

# Package ‘motifcounter’

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**Type** Package

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**Maintainer** Wolfgang Kopp <wolfgang.kopp@mdc-berlin.de>

**Description** 'motifcounter' provides motif matching, motif counting and motif enrichment functionality based on position frequency matrices.

The main features of the packages include the utilization of higher-order background models and accounting for self-overlapping motif matches when determining motif enrichment. The background model allows to capture dinucleotide (or higher-order nucleotide) composition adequately which may reduced model biases and misleading results compared to using simple GC background models.

When conducting a motif enrichment analysis based on the motif match count, the package relies on a compound Poisson distribution or alternatively a combinatorial model. These distribution account for self-overlapping motif structures as exemplified by repeat-like or palindromic motifs, and allow to determine the p-value and fold-enrichment for a set of observed motif matches.

**License** GPL-2

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 'combinatorial\_wrapper.R' 'score\_wrapper.R' 'count\_wrapper.R'  
 'enrichmentTest.R' 'foreground\_wrapper.R' 'markovmodel.R'  
 'motifcounter-package.R' 'observed\_wrapper.R' 'option.R'  
 'overlap.R' 'simulate\_wrapper.R' 'wrapper.R'

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motifcounter-package    *TFBSs analysis in DNA sequences*

---

## Description

The package provides functions for determining the positions of motif hits as well as motif hit enrichment for a given position frequency matrix (PFM) in a DNA sequence of interest. The following examples guides you through the main functions of the ‘motifcounter’ package.

## Details

For an analysis with ‘motifcounter’, the user is required to provide 1) a PFM, 2) a DNA sequence which is used to estimate a background model (see `link{readBackground}`), 3) a DNA sequence of interest that shall be scanned for motif hits (can be the same as the one used for point 2), and 4) (optionally) a desired false positive probability of motif hits in random DNA sequences (see `motifcounterOptions`).

Package: motifcounter  
 Type: Package  
 Version: 1.0  
 Date: 2016-11-04  
 License: GPL-2

## Author(s)

Wolfgang Kopp

Maintainer: Wolfgang Kopp <kopp@molgen.mpg.de>

**Examples**

```
# Load sequences
file = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(file)

# Estimate an order-1 background model
order = 1
bg = readBackground(seqs, order)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Normalize the motif
# Normalization is sometimes necessary to prevent zeros in
# the motif
motif = normalizeMotif(motif)

# Use subset of the sequences
seqs = seqs[1:10]

# Optionally, set the false positive probability
#alpha=0.001 # is also the default
#motifcounterOptions(alpha)

# Investigate the per-position and per-strand scores in a given sequence
scores = scoreSequence(seqs[[1]], motif, bg)

# Investigate the per-position and per-strand motif hits in a given sequence
hits = motifHits(seqs[[1]], motif, bg)

# Determine the average score profile across a set of sequences
scores = scoreProfile(seqs, motif, bg)

# Determine the average motif hit profile across a set of sequences
hits = motifHitProfile(seqs, motif, bg)

# Determine the empirical score distribution
scoreHistogram(seqs, motif, bg)

# Determine the theoretical score distribution in random sequences
scoreDist(motif, bg)

# Determine the motif hit enrichment in a set of DNA sequences
# 1. Use the compound Poisson approximation
# and scan only a single strand for motif hits
result = motifEnrichment(seqs, motif, bg,
  singlestranded = TRUE, method = "compound")

# Determine the motif hit enrichment in a set of DNA sequences
# 2. Use the compound Poisson approximation
```

```

# and scan both strands for motif hits
result = motifEnrichment(seqs, motif, bg,
                        singlestranded = FALSE, method = "compound")

# Determine the motif hit enrichment in a set of DNA sequences
# 3. Use the combinatorial model
# and scan both strands for motif hits
result = motifEnrichment(seqs, motif, bg, singlestranded = FALSE,
                        method = "combinatorial")

```

---

Background-class	<i>Background class definition</i>
------------------	------------------------------------

---

### Description

Objects of this class serve as a container that holds parameters for the Background model.

### Details

A Background model is constructed via [readBackground](#).

### Slots

station Stationary probabilities  
trans Transition probabilities  
counts k-mer counts  
order Background model order

---

clumpSizeDist	<i>Clump size distribution</i>
---------------	--------------------------------

---

### Description

This function approximates the distribution of the clump sizes.

### Usage

```
clumpSizeDist(maxclump, overlap, method = "kopp")
```

### Arguments

maxclump	Maximal clump size
overlap	An Overlap object.
method	String that defines which method shall be invoked: 'pape' or 'kopp' (see description). Default: method = 'kopp'.

## Details

The clump size distribution can be determined in two alternative ways:

1. A re-implemented version of the algorithm that was described in Pape et al. *Compound poisson approximation of the number of occurrences of a position frequency matrix (PFM) on both strands*. 2008 can be invoked using `method='pape'`.
2. An improved approximation of the clump size distribution uses more appropriate statistical assumptions concerning overlapping motif hits and that can be used with order-d background models as well. The improved version is used by default with `method='kopp'`.

## Value

List containing

**dist** Distribution of the clump size

## See Also

[probOverlapHit](#)

## Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Load background model
bg = readBackground(seqs, 1)

# Use 100 individual sequences of length 150 bp each
seqlen = rep(150, 100)

# Compute overlapping probabilities
# for scanning the forward DNA strand only
op = motifcounter::probOverlapHit(motif, bg, singlestranded = FALSE)

# Computes the compound Poisson distribution
dist = motifcounter::clumpSizeDist(20, op)
```

---

combinatorialDist	<i>Combinatorial model approximation of the number of motif hits</i>
-------------------	--

---

## Description

This function approximates the distribution of the number of motif hits. To this end, it sums over all combinations of obtaining  $k$  hits in a random sequence of a given length using an efficient dynamic programming algorithm.

## Usage

```
combinatorialDist(seqlen, overlap)
```

## Arguments

seqlen	Integer-valued vector that defines the lengths of the individual sequences. For a given DNASTringSet, this information can be retrieved using <a href="#">numMotifHits</a> .
overlap	An Overlap object.

## Details

This function is an alternative to [compoundPoissonDist](#) which requires fixed-length sequences and currently only supports the computation of the distribution of the number of hits when both DNA strands are scanned for motif hits.

## Value

List containing

**dist** Distribution of the number of hits

## See Also

[compoundPoissonDist](#)  
[numMotifHits](#)  
[probOverlapHit](#)

## Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Load background model
```

```

bg = readBackground(seqs, 1)

# Compute overlap probabilities
op = motifcounter:::probOverlapHit(motif, bg, singlestranded = FALSE)

# Use 2 sequences of length 100 bp each
seqlen = rep(100, 2)

# Computes the combinatorial distribution of the number of motif hits
dist = motifcounter:::combinatorialDist(seqlen, op)

```

---

compoundPoissonDist    *Compound Poisson Approximation*

---

### Description

This function approximates the distribution of the number of motif hits that emerges from a random DNA sequence of a given length.

### Usage

```
compoundPoissonDist(seqlen, overlap, method = "kopp")
```

### Arguments

seqlen	Integer-valued vector that defines the lengths of the individual sequences. For a given DNASTringSet, this information can be retrieved using <code>numMotifHits</code> .
overlap	An Overlap object.
method	String that defines which method shall be invoked: 'pape' or 'kopp' (see description). Default: method = 'kopp'.

### Details

The distribution can be determined in two alternative ways:

1. A re-implemented version of the algorithm that was described in Pape et al. *Compound poisson approximation of the number of occurrences of a position frequency matrix (PFM) on both strands*. 2008 can be invoked using `method='pape'`. The main purpose of this implementation concerns benchmarking an improved approximation. In contrast to the original model, this implementation can be used with general order-d Markov models.
2. We provide an improved compound Poisson approximation that uses more appropriate statistical assumptions concerning overlapping motif hits and that can be used with order-d background models as well. The improved version is used by default with `method='kopp'`. Note: Only `method='kopp'` supports the computation of the distribution of the number of motif hits w.r.t. scanning a single DNA strand (see `probOverlapHit`).



**Value**

List containing

**dist** Distribution of the number of hits

**See Also**

[combinatorialDist](#)

[probOverlapHit](#)

[numMotifHits](#)

**Examples**

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNAStringSet(seqfile)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Load background model
bg = readBackground(seqs, 1)

# Use 100 individual sequences of length 150 bp each
seqlen = rep(150, 100)

# Compute overlapping probabilities
# for scanning the forward DNA strand only
op = motifcounter:::probOverlapHit(motif, bg, singlestranded = TRUE)

# Computes the compound Poisson distribution
dist = motifcounter:::compoundPoissonDist(seqlen, op)
#plot(1:length(dist$dist)-1, dist$dist)

# Compute overlapping probabilities
# for scanning the forward DNA strand only
op = motifcounter:::probOverlapHit(motif, bg, singlestranded = FALSE)

# Computes the compound Poisson distribution
dist = motifcounter:::compoundPoissonDist(seqlen, op)
#plot(1:length(dist$dist)-1, dist$dist)
```

---

computeClumpStartProb *Computes the Clump start probability based on a Markov model*

---

**Description**

This function leverages a Markov model in order to determine the clump start probability. The computation depends on the selected false positive probability for calling motif matches 'alpha' and the pre-determined overlapping match probabilities 'beta'.

**Usage**

```
computeClumpStartProb(overlap)
```

**Arguments**

overlap            An Overlap object.

**Details**

The general idea of the method relies on the fact that for the stationary distribution of the Markov model, motif matches must be observed with probability 'alpha'. Hence, the clump start probability 'tau' is optimized to achieve that goal.

The R interface is only used for the purpose of testing the correctness of the model.

**Value**

Clump start probability 'tau'

**See Also**

[compoundPoissonDist](#)  
[numMotifHits](#)  
[probOverlapHit](#)

**Examples**

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNAStringSet(seqfile)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Load background model
bg = readBackground(seqs, 1)

# Compute overlap probabilities
op = motifcounter:::probOverlapHit(motif, bg, singlestranded = FALSE)

# Computes the clump start probability
dist = motifcounter:::computeClumpStartProb(op)
```

---

generatedDNAStrng	<i>Generate DNAStrng</i>
-------------------	--------------------------

---

### Description

This function generates a random DNAStrng of a given length by sampling from the background model.

### Usage

```
generatedDNAStrng(len, bg)
```

### Arguments

len	Integer length of the sequence
bg	A Background object

### Value

A DNAStrng object

### See Also

[generatedDNAStrngSet](#)

### Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNAStrngSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Generate a 1 kb random sequence
motifcounter::generatedDNAStrng(1000, bg)
```

---

generatedDNAStrngSet *Generate DNAStrngSet*

---

### Description

This function generates a DNAStrngSet-object of the given individual sequence lengths by sampling from the background model.

### Usage

```
generateDNAStrngSet(seqlen, bg)
```

### Arguments

seqlen	Integer-valued vector that defines the lengths of the individual sequences. For a given DNAStrngSet, this information can be retrieved using <code>numMotifHits</code> .
bg	A Background object

### Value

A DNAStrngSet object

### See Also

[generateDNAStrngSet](#)

### Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNAStrngSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Generate random sequences of various lengths
motifcounter::generateDNAStrngSet(10:50, bg)
```

---

<code>getAlpha</code>	<i>Accessor to slot alpha</i>
-----------------------	-------------------------------

---

**Description**

Accessor to slot alpha

**Usage**

`getAlpha(obj)`

**Arguments**

`obj`            An Overlap object

**Value**

alpha slot

---

<code>getBeta</code>	<i>Accessor to slot beta</i>
----------------------	------------------------------

---

**Description**

Accessor to slot beta

**Usage**

`getBeta(obj)`

**Arguments**

`obj`            An Overlap object

**Value**

beta slot

---

getBeta3p	<i>Accessor to slot beta3p</i>
-----------	--------------------------------

---

**Description**

Accessor to slot beta3p

**Usage**

```
getBeta3p(obj)
```

**Arguments**

obj	An Overlap object
-----	-------------------

**Value**

beta3p slot

---

getBeta5p	<i>Accessor to slot beta</i>
-----------	------------------------------

---

**Description**

Accessor to slot beta

**Usage**

```
getBeta5p(obj)
```

**Arguments**

obj	An Overlap object
-----	-------------------

**Value**

beta5p slot

---

getCounts                      *Accessor to slot counts*

---

**Description**

Accessor to slot counts

**Usage**

getCounts(obj)

**Arguments**

obj                      A Background object

**Value**

counts slot

---

getGamma                      *Accessor to slot gamma*

---

**Description**

Accessor to slot gamma

**Usage**

getGamma(obj)

**Arguments**

obj                      An Overlap object

**Value**

gamma slot

---

getOrder	<i>Accessor to slot order</i>
----------	-------------------------------

---

**Description**

Accessor to slot order

**Usage**

getOrder(obj)

**Arguments**

obj            A Background object

**Value**

order slot

---

getSinglestranded	<i>Accessor to slot singlestranded</i>
-------------------	--

---

**Description**

Accessor to slot singlestranded

**Usage**

getSinglestranded(obj)

**Arguments**

obj            An Overlap object

**Value**

singlestranded slot



---

*getStation*                      *Accessor to slot station*

---

**Description**

Accessor to slot station

**Usage**

`getStation(obj)`

**Arguments**

`obj`                      A Background object

**Value**

station slot

---

*getTrans*                      *Accessor to slot trans*

---

**Description**

Accessor to slot trans

**Usage**

`getTrans(obj)`

**Arguments**

`obj`                      A Background object

**Value**

trans slot

---

hitStrand	<i>Hit strand</i>
-----------	-------------------

---

### Description

This function computes the per-position motif matches in a given DNA strand.

### Usage

```
hitStrand(seq, pfm, bg, threshold = NULL)
```

### Arguments

seq	A DNASTring object
pfm	An R matrix that represents a position frequency matrix
bg	A Background object
threshold	Score threshold for calling motif matches. If NULL, the threshold will be determined from alpha.

### Details

The function returns the per-position scores for the given strand. If the sequence is too short, it contains an empty vector.

### Value

**hits** Vector of motif hits on the given strand

### Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute the per-position and per-strand scores
motifcounter::hitStrand(seqs[[1]], motif, bg)
```

---

lenSequences	<i>Length of sequences in a given fasta file</i>
--------------	--

---

**Description**

The function returns a vector containing the lengths of each sequence contained in a set of sequences. Sequences containing 'N' or 'n' are skipped from the analysis and are set to length zero.

**Usage**

```
lenSequences(seqs)
```

**Arguments**

seqs                    A DNASTringSet object

**Value**

A vector containing the lengths of each individual sequences

**Examples**

```
# Load sequences
file = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(file)

# Retrieve sequence lengths
motifcounter::lenSequences(seqs)
```

---

markovModel	<i>Markov model for generating Y_1Y_2_Y3 ...</i>
-------------	--

---

**Description**

This function implements the Markov model for producing motif matches. The function takes a state probability vector and uses the transition probabilities in order to obtain the state probability at the next time point. This function is used used to determine the stationary distribution of the states.

**Usage**

```
markovModel(overlap, nsteps = 1)
```

**Arguments**

overlap                An Overlap object.  
nsteps                 Number of state transitions to perform

## Details

The R interface is only used for the purpose of testing the correctness of the model.

## Value

List containing

**dist** State probability distribution after the given number of steps

## See Also

[compoundPoissonDist](#)

[numMotifHits](#)

[probOverlapHit](#)

## Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Load background model
bg = readBackground(seqs, 1)

# Compute overlap probabilities
op = motifcounter:::probOverlapHit(motif, bg, singlestranded = FALSE)

# Computes the state probabilities of the Markov model
# (default: after one step)
dist = motifcounter:::markovModel(op)
```

---

motifAndBackgroundValid

*Check validity of PFM with background*

---

## Description

This function checks if the PFM x background combination is valid. The function throws an error if this is not the case.

## Usage

```
motifAndBackgroundValid(pfm, bg)
```

**Arguments**

pfm	An R matrix that represents a position frequency matrix
bg	A Background object

**Value**

None

**Examples**

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x1.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Check validity
motifcounter::motifAndBackgroundValid(motif, bg)
```

---

motifcounterOptions    *Set parameters for the enrichment analysis*

---

**Description**

This function sets some global parameters for the ‘motifcounter’ package.

**Usage**

```
motifcounterOptions(alpha = 0.001, gran = 0.1, ncores = 1)
```

**Arguments**

alpha	Numeric False positive probability for calling motif hits by chance. Default: alpha = 0.001
gran	Numeric score granularity which is used for discretizing the score range. Default: gran = 0.1
ncores	Integer number of cores used for parallel processing, if openMP is available. Default: ncores = 1

**Details**

alpha=0.001 amounts to calling one motif hit per strand by chance in a sequence of length 1000 bp. Decreasing gran will increase number of discrete bins that represent the real-valued score range. This will yield more a accurate score distribution due to less discretization noise, however, it incurs an increase of the computational burden.

**Value**

None

**Examples**

```
# Prescribe motifcounter Options
motifcounterOptions(alpha = 0.001, gran = 0.1, ncores = 1)
```

---

motifEnrichment	<i>Enrichment of motif hits</i>
-----------------	---------------------------------

---

**Description**

This function determines whether a given motif is enriched in a given DNA sequences.

**Usage**

```
motifEnrichment(seqs, pfm, bg, singlestranded = FALSE, method = "compound")
```

**Arguments**

seqs	A DNASTringSet or DNASTring object
pfm	An R matrix that represents a position frequency matrix
bg	A Background object
singlestranded	Boolean that indicates whether a single strand or both strands shall be scanned for motif hits. Default: singlestranded = FALSE.
method	String that defines whether to use the 'compound' Poisson approximation' or the 'combinatorial' model. Default: method='compound'.

**Details**

Enrichment is tested by comparing the observed number of motif hits against a theoretical distribution of the number of motif hits in random DNA sequences. Optionally, the theoretical distribution of the number of motif hits can be evaluated by either a 'compound Poisson model' or the 'combinatorial model'. Additionally, the enrichment test can be conducted with respect to scanning only the forward strand or both strands of the DNA sequences. The latter option is only available for the 'compound Poisson model'

**Value**

List that contains

**pvalue** P-value for the enrichment test

**fold** Fold-enrichment with respect to the expected number of hits

**See Also**

[compoundPoissonDist](#), [combinatorialDist](#)

**Examples**

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# 1 ) Motif enrichment test w.r.t. scanning a *single* DNA strand
# based on the 'Compound Poisson model'

result = motifEnrichment(seqs, motif, bg,
                        singlestranded = TRUE, method = "compound")

# 2 ) Motif enrichment test w.r.t. scanning *both* DNA strand
# based on the 'Compound Poisson model'

result = motifEnrichment(seqs, motif, bg, method = "compound")

# 3 ) Motif enrichment test w.r.t. scanning *both* DNA strand
# based on the *combinatorial model*

result = motifEnrichment(seqs, motif, bg, singlestranded = FALSE,
                        method = "combinatorial")
```

---

motifHitProfile

*Motif hit profile across multiple sequences*

---

**Description**

This function computes the per-position average motif hit profile across a set of fixed-length DNA sequences. It can be used to reveal positional constraints of TFBSs.

**Usage**

```
motifHitProfile(seqs, pfm, bg)
```

**Arguments**

seqs	A DNASTringSet or DNASTring object
pfm	An R matrix that represents a position frequency matrix
bg	A Background object

**Value**

List containing

**fscores** Per-position average forward strand motif hits

**rscores** Per-position average reverse strand motif hits

**Examples**

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)
seqs = seqs[1:10]

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute the motif hit profile
motifHitProfile(seqs, motif, bg)
```

---

motifHits

*Motif hit observations*

---

**Description**

This function determines per-position motif hits in a given DNA sequence.

**Usage**

```
motifHits(seq, pfm, bg, threshold = NULL)
```



**Arguments**

seq	A DNASTring object
pfm	An R matrix that represents a position frequency matrix
bg	A Background object
threshold	Score threshold for calling motif matches. If NULL, the threshold will be determined from alpha.

**Value**

List containing

**fhits** Per-position motif hits on the forward strand

**rhits** Per-position motif hits on the reverse strand

**Examples**

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seq = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seq, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Determine the motif hits
motifHits(seq[[1]], motif, bg)
```

---

motifValid

*Check validity of PFM*

---

**Description**

This function checks if the PFM is valid. The function throws an error if the R matrix does not represent a PFM.

**Usage**

```
motifValid(pfm)
```

**Arguments**

pfm	An R matrix that represents a position frequency matrix
-----	---

**Value**

None

**Examples**

```
# Load motif
motiffile = system.file("extdata", "x1.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Check validity
motifcounter::motifValid(motif)
```

---

normalizeMotif	<i>Normalizes a PFM</i>
----------------	-------------------------

---

**Description**

This function normalizes a PFM and optionally adds pseudo-evidence to each entry of the matrix.

**Usage**

```
normalizeMotif(pfm, pseudo = 0.01)
```

**Arguments**

pfm	An R matrix that represents a position frequency matrix
pseudo	Small numeric pseudo-value that is added to each entry in the PFM in order to ensure strictly positive entries. Default: pseudo = 0.01

**Value**

A normalized PFM

**Examples**

```
# Load motif
motiffile = system.file("extdata", "x1.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Normalize motif
new_motif = normalizeMotif(motif)
```

---

numMotifHits	<i>Number of motif hits in a set of DNA sequences</i>
--------------	---

---

### Description

This function counts the number of motif hits that are found in a given set of DNA sequences.

### Usage

```
numMotifHits(seqs, pfm, bg, singlestranded = FALSE)
```

### Arguments

seqs	A DNASTringSet or DNASTring object
pfm	An R matrix that represents a position frequency matrix
bg	A Background object
singlestranded	Boolean that indicates whether a single strand or both strands shall be scanned for motif hits. Default: singlestranded = FALSE.

### Details

Optionally, it can be used to count motif hits on one or both strands, respectively.

### Value

A list containing

**nseq** Number of individual sequences

**lseq** Vector of individual sequence lengths

**numofhits** Vector of the number of hits in each individual sequence

### Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Count motif hits both strands
noc = motifcounter:::numMotifHits(seqs, motif, bg)
noc$numofhits
```

```
# Count motif hits on a single strand
noc = motifcounter::numMotifHits(seqs, motif, bg, singlestranded = TRUE)
noc$numofhits
```

---

Overlap-class	<i>Overlap class definition</i>
---------------	---------------------------------

---

### Description

Objects of this class serve as a container that holds parameters for the overlapping hit probabilities.

### Details

An Overlap object is constructed via the [probOverlapHit](#)

### Slots

alpha Scalar numeric significance level to call motif matches  
 beta Numeric vector of principal overlapping hit probabilities on the same strand.  
 beta3p Numeric vector of principal overlapping hit probabilities with 3'-overlap.  
 beta5p Numeric vector of principal overlapping hit probabilities with 5'-overlap.  
 gamma Numeric vector of marginal overlapping hit probabilities.  
 singlestranded logical flag to indicate whether one or both strands are scanned for motif matches.

---

probOverlapHit	<i>Overlapping motif hit probabilities</i>
----------------	--

---

### Description

This function computes a set of self-overlapping probabilities for a motif and background model.

### Usage

```
probOverlapHit(pfm, bg, singlestranded = FALSE)
```

### Arguments

pfm An R matrix that represents a position frequency matrix  
 bg A Background object  
 singlestranded Boolean that indicates whether a single strand or both strands shall be scanned for motif hits. Default: singlestranded = FALSE.

## Details

The ‘gamma’s are determined based on two-dimensional score distributions (similar as described in Pape et al. 2008), however, they are computed based on an order-d background model. On the other hand, the ‘beta’s represent overlapping hit probabilities that were corrected for intermediate hits.

## Value

An Overlap object

## Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute overlapping hit probabilities for scanning both DNA strands
op = motifcounter:::probOverlapHit(motif, bg, singlestranded = FALSE)

# Compute overlapping hit probabilities for scanning a single DNA strand
op = motifcounter:::probOverlapHit(motif, bg, singlestranded = TRUE)
```

---

readBackground	<i>Estimates a background model from a set of DNA sequences</i>
----------------	---

---

## Description

Given a set of DNA sequences and an order, this function estimates an order-d Markov model which is used to characterize random DNA sequences.

## Usage

```
readBackground(seqs, order = 1)
```

## Arguments

seqs	A DNASTringSet object
order	Order of the Markov models that shall be used as the background model. Default: order = 1.

**Value**

A Background object

**Examples**

```
# Load sequences
file = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(file)

# Estimate an order-1 Markov model
bg = readBackground(seqs, 1)
```

---

revcompMotif

*Reverse complements a PFM*

---

**Description**

This function computes the reverse complement of a given PFM.

**Usage**

```
revcompMotif(pfm)
```

**Arguments**

pfm                    An R matrix that represents a position frequency matrix

**Value**

Reverse complemented PFM

**Examples**

```
# Load motif
motiffile = system.file("extdata", "x1.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Reverse complement motif
revcompmotif = motifcounter:::revcompMotif(motif)
```

---

scoreDist	<i>Score distribution</i>
-----------	---------------------------

---

### Description

This function computes the score distribution for the given PFM and background. The Score distribution is computed based on an efficient dynamic programming algorithm.

### Usage

```
scoreDist(pfm, bg)
```

### Arguments

pfm	An R matrix that represents a position frequency matrix
bg	A Background object

### Value

List that contains

**scores** Vector of scores

**dist** Score distribution

### Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute the score distribution
dp = scoreDist(motif, bg)
```

---

scoreDistBf	<i>Score distribution</i>
-------------	---------------------------

---

### Description

This function computes the score distribution for a given PFM and a background model.

### Usage

```
scoreDistBf(pfm, bg)
```

### Arguments

pfm	An R matrix that represents a position frequency matrix
bg	A Background object

### Details

The result of this function is identical to [scoreDist](#), however, the method employs a less efficient algorithm that enumerates all DNA sequences of the length of the motif. This function is only used for debugging and testing purposes and might require substantial computational resources for long motifs.

### Value

List containing

- scores** Vector of scores
- dist** Score distribution

### See Also

[scoreDist](#)

### Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute the score distribution
dp = motifcounter::scoreDistBf(motif, bg)
```



---

scoreDistEmpirical      *Empirical score distribution*

---

### Description

This function estimates the empirical score distribution on a set of randomly generated DNA sequences based on the background model. This function is only used for benchmarking analysis.

### Usage

```
scoreDistEmpirical(pfm, bg, seqlen, nsim)
```

### Arguments

pfm	An R matrix that represents a position frequency matrix
bg	A Background object
seqlen	Integer-valued vector that defines the lengths of the individual sequences. For a given DNASTringSet, this information can be retrieved using <a href="#">numMotifHits</a> .
nsim	Integer number of random samples.

### Value

List containing

**scores** Vector of scores

**dist** Score distribution

### See Also

[scoreDist](#)

### Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute the empirical score distribution in
# sequences of length 1kb using 1000 samples
motifcounter::scoreDistEmpirical(motif, bg, seqlen = 1000, nsim = 1000)
```

---

scoreHistogram	<i>Score histogram</i>
----------------	------------------------

---

### Description

This function computes the empirical score distribution for a given set of DNA sequences.

### Usage

```
scoreHistogram(seqs, pfm, bg)
```

### Arguments

seqs	A DNASTringSet or DNASTring object
pfm	An R matrix that represents a position frequency matrix
bg	A Background object

### Details

It can be used to compare the empirical score distribution against the theoretical one (see [scoreDist](#)).

### Value

List containing

**scores** Vector of scores

**dist** Score distribution

### See Also

[scoreDist](#)

### Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute the empirical score histogram
scoreHistogram(seqs, motif, bg)
```

---

`scoreHistogramSingleSeq`*Score histogram on a single sequence*

---

### Description

This function computes the empirical score distribution by normalizing the observed score histogram for a given sequence.

### Usage

```
scoreHistogramSingleSeq(seq, pfm, bg)
```

### Arguments

<code>seq</code>	A DNASTring object
<code>pfm</code>	An R matrix that represents a position frequency matrix
<code>bg</code>	A Background object

### Value

List containing

**scores** Vector of scores

**dist** Score distribution

### Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute the per-position and per-strand scores
motifcounter::scoreHistogramSingleSeq(seqs[[1]], motif, bg)
```

---

scoreProfile	<i>Score profile across multiple sequences</i>
--------------	--

---

### Description

This function computes the per-position and per-strand average score profiles across a set of DNA sequences. It can be used to reveal positional constraints of TFBSs.

### Usage

```
scoreProfile(seqs, pfm, bg)
```

### Arguments

seqs	A DNASTringSet or DNASTring object
pfm	An R matrix that represents a position frequency matrix
bg	A Background object

### Value

List containing

**fscores** Vector of per-position average forward strand scores

**rscores** Vector of per-position average reverse strand scores

### Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute the score profile
scoreProfile(seqs, motif, bg)
```

---

scoreSequence	<i>Score observations</i>
---------------	---------------------------

---

## Description

This function computes the per-position and per-strand score in a given DNA sequence.

## Usage

```
scoreSequence(seq, pfm, bg)
```

## Arguments

seq	A DNASTring object
pfm	An R matrix that represents a position frequency matrix
bg	A Background object

## Value

List containing

**fscores** Vector of scores on the forward strand

**rscores** Vector of scores on the reverse strand

## Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute the per-position and per-strand scores
scoreSequence(seqs[[1]], motif, bg)
```

---

scoreStrand	<i>Score strand</i>
-------------	---------------------

---

## Description

This function computes the per-position score in a given DNA strand.

## Usage

```
scoreStrand(seq, pfm, bg)
```

## Arguments

seq	A DNASTring object
pfm	An R matrix that represents a position frequency matrix
bg	A Background object

## Details

The function returns the per-position scores for the given strand. If the sequence is too short, it contains an empty vector.

## Value

**scores** Vector of scores on the given strand

## Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute the per-position and per-strand scores
motifcounter::scoreStrand(seqs[[1]], motif, bg)
```

---

scoreThreshold	<i>Score threshold</i>
----------------	------------------------

---

### Description

This function computes the score threshold for a desired false positive probability ‘alpha’.

### Usage

```
scoreThreshold(pfm, bg)
```

### Arguments

pfm	An R matrix that represents a position frequency matrix
bg	A Background object

### Details

Note that the returned alpha usually differs slightly from the one that is prescribed using [motifcounterOptions](#), because of the discrete nature of the sequences.

### Value

List containing

**threshold** Score threshold

**alpha** False positive probability

### Examples

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Compute the score threshold
motifcounter::scoreThreshold(motif, bg)
```

---

sigLevel	<i>Retrieve the false positive probability</i>
----------	--

---

### Description

This function returns the current false positive level for calling motif hits in random sequences.

### Usage

```
sigLevel()
```

### Details

The returned value is usually slightly smaller than the prescribed ‘alpha’ in ‘motifcounterOptions’, because of the discrete nature of sequences.

### Value

False positive probability

### Examples

```
motifcounter:::sigLevel()
```

---

simulateClumpSizeDist	<i>Empirical clump size distribution</i>
-----------------------	--

---

### Description

This function repeatedly simulates random DNA sequences according to the background model and subsequently counts the number of k-clump occurrences, where denotes the clump size. This function is only used for benchmarking analysis.

### Usage

```
simulateClumpSizeDist(pfm, bg, seqlen, nsim = 10, singlestranded = FALSE)
```

### Arguments

pfm	An R matrix that represents a position frequency matrix
bg	A Background object
seqlen	Integer-valued vector that defines the lengths of the individual sequences. For a given DNASTringSet, this information can be retrieved using <a href="#">numMotifHits</a> .
nsim	Integer number of random samples.
singlestranded	Boolean that indicates whether a single strand or both strands shall be scanned for motif hits. Default: singlestranded = FALSE.



**Value**

A List that contains

**dist** Empirical distribution of the clump sizes

**See Also**

[compoundPoissonDist](#), [combinatorialDist](#)

**Examples**

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Study the clump size frequencies in one sequence of length 1 Mb
seqlen = 1000000

# scan both strands
simc = motifcounter::simulateClumpSizeDist(motif, bg, seqlen)

# scan a single strand
simc = motifcounter::simulateClumpSizeDist(motif, bg,
  seqlen, singlestranded = TRUE)
```

---

simulateNumHitsDist     *Empirical number of motif hits distribution*

---

**Description**

This function repeatedly simulates random DNA sequences according to the background model and subsequently counts how many motif hits occur in them. Thus, this function gives rise to the empirical distribution of the number of motif hits. This function is only used for benchmarking analysis.

**Usage**

```
simulateNumHitsDist(pfm, bg, seqlen, nsim, singlestranded = FALSE)
```

**Arguments**

pfm	An R matrix that represents a position frequency matrix
bg	A Background object
seqlen	Integer-valued vector that defines the lengths of the individual sequences. For a given DNASTringSet, this information can be retrieved using <a href="#">numMotifHits</a> .
nsim	Integer number of random samples.
singlestranded	Boolean that indicates whether a single strand or both strands shall be scanned for motif hits. Default: singlestranded = FALSE.

**Value**

A List that contains

**dist** Empirical distribution of the number of motif hits

**See Also**

[compoundPoissonDist](#), [combinatorialDist](#)

**Examples**

```
# Load sequences
seqfile = system.file("extdata", "seq.fasta", package = "motifcounter")
seqs = Biostrings::readDNASTringSet(seqfile)

# Load background
bg = readBackground(seqs, 1)

# Load motif
motiffile = system.file("extdata", "x31.tab", package = "motifcounter")
motif = t(as.matrix(read.table(motiffile)))

# Study the counts in one sequence of length 150 bp
seqlen = rep(150, 1)

# Compute empirical distribution of the number of motif hits
# by scanning both strands using 100 samples
simc = motifcounter::simulateNumHitsDist(motif, bg,
    seqlen, nsim = 100, singlestranded = FALSE)

# Compute empirical distribution of the number of motif hits
# by scanning a single strand using 100 samples
simc = motifcounter::simulateNumHitsDist(motif, bg,
    seqlen, nsim = 100, singlestranded = TRUE)
```

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