

Package ‘deepSNV’

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Maintainer Moritz Gerstung <moritz.gerstung@ebi.ac.uk>

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Title Detection of subclonal SNVs in deep sequencing data.

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Type Package

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Description This package provides provides quantitative variant callers for detecting subclonal mutations in ultra-deep (>=100x coverage) sequencing experiments. The deepSNV algorithm is used for a comparative setup with a control experiment of the same loci and uses a beta-binomial model and a likelihood ratio test to discriminate sequencing errors and subclonal SNVs. The shearwater algorithm computes a Bayes classifier based on a beta-binomial model for variant calling with multiple samples for precisely estimating model parameters - such as local error rates and dispersion - and prior knowledge, e.g. from variation data bases such as COSMIC.

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Author Niko Beerenwinkel [ths],
 Raul Alcantara [ctb],
 David Jones [ctb],
 Inigo Martincorena [ctb],
 Moritz Gerstung [aut, cre]

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Description

Detection of subclonal SNVs in deep sequencing experiments

Details

This package 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 an overdispersed beta-binomial model. The statistic can be tuned across genomic sites by a shared Dirichlet prior and this package provides procedures for normalizing sequencing data from different runs.

Author(s)

Moritz Gerstung, Wellcome Trust Sanger Institute, <moritz.gerstung@sanger.ac.uk>

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", "control.bam", package="deepSNV"))
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.bsse.ethz.ch/cbg/software/deepSNV/data/control.bam")
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,
  mq = 0,
  s = 2,
  head.clip = 0,
  max.depth = 1e+06,
  verbose = FALSE,
  mask = 0,
  keepflag = 0,
  max.mismatches = NULL
)

```

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'. |
| mq | An optional cutoff for the read mapping quality. Default mq = 0 (no filter). reads with MQ < mq will be discarded. |
| 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. |
| verbose | Boolean. Set to TRUE if you want to get additional output. |
| mask | Integer indicating which flags to filter. Default 0 (no mask). Try 3844 (UN- MAP SECONDARY QCFAIL DUP SUPPLEMENTARY). |

keepflag Integer indicating which flags to keep. Default 0 (no mask). Try 3 (PAIRED|PROPERLY_PAURED).
 max.mismatches Integer indicating maximum NM value to allow in a read. Default NULL (no filter).

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_BRU_K034")
# head(bam)
```

bbb

Bayesian beta-binomial test, codename shearwater

Description

This is the workhorse of the shearwater test. It computes the Bayes factor for each sample, nucleotide and position of the null-model vs. the alternative of a real variant.

Usage

```
bbb(
  counts,
  rho = NULL,
  alternative = "greater",
  truncate = 0.1,
  rho.min = 1e-04,
  rho.max = 0.1,
  pseudo = .Machine$double.eps,
  return.value = c("BF", "P0", "err"),
  model = c("OR", "AND", "adaptive"),
  min.cov = NULL,
  max.odds = 10,
```

```

    mu.min = 1e-06,
    mu.max = 1 - mu.min
)

```

Arguments

| | |
|--------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| counts | An array of nucleotide counts (samples x positions x 10 nucleotides in forward and reverse orientation), typically from loadAllData |
| rho | Disperision factor. If NULL, estimated from the data. |
| alternative | The alternative. Currently only "greater" is implemented. |
| truncate | The model uses a compound control sample which is the sum of all samples with a relative nucleotide frequency below truncate at this locus. Default = 0.1. |
| rho.min | Lower bound for the method of moment estimate of the dispersion factor rho. |
| rho.max | Upper bound for the method of moment estimate of the dispersion factor rho. |
| pseudo | A pseudo count to be added to the counts to avoid problems with zeros. |
| return.value | Return value. Either "BF" for Bayes Factor or "P0" for the posterior probability (assuming a prior of 0.5). |
| model | The null model to use. For "OR" it requires the alternative model to be violated on either of the strands, for "AND" the null is specified such that the error rates of the sample of interest and the compound control sample are identical on both strands. "AND" typically yield many more calls. The most recent addition is "adaptive", which switches from "OR" to "AND", if the coverage is less than min.cov, or if the odds of forward and reverse coverage is greater than max.odds. Default = "OR". |
| min.cov | Minimal coverage to swith from OR to AND, if model is "adaptive" |
| max.odds | Maximal odds before switching from OR to AND if model is "adaptive" and min.cov=NULL. |
| mu.min | Minimum of the error rate mu. |
| mu.max | Maximal error rate mu. |

Value

An [array](#) of Bayes factors

Note

Experimental code, subject to changes

Author(s)

mg14

Examples

```

## Load data from deepSNV example
regions <- GRanges("B.FR.83.HXB2_LAI_IIIB_BRU_K034", IRanges(start = 3120, end=3140))
files <- c(system.file("extdata", "test.bam", package="deepSNV"), system.file("extdata", "control.bam", package="
counts <- loadAllData(files, regions, q=10)

## Run (bbb) computes the Bayes factor
bf <- bbb(counts, model = "OR", rho=1e-4)
vcf <- bf2Vcf(bf, counts, regions, samples = files, prior = 0.5, mvcf = TRUE)

## Compare to deepSNV
bf <- bbb(counts, model = "AND", rho=1e-4)
dpSNV <- deepSNV(test = files[1], control = files[2], regions=regions, q=10)
plot(p.val(dpSNV), bf[,1,]/(1+bf[,1,]), log="xy")

```

betabinLRT

*ShearwaterML***Description**

Maximum likelihood version of Shearwater producing p-values instead of Bayes factors.

Usage

```

betabinLRT(
  counts,
  rho = NULL,
  truncate = 0.05,
  rho.min = 1e-04,
  rho.max = 0.8,
  maxvaf = 0.3,
  mindepth = 10,
  maxtruncate = 0.5
)

```

Arguments

| | |
|----------|-------------------------------------------------------------------------------------------------------------------------------|
| counts | The array of counts typically generated by loadAllData. |
| rho | Use this variable to fix the dispersion parameter to a value of interest. Default: NULL, rho will be estimated from the data. |
| truncate | Samples with variant allele frequencies higher than "truncate" will be excluded from the background error model. |
| rho.min | If rho=NULL, rho will be estimated from the data in the interval [rho.min,rho.max]. |
| rho.max | If rho=NULL, rho will be estimated from the data in the interval [rho.min,rho.max]. |
| maxvaf | Sites with an average rate of mismatches higher than maxvaf will not be considered (e.g. SNPs or reference sites). |

| | |
|-------------|---------------------------------------------------------------------------------------------------------------------|
| mindepth | Minimum coverage required to test a site. |
| maxtruncate | Maximum number of samples that can be excluded from the background error model by truncate for a site to be tested. |

Value

A list with two arrays for P- and Q-values.

Author(s)

Inigo Martincorena and Moritz Gerstung

References

Martincorena I, Roshan A, Gerstung M, et al. (2015). High burden and pervasive positive selection of somatic mutations in normal human skin. *_Science_* (Under consideration).

Examples

```
# code to be added
```

| | |
|--------|-----------------------------------------------------------------------------------------------------------|
| bf2Vcf | <i>Function to create a VCF object with variant calls from an array of Bayes factors.</i> |
|--------|-----------------------------------------------------------------------------------------------------------|

Description

This function thresholds the Bayes factors computed by the shearwater algorithm and creates a [VCF](#) object as output.

Usage

```
bf2Vcf(  
  BF,  
  counts,  
  regions,  
  samples = 1:nrow(counts),  
  err = NULL,  
  mu = NULL,  
  cutoff = 0.05,  
  prior = 0.5,  
  mvcf = TRUE  
)
```


Arguments

| | |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| BF | array of Bayes factors from bbb . |
| counts | array of counts from loadAllData . |
| regions | GRanges with the regions corresponding to counts and BF. |
| samples | vector of samples names. |
| err | Optional matrix of error rates, otherwise recomputed from counts. |
| mu | Optional matrix of relative frequencies, otherwise recomputed from counts. |
| cutoff | Cutoff for the posterior artifact probability below which a variant is considered to be true (default = 0.05) |
| prior | matrix of prior probabilities for finding a true call, typically from makePrior . Alternatively a single fixed number. |
| mvcf | boolean flag, if TRUE compute a large VCF with as many genotype columns as samples. Default TRUE. Otherwise use duplicate rows and only one genotype column. The sample is then provided by the info:PD field. Can be inefficient for large sample sizes. |

Value

A [VCF](#) object

Note

Experimental code, subject to changes

Author(s)

mg14

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

```
consensusSequence(x, ...)  
  
## 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 <code>deepSNV-class</code> object, or a named matrix with nucleotide counts. |
| ... | Additional arguments passed to methods. |
| 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 <code>het.cut</code> are reported. Default <code>haploid = TRUE</code> . |
| het.cut | Heterozygous cutoff. If <code>haploid = FALSE</code> , report all nucleotides with relative frequency larger than <code>het.cut</code> . Default = 0.333. |

Value

A `DNAStrng` 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

```
control(deepSNV, ...)

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

Arguments

| | |
|---------|------------------------------------------------------|
| deepSNV | a <code>deepSNV-class</code> object |
| ... | Additional param passed to specific methods |
| 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 | <i>Get coordinates</i> |
|-------------|------------------------|

Description

Convenience function to get the coordinates from a deepSNV object.

Usage

```
coordinates(deepSNV, ...)

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

Arguments

| | |
|---------|---------------------------------------------|
| deepSNV | a deepSNV-class object |
| ... | Additional param passed to specific methods |

Value

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

Examples

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

| | |
|--------|----------------------------|
| counts | <i>Example count table</i> |
|--------|----------------------------|

Description

A table with counts of the HIVmix data set. Used for minimal unit testing.

Examples

```
data("counts", package="deepSNV")
countsFromBam <- bam2R(file = system.file("extdata", "test.bam", package="deepSNV"), chr="B.FR.83.HXB2_LAI_IIIB_E
all(counts == countsFromBam)
```

| | |
|------------|-----------------------------------------------|
| dbetabinom | <i>Beta-binomial probability distribution</i> |
|------------|-----------------------------------------------|

Description

Beta-binomial probability distribution

Usage

```
dbetabinom(x, n, mu, rho, log = FALSE)
```

Arguments

| | |
|-----|---------------------------|
| x | Counts |
| n | Size |
| mu | Probability |
| rho | Dispersion. rho in (0,1) |
| log | Return logarithmic values |

Value

d

Author(s)

mg14

| | |
|---------|-----------------------------------------------------------------------------|
| deepSNV | <i>Test two matched deep sequencing experiments for low-frequency SNVs.</i> |
|---------|-----------------------------------------------------------------------------|

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_1^2 . 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

```

deepSNV(test, control, ...)

## S4 method for signature 'matrix,matrix'
deepSNV(test,control, alternative = c('greater', 'less', 'two.sided'), dirichlet.prior = NULL, pseudo.

## 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. |
| ... | Additional arguments. |
| alternative | The alternative to be tested. One of greater, less, or two.sided. |
| 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. |
| combine.method | The method to combine p-values. One of "fisher" (default), "max", or "average". See p.combine for details. |
| over.dispersion | A numeric factor for the over.dispersion, if the model is beta-binomial. Default 100. |
| model | Which model to use. Either "bin", or "betabin". Default "bin". |
| 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 argument 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. |

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", "control.bam", package="deepSNV"))
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.bsse.ethz.ch/cbg/software/deepSNV/data/control.bam")
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 positive 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

pseudo.count The pseudo count if used)

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", "control.bam", package="deepSNV"))
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.bsse.ethz.ch/cbg/software/deepSNV/data/control.bam")
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 separately 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

```
estimateDirichlet(control)

## 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 $X_{i,j} \sim \text{BB}(n_i; \alpha, \beta_{ij})$, 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

```
estimateDispersion(test, control, ...)

## 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. |
| ... | Additional param passed to specific methods |
| 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)
```

estimateRho

Helper function for estimating the dispersion factor rho

Description

It uses a method of moments approximation to estimate rho from the variances of the relative frequencies nu across samples

Usage

```
estimateRho(x, mu, ix, pseudo.rho = .Machine$double.eps)
```

Arguments

| | |
|------------|-------------------------------------------------------------------------------------------------------------------|
| x | counts |
| mu | relative frequency across all samples |
| ix | index indicating the set of samples to use (typically indicating those with relative frequency smaller than 0.1). |
| pseudo.rho | a pseudo count added to each sample to avoid problems with zeros. Default = <code>.Machine\$double.eps</code> |

Value

rho

Note

Experimental code, subject to changes

Author(s)

mg14

Extract

Subsetting for deepSNV objects.

Description

Subsetting for deepSNV objects.

Usage

```
## S4 method for signature 'deepSNV,ANY,ANY,ANY'  
x[i, j]
```

Arguments

| | |
|---|-----------------------------------------|
| x | A deepSNV-class object. |
| i | Row indices. |
| j | Column (nucleotide) indices. |

Value

A [deepSNV-class](#) object.

Author(s)

Moritz Gerstung

Examples

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

| | |
|-------------|-----------------------------------------------------------|
| loadAllData | <i>Function to load all data from a list of bam files</i> |
|-------------|-----------------------------------------------------------|

Description

This function uses the parallel package and the bam2R interface to load all nucleotide counts from a list of bam files and a set of regions into a large array.

Usage

```
loadAllData(files, regions, ..., mc.cores = 1)
```

Arguments

| | |
|----------|-----------------------------------------------------------------|
| files | A character vector with the paths to all bam files |
| regions | Either a GRanges or data.frame with the coordinates of interest |
| ... | Arguments passed to bam2R |
| mc.cores | Number of cores used for loading, default = 1 |

Value

counts

Note

Experimental code, subject to changes

Author(s)

mg14

| | |
|-----------|-------------------------------------------------|
| makePrior | <i>Compute a prior from a COSMIC VCF object</i> |
|-----------|-------------------------------------------------|

Description

This function computes the prior probability of detecting a true variant from a variation data base. It assumes a VCF file with a CNT slot for the count of a given base substitution. Such a VCF file can be downloaded at <ftp://ngs.sanger.ac.uk/production/cosmic/>. The prior probability is simply defined as $\pi_i \cdot \text{CNT}[i] / \sum(\text{CNT})$. On sites with no count, a background probability of π_0 is used.

Usage

```
makePrior(COSMIC, regions, pi.gene = 0.1, pi.backgr = 1e-04)
```

Arguments

| | |
|-----------|---------------------------------------------------------------------------------------------------------------------------|
| COSMIC | A VCF object from COSMIC VCF export. |
| regions | A GRanges object with the regions (gene) of interest. |
| pi.gene | Probability that a gene is mutated |
| pi.backgr | Background probability of a locus being mutated. Default 1e-4, corresponding to an expected value of 1 SNV per 1e4 bases. |

Value

A vector of prior values with length given by the length of the regions GRanges object.

Note

Experimental code, subject to changes

Author(s)

mg14

Examples

```
## Make prior (not run)
#COSMIC <- readVcf("PATHTO/CosmicCodingMuts_v64_02042013_noLimit.vcf.gz", genome="GChr37")
#prior <- makePrior(COSMIC[info(COSMIC)$GENE=="TP53"], regions=GRanges(17, IRanges(7571720,7578811)))
#plot(prior[,1], type="h")
```

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)
```

`mcChunk`*Little helper function to split the count objects into a smaller digestible chunks and run function FUN on each subset*

Description

Little helper function to split the count objects into a smaller digestible chunks and run function FUN on each subset

Usage

```
mcChunk(FUN, X, split = 250, mc.cores = 1, ...)
```

Arguments

| | |
|-----------------------|----------------------------------------------------|
| <code>FUN</code> | The function to call on each chunk |
| <code>X</code> | The object to be subsetted using <code>[,i]</code> |
| <code>split</code> | The size of each chunk |
| <code>mc.cores</code> | The number of cores to use |
| <code>...</code> | Additional arguments passed to FUN |

Value

The value of FUN

Note

Experimental code, subject to changes

Author(s)

mg14

| | |
|-----------|-------------------------------------|
| normalize | <i>Normalize nucleotide counts.</i> |
|-----------|-------------------------------------|

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.

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 <code>link{deepSNV-class}</code> object, otherwise a matrix with nucleotide counts. |
| ... | Parameters passed to loess . |
| round | Logical. Should normalized counts be rounded to integers? Default=TRUE |

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_4^2 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. "negfisher" is the negative of Fisher's method using $1 - F(1 - P_1, 1 - P_2)$, where F is the combination function of Fisher's method; for small P_1, P_2 , the result is very similar to method="average". Fisher's method behaves a bit like a logical AND of the joint null-hypothesis, whereas negative Fisher is like an OR.

Usage

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

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

```
p.val(deepSNV, ...)
```

S4 method for signature 'deepSNV'

```
p.val(deepSNV)
```

Arguments

deepSNV a [deepSNV-class](#) object

... Additional param passed to specific methods

Value

A matrix with the p-values.

Examples

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

pbetabinom *Cumulative beta-binomial probability distribution*

Description

Cumulative beta-binomial probability distribution

Usage

```
pbetabinom(x, n, mu, rho, log = FALSE)
```

Arguments

x Counts

n Sample size

mu Probability

rho Dispersion. rho in (0,1)

log Return logarithmic values

Value

Probability

Author(s)

mg14

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)
```

pi

Example prior

Description

Prior from COSMIC v63 for the TP53 gene

Examples

```
data("pi", package="deepSNV")
plot(pi[,1], type="h")
```

`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 logarithmic 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

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

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", "control.bam", package="deepSNV"))
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.bsse.ethz.ch/cbg/software/deepSNV/control/control.bam")
data(HIVmix) # attach data instead..
show(HIVmix)
plot(HIVmix)
head(summary(HIVmix))
```

| | |
|-----------|------------------------------------------------------------------------------------------------------|
| qvals2Vcf | <i>Function to create a VCF object with variant calls from an array of q-values.</i> |
|-----------|------------------------------------------------------------------------------------------------------|

Description

This function thresholds the q-values computed by the shearwater algorithm and creates a [VCF](#) object as output.

Usage

```
qvals2Vcf(
  qvals,
  counts,
  regions,
  samples = 1:nrow(counts),
  err = NULL,
  mu = NULL,
  cutoff = 0.05,
  mvcf = TRUE
)
```

Arguments

| | |
|---------|-----------------------------------------------------------------------------|
| qvals | array of q-values from betabinLRT . |
| counts | array of counts from loadAllData . |
| regions | GRanges with the regions corresponding to counts and qvals. |

| | |
|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>samples</code> | vector of samples names. |
| <code>err</code> | Optional matrix of error rates, otherwise recomputed from counts. |
| <code>mu</code> | Optional matrix of relative frequencies, otherwise recomputed from counts. |
| <code>cutoff</code> | Cutoff for the q-values below which a variant is considered to be true (default = 0.05) |
| <code>mvcf</code> | boolean flag, if TRUE compute a large VCF with as many genotype columns as samples. Default TRUE. Otherwise use duplicate rows and only one genotype column. The sample is then provided by the info:PD field. Can be inefficient for large sample sizes. |

Value

A [VCF](#) object

Note

Experimental code, subject to changes

Author(s)

mg14

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 | <i>Mask homopolymeric repeats.</i> |
|------------|------------------------------------|

Description

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

Usage

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

Arguments

| | |
|-------|----------------------------------------------------------------------------------------------------------------|
| x | An object. Either a <code>deepSNV-class</code> object or a <code>DNAStrng</code> with the nucleotide sequence. |
| ... | Additional param passed to specific methods |
| w | Integer. The minimal length at which repeats should be masked. Default <code>w=0</code> . |
| flank | Boolean. Indicates whether the sites adjacent to the repeat should also be masked. |

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 separately (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

Usage

```
## S4 method for signature 'deepSNV'
show(object)
```

Arguments

| | |
|--------|--------------------------------------|
| object | A <code>deepSNV-class</code> 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", "control.bam", package="deepSNV"))
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.bsse.ethz.ch/cbg/software/deepSNV/control/control.bam")
data(HIVmix) # attach data instead..
show(HIVmix)
plot(HIVmix)
head(summary(HIVmix))
```

summary

*Summary of a deepSNV object***Description**

Tabularize significant SNVs by evaluating the p-values of the [deepSNV](#) test.

Usage

```
## S4 method for signature 'deepSNV'
summary(
  object,
  sig.level = 0.05,
  adjust.method = "bonferroni",
  fold.change = 1,
  value = c("data.frame", "VCF")
)
```

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 | String. The type of the returned object. Either "data.frame" for a data.frame (default) or "VCF" for an ExtendedVCF-class object. |

Value

If value="data.frame", 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 |

If value = "VCF", this functions returns a [VCF-class](#) object with the following entries: FIXED:

| | |
|-------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| REF | Reference allele in control sample. Note that deletions in the control sample will be reported like insertions, e.g. if the consensus of the control is A,- at positions 1 and 2 (relative to the reference) and the test was A,A, then this would be denoted as REF="A" and VAR="AA" with coordinate IRanges(1,2). This may cause ambiguities when the VCF object is written to text with <code>writeVcf()</code> , which discards the width of the coordinate, and this variant remains indistinguishable from an insertion to the <code>_reference_</code> genome. |
| VAR | Variant allele in test sample |
| QUAL | $-10 \cdot \log_{10}(\text{raw.p.val})$ |
| INFO: | |
| VF | Variant frequency. Variant allele frequency in the test minus variant allele frequency in the control. |
| VFV | Variant frequency variance. Variance of the variant frequency; can be thought of as confidence interval. |

GENO (one column for test and one column for control):

| | |
|-----|-----------------------|
| FW | Forward allele count |
| BW | Backward allele count |
| DFW | Forward read depth |
| DBW | Backward read depth |

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", "control.bam", package="deepSNV"))
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.bsse.ethz.ch/cbg/software/deepSNV/data/control.bam")
data(HIVmix) # attach data instead..
show(HIVmix)
plot(HIVmix)
head(summary(HIVmix))
```

| | |
|------|------------------------|
| test | <i>Get test counts</i> |
|------|------------------------|

Description

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

Usage

```
test(deepSNV, ...)

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

Arguments

deepSNV a `deepSNV-class` object
... Additional param passed to specific methods
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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