Package 'deepSNV'

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Imports Rsamtools

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Description This package provides provides a quantitative variant caller for detecting subclonal mutations in ultra-deep (>=100x coverage) sequencing experiments. It assumes a comparative setup with a control experiment of the same loci and a beta-binomial model to discriminate sequencing errors and subclonal SNVs.

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URL http://www.cbg.ethz.ch/software/deepSNV

Depends R (>= 2.13.0), Rsamtools (>= 1.4.3), GenomicRanges, IRanges,Biostrings, VGAM, methods, graphics

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R topics documented:

`deepSNV-package` *Detection of subclonal SNVs in deep sequencing experiments*

Description

Detection of subclonal SNVs in deep sequencing experiments

Details

This packages provides algorithms for detecting subclonal single nucleotide variants (SNVs) and their frequencies from ultra-deep sequencing data. It retrieves the nucleotide counts at each position and each strand from two .bam files and tests for differences between the two experiments with a likelihood ratio test using either a binomial or and overdispersed beta-binomial model. The statistic can be tuned across genomic sites by a shared Dirichlet prior and there package provides procedures for normalizing sequencing data from different runs.

Author(s)

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References

Gerstung M, Beisel C, Rechsteiner M, Wild P, Schraml P, Moch H, and Beerenwinkel N. Reliable detection of subclonal single-nucleotide variants in tumour cell populations. Nat Commun 3:811 (2012). DOI:10.1038/ncomms1814.

See Also

deepSNV

Examples

```
## Short example with 2 SNVs at frequency ~10%
regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 3120, stop=3140)
ex <- deepSNV(test = system.file("extdata", "test.bam", package="deepSNV"), control = system.file("extdata", "cont
show(ex)   # show method
plot(ex)   # scatter plot
summary(ex)   # summary with significant SNVs
ex[1:3,]   # subsetting the first three genomic positions
tail(test(ex, total=TRUE))   # retrieve the test counts on both strands
tail(control(ex, total=TRUE))

## Not run: Full example with ~ 100 SNVs. Requires an internet connection, but try yourself.
# regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 2074, stop=3585)
# HIVmix <- deepSNV(test = "http://www.bsse.ethz.ch/cbg/software/deepSNV/data/test.bam", control = "http://www.bs
data(HIVmix) # attach data instead..
show(HIVmix)
plot(HIVmix)
head(summary(HIVmix))
```

---

bam2R *Read nucleotide counts from a .bam file*

Description

This function uses a C interface to read the nucleotide counts on each position of a .bam alignment. The counts of both strands are reported separately and nucleotides below a quality cutoff are masked. It is called by deepSNV to parse the alignments of the test and control experiments, respectively.

Usage

```
bam2R(file, chr, start, stop, q = 25, s = 2,
  head.clip = 0, max.depth = 1e+06)
```

Arguments

file	The name of the .bam file as a string.
chr	The chromosome as a string.
start	The start position (1-indexed).
stop	The end position (1-indexed).
q	An optional cutoff for the nucleotide Phred quality. Default q = 25. Nucleotides with Q < q will be masked by 'N'.
s	Optional choice of the strand. Defaults to s = 2 (both).
head.clip	Should n nucleotides from the head of reads be clipped? Default 0.
max.depth	The maximal depth for the pileup command. Default 1,000,000.

Value

A named matrix with rows corresponding to genomic positions and columns for the nucleotide counts (A, T, C, G, -), masked nucleotides (N), (INS)ertions, (DEL)etions, (HEAD)s and (TAIL)s that count how often a read begins and ends at the given position, respectively, and the sum of alignment (QUAL)ities, which can be indicative of alignment problems. Counts from matches on the reference strand (s=0) are uppercase, counts on the complement (s=1) are lowercase. The returned matrix has 11 * 2 (strands) = 22 columns and (stop - start + 1) rows.

Author(s)

Moritz Gerstung

Examples

```
## Simple example:
counts <- bam2R(file = system.file("extdata", "test.bam", package="deepSNV"), chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034
show(counts)
## Not run: Requires an internet connection, but try yourself.
# bam <- bam2R(file = "http://www.bsse.ethz.ch/cbg/software/deepSNV/data/test.bam", chr="B.FR.83.HXB2_LAI_IIIB_B
# head(bam)
```

---

consensusSequence *Calculate the consensus sequence.*

Description

This function computes the consensus sequence from a matrix of nucleotide counts, or the control slot of a deepSNV object.

Usage

```
  ## S4 method for signature 'matrix'
consensusSequence(x, vector=FALSE,
    haploid=TRUE, het.cut = .333)

  ## S4 method for signature 'deepSNV'
consensusSequence(x, vector=FALSE,
    haploid=TRUE, het.cut = .333)
```

Arguments

x	An object. Either an deepSNV-class object, or a named matrix with nucleotide counts.
vector	Boolean where TRUE indicates that a character vector should be returned.
haploid	Should the consensus be called for a haploid control? Otherwise, also all bases larger than het.cut are rerported. Default haploid = TRUE.
het.cut	Heterozygous cutoff. If haploid = FALSE, report all nucleotides with relative frequency larger than het.cut. Default = 0.333.
...	Additional arguments passed to methods.

Value

A DNAString with the consensus sequence, or if vector = TRUE, a character vector.

Author(s)

Moritz Gerstung

Examples

```
data(HIVmix)
seq = consensusSequence(HIVmix)
consensusSequence(HIVmix, vector=TRUE)[1:10]
```

---

control *Get control counts*

Description

Convenience function to obtain the control counts from a deepSNV object.

Usage

```
  ## S4 method for signature 'deepSNV'
control(deepSNV, total = FALSE)
```

Arguments

deepSNV	a deepSNV-class object
total	Logical. If true the sum of both strands is returned

Value

A matrix with the absolute frequencies summed over both strands.

Examples

```
data(HIVmix)
control(HIVmix)[1:10,]
control(HIVmix, total=TRUE)[1:10,]
```

---

coordinates *Get coordinates*

Description

Convenience function to get the coordinates from a deepSNV object.

Usage

```
  ## S4 method for signature 'deepSNV'
coordinates(deepSNV)
```

Arguments

deepSNV	a deepSNV-class object

Value

A data.frame with columns "chrom(osome)" and "pos(ition)".

Examples

```
data(HIVmix)
coordinates(HIVmix)[1:10,]
```

deepSNV *Test two matched deep sequencing experiments for low-frequency SNVs.*

Description

This generic function can handle different types of inputs for the test and control experiments. It either reads from two .bam files, uses two matrices of nucleotide counts, or re-evaluates the test results from a deepSNV-class object. The actual test is a likelihood ratio test of a (beta-)binomial model for the individual nucleotide counts on each position under the hypothesis that both experiments share the same parameter, and the alternative that the parameters differ. Because the difference in degrees of freedom is 1, the test statistic $D = -2 \log \max L_0 / \max L_1$ is asymptotically distributed as χ^2_1. The statistic may be tuned by a nucleotide specific Dirichlet prior that is learned across all genomic sites, see estimateDirichlet. If the model is beta-binomial, a global dispersion parameter is used for all sites. It can be learned with estimateDispersion.

Usage

```
  ## S4 method for signature 'matrix,matrix'
deepSNV(test,control,
    alternative = c('greater', 'less', 'two.sided'),
    dirichlet.prior = NULL, pseudo.count=1, combine.method
    = c("fisher", "max", "average"), over.dispersion = 100,
    model = c("bin", "betabin"), ...)

  ## S4 method for signature 'deepSNV,missing'
deepSNV(test, control, ...)

  ## S4 method for signature 'character,character'
deepSNV(test, control,
    regions, q=25, s=2, head.clip=0, ...)

  ## S4 method for signature 'matrix,character'
deepSNV(test, control,
    regions, q=25, s=2, ...)

  ## S4 method for signature 'character,matrix'
deepSNV(test, control,
    regions, q=25, s=2, ...)
```

Arguments

test	The test experiment. Either a .bam file, or a matrix with nucleotide counts, or a deepSNV-class object.
control	The control experiment. Must be of the same type as test, or missing if test is a deepSNV-class object.
alternative	The alternative to be tested. One of greater, less, or two.sided.

model	Which model to use. Either "bin", or "betabin". Default "bin".
dirichlet.prior	A base-sepecific Dirichlet prior specified as a matrix. Default NULL.
pseudo.count	If dirichlet.prior=NULL, a pseudocount can be used to define a flat prior.
over.dispersion	A numeric factor for the over.dispersion, if the model is beta-binomial. Default 100.
combine.method	The method to combine p-values. One of "fisher" (default), "max", or "average". See p.combine for details.
regions	The regions to be parsed if test and control are .bam files. Either a data.frame with columns "chr" (chromosome), "start", "stop", or a GRanges object. If multiple regions are specified, the appropriate slots of the returned object are concatenated by row.
q	The quality arguement passed to bam2R if the experiments are .bam files.
s	The strand argument passed to bam2R if the experiments are .bam files.
head.clip	The head.clip argument passed to bam2R if the experiments are .bam files.
...	Additional arguments.

Value

A deepSNV object

Author(s)

Moritz Gerstung

Examples

```
## Short example with 2 SNVs at frequency ~10%
regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 3120, stop=3140)
ex <- deepSNV(test = system.file("extdata", "test.bam", package="deepSNV"), control = system.file("extdata", "cont
show(ex)   # show method
plot(ex)   # scatter plot
summary(ex)   # summary with significant SNVs
ex[1:3,]   # subsetting the first three genomic positions
tail(test(ex, total=TRUE))   # retrieve the test counts on both strands
tail(control(ex, total=TRUE))

## Not run: Full example with ~ 100 SNVs. Requires an internet connection, but try yourself.
# regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 2074, stop=3585)
# HIVmix <- deepSNV(test = "http://www.bsse.ethz.ch/cbg/software/deepSNV/data/test.bam", control = "http://www.bs
data(HIVmix) # attach data instead..
show(HIVmix)
plot(HIVmix)
head(summary(HIVmix))
```

`deepSNV-class` *deepSNV class.*

Description

This class stores the contents of the deepSNV test. It is typically initialized with `deepSNV`. This class has the following slots:

p.val The P-values of the test.

test A matrix with the nucleotide counts in the test experiment. The column names of the nucleotide counts are A, T, C, G, - for the positivie strand and a, t, c, g, _ for the reverse.

control A matrix with the nucleotide counts in the control experiment. The column names must be the same as for the test.

coordinates A `data.frame` with the genomic coordinates chr and pos, and other columns, if desired.

dirichlet.prior A matrix with the nucleotide-specific Dirichlet prior

alternative A string with the alternative used in the test.

nucleotides A character vector with the nucleotides tested.

regions A `data.frame` with columns chr, start, and stop.

files A list with two entries test and control storing the filenames (if the object was initialized from two bam-files).

combine.method The method for combining p-values as a character string.

model The statistical model, either bin for binomial, or betabin for beta-binomial

over.dispersion If the model is beta-binomial, the first parameter for the beta-binomial model, which is shared across sites.

call The last function call to deepSNV.

log.lik The log likelihood of the data under the null hypothesis. (Excluding zeros on the opposite site under a one-sided test.)

Author(s)

Moritz Gerstung

See Also

`deepSNV`

Examples

```
## Short example with 2 SNVs at frequency ~10%
regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 3120, stop=3140)
ex <- deepSNV(test = system.file("extdata", "test.bam", package="deepSNV"), control = system.file("extdata", "cont
show(ex)   # show method
plot(ex)   # scatter plot
summary(ex)   # summary with significant SNVs
ex[1:3,]   # subsetting the first three genomic positions
tail(test(ex, total=TRUE))   # retrieve the test counts on both strands
tail(control(ex, total=TRUE))

## Not run: Full example with ~ 100 SNVs. Requires an internet connection, but try yourself.
# regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 2074, stop=3585)
# HIVmix <- deepSNV(test = "http://www.bsse.ethz.ch/cbg/software/deepSNV/data/test.bam", control = "http://www.bs
data(HIVmix) # attach data instead..
show(HIVmix)
plot(HIVmix)
head(summary(HIVmix))
```

`estimateDirichlet` *Learn a base-specific Dirichlet prior.*

Description

The prior learns the parameters of a Dirichlet distribution seperately for each consensus base. The expected value of the Dirichlet distributions is the base-substitution matrix, where rows correspond to the initial nucleotide and columns to the substituted nucleotide. The absolute values determine the higher moments of the Dirichlet distributions. After having learned the prior the `deepSNV-class` test is recomputed.

Usage

```
  ## S4 method for signature 'matrix'
estimateDirichlet(control)

  ## S4 method for signature 'deepSNV'
estimateDirichlet(control)
```

Arguments

`control`	Either a matrix with nucleotide counts or a `deepSNV-class` object.

Value

An `deepSNV-class` object.

Author(s)

Moritz Gerstung

Examples

```
data(phiX)
estimateDirichlet(phiX)
```

`estimateDispersion` *Estimate the Dispersion factor in a beta-binomial model.*

Description

This function estimates the dispersion factor in a beta-binomial model of the nucleotide counts. This model assumes that the count for nucleotide j at position i is distributed after a beta-binomial $X_{i,j} \sim \mathrm{BB}(n_i; \alpha, \beta_{ij})$, where n_i is the coverage. The base and nucleotide specific parameter β_{ij} is estimated from the local mean by the method-of-moments estimate, α is a shared overdispersion parameter. It is estimated via a numerical optimization of the likelihood under the null-hypothesis.

Usage

```
  ## S4 method for signature 'deepSNV,missing'
estimateDispersion(test,
    control, alternative = NULL, interval = c(0,1000))

  ## S4 method for signature 'matrix,matrix'
estimateDispersion(test,
    control, alternative = NULL, interval = c(0,1000))
```

Arguments

`test`	Either a deepSNV object, or a matrix with the test counts.
`control`	Missing if test is a deepSNV object, otherwise missing.
`alternative`	The alternative to be tested. One of "greater", "less", "two-sided" (default). If test is a deepSNV object, automatically taken from the corresponding slot if unspecified.
`interval`	The interval to be screened for the overdispersion factor. Default (0,1000).

Value

A `deepSNV-class` object if the input was a deepSNV object. Otherwise the loglikelihood and the estimated parameter.

Author(s)

Moritz Gerstung

Examples

```
data("RCC", package="deepSNV")
plot(RCC)
summary(RCC)[,1:6]
RCC.bb = estimateDispersion(RCC, alternative = "two.sided")
summary(RCC.bb)
```

`Extract` *Subsetting for deepSNV objects.*

Description

Subsetting for deepSNV objects.

Arguments

`x`	A `deepSNV-class` object.
`i`	Row indeces.
`j`	Column (nucleotide) indeces.
`drop`	For matrices and arrays. If `TRUE` the result is coerced to the lowest possible dimension (see the examples). This only works for extracting elements, not for the replacement. See `drop` for further details.

Value

A `deepSNV-class` object.

Author(s)

Moritz Gerstung

Examples

```
data(HIVmix)
HIVmix[1:10,]
```

manhattanPlot *Manhattan plot.*

Description

This functions performs a Manhattan plot of the p-values of a deepSNV test against the position

Usage

```
manhattanPlot(x, col = nt.col)
```

Arguments

x	An deepSNV object.
col	An optional vector of colors for the nucleotides.

Value

NULL.

Author(s)

Moritz Gerstung

Examples

```
data(HIVmix)
manhattanPlot(HIVmix)
```

normalize *Normalize nucleotide counts.*

Description

This functions performs a loess normalization of the nucleotide. This experimental feature can be used to compare experiments from different libraries or sequencing runs that may have differing noise characteristics.

Normalize nucleotide counts.

Normalize nucleotide counts.

Usage

```
normalize(test, control, ...)

## S4 method for signature 'matrix,matrix'
normalize(test, control,
  round=TRUE, ...)

## S4 method for signature 'deepSNV,missing'
normalize(test, control, ...)
```

Arguments

test	Either an deepSNV-class object or a named matrix with nucleotide counts.
control	Missing if test is an link{deepSNV-class} object, otherwise a matrix with nucleotide counts.
round	Logical. Should normalized counts be rounded to integers? Default=TRUE
...	Parameters passed to loess.

Value

A deepSNV-class object.

Note

This feature is somewhat experimental and the results should be treated with care. Sometimes it can be better to leave the data unnormalized and use a model with greater dispersion instead.

Author(s)

Moritz Gerstung

Examples

```
data(phiX, package = "deepSNV")
plot(phiX)
phiN <- normalize(phiX, round = TRUE)
plot(phiN)
```

---

p.combine *Combine two p-values*

Description

This function combines two P-values into a single one using a statistic defined by method. "fisher" uses the product of the two, in this case the logarithm of the product is χ^2_4 distributed. If the method = "max", the resulting P-value is $\max\{P_1, P_2\}^2$. For method = "average" the mean is used, yielding a P-value of $2x^2$ if $x = (P_1 + P_2)/2 < .5$ and $1 - 2x^2$ otherwise.

Usage

```
p.combine(p1, p2,
  method = c("fisher", "max", "average", "prod"))
```

Arguments

p1	P-value 1
p2	P-value 2
method	One of "fisher" (default), "max" or "average"

Value

p-values

Author(s)

Moritz Gerstung

Examples

```
p1 <- runif(1000)
p2 <- runif(1000)
hist(p1)
p.avg = p.combine(p1,p2, method="average")
hist(p.avg)
p.fish = p.combine(p1,p2, method="fisher")
hist(p.fish)
p.max = p.combine(p1,p2, method="max")
hist(p.max)
pairs(data.frame(p1,p2,p.fish,p.max,p.avg))
```

---

p.val *Get p-values*

Description

Convenience function to get the p-values from a deepSNV object.

Usage

```
  ## S4 method for signature 'deepSNV'
p.val(deepSNV)
```

Arguments

deepSNV	a deepSNV-class object

Value

A matrix with the p-values.

Examples

```
data(HIVmix)
p.val(HIVmix)[1:10,]
```

---

phiX *Example phiX data*

Description

Data from two phiX experiments sequenced on a GAIIx.

Examples

```
data(phiX, package="deepSNV")
plot(phiX)
phiN <- normalize(phiX, round=TRUE)
plot(phiN)
```

---

plot.deepSNV *Scatter plot of relative nucleotide frequencies.*

Description

This function plots the relative nucleotide frequencies of the test against the control experiment on a logarithmit scale. The color of the symbols denotes the nucleotide, and the area of the circle is proportional to the $-\log$ of the p-value.

Usage

```
  ## S3 method for class 'deepSNV'
plot(x, sig.level = NULL, col = NULL,
   col.null = "grey", cex.min = 0.2,
   ylab = "Relative Frequency in Test",
   xlab = "Relative Frequency in Control", pch = 16, ...)
```

Arguments

x	A deep SNV object.
sig.level	By default, p-values below sig.level are drawn as filled circles.
col	Color of the nucleotides.
col.null	Color of insignificant nucleotides.
cex.min	The minimal size of the points.
xlab	The x-axis label.
ylab	The y-axis label.
pch	The plotting symbol. Default = 16 (filled circle)
...	Additional arguments passed to plot.

Value

NULL

Author(s)

Moritz Gerstung

Examples

```
## Short example with 2 SNVs at frequency ~10%
regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 3120, stop=3140)
ex <- deepSNV(test = system.file("extdata", "test.bam", package="deepSNV"), control = system.file("extdata", "cont
show(ex)   # show method
plot(ex)   # scatter plot
summary(ex)   # summary with significant SNVs
ex[1:3,]   # subsetting the first three genomic positions
tail(test(ex, total=TRUE))   # retrieve the test counts on both strands
tail(control(ex, total=TRUE))

## Not run: Full example with ~ 100 SNVs. Requires an internet connection, but try yourself.
# regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 2074, stop=3585)
# HIVmix <- deepSNV(test = "http://www.bsse.ethz.ch/cbg/software/deepSNV/data/test.bam", control = "http://www.bs
data(HIVmix) # attach data instead..
show(HIVmix)
plot(HIVmix)
head(summary(HIVmix))
```

RCC *Example RCC data*

Description

Deep sequencing experiments of a renal cell carcinoma and healthy control tissue.

Examples

```
data("RCC", package="deepSNV")
summary(RCC, adjust.method="bonferroni")[,1:6]
plot(RCC)
RCC.bb <- estimateDispersion(RCC, alternative="two.sided")
summary(RCC.bb, adjust.method="bonferroni")[,1:6]
plot(RCC.bb)
```

repeatMask *Mask homopolymeric repeats.*

Description

This function masks homopolymeric repeats longer than a given width. These are hot-spots of sequencing error and can confound the analysis.

Usage

```
  ## S4 method for signature 'DNAString'
repeatMask(x, w=5, flank=TRUE)

  ## S4 method for signature 'deepSNV'
repeatMask(x, w=5, flank=TRUE)
```

Arguments

x	An object. Either a deepSNV-class object or a DNAString with the nucleotide sequence.
flank	Boolean. Indicates whether the sites adjacent to the repeat should also be masked.
w	Integer. The minimal length at which repeats should be masked. Default w=0.

Value

A boolean vector where TRUE indicates a non-homopolymeric region.

Author(s)

Moritz Gerstung

Examples

```
data(HIVmix)
which(repeatMask(HIVmix))
```

RF *Relative frequencies.*

Description

Convenience function to compute the relative frequencies from a matrix with absolute counts.

Usage

```
RF(freq, total = FALSE)
```

Arguments

freq	A matrix with nucleotide counts.
total	If the nucleotide counts have columns for forward and reverse direction, return each strand sepratatelu (FALSE), or add the two (TRUE).

Value

A matrix with the relative frequencies.

Author(s)

Moritz Gerstung

Examples

```
data(HIVmix)
RF(test(HIVmix))[1:10,]
RF(test(HIVmix), total=TRUE)[1:10,]
```

show,deepSNV-method *Show method for deepSNV objects*

Description

Show method for deepSNV objects

Arguments

object	A deepSNV-class object.

Value

NULL

Author(s)

Moritz Gerstung

Examples

```
## Short example with 2 SNVs at frequency ~10%
regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 3120, stop=3140)
ex <- deepSNV(test = system.file("extdata", "test.bam", package="deepSNV"), control = system.file("extdata", "cont
show(ex)   # show method
plot(ex)   # scatter plot
summary(ex)   # summary with significant SNVs
ex[1:3,]   # subsetting the first three genomic positions
tail(test(ex, total=TRUE))   # retrieve the test counts on both strands
tail(control(ex, total=TRUE))

## Not run: Full example with ~ 100 SNVs. Requires an internet connection, but try yourself.
# regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 2074, stop=3585)
# HIVmix <- deepSNV(test = "http://www.bsse.ethz.ch/cbg/software/deepSNV/data/test.bam", control = "http://www.bs
data(HIVmix) # attach data instead..
show(HIVmix)
plot(HIVmix)
head(summary(HIVmix))
```

summary *Summary of a deepSNV object*

Description

Tabularize significant SNVs by evalutating the p-values of the deepSNV test.

Summary for deepSNV object

Arguments

object	A deepSNV-class object.
sig.level	The desired significance level.
adjust.method	The adjustment method for multiple testing corrections. See p.adjust for details. Set to NULL, for no adjustment. Default "bonferroni".
fold.change	The minimal fold change required of the relative frequency. Default 1.

Value

A data.frame with the following columns:

chr	The chromosome
pos	The position (1-based)
ref	The reference (consensus) nucleotide
var	The variant nucleotide
p.val	The (corrected) p-value
freq.var	The relative frequency of the SNV
sigma2.freq.var	The estimated variance of the frequency
n.tst.fw	The variant counts in the test experiment, forward strand
cov.tst.fw	The coverage in the test experiment, forward strand
n.tst.bw	The variant counts in the test experiment, backward strand
cov.tst.bw	The coverage in the test experiment, backward strand
n.ctrl.fw	The variant counts in the control experiment, forward strand
cov.ctrl.fw	The coverage in the control experiment, forward strand
n.ctrl.bw	The variant counts in the control experiment, backward strand
cov.ctrl.bw	The coverage in the control experiment, backward strand
raw.p.val	The raw p-value

Author(s)

Moritz Gerstung

Examples

```
## Short example with 2 SNVs at frequency ~10%
regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 3120, stop=3140)
ex <- deepSNV(test = system.file("extdata", "test.bam", package="deepSNV"), control = system.file("extdata", "cont
show(ex)   # show method
plot(ex)   # scatter plot
summary(ex)   # summary with significant SNVs
ex[1:3,]   # subsetting the first three genomic positions
tail(test(ex, total=TRUE))   # retrieve the test counts on both strands
tail(control(ex, total=TRUE))

## Not run: Full example with ~ 100 SNVs. Requires an internet connection, but try yourself.
# regions <- data.frame(chr="B.FR.83.HXB2_LAI_IIIB_BRU_K034", start = 2074, stop=3585)
# HIVmix <- deepSNV(test = "http://www.bsse.ethz.ch/cbg/software/deepSNV/data/test.bam", control = "http://www.bs
data(HIVmix) # attach data instead..
show(HIVmix)
plot(HIVmix)
head(summary(HIVmix))
```

`test` *Get test counts*

Description

Convenience function to obtain the test counts from a deepSNV object.

Usage

```
  ## S4 method for signature 'deepSNV'
test(deepSNV, total = FALSE)
```

Arguments

`deepSNV`	a `deepSNV-class` object
`total`	Logical. If true the sum of both strands is returned

Value

A matrix with the absolute frequencies summed over both strands.

Examples

```
data(HIVmix)
test(HIVmix)[1:10,]
test(HIVmix, total=TRUE)[1:10,]
```

trueSNVs *Example .bam data and true SNVs.*

Description

Two .bam alignments as example data sets are downloaded remotely via http. Sequenced were a 1,512 nt fragment of the HIV genome and a mixture (90% + 10%) with another variants. The two sequences were confirmed by Sanger sequencing and stored in the table trueSNVs.

Examples

```
data(HIVmix)
data(trueSNVs)
table(p.adjust(p.val(HIVmix), method="BH") < 0.05, trueSNVs)
```

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