

The genomic sequences obtained from the ar\_er\_peaks

**Usage**

```
data("ar_er_seq")
```

**Format**

An object of class DNASTringSet of length 229.

**Details**

These sequences represent the sequences obtained from BSgenome.Hsapiens.UCSC.hg19 for the peaks supplied as ar\_er\_peaks

Generation of these sequences is documented in `system.file("scripts/ar_er_peaks.R", package = "motifTestR")`

**Examples**

```
data("ar_er_seq")
ar_er_seq
```

---

countPwmMatches	<i>Count the matches to a PWM within an XStringSet</i>
-----------------	--

---

**Description**

Count the matches to a PWM within an XStringSet

**Usage**

```
countPwmMatches(  
  pwm,  
  stringset,  
  rc = TRUE,  
  min_score = "80%",  
  mc.cores = 1,  
  ...  
)
```

**Arguments**

pwm	A Position Weight Matrix
stringset	An XStringSet
rc	logical(1) Also find matches using the reverse complement of pwm
min_score	The minimum score to return a match
mc.cores	Passed to <a href="#">mclapply</a> when analysing a list of PWMs
...	Passed to <a href="#">countPWM</a>

**Details**

Will simply count the matches within an XStringSet and return an integer. All matches are included.

**Value**

An integer vector

**Examples**

```
## Load the example PWM
data("ex_pwm")
esr1 <- ex_pwm$ESR1

## Load the example Peaks
data("ar_er_seq")
countPwmMatches(esr1, ar_er_seq)

## Count all PWMs
countPwmMatches(ex_pwm, ar_er_seq)
```

---

ex\_pwm

*Example Position Weight Matrices*

---

**Description**

Example Position Weight Matrices

**Usage**

```
data("ex_pwm")
```

**Format**

An object of class `list` of length 5.

**Details**

This object contains 5 PWMs taken from HOCOMOCOv11-coreA for examples and testing

Generation of this motif list is documented in `system.file("scripts/ex_pwm.R", package = "motifTestR")`

**Examples**

```
data("ex_pwm")
ex_pwm$ESR1
```

---

getPwmMatches	<i>Find all PWM matches within an XStringSet</i>
---------------	--

---

**Description**

Find all PWM matches within a set of sequences

**Usage**

```
getPwmMatches(
  pwm,
  stringset,
  rc = TRUE,
  min_score = "80%",
  best_only = FALSE,
  break_ties = c("all", "random", "first", "last", "central"),
  mc.cores = 1,
  ...
)
```

**Arguments**

pwm	A Position Weight Matrix, list of PWMs or universal motif list
stringset	An XStringSet
rc	logical(1) Also find matches using the reverse complement of pwm
min_score	The minimum score to return a match
best_only	logical(1) Only return the best match
break_ties	Method for breaking ties when only returning the best match Ignored when all matches are returned (the default)
mc.cores	Passed to <a href="#">mclapply</a> if passing multiple PWMs
...	Passed to <a href="#">matchPWM</a>

## Details

Taking a set of sequences as an `XStringSet`, find all matches above the supplied score (i.e. threshold) for a single Position Weight Matrix (PWM), generally representing a transcription factor binding motif. By default, matches are performed using the PWM as provided and the reverse complement, however this can easily be disabled by setting `rc = FALSE`.

The function relies heavily on `matchPWM` and `Views` for speed.

When choosing to return the best match (`best_only = TRUE`), only the match with the highest score is returned for each sequence. Should there be tied scores, the best match can be chosen as either the first, last, most central, all tied matches, or choosing one at random (the default).

## Value

A `DataFrame` with columns: `seq`, `score`, `direction`, `start`, `end`, `fromCentre`, `seq_width`, and `match`

The first three columns describe the sequence with matches, the score of the match and whether the match was found using the forward or reverse PWM. The columns `start`, `end` and `width` describe the where the match was found in the sequence, whilst `from_centre` defines the distance between the centre of the match and the centre of the sequence being queried. The final column contains the matching fragment of the sequence as an `XStringSet`.

When passing a list of PWMs, a list of the above `DataFrames` will be returned.

## Examples

```
## Load the example PWM
data("ex_pwm")
esr1 <- ex_pwm$ESR1

## Load the example Peaks
data("ar_er_seq")

## Return all matches
getPwmMatches(esr1, ar_er_seq)

## Just the best match
getPwmMatches(esr1, ar_er_seq, best_only = TRUE)

## Apply multiple PWMs as a list
getPwmMatches(ex_pwm, ar_er_seq, best_only = TRUE)
```

---

hg19\_mask

*Regions from hg19 with high N content*

---

## Description

A `GRanges` object with regions annotated as telomeres or centromeres

**Usage**

```
data("hg19_mask")
```

**Format**

An object of class GRanges of length 345.

**Details**

The regions defined as centromeres or telomeres in hg19, taken from AnnotationHub objects "AH107360" and "AH107361". These were combined with regions containing Ns from the UCSC 2bit file, and regions with Ns in the BSgenome.Hsapiens.UCSC.hg19 were retained.

Generation of these ranges is documented in `system.file("scripts/hg19_mask.R", package = "motifTestR")`

**Source**

The package AnnotationHub and <https://hgdownload.cse.ucsc.edu/goldenpath/hg19/bigZips/hg19.fa.masked.gz>

**Examples**

```
data("hg19_mask")
hg19_mask
```

---

makeRMRanges	<i>Form a set of random, matching ranges for bootstrapping or permuting</i>
--------------	---

---

**Description**

Form a set of ranges from y which (near) exactly match those in x for use as a background set requiring matching

**Usage**

```
makeRMRanges(x, y, ...)

## S4 method for signature 'GRanges,GRanges'
makeRMRanges(
  x,
  y,
  exclude = GRanges(),
  n_iter = 1,
  n_total = NULL,
  replace = TRUE,
  ...,
```



```

    force_ol = TRUE
  )

## S4 method for signature 'GRangesList,GRangesList'
makeRMRanges(
  x,
  y,
  exclude = GRanges(),
  n_iter = 1,
  n_total = NULL,
  replace = TRUE,
  mc.cores = 1,
  ...,
  force_ol = TRUE,
  unlist = TRUE
)

```

### Arguments

x	GRanges/GRangesList with ranges to be matched
y	GRanges/GRangesList with ranges to select random matching ranges from
...	Not used
exclude	GRanges of ranges to omit from testing
n_iter	The number of times to repeat the random selection process
n_total	Setting this value will over-ride anything set using n_iter. Can be vector of any length, corresponding to the length of x, when x is a GRangesList
replace	logical(1) Sample with or without replacement when creating the set of random ranges.
force_ol	logical(1) Enforce an overlap between every site in x and y
mc.cores	Passed to <a href="#">mclapply</a>
unlist	logical(1) Return as a sorted GRanges object, or leave as a GRangesList

### Details

This function uses the width distribution of the 'test' ranges (i.e. x) to randomly sample a set of ranges with matching width from the ranges provided in y. The width distribution will clearly be exact when a set of fixed-width ranges is passed to x, whilst random sampling may yield some variability when matching ranges of variable width.

When both x and y are GRanges objects, they are implicitly assumed to both represent similar ranges, such as those overlapping a promoter or enhancer. When passing two GRangesList objects, both objects are expected to contain ranges annotated as belonging to key features, such that the list elements in y must encompass all elements in x. For example if x contains two elements named 'promoter' and 'intron', y should also contain elements named 'promoter' and 'intron' and these will be sampled as matching ranges for the same element in x. If elements of x and y are not named, they are assumed to be in matching order.

The default behaviour is to assume that randomly-generated ranges are for iteration, and as such, ranges are randomly formed in multiples of the number of 'test' ranges provided in x. The column iteration will be added to the returned ranges. Placing any number into the n\_total argument will instead select a total number of ranges as specified here. In this case, no iteration column will be included in the returned ranges.

Sampling is assumed to be with replacement as this is most suitable for bootstrapping and related procedures, although this can be disabled by setting replace = FALSE

### Value

A GRanges or GRangesList object

### Examples

```
## Load the example peaks
data("ar_er_peaks")
sq <- seqinfo(ar_er_peaks)
## Now sample size-matched ranges for two iterations from chr1
makeRMRanges(ar_er_peaks, GRanges(sq)[1], n_iter = 2)

## Or simply sample 100 ranges if not planning any iterative analyses
makeRMRanges(ar_er_peaks, GRanges(sq)[1], n_total = 100)
```

---

plotMatchPos

*Plot Motif Match Positions*

---

### Description

Plot the distribution of motif matches across sequences

### Usage

```
plotMatchPos(
  matches,
  binwidth = 10,
  abs = FALSE,
  use_totals = FALSE,
  type = c("density", "cdf", "heatmap"),
  geom = c("smooth", "line", "point", "col"),
  cluster = FALSE,
  w = 0.1,
  heat_fill = NULL,
  ...
)
```

## Arguments

matches	Output from <a href="#">getPwmMatches</a>
binwidth	Width of bins to use when plotting
abs	logical(1) Plot absolute distances from centre
use_totals	logical(1). If TRUE, plots will use total counts. The default (FALSE) plots probabilities.
type	Plot match density, the CDF or a binned heatmap
geom	Type of geom to be used for line plots. Ignored for heatmaps
cluster	logical(1) Cluster motifs when drawing a heatmap. If TRUE a dendrogram will be added to the LHS of the plot
w	Relative width of the dendrogram on (0, 1)
heat_fill	scale_fill_continuous object for heatmaps. If not provided, scale_fill_viridis_c() will be added to the heatmap.
...	Passed to individual geom* functions

## Details

Multiple options are provided for showing the distribution of PWM matches within a set of sequences, using either the smoothed probability density, the probability CDF or as a heatmap. Distances can be shown as symmetrical around the centre or using absolute distances from the central position within the sequences.

Heatmaps are only enabled for comparisons across multiple PWMs, with optional clustering enabled. If adding a dendrogram for clustering, the returned plot object will be a patchwork object.

## Value

A ggplot2 object

## Examples

```
## Load the example PWM
data("ex_pwm")
esr1 <- ex_pwm$ESR1

## Load the example sequences from the peaks
data("ar_er_seq")

## Just the best match
bm <- getPwmMatches(esr1, ar_er_seq, best_only = TRUE)
plotMatchPos(bm, se = FALSE)

## Matches can also be shown by distance from centre
plotMatchPos(bm, abs = TRUE)

## Cumulative Probability plots are also implemented
plotMatchPos(bm, type = "cdf", geom = "line", colour = "red") +
  geom_abline(intercept = 0.5, slope = 1/400)
```

---

testMotifEnrich	<i>Test motif enrichment using a background set of sequences</i>
-----------------	--

---

### Description

Test for motif enrichment within a set of sequences using a background set to derive a NULL hypothesis

### Usage

```
testMotifEnrich(
  pwm,
  stringset,
  bg,
  var = "iteration",
  model = c("quasipoisson", "hypergeometric", "poisson", "iteration"),
  sort_by = c("p", "none"),
  mc.cores = 1,
  ...
)
```

### Arguments

pwm	A Position Weight Matrix or list of PWMs
stringset	An XStringSet with equal sequence widths
bg	An XStringSet with the same sequence widths as the test XStringset
var	A column in the mcols element of bg, usually denoting an iteration number
model	The model used for analysis
sort_by	Column to sort results by
mc.cores	Passed to <a href="#">mclapply</a>
...	Passed to <a href="#">getPwmMatches</a> or <a href="#">countPwmMatches</a>

### Details

This function offers four alternative models for assessing the enrichment of a motif within a set of sequences, in comparison to a background set of sequences. Selection of the BG sequences plays an important role and, in conjunction with the question being addressed, determines the most appropriate model to use for testing, as described below.

It should also be noted that the larger the BG set of sequences, the larger the computational burden, and results can take far longer to return. For many millions of background sequences, this may run beyond an hour

#### Descriptions of Models and Use Cases:

*Hypergeometric Tests:*

Hypergeometric tests are best suited to the use case where the test set of sequences represents a subset of a larger set, with a specific feature or behaviour, whilst the BG set may be the remainder of the set without that feature. For example, the test set may represent ChIP-Seq binding sites where signal changes in response to treatment, whilst the BG set represents the sites where no changed signal was observed. Testing is one-sided, for enrichment of motifs within the test set.

Due to these relatively smaller sized datasets, setting `model = "hypergeometric"`, will generally return results quickly

*Poisson Tests:*

This approach requires a set of background sequences which should be much larger than the test set of sequences. The parameters for a Poisson model are estimated in a per-sequence manner on the set of BG sequences, and the observed rate of motif-matches within the test set is then tested using `poisson.test`.

This approach assumes that all matches follow a Poisson distribution, which is often true, but data can also be overdispersed. Given that this model can also return results relatively quickly, it is primarily suitable for data exploration, but not for final results.

*Quasi-Poisson Test:*

The quasipoisson model allows for over-dispersion and will return more conservative results than using the standard Poisson model. Under the method currently implemented here, BG sequences should be divided into blocks (i.e. iterations), identical in size to the test set of sequences. Model parameters are estimated per iteration across the BG set of sequences, with the rate of matches in the test being compared against these.

It is expected that the BG set will be matched for the features of interest and chosen using `makeRMRanges` with a large number of iterations, e.g. `n_iter = 1000`. Due to this parameterisation, quasipoisson approaches can be computationally time-consuming, as this is effectively an iterative approach.

*Iteration:*

Setting the model as "iteration" performs a non-parametric analysis, with the exception of returning Z-scores under the Central Limit Theorem. Mean and SD of matches is found for each iteration, and used to return Z scores, with p-values returned from both a Z-test and from comparing observed values directly to sampled values obtained from the BG sequences. Sampled values are calculated directly and as such, are limited in precision.

As for the QuasiPoisson model, a very large number of iterations is expected to be used, to ensure the CLT holds, again making this a computationally demanding test. Each iteration/block is expected to be identically-sized to the test set, and matched for any features as appropriate using `makeRMRanges()`.

**Value**

A data.frame with columns: `sequences`, `matches`, `expected`, `enrichment`, and `p`, with additional columns `Z`, `est_bg_rate` (Poisson), `odds_ratio` (Hypergeometric) or `Z`, `sd_bg`, `n_iter` and `iter_p` (Iterations). The numbers of sequences and matches refer to the test set of sequences, whilst `expected` is the expected number of matches under the Poisson or iterative null distribution. The ratio of matches to expected is given as `enrichment`, along with the Z score and p-value. Whilst the Z score is only representative under the Poisson model, it is used to directly estimate p-values under the iterative approach. Under this latter approach, the sd of the matches found in the background

sequences is also given, along with the number of iterations and the p-values from permutations testing the one-sided hypothesis hypothesis for enrichment.

It may also be worth noting that if producing background sequences using `makeRMRanges` with `replace = TRUE` and `force_ol = TRUE`, the iterative model corresponds to a bootstrap, given that the test sequences will overlap the background sequences and background ranges are able to be sampled with replacement.

### See Also

`makeRMRanges()`, `getPwmMatches()`, `countPwmMatches()`

### Examples

```
## Load the example peaks & the sequences
data("ar_er_peaks")
data("ar_er_seq")
sq <- seqinfo(ar_er_peaks)
## Now sample size-matched ranges 10 times larger. In real-world analyses,
## this set should be sampled as at least 1000x larger, ensuring features
## are matched to your requirements. This example masks regions with known N
## content, including centromeres & telomeres
data("hg19_mask")
set.seed(305)
bg_ranges <- makeRMRanges(
  ar_er_peaks, GRanges(sq)[1], exclude = hg19_mask, n_iter = 10
)

## Convert ranges to DNASTringSets
library(BSgenome.Hsapiens.UCSC.hg19)
genome <- BSgenome.Hsapiens.UCSC.hg19
bg_seq <- getSeq(genome, bg_ranges)

## Test for enrichment of the ESR1 motif
data("ex_pwm")
esr1 <- ex_pwm$ESR1
testMotifEnrich(esr1, ar_er_seq, bg_seq, model = "poisson")

## Test all motifs
testMotifEnrich(ex_pwm, ar_er_seq, bg_seq, model = "poisson")
```

---

testMotifPos

*Test for a Uniform Distribution across a set of best matches*

---

### Description

Test for a Uniform Distribution across a set of best matches

**Usage**

```
testMotifPos(
  x,
  stringset,
  binwidth = 10,
  abs = FALSE,
  rc = TRUE,
  min_score = "80%",
  break_ties = "all",
  alt = c("greater", "less", "two.sided"),
  sort_by = c("p", "none"),
  mc.cores = 1,
  ...
)
```

**Arguments**

x	A Position Weight Matrix, universalmotif object or list thereof. Alternatively can be a single DataFrame or list of DataFrames as returned by <a href="#">getPwmMatches</a> with <code>best_only = TRUE</code>
stringset	An XStringSet. Not required if matches are supplied as x
binwidth	Width of bins across the range to group data into
abs	Use absolute positions around zero to find symmetrical enrichment
rc	logical(1) Also find matches using the reverse complement of pwm
min_score	The minimum score to return a match
break_ties	Choose how to resolve matches with tied scores
alt	Alternative hypothesis for the binomial test
sort_by	Column to sort results by
mc.cores	Passed to <a href="#">mclapply</a>
...	Passed to <a href="#">matchPWM</a>

**Details**

This function tests for an even positional spread of motif matches across a set of sequences, using the assumption (i.e.  $H_0$ ) that if there is no positional bias, matches will be evenly distributed across all positions within a set of sequences. Conversely, if there is positional bias, typically but not necessarily near the centre of a range, this function intends to detect this signal, as a rejection of the null hypothesis.

Input can be provided as the output from [getPwmMatches](#) setting `best_only = TRUE` if these matches have already been identified. If choosing to provide this object to the argument `matches`, nothing is required for the arguments `pwm`, `stringset`, `rc`, `min_score` or `break_ties`. Otherwise, a Position Weight Matrix (PWM) and an XStringSet are required, along with the relevant arguments, with best matches identified within the function.

The set of best matches are then grouped into bins along the range, with the central bin containing zero, and tallied. Setting `abs` to `TRUE` will set all positions from the centre as *absolute values*,

returning counts purely as bins with distances from zero, marking this as an inclusive lower bound. Motif alignments are assigned into bins based on the central position of the match, as provided in the column `from_centre` when calling `getPwmMatches`.

The `binom.test` is performed on each bin using the alternative hypothesis, with the returned p-values across all bins combined using the Harmonic Mean p-value (HMP) (See [p.hmp](#)). All bins with raw p-values below the HMP are identified and the returned values for start, end, centre, width, matches in region, expected and enrichment are across this set of bins. The expectation is that where a positional bias is evident, this will be a narrow range containing a non-trivial proportion of the total matches.

It should also be noted that `binom.test()` can return p-values of zero, as beyond machine precision. In these instances, zero p-values are excluded from calculation of the HMP. This will give a very slight conservative bias, and assumes that for these extreme cases, neighbouring bins are highly likely to also return extremely low p-values and no significance will be lost.

## Value

A data.frame with columns `start`, `end`, `centre`, `width`, `total_matches`, `matches_in_region`, `expected`, `enrichment`, `prop_total`, `p` and `consensus_motif`. The total matches represent the total number of matches within the set of sequences, whilst the number observed in the final region are also given, along with the proportion of the total this represents. Enrichment is simply the ratio of observed to expected based on the expectation of the null hypothesis.

The consensus motif across all matches is returned as a Position Frequency Matrix (PFM) using [consensusMatrix](#).

## Examples

```
## Load the example PWM
data("ex_pwm")
esr1 <- ex_pwm$ESR1

## Load the example sequences
data("ar_er_seq")

## Get the best match and use this data
matches <- getPwmMatches(esr1, ar_er_seq, best_only = TRUE)
## Test for enrichment in any position
testMotifPos(matches)

## Provide a list of PWMs, testing for distance from zero
testMotifPos(ex_pwm, ar_er_seq, abs = TRUE, binwidth = 10)
```



**Description**

The chr1 subset of candidate enhancers for ZR-75-1 cells

**Usage**

```
data("zr75_enh")
```

**Format**

An object of class GRanges of length 5237.

**Details**

These enhancers are the chr1 subset of enhancer regions for ZR-75-1 cells as identified by EnhancerAtlas 2.0

```
#' Generation of these ranges is documented in system.file("scripts/zr75_enh.R", package = "motifTestR")
```

**Source**

<http://www.enhanceratlas.org/index.php>

**Examples**

```
data("zr75_enh")
zr75_enh
```

# Index

- \* **datasets**
  - ar\_er\_peaks, [3](#)
  - ar\_er\_seq, [4](#)
  - ex\_pwm, [5](#)
  - hg19\_mask, [7](#)
  - zr75\_enh, [16](#)
- \* **internal**
  - motifTestR-package, [2](#)
  
- ar\_er\_peaks, [3](#)
- ar\_er\_seq, [4](#)
  
- binom.test, [16](#)
  
- consensusMatrix, [16](#)
- countPWM, [5](#)
- countPwmMatches, [4](#), [12](#)
- countPwmMatches(), [2](#), [14](#)
  
- ex\_pwm, [5](#)
  
- getPwmMatches, [6](#), [11](#), [12](#), [15](#), [16](#)
- getPwmMatches(), [2](#), [14](#)
  
- hg19\_mask, [7](#)
  
- makeRMRanges, [8](#), [13](#), [14](#)
- makeRMRanges(), [2](#), [13](#), [14](#)
- makeRMRanges, GRanges, GRanges-method  
    (makeRMRanges), [8](#)
- makeRMRanges, GRangesList, GRangesList-method  
    (makeRMRanges), [8](#)
- matchPWM, [6](#), [7](#), [15](#)
- mclapply, [5](#), [6](#), [9](#), [12](#), [15](#)
- motifTestR (motifTestR-package), [2](#)
- motifTestR-package, [2](#)
  
- p.hmp, [16](#)
- plotMatchPos, [10](#)
- plotMatchPos(), [2](#)
- poisson.test, [13](#)
  
- testMotifEnrich, [12](#)
- testMotifEnrich(), [2](#)
- testMotifPos, [14](#)
- testMotifPos(), [2](#)
  
- Views, [7](#)
  
- zr75\_enh, [16](#)