Package 'h5vc'

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Description This package contains functions to interact with tally data from NGS experiments that is stored in HDF5 files.

License GPL (>= 3)

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h5vc-package *Managing alignment tallies using a hdf5 backend*

Description

This package contains functions to interact with tally data from NGS experiments that is stored in HDF5 files. For detail see vignettes shipped with this package.

Details

Package:	h5vc
Type:	Package
Version:	1.0.4
Date:	2013-10-11
License:	GPL (>= 3)

This package is desgned to facilitate the analysis of genomics data through tallies stored in a HDF5 file. Within a HDF5 file the tally is simply a table of bases times genomic positions listing for each position the count of each base observed as a mismatch in the sample at any given position. Strand and sample are additional dimension in this array, which leads to a 4D-array called 'Counts'. The total coverage is stored in a separate array of 3 dimensions (Sample x Strand x Genomic Position)

called 'Coverages', there is a 3 dimensional 'Deletions' array and a 1D-vector encoding the reference base ('Reference'). Those 4 arrays are stored as datasets within a HDF5 tally file in which the group-structure of the tally file encodes for the organisatorial levels of 'Study' and 'Chromosome'. For details on the layout of HDF5 files visit (http://www.hdfgroup.org), a short description is given in the vignettes.

Creating those HDF5 tally files can be accomplished from within R or through a Python script that will generate a tally file from a set of .bam files. The workflow is described in the vignettes `h5vc.creating.tallies` and `h5vc.creating.tallies.within.R`.

Author(s)

Paul Pyl Maintainer: Paul Pyl pyl@embl.de

`applyTallies` *Preparing the results of tallyBAM for writing to an HDF5 tally file*

Description

This function tallies a set of bam files and prepares the data for writing to an HDF5 tally file.

Usage

```
applyTallies( bamfiles, chrom, start, stop, q=25, ncycles = 0, max.depth=1000000, prepForHDF5 = TRUE, re
```

Arguments

`bamfiles`	A character vector of filenames of the bam files that should be tallies. Note that for writing to an HDF5 file the order of this vector must match the order of the Column field in the sampledata object that corresponds to the dataset - see `setSampleData` for details.
`prepForHDF5`	Boolean flag to specify whether the data shall be structured for compatibility with the HDF5 tally file format. See the details section of this manual page.
`reference`	A DNAString object containing the reference sequence corresponding to the region that is described in the counts array – if this is `NULL` a consensus vote will be used to estimate the reference at any given position, this means you cannot detect variants with AF >= 0.5 anymore
`chrom`	Chromosome in which to tally
`start`	First position of the tally
`stop`	Last position of the tally
`q`	quality cut-off for considering a base call
`ncycles`	number of sequencing cycles form the front and back of the read that should be considered unreliable - used for stratifying the nucleotide counts
`max.depth`	only tally a position if there are less than this many reads overlapping it - can prevent long runtimes in unreliable regions

Details

This is a wrapper function for applying tallyBAM to a set of bam files specified in the bamfiles argument. If prepForHDF5 is not true the result is equivalent to calling tallyBAM with lapply on the file names, otherwise the resulting data structure has the same layout as the return value of h5readBlock and can be written to an HDF5 tally file directly. The order or samples along the sample dimension is the same as the order of the file names (i.e. the order of the bamfiles argument).

Value

A list with slots containing the Counts,Coverages,Deletions and Reference datasets for the given sample if prepForHDF5 is true, a list of 3D-arrays (Nucleotide x Strand x Position) otherwise.

Author(s)

Paul Pyl

Examples

```
library(h5vc)
library(BSgenome.Hsapiens.UCSC.hg19)
files <- c("NRAS.AML.bam","NRAS.Control.bam")
bamFiles <- file.path( system.file("extdata", package = "h5vcData"), files)
chrom = "1"
startpos <- 115247090
endpos <- 115259515
theData <- applyTallies( bamFiles, reference = Hsapiens[["chr1"]][startpos:endpos], chr = chrom, start = startpos,
str(theData)
```

---

batchTallies *Tallying bam files in parallel using* BatchJobs *on high performance compute clusters (HPC)*

Description

These function tally a set of bam files in blocks spanning a specified region and write the results to an HDF5 tally file; uses BatchJobs for parallel computation on HPCs

Usage

```
batchTallyParam(
  bamFiles,
  destination,
  group,
  chrom, start, stop,
  blocksize = 100000,
  registryDir = tempdir(),
 resources = list("queue" = "research-rh6", "memory"="4000", "ncpus"="4", walltime="90:00"),
```

```
  q=25, ncycles = 0, max.depth=1000000,
  reference = NULL,
  sleep = 5
)

batchTallies( confList = batchTallyParam() )

rerunBatchTallies( confList, tryCollect = TRUE )

collectTallies(blocks, confList, registries )
```

Arguments

bamFiles	A character vector of filenames of the bam files that should be tallies. Note that for writing to an HDF5 file the order of this vector must match the order of the Column field in the sampledata object that corresponds to the dataset - see setSampleData for details.
reference	A DNAString object containing the reference sequence corresponding to the region that is to be tallied – if this is NULL a consensus vote will be used to estimate the reference at any given position, this means you cannot detect variants with AF >= 0.5 anymore – especially when tallying more than one bamFile you really should specify this
destination	Filename of the HDF5 tally file that will be written to – this needs to contain all the groups and datasets already – see prepareTallyFile for details
group	Location within the tally file where the data will be written – e.g. "/ExampleStudy/22"
chrom	Chromosome in which to tally
start	First position of the tally
stop	Last position of the tally
q	quality cut-off for considering a base call
ncycles	number of sequencing cycles form the front and back of the read that should be considered unreliable - used for stratifying the nucleotide counts
max.depth	only tally a position if there are less than this many reads overlapping it - can prevent long runtimes in unreliable regions
blocksize	Size of the blocks in bases that the tallying will be performed in, this influences the number of jobs send to the cluster
registryDir	Directory in which the registries created by BatchJobs wil be held, this can be temporary since we delete them when we are done
resources	A named list specifying the resource requirements of the cluster jobs, this must contain names for the fields specified in the cluster configuration file – see the documentation of BatchJobs for details
confList	A configuration list as returned by a call to batchTallyParam()
sleep	Number of seconds to sleep before checking if blocks are finshed, increase this if you have large blocks and find the output of batchTallies to verbose
tryCollect	Boolean flag specifying whether the rerunBatchTallies function should try to collect data from the specified registries before re-submitting.

blocks	data.frame defining blocks to tally in, result of a cal to defineBlocks
registries	A list mapping registry IDs to the work paths of the corresponding registries

Details

This is a wrapper function for applying tallyBAM to a set of bam files specified in the bamFiles argument. The order or samples along the sample dimension is the same as the order of the file names (i.e. the order of the bamfiles argument). The function uses BatchJobs to dispatch tallying in blocks along the genome to a HPC and collects the results and writes them into the HDF5 tally file specified in the destination parameter.

rerunBatchTallies can be used to re-submit failed blocks.

collectTallies can be used to manually collect tally data from the registries created by batchTallies

Value

[None] – prints progress messages along the way.

Author(s)

Paul Pyl

Examples

```
## Not run:
library(h5vc)
files <- c("NRAS.AML.bam","NRAS.Control.bam")
bamFiles <- file.path( system.file("extdata", package = "h5vcData"), files)
chrom = "1"
startpos <- 115247090
endpos <- 115259515
batchTallies( batchTallyParam(bamFiles, chrom, startpos, endpos) )

## End(Not run)
```

---

binGenome *Function for binning a genome.*

Description

Function for generating a GRanges representation of a binning of the genome given in the reference object.

Usage

```
binGenome(reference, binsize = 1e+06, chroms = seqnames(reference))
```

Arguments

reference	A BSgenome object.
binsize	Size of bins along the genome.
chroms	Which chromosomes to use, defaults to all chromosome described as seqnames of the reference object.

Details

This function creates a GRanges object that represents bins of size binsize along the genome represented by the reference object.

Value

A GRanges object that represents bins of size binsize along the genome represented by the reference object, includes special handling of chromosomes shorter than binsize and the last bin of each chromosome.

Author(s)

Paul Theodor Pyl

See Also

defineBlocks

Examples

```
library(BSgenome.Hsapiens.NCBI.GRCh38)
bins <- binGenome(Hsapiens, binsize = 100e6, chroms = c("1","2","3","X","MT"))
bins
```

---

binnedAFs *Estimate allelic frequency distributions in bins along the genome*

Description

This function is used to give estimates of the ditribution of observed allelic freuqencies in a regions of the genome, use in conjunction with h5dapply

Usage

```
binnedAFs(data, sampledata, normalise = TRUE, binWidth = 0.05, minCov = 10, minCount = 2)
```

Arguments

data	A list object returned by a call to h5dapply or h5readBlock.
sampledata	Sample metadata describing the cohort, can be extracted from an HDF5 tally file using the getSampleData function.
normalise	Boolean flag to specify whether the counts or percentages of observed allelic frequencies should be returned.
binWidth	Width of bins in allelic frequency space, defaults to 0.05.
minCov	Minimum required coverage for a position to be considered.
minCount	Minimum required number of mismatches for a position to be considered.

Value

A matrix of AF bins times samples.

Author(s)

Paul Theodor Pyl

Examples

```
library(h5vc)
tallyFile <- system.file( "extdata", "example.tally.hfs5", package = "h5vcData" )
sampleData <- getSampleData( tallyFile, "/ExampleStudy/16" )
afs <- h5dapply(
  filename = tallyFile,
  group = "/ExampleStudy/16",
  names = c("Counts", "Coverages"),
  range = c(29e6, 29.05e6),
  blocksize = 1e4,
  FUN = binnedAFs,
  sampledata = sampleData
)
afs[[3]]
```

---

callVariants *Variant calling*

Description

These functions implement various attempts at variant calling.

Usage

```
callVariantsPaired( data, sampledata, cl = vcConfParams() )

vcConfParams(
  minStrandCov = 5,
  maxStrandCov = 200,
  minStrandAltSupport = 2,
  maxStrandAltSupportControl = 0,
  minStrandDelSupport = minStrandAltSupport,
  maxStrandDelSupportControl = maxStrandAltSupportControl,
  minStrandInsSupport = minStrandAltSupport,
  maxStrandInsSupportControl = maxStrandAltSupportControl,
  minStrandCovControl = 5,
  maxStrandCovControl = 200,
  bases = 5:8,
  returnDataPoints = TRUE,
  annotateWithBackground = TRUE,
  mergeCalls = TRUE,
  mergeAggregator = mean,
  pValueAggregator = max
)
```

Arguments

`data`	A `list` with elements `Counts` (a 4d `integer` array of size [1:12, 1:2, 1:k, 1:n]), `Coverage` (a 3d `integer` array of size [1:2, 1:k, 1:n]), `Deletions` (a 3d `integer` array of size [1:2, 1:k, 1:n]), `Reference` (a 1d `integer` vector of size [1:n]) – see Details.
`sampledata`	A `data.frame` with `k` rows (one for each sample) and columns `Type`, `Column` and (`SampleGroup` or `Patient`). The tally file should contain this information as a group attribute, see `getSampleData` for an example.
`cl`	A list with parameters used by the variant calling functions. Such a list can be produced, for instance, by a call to `vcConfParams`.
`minStrandCov`	Minimum coverage per strand in the case sample.
`maxStrandCov`	Maximum coverage per strand in the case sample.
`minStrandCovControl`	Minimum coverage per strand in the control sample.
`maxStrandCovControl`	Maximum coverage per strand in the control sample.
`minStrandAltSupport`	Minimum support for the alternative allele per strand in the case sample. This should be 1 or higher.
`maxStrandAltSupportControl`	Maximum support for the alternative allele per strand in the control sample. This should usually be 0.

minStrandDelSupport
: Minimum support for the deletion per strand in the case sample. This should be 1 or higher.

maxStrandDelSupportControl
: Maximum support for the deletion per strand in the control sample. This should usually be 0.

minStrandInsSupport
: Minimum support for the insertion per strand in the case sample. This should be 1 or higher.

maxStrandInsSupportControl
: Maximum support for the insertion per strand in the control sample. This should usually be 0.

bases
: Indices for subsetting in the bases dimension of the Counts array, 5:8 extracts only those calls made in the middle one of the sequencing cycle bins.

returnDataPoints
: Boolean flag to specify that a data.frame with the variant calls should be returned, otherwise only position are returned as a numeric vector. If `returnDataPoints == FALSE` only the variant positions are returned.

annotateWithBackground
: Boolean flag to specify that the background mismatch / deletion frequency estimated from all control samples in the cohort should be added to the output. A simple binomial test will be performed as well. Only usefull if `returnDataPoints == TRUE`

mergeCalls
: Boolean flag to specify that adjacent calls should be merged where appropriate (used by `callDeletionsPaired`). Only usefull applied if `returnDataPoints == TRUE`

mergeAggregator
: Aggregator function for merging adjacent calls, defaults to `mean`, which means that a deletion larger than 1bp will be annotated with the means of the counts and coverages

pValueAggregator
: Aggregator function for combining the p-values of adjacent calls when merging, defaults to `max`. Is only applied if `annotateWithBackground == TRUE`

Details

`data` is a list of datasets which has to at least contain the `Counts` and `Coverages` for variant calling respectively `Deletions` for deletion calling. This list will usually be generated by a call to the `h5dapply` function in which the tally file, chromosome, datasets and regions within the datasets would be specified. See `?h5dapply` for specifics. In order for `callVariantsPaired` to return the correct locations of the variants there must be the `h5dapplyInfo` slot present in `data` as well. This is itself a list (being automatically added by `h5dapply` and `h5readBlock` respectively) and contains the slots `Group` (location in the HDF5 file) and `Blockstart`, which are used to set the chromosome and the genomic positions of variants.

`vcConfParams` is a helper function that builds a set of variant calling parameters as a list. This list is provided to the calling functions e.g. `callVariantsPaired` and influences their behavior.

callVariantsPaired implements a simple pairwise variant callign approach applying the filters specified in cl, and might additionally computes an estimate of the background mismatch rate (the mean mismatch rate of all samples labeled as 'Control' in the sampledata and annotate the calls with p-values for the binom.test of the observed mismatch counts and coverage at each of the samples labeled as 'Case'.

Value

The result is either a list of positions with SNVs / deletions or a data.frame containing the calls themselves which might contain annotations. Adjacent calls might be merged and calls might be annotated with p-values depending on configuration parameters.

When the configuration parameter returnDataPoints is FALSE the functions return the positions of potential variants as a list containing one integer vector of positions for each sample, if no positions were found for a sample the list will contain NULL instead. In the case of returnDatapoints == TRUE the functions return either NULL if no poisitions were found or a data.frame with the following slots:

Chrom	The chromosome the potential variant / deletion is on
Start	The starting position of the variant / deletion
End	The end position of the variant / deletions (equal to Start for SNVs and single basepair deletions)
Sample	The Case sample in which the variant was observed
altAllele	The alternate allele for SNVs (skipped for deletions, would be "-")
refAllele	The reference allele for SNVs (skipped for deletions since the tally file might not contain all the information necessary to extract it)
caseCountFwd	Support for the variant in the Case sample on the forward strand
caseCountRev	Support for the variant in the Case sample on the reverse strand
caseCoverageFwd	Coverage of the variant position in the Case sample on the forward strand
caseCoverageRev	Coverage of the variant position in the Case sample on the reverse strand
controlCountFwd	Support for the variant in the Control sample on the forward strand
controlCountRev	Support for the variant in the Control sample on the reverse strand
controlCoverageFwd	Coverage of the variant position in the Control sample on the forward strand
controlCoverageRev	Coverage of the variant position in the Control sample on the reverse strand

If the annotateWithBackground option is set the following extra columns are returned

backgroundFrequencyFwd	The averaged frequency of mismatches / deletions at the position of all samples of type Control on the forward strand

backgroundFrequencyRev
: The averaged frequency of mismatches / deletions at the position of all samples of type Control on the reverse strand

pValueFwd
: The p.value of the test binom.test(caseCountFwd, caseCoverageFwd, p = backgroundFrequencyFwd, alternative = "greater")

pValueRev
: The p.value of the test binom.test(caseCountRev, caseCoverageRev, p = backgroundFrequencyRev, alternative = "greater")

The function callDeletionsPaired merges adjacent single-base deletion calls if the option mergeCalls is set to TRUE, in that case the counts and coverages (e.g. caseCountFwd) are aggregated using the function supplied in the mergeAggregator option of the configuration list (defaults to mean) and the p-values pValueFwd and pValueFwd (if annotateWithBackground is TRUE), are aggregated using the function supplied in the pValueAggregator option (defaults to max).

Author(s)

Paul Pyl

Examples

```
library(h5vc) # loading library
tallyFile <- system.file( "extdata", "example.tally.hfs5", package = "h5vcData" )
sampleData <- getSampleData( tallyFile, "/ExampleStudy/16" )
position <- 29979629
windowsize <- 1000
vars <- h5dapply( # Calling Variants
  filename = tallyFile,
  group = "/ExampleStudy/16",
  blocksize = 500,
  FUN = callVariantsPaired,
  sampledata = sampleData,
  cl = vcConfParams(returnDataPoints=TRUE),
  names = c("Coverages", "Counts", "Reference", "Deletions"),
  range = c(position - windowsize, position + windowsize)
)
vars <- do.call( rbind, vars ) # merge the results from all blocks by row
vars # We did find a variant
```

---

callVariantsFisher *Paired variant calling using fisher tests*

Description

This function implements a simple paired variant calling strategy based on the fisher test

Usage

```
callVariantsPairedFisher(data, sampledata, pValCutOff = 0.05, minCoverage = 5, mergeDels = TRUE, mergeA
```

Arguments

data	A list with elements Counts (a 4d integer array of size [1:12, 1:2, 1:k, 1:n]), Coverage (a 3d integer array of size [1:2, 1:k, 1:n]), Reference (a 1d integer vector of size [1:n]) – see Details.
sampledata	A data.frame with k rows (one for each sample) and columns Type, Column and (Group or Patient). The tally file should contain this information as a group attribute, see getSampleData for an example.
pValCutOff	Maximum allowed p-Value for the fisher test on contingency matrix matrix(c(caseCounts, caseCoverage, controlCounts, controlCoverage), nrow=2).
minCoverage	Required coverage in both sample for a call to be made
mergeDels	Boolean flag specifying whether adjacent deletions should be merged
mergeAggregator	Which function to use for aggregating the values associated with adjacent deletions that are being merged

Details

data is a list which has to at least contain the Counts, Coverages and Reference datasets. This list will usually be generated by a call to the h5dapply function in which the tally file, chromosome, datasets and regions within the datasets would be specified. See h5dapply for specifics.

callVariantsPairedFisher implements a simple pairwise variant callign approach based on using the fisher.test on the following contingency matrix:

caseSupport	caseCoverage - caseSupport
conttrolSupport	controlCoverage - controlSupport

The results are filtered by pValCutOff and minCoverage.

Value

The return value is a data.frame with the following slots:

Chrom	The chromosome the potential variant is on
Start	The starting position of the variant
End	The end position of the variant
Sample	The Case sample in which the variant was observed
refAllele	The reference allele
altAllele	The alternate allele
caseCount	Support for the variant in the Case sample
caseCoverage	Coverage of the variant position in the Case sample
controlCount	Support for the variant in the Control sample
controlCoverage	Coverage of the variant position in the Control sample
pValue	The p.value of the fisher.test

Author(s)

Paul Pyl

Examples

```
library(h5vc) # loading library
tallyFile <- system.file( "extdata", "example.tally.hfs5", package = "h5vcData" )
sampleData <- getSampleData( tallyFile, "/ExampleStudy/16" )
position <- 29979629
windowsize <- 2000
vars <- h5dapply( # Calling Variants
  filename = tallyFile,
  group = "/ExampleStudy/16",
  blocksize = 1000,
  FUN = callVariantsPairedFisher,
  sampledata = sampleData,
  pValCutOff = 0.1,
  names = c("Coverages", "Counts", "Reference"),
  range = c(position - windowsize, position + windowsize),
  verbose = TRUE
)
vars <- do.call(rbind, vars)
vars
```

---

callVariantsSingle *Single sample variant calling*

Description

A simple single sample variant calling function (calling SNVs and deletions)

Usage

```
callVariantsSingle( data, sampledata, samples = sampledata$Sample, errorRate = 0.001, minSupport = 2, m
```

Arguments

data	A list with elements Counts (a 4d integer array of size [1:12, 1:2, 1:k, 1:n]), Coverage (a 3d integer array of size [1:2, 1:k, 1:n]), Deletions (a 3d integer array of size [1:2, 1:k, 1:n]), Reference (a 1d integer vector of size [1:n]) – see Details.
sampledata	A data.frame with k rows (one for each sample) and columns Column and (Sample. The tally file should contain this information as a group attribute, see getSampleData for an example.
samples	The samples on which variants should be called, by default all samples specified in sampledata are used
errorRate	The expected error rate of the sequencing technology that was used, for illumina this should be 1/1000

minSupport	minimal support required for a position to be considered variant
minAF	minimal allelic frequency for an allele at a position to be cosidered a variant
minStrandSupport	minimal per-strand support for a position to be considered variant
mergeDels	Boolean flag to specify that adjacent deletion calls should be merged
aggregator	Aggregator function for merging statistics of adjacent deletion calls, defaults to mean, which means that a deletion larger than 1bp will be annotated with the means of the counts and coverages etc.

Details

data is a list of datasets which has to at least contain the Counts and Coverages for variant calling respectively Deletions for deletion calling (if Deletions is not present no deletion calls will be made). This list will usually be generated by a call to the h5dapply function in which the tally file, chromosome, datasets and regions within the datasets would be specified. See h5dapply for specifics.

callVariantsSingle implements a simple single sample variant callign approach for SNVs and deletions (if Deletions is a dataset present in the data parameter. The function applies three essential filters to the provided data, requiring:

- minSupport total support for the variant at the position - minStrandSupport support for the variant on each strand - an allele freqeuncy of at least minAF (for pure diploid samples this can be set relatively high, e.g. 0.3, for calling potentially homozygous variants a value of 0.8 or higher might be used)

Calls are annotated with the p-Value of a binom.test of the present support and coverage given the error rate provided in the errorRate parameter, no filtering is done on this annotation.

Adjacent deletion calls are merged based in the value of the mergeDels parameter and their statistics are aggregated with the function supplied in the aggregator parameter.

Value

This function returns a data.frame containing annotated calls with the following slots:

Chrom	The chromosome the potential variant / deletion is on
Start	The starting position of the variant / deletion
End	The end position of the variant / deletions (equal to Start for SNVs and single basepair deletions)
Sample	The sample in which the variant was called
altAllele	The alternate allele for SNVs (deletions will have a "-" in that slot)
refAllele	The reference allele for SNVs (deletions will have the deleted sequence here as extracted from the Reference dataset, if the tally file contains a sparse representation of the reference, i.e. only positions with mismatches show a reference value the missing values are substituted with "N"'s. It is strongly suggested to write the whole reference into the tally file prior to deletion calling - see writeReference for details)
SupFwd	Support for the variant in the sample on the forward strand

SupRev	Support for the variant in the sample on the reverse strand
CovFwd	Coverage of the variant position in the sample on the forward strand
CovRev	Coverage of the variant position in the sample on the reverse strand
AF_Fwd	Allele frequency of the variant in the sample on the forward strand
AF_Rev	Allele frequency of the variant in the sample on the reverse strand
Support	Total Support of the variant - i.e. SupFwd + SupRev
Coverage	Total Coverage of the variant position - i.e. CovFwd + CovRev
AF	Total allele frequency of the variant, i.e. Support / Coverage
fBackground	Background frequency of the variant in all samples but the one the variant is called in
pErrorFwd	Probablity of the observed support and coverage given the error rate on the forward strand
pErrorRev	Probablity of the observed support and coverage given the error rate on the reverse strand
pError	Probablity of the observed support and coverage given the error rate on both strands combined
pError	Coverage of the variant position in the Control sample on the forward strand
pStrand	p-Value of a fisher.test on the contingency matrix matrix(c(CovFwd,CovRev,SupFwd,SupRev), nrow = 2) at this position - low values could indicate strand bias

Author(s)

Paul Pyl

Examples

```
library(h5vc) # loading library
tallyFile <- system.file( "extdata", "example.tally.hfs5", package = "h5vcData" )
sampleData <- getSampleData( tallyFile, "/ExampleStudy/16" )
position <- 29979629
windowsize <- 1000
vars <- h5dapply( # Calling Variants
  filename = tallyFile,
  group = "/ExampleStudy/16",
  blocksize = 500,
  FUN = callVariantsSingle,
  sampledata = sampleData,
  names = c("Coverages", "Counts", "Reference", "Deletions"),
  range = c(position - windowsize, position + windowsize)
)
vars <- do.call( rbind, vars ) # merge the results from all blocks by row
vars # We did find a variant
```

Coverage *Coverage analysis*

Description

Functions to do analyses based on coverage

Usage

```
binnedCoverage( data, sampledata, gccount = FALSE )
```

Arguments

data	A list with element Coverage (a 3d integer array of size [1:2, 1:k, 1:n])
sampledata	A data.frame with k rows (one for each sample) and columns Type, Column and (SampleGroup or Patient). The tally file should contain this information as a group attribute, see getSampleData for an example.
gccount	Boolean flag to specify whether the gc count of the bin should be reported as well, Reference must be a slot in the data object

Details

Explanations:

This computes the per sample coverage in a given bin (determined by the width of data). This feature is not implemented yet!

Value

Returns a data.frame with columns containing the coverage with the current bin for all samples provided in sampledata. The binsize is determined by the blocksize argument given to h5dapply when this function is run directly on a tally file.

Author(s)

Paul Pyl

Examples

```
# loading library and example data
library(h5vc)
tallyFile <- system.file( "extdata", "example.tally.hfs5", package = "h5vcData" )
sampleData <- getSampleData( tallyFile, "/ExampleStudy/22" )
data <- h5dapply( # extractting coverage binned at 1000 bases
  filename = tallyFile,
  group = "/ExampleStudy/22",
  blocksize = 1000,
  FUN = binnedCoverage,
  sampledata = sampleData,
```

```
  gccount = TRUE,
  names = c( "Coverages", "Reference" ),
  range = c(38900000,39000000)
)
data <- do.call(rbind, data)
rownames(data) <- NULL
head(data)
```

---

geom_h5vc *geom_h5vc*

Description

Plotting function that returns a ggplot2 layer representing the specified dataset for the specified samples in the region [positon - windowsize, position + windowsize].

Usage

```
geom_h5vc( data, sampledata, samples=sampledata$Sample, windowsize, position, dataset, ... )
```

Arguments

data	The data to be plotted. Returned by h5dapply. Must be centered on position, extend by windowsize in each direction and contain a slot named like the dataset argument
sampledata	The sampledata for the cohort represented by data. Returned by getSampleData
samples	A character vector listing the names of samples to be plotted, defaults to all samples as described in sampledata
windowsize	Size of the window in which to plot on each side. The total interval that is plotted will be [position-windowsize,position+windowsize]
position	The position at which the plot shall be centered
dataset	The slot in the data argument that should be plotted
...	Paramteters to be passed to the internally used geom_rect, see geom_rect for details

Details

Creates a ggplot layer centered on position using the specified dataset from list data, annotating it with sample information provided in the data.frame sampledata and showing all samples listed in sample. The resulting plot uses ggplot2's geom_rect to draw boxes representing the values from dataset. The x-axis is the position and will span the interval [positon - windowsize, position + windowsize]. The x-axis is centered at 0 and additional layers to be added to the plot should be centered at 0 also.

Ths function allows for fast creation of overview plots similar to mismatchPlot (without the stacking of tracks). The example below shows how one can create a plot showing the coverage and number of mismatches per position (but not the alternative allele) for a given region.

Value

A ggplot layer object containing the plot of the specified dataset, this can be used like any other ggplot layer, i.e. it may be added to another plot.

Author(s)

Paul Pyl

Examples

```
# loading library and example data
library(h5vc)
library(ggplot2)
tallyFile <- system.file( "extdata", "example.tally.hfs5", package = "h5vcData" )
sampleData <- getSampleData( tallyFile, "/ExampleStudy/16" )
position <- 29979629
windowsize <- 30
samples <- sampleData$Sample[sampleData$Patient == "Patient8"]
data <- h5dapply(
  filename = tallyFile,
  group = "/ExampleStudy/16",
 blocksize = windowsize * 3, #choose blocksize larger than range so that all needed data is collected as one block
  names = c("Coverages", "Counts", "Deletions"),
  range = c(position - windowsize, position + windowsize)
)[[1]]
# Summing up all mismatches irrespective of the alternative allele
data$CountsAggregate = colSums(data$Counts)
# Simple overview plot showing number of mismatches per position
p <- ggplot() +
geom_h5vc( data=data, sampledata=sampleData, windowsize = 35, position = 500, dataset = "Coverages", fill = "gray'
geom_h5vc( data=data, sampledata=sampleData, windowsize = 35, position = 500, dataset = "CountsAggregate", fill =
 facet_wrap( ~ Sample, ncol = 2 )
 print(p)
```

---

getSampleData *Reading and writing sample data from / to a tally file*

Description

These functions allow reading and writing of sample data to the HDF5-based tally files. The sample data is stored as group attribute.

Usage

```
getSampleData( filename, group )
setSampleData( filename, group, sampleData, largeAttributes = FALSE, stringSize = 64 )
```

Arguments

filename	The name of a tally file
group	The name of a group within that tally file, e.g. /ExampleStudy/22
sampleData	A data.frame with k rows (one for each sample) and columns Type, Column and (SampleGroup or Patient. Additional column will be added as well but are not required.)
largeAttributes	HDF5 limits the size of attributes to 64KB, if you have many samples setting this flag will write the attributes in a separate dataset instead. getSampleData is aware of this and automatically chooses the dataset-stored attributes if they are present
stringSize	Maximum length for string attributes (number of characters) - default of 64 characters should be fine for most cases; This has to be specified since we do not support variable length strings as of now.

Details

The returned data.frame contains information about the sample ids, sample columns in the sample dimension of the dataset. The type of sample must be one of c("Case","Control") to be used with the provided SNV calling function. Additional relevant per-sample information may be stored here.

Note that the following columns are required in the sample data where the rows represent samples in the cohort:

Sample: the sample id of the corresponding sample

Column: the index within the genomic position dimension of the corresponding sample, be aware that getSampleData and setSampleData automatically add / remove 1 from this value since internally the tally files store the dimension 0-based whereas within R we count 1-based.

Patient the patient id of the corresponding sample

Type the type of sample

Value

sampledata	A data.frame with k rows (one for each sample) and columns Type, Column and (SampleGroup or Patient).

Author(s)

Paul Pyl

Examples

```
 # loading library and example data
 library(h5vc)
# We make a copy of the file to tmp here, this is only needed if we want to keep the original intact.
 tallyFile <- tempfile()
stopifnot(file.copy(system.file( "extdata", "example.tally.hfs5", package = "h5vcData" ), tallyFile))
```

```
sampleData <- getSampleData( tallyFile, "/ExampleStudy/16" )
sampleData
# modify  the sample data
sampleData$AnotherColumn <- paste( sampleData$Patient, "Modified" )
# write to tallyFile
setSampleData( tallyFile, "/ExampleStudy/16", sampleData )
# re-load and check if it worked
sampleData <- getSampleData( tallyFile, "/ExampleStudy/16" )
sampleData
```

---

h5dapply *h5dapply*

Description

This is the central function of the h5vc package, allows an apply operation along common dimensions of datasets in a tally file.

Usage

```
## S4 method for signature 'numeric'
h5dapply( ..., blocksize, range)
## S4 method for signature 'GRanges'
h5dapply( ..., group, range)
## S4 method for signature 'IRanges'
h5dapply( ..., range)
```

Arguments

`blocksize`	The size of the blocks in which to process the data (integer)
`...`	Further parameters to be handed over to `FUN`
`range`	The range along the specified dimensions which should be processed, this allows for limiting the apply to a specific region or set of samples, etc. - optional (defaults to the whole chromosome); This can be a `GRanges`, `IRanges` or numerical vector of length 2 (i.e [start, stop])
`group`	The group (location) within the HDF5 file, note that when range is `numeric` or `IRanges` this has to point to the location of the chromosome, e.g. `/ExampleTally/Chr7`. When range is a `GRanges` object, the chromosome information is encoded in the `GRanges` directly and `group` should only point to the root-group of the study, i.e. `/ExampleTally`

Details

Additional function parameters are:

filename The name of a tally file to process

group The name of a group in that tally file

FUN The function to apply to each block, defaults to `function(x) x`, which returns the data as is (a list of arrays)

names The names of the datasets to extract, e.g. `c("Counts","Coverages")` - optional (defaults to all datasets)

dims The dimension to apply along for each dataset in the same order as `names`, these should correspond to compatible dimensions between the datsets. - optional (defaults to the genomic position dimension)

samples Character vector of sample names - must match contents of sampleData stored in the `tallyFile`

sampleDimMap A list mapping dataset names to their respective sample dimensions - default provides values for "Counts", "Coverages", "Deletions" and "Reference"

verbose Boolean flag that controls the amount of messages being printed by `h5dapply`

BPPARAM BPPARAM object to be passed to the `bplapply` call used to apply `FUN` to the blocks - see `BiocParallel` documentation for details; if this is `NULL` a normal `lapply` will be used instead of `bplapply`.

This function applys parameter `FUN` to blocks along a specified axis within the tally file, group and specified datasets. It creates a list of arrays (one for each dataset) and processes that list with the function `FUN`.

This is by far the most essential and powerful function within this package since it allows the user to execute their own analysis functions on the tallies stored within the HDF5 tally file.

The supplied function `FUN` must have a parameter `data` or `...` (the former is the expected behaviour), which will be supplied to `FUN` from `h5dapply` for each block. This structure is a `list` with one slot for each dataset specified in the `names` argument to `h5dapply` containing the array corresponding to the current block in the given dataset. Furthemore the slot `h5dapplyInfo` is reserved and contains another `list` with the following content:

`Blockstart` is an integer specifying the starting position of the current block (in the dimension specified by the `dims` argument to `h5dapply`)

`Blockend` is an integer specifying the end position of the current block (in the dimension specified by the `dims` argument to `h5dapply`)

`Datasets` Contains a `data.frame` as it is returned by `h5ls` listing all datasets present in the other slots of `data` with their group, name, dimensions, number of dimensions (`DimCount`) and the dimension that is used for splitting into blocks (`PosDim`)

`Group` contains the name of the group as specified by the `group` argument to `h5dapply`

Value

A list with one entry per block, which is the result of applying `FUN` to the datasets specified in the parameter `names` within the block.

Author(s)

Paul Pyl

Examples

```
# loading library and example data
library(h5vc)
tallyFile <- system.file( "extdata", "example.tally.hfs5", package = "h5vcData" )
sampleData <- getSampleData( tallyFile, "/ExampleStudy/16" )
# check the available samples and sampleData
print(sampleData)
data <- h5dapply( #extracting coverage using h5dapply
  filename = tallyFile,
  group = "/ExampleStudy/16",
  blocksize = 1000,
  FUN = function(x) rowSums(x$Coverages),
  names = c( "Coverages" ),
  range = c(29000000,29010000),
  verbose = TRUE
  )
coverages <- do.call( rbind, data )
colnames(coverages) <- sampleData$Sample[order(sampleData$Column)]
coverages
#Subsetting by Sample
sampleData <- sampleData[sampleData$Patient == "Patient5",]
data <- h5dapply( #extracting coverage using h5dapply
  filename = tallyFile,
  group = "/ExampleStudy/16",
  blocksize = 1000,
  FUN = function(x) rowSums(x$Coverages),
  names = c( "Coverages" ),
  range = c(29000000,29010000),
  samples = sampleData$Sample,
  verbose = TRUE
  )
coverages <- do.call( rbind, data )
colnames(coverages) <- sampleData$Sample[order(sampleData$Column)]
coverages
#Using GRanges and IRanges
library(GenomicRanges)
library(IRanges)
granges <- GRanges(
c(rep("16", 10), rep("22", 10)),
ranges = IRanges(
  start = c(seq(29000000,29009000, 1000), seq(39000000,39009000, 1000)),
  width = 1000
))
data <- h5dapply( #extracting coverage using h5dapply
  filename = tallyFile,
  group = "/ExampleStudy",
  blocksize = 1000,
  FUN = function(x) rowSums(x$Coverages),
  names = c( "Coverages" ),
  range = granges,
  verbose = TRUE
  )
```

```
lapply( data, function(x) do.call(rbind, x) )
```

---

h5readBlock *h5readBlock*

Description

A simple access function for extracting a single block of data from a tally file, use h5dapply for applying functions on multiple blocks / extracting multiple blocks form a tally file.

Usage

```
h5readBlock( filename, group, names, dims, range, samples = NULL, sampleDimMap = .sampleDimMap, verbose
```

Arguments

filename	The name of a tally file to process
group	The name of a group in that tally file
names	The names of the datasets to extract, e.g. c("Counts","Coverages") - optional (defaults to all datasets)
dims	The dimension in which the block shall be extracted for each dataset in the same order as names, these should correspond to compatible dimensions between the datsets. - optional (defaults to the genomic position dimension)
range	The range along the specified dimensions which should be extracted
samples	Character vector of sample names - must match contents of sampleData stored in the tallyFile
sampleDimMap	A list mapping dataset names to their respective sample dimensions - default provides values for "Counts", "Coverages", "Deletions" and "Reference"
verbose	Boolean flag that controls the amount of messages being printed by h5dapply

Details

This function extracts a block along the dimensions specified in dims (default: genomic position) from the datasets specified in names and returns it. The block is defined by the parameter range.

The function returns a list with one slot for each dataset specified in the names argument to containing the array corresponding to the specified block in the given dataset. Furthemore the slot h5dapplyInfo is reserved and contains another list with the following content:

Blockstart is an integer specifying the starting position of the current block (in the dimension specified by the dims argument to h5dapply)

Blockend is an integer specifying the end position of the current block (in the dimension specified by the dims argument to h5dapply)

Datasets Contains a data.frame as it is returned by h5ls listing all datasets present in the other slots of data with their group, name, dimensions, number of dimensions (DimCount) and the dimension that is used for splitting into blocks (PosDim)

Group contains the name of the group as specified by the group argument to h5dapply

Value

A list with one entry per dataset and an additional slot `h5dapplyInfo` containing auxiliary information.

Author(s)

Paul Pyl

Examples

```
library(h5vc) # loading the library
tallyFile <- system.file( "extdata", "example.tally.hfs5", package = "h5vcData" )
data <- h5readBlock( #extracting coverage, deletions and reference using h5dreadBlock
  filename = tallyFile,
  group = "/ExampleStudy/16",
  names = c( "Coverages", "Deletions", "Reference" ),
  range = c(29000000,29010000),
  verbose = TRUE
)
str(data)
sampleData <- getSampleData( tallyFile, "/ExampleStudy/16" )
#Subsetting by Sample
sampleData <- sampleData[sampleData$Patient == "Patient8",]
data <- h5readBlock( #extracting coverage, deletions and reference using h5dreadBlock
  filename = tallyFile,
  group = "/ExampleStudy/16",
  names = c( "Coverages", "Deletions", "Reference" ),
  range = c(29000000,29010000),
  samples = sampleData$Sample,
  verbose = TRUE
)
str(data)
```

---

helpers *helper functions*

Description

These functions are helpers for dealing with tally data stored in HDF5 files.

Usage

```
formatGenomicPosition( x, unit = "Mb", divisor = 1000000, digits = 3,
nsmall = 1 )
encodeDNAString( ds )
defineBlocks( start, stop, blocksize )
getChromSize( tallyFile, group, dataset = "Reference", posDim = 1 )
```

Arguments

x	Numerical genomic position
unit	Which unit to convert the position to
divisor	divisor corresponding to the unit, i.e. 'Mb' -> 1e6, 'Kb' -> 1e3
digits	number of digits to keep
nsmall	nsmall parameter to the format function
ds	A DNAString object to be encoded in the HDF5 tally file specific encoding of nucleotides.
start	first position
stop	last position
blocksize	size of blocks
tallyFile	Tally file to work on
group	Group within tallyFile that we want to find the chromosome size for
dataset	Datset to extract chromosome size from - default is "Reference"
posDim	Which dimension of the dataset describes the genomic position

Details

formatGenomicPosition: Helps formatting genomic positions for annotating axes in mismatch plots etc.

encodeDNAString: This translates a DNAString object into a comaptible encoding that can be written to a HDF5 based tally file in the Reference dataset. Since the Python script for generating tallies only sets the Reference dataset in positions where mismatches exists updating the Reference dataset becomes necessary if one would like to perform analysis involving sequence context (GC-bias, mutationSpectrum, etc.)

defineBlocks: This function returns a data.frame with the columns Start and End for blocks of size blocksize spanning the interval [start, stop].

getChromSize: This function is a helper to quickly look-up the chromosome size of a given group and tally file.

Value

formatGenomicPosition: formatted genomic position, e.g. "123.4 Mb"

encodeDNAString: A numeric vector encoding the nucleotide sequence provided in ds according to the scheme c("A"=0,"C"=1,"G"=2,"T"=3).

defineBlocks: A data.frame with the columns Start and End for blocks of size blocksize spanning the interval [start, stop].

getChromSize: Returns a numeric that is the size of the chromosome.

Author(s)

Paul Pyl

Examples

```
formatGenomicPosition(123456789)
library(Biostrings)
lapply( DNAStringSet( c("simple"="ACGT", "movie"="GATTACA") ), encodeDNAString )
getChromSize( system.file("extdata", "example.tally.hfs5", package="h5vcData"), "/ExampleStudy/16" )
```

`mergeTallies` *Merging the prepared results from multiple bam file tallies into one block that can be written to the HDF5 tally file*

Description

This function merges a set of tallies that have been processed with `prepareForHDF5` into one block of data.

Usage

```
mergeTallies( tallies )
```

Arguments

`tallies`	A list of prepared talies, i.e. a list of lists with slots for the datasets "Counts", "Coverage", "Deletions" and "Reference" in each sub-list

Details

This function merges tallies from a set of bam files / samples, note that the order of samples in the sample column will be the same as the order of samples in the provided list, so ake sure this matches your sampledata.

Value

A list with slots containing the `Counts`,`Coverages`,`Deletions` and `Reference` datasets for the samples given in `tallies`. Each of the slots contains an array with the contents of the provided sub-lists merged along the "sample" axis. The `Reference` slot os filled from the first element of `tallies` and it is up to the user to make sure that the tallies provided for merging have compatible references.

Author(s)

Paul Pyl

Examples

```
library(h5vc)
library(BSgenome.Hsapiens.UCSC.hg19)
files <- c("NRAS.AML.bam","NRAS.Control.bam")
bamFiles <- file.path( system.file("extdata", package = "h5vcData"), files)
chrom = "1"
startpos <- 115247090
endpos <- 115259515
theData <- lapply( bamFiles, function(bamf){ tallyBAM(bamf, chrom, startpos, endpos) } )
str(theData)
reference <- getSeq(BSgenome.Hsapiens.UCSC.hg19, "chr1", startpos, endpos)
theMergedData <- mergeTallies(lapply(theData, prepareForHDF5, reference))
str(theMergedData)
```

`mergeTallyFiles` *Merging multiple tally files into one*

Description

Function to merge multiple tally files by genomic position (i.e. gluing samples together)

Usage

```
mergeTallyFiles( inputFiles, destFile, destGroup, blockSize = 1e6, sampleDims = c(), positionDims = c()
```

Arguments

`inputFiles`	A list mapping input file names to the groups within them from which the data shall be taken (e.g. "example.tally.hfs5" -> "/ExampleStudy/16")
`destFile`	Name of the file that should be created
`destGroup`	Group within `destFile` that will hold the merged data
`blockSize`	Size of the blocks in bases that the merging will be performed in
`sampleDims`	List mapping dataset names to their respective sample dimension, e.g. "Counts" -> 2 - has the standard datasets included by default
`positionDims`	List mapping dataset names to their respective position dimension, e.g. "Counts" -> 4 - has the standard datasets included by default

Details

This function merges tally data from a list of tally files into a new destination file.

Value

[None] – prints progress messages along the way.

Author(s)

Paul Pyl

Examples

```
## Not run:
mergeTallyFiles{ # merging a file to itself, i.e. "doubling" it
  list(
    "example.tally.hfs5" = "/ExampleStudy/16",
    "example.tally.hfs5" = "/ExampleStudy/16"
  ),
  "test.merge.hfs5",
  "/MergedStudy/16"}

## End(Not run)
```

`mismatchPlot` *mismatchPlot*

Description

Plotting function that returns a ggplot2 object representing the mismatches and coverages of the specified samples in the specified region.

Usage

```
mismatchPlot( data, sampledata, samples=sampledata$Sample, windowsize = NULL, position = NULL, range =
```

Arguments

`data`	The data to be plotted. Returned by `h5dapply` or `h5readBlock`.
`sampledata`	The sampledata for the cohort represented by `data`. Returned by `getSampleData`
`samples`	A character vector listing the names of samples to be plotted, defaults to all samples as described in `sampledata`
`windowsize`	Size of the window in which to plot on each side. The total interval that is plotted will be [position-windowsize,position+windowsize]
`position`	The position at which the plot shall be centered
`range`	Integer vector of two elements specifying a range of coordinates to be plotted, use either position + windowsize or range; if both are provided range overwrites position and windowsize.
`plotReference`	This boolean flag specifies if a reference track should be plotted, only takes effect if there is a slot named `Reference` in the `data` object passed to the function
`refHeight`	Height of the reference track in coverage units (default of 8 = reference track is as high as 8 reads coverage would be in the plot of a sample.)

printReference	Boolean parameter to indicate whether a text representation of the reference should be overlayed to the reference track, can only be true if plotReference is true.
printRefSize	Size parameter of the geom_text layer used to print the reference. This value is unitless and needs to be manually optimised for a given plot.
tickSpacing	Integer vector of two elements, specifying the spacing of ticks along the x and y axes respectively.

Details

If position and windowsize are specified this function creates a plot centered on position using the coverage and mismatch counts stored in data, annotating it with sample information provided in the data.frame sampledata and showing all samples listed in sample. If range is specified, the plot will cover the positions from range[1] to range[2]. The difference between specifying range or position plus windowsize lies only in the labelling of the x-axis and the coordinate system used on the x-axis. In the former case the coordinate system is that of genomic coordinates as specified in range, when using the latter the x-axis coordinates go from -windowsize through +windowsize and position 0 is marked with the calue provided in the position parameter. Furthermore when a position and windowsize are provided two black lines marking the center position are drawn (this is usefull for visualising SNVs)

If neither range, nor position and windowsize are specified the function will try to extract the information from the data object. If data is the return value of a call to h5dapply or h5readBlock this will work automagically.

The plot has the genomic position on the x-axis. The y-axis encodes values where positive values are on the forward strand and negative values on the reverse. The coverage is shown in grey, deletions in purple and the mismatches in the colors specified in the legend. Note that for each possible mismatch there is an additional color for low-quality counts (coming from the first and last sequencing cycles), so e.g. C is filled dark red and C_lq light red.

If data is the result of a call to h5dapply representing multiple blocks of data as defined in the range parameter to h5dapply then the plot will contain the mismatchPlots of each of the ranges plotted next to each other.

Value

A ggplot object containing the mismatch plot, this can be used like any other ggplot object, i.e. additional layers and styles my be applied by simply adding them to the plot.

Author(s)

Paul Pyl

Examples

```
# loading library and example data
library(h5vc)
tallyFile <- system.file( "extdata", "example.tally.hfs5", package = "h5vcData" )
sampleData <- getSampleData( tallyFile, "/ExampleStudy/16" )
position <- 29979628
```

```
windowsize <- 30
samples <- sampleData$Sample[sampleData$Patient == "Patient8"]
data <- h5readBlock(
  filename = tallyFile,
  group = "/ExampleStudy/16",
  names = c("Coverages", "Counts", "Deletions", "Reference"),
  range = c(position - windowsize, position + windowsize)
)
#Plotting with position and windowsize
p <- mismatchPlot(
  data = data,
  sampledata = sampleData,
  samples = samples,
  windowsize = windowsize,
  position = position
)
print(p)
#plotting with range and modified tickSpacing and refHeight
p <- mismatchPlot(
  data = data,
  sampledata = sampleData,
  samples = samples,
  range = c(position - windowsize, position + windowsize),
  tickSpacing = c(20, 5),
  refHeight = 5
)
print(p)
#plotting without specfiying range or position
p <- mismatchPlot(
  data = data,
  sampledata = sampleData,
  samples = samples
)
print(p)
#Plotting multiple regions (with small overlaps)
library(IRanges)
dataList <- h5dapply(
  filename = tallyFile,
  group = "/ExampleStudy/16",
  names = c("Coverages", "Counts", "Deletions", "Reference"),
 range = IRanges(start = seq( position - windowsize, position + windowsize, 20), width = 30 )
)
p <- mismatchPlot(
  data = dataList,
  sampledata = sampleData,
  samples = samples
)
print(p)
```

`mutationSpectra` *Mutation spectrum analyses*

Description

These functions help in analyses of mutation spectra

Usage

```
mutationSpectrum( variantCalls, tallyFile, study, context = 1 )
```

Arguments

variantCalls	A data.frame object that can be the output of a call to a callVariantsPaired or callDeletionsPaired function. The following columns are required: - altAllele - refAllele - Sample - Start - End - Chrom
tallyFile	filename of a tally file matching the variant calls
study	the study id used in the tally file
context	An integer specifying the size of the context that should be considered (i.e. the length of the prefix and suffix of the variant call)

Details

This function takes a set of variant calls (SNVs/Deletions) and a tallyFile as well as a context size and tabulates the number of observed mutations stratified by type (refAllele->altAllele) and sequence context (i.e. the prefix and suffix of size context around the variant position in the genome)

bases serves to map character representations to numeric encoding of bases

variantCalls is an example dataset of variant calls created by running callVariantsPaired on the example.tally.hfs5 file.

Value

A table listing the counts of mutations stratified by allele, sequence context and sample.

Author(s)

Paul Pyl

Examples

```
library(h5vc)
tallyFile <- system.file( "extdata", "example.tally.hfs5", package = "h5vcData" )
data( "example.variants", package = "h5vcData" )
head( mutationSpectrum( variantCalls, tallyFile, "/ExampleStudy" ) )
```

plotMutationSpectrum *Plotting a mutation spectrum*

Description

This function generates a mutation spectrum plot from a mutation spectrum returned by a call to mutationSPectrum

Usage

```
plotMutationSpectrum( ms, plotCounts = TRUE )
```

Arguments

ms	A mutation spectrum as returned by mutationSpectrum
plotCounts	Boolean flag specifying whether ms contains one row per variant (default) or already contains summarized counts per type of mutation

Details

The plot is inspired by the one shown in figure 1b of Signatures of mutational processes in human cancer -- Alexandrov et. al.

Value

A ggplot object containing the mutation spectrum plot

Author(s)

Paul Pyl

Examples

```
library(h5vc)
tallyFile <- system.file( "extdata", "example.tally.hfs5", package = "h5vcData" )
data( "example.variants", package = "h5vcData" )
plotMutationSpectrum( mutationSpectrum( variantCalls, tallyFile, "/ExampleStudy" ) )
```

prepareForHDF5 *Preparing the results of tallyBAM for writing to an HDF5 tally file*

Description

This function prepares the resulting array of a call to tallyBAM for writing to an HDF5 tally file.

Usage

```
prepareForHDF5( counts, reference )
```

Arguments

counts	An array as produced by a call to tallyBAM
reference	A DNAString object containing the reference sequence corresponding to the region that is described in the counts array – if this is NULL a consensus vote will be used to estimate the reference at any given position, this means you cannot detect variants with AF >= 0.5 anymore

Details

This function performs the neccessary transformation to the array returned by tallyBAM to be compatible with the HDF5 tally file data structure.

Value

A list with slots containing the Counts,Coverages,Deletions and Reference datasets for the given sample.

Author(s)

Paul Pyl

Examples

```
library(h5vc)
library(BSgenome.Hsapiens.UCSC.hg19)
files <- c("NRAS.AML.bam","NRAS.Control.bam")
bamFiles <- file.path( system.file("extdata", package = "h5vcData"), files)
chrom = "1"
startpos <- 115247090
endpos <- 115259515
theData <- lapply( bamFiles, function(bamf){
  tallyBAM( file = bamf, chr = chrom, start = startpos, stop = endpos, ncycles = 10 )
})
reference <- getSeq(BSgenome.Hsapiens.UCSC.hg19, "chr1", startpos, endpos)
theData <- lapply(theData, prepareForHDF5, reference)
str(theData)
```

prepareTallyFile *prepareTallyFile*

Description

Functions for preparing an HDF5 file for storing tally data and / or modifying an existing file

Usage

```
prepareTallyFile( filename, study, chrom, chromlength, nsamples, maxsamples = nsamples, chunkSize = 500
resizeCohort( filename, study, chrom, newNumberOfSamples, dimmap = .sampleDimMap, force = FALSE )
```

Arguments

filename	Filename of the HDF5 file that should store the tallies
study	Study identifier which will be used in structuring the file
chrom	Chromosome for which the structure should be generated
chromlength	The length of the chromosom, this will be the size of genomic position dimension
nsamples	Number of samples that will be stored in the file
maxsamples	Maximum Number of samples that can be stored in the file, this relatesto the maxdim property of HDF5 datasets, which is used to specify possible re-sizing of datasets after creation - see http://www.hdfgroup.org for details
chunkSize	The size of the chunks used in HDF5 storage, this is specified along the genomic position dimension, by default chunks will always be all data from all samples with the given width along the genomic position dimension
compressionLevel	Compression level to use in the HDF5 file, defaults to 9 (highest), use lower numbers to improve access time at the cost of disk space usage
sampleChunkSize	Size of the HDF5 chunks along the sample dimension, the dafault value is the whole dataset, i.e. all samples. For larger datasets where the typical use-case is to extract only data corresponding to a specific sample and genomic position, smaller values of sampleChunkSize should be used.
referenceFillValue	Default value to be used for the Reference dataset, this is set to 5 by default, which corresponds to the nucleotide N
newNumberOfSamples	New cohort size, this must be smaller than the value of maxsamples that was provided when the file was created
dimmap	A list mapping dataset names to the dimension in which the samples are stored (e.g. "Counts" -> 2)
force	Boolean parameter that controls whether a shrinking operation (i.e. newNumberOfSamples is smaller than the current number of samples) should be performed or throw an error. Shrinking will result in data loss.

Details

prepareTallyFile prepares (and creates if neccessary) an HDF5 file for storing the datasets that are associated with a tally. It creates the required groups and datasets (filled with 0's). resizeCohortResizes the datasets to a new number of samples, this is limited by the value of maxsamples that was provided in the initial call to prepareTallyFile

Value

Returns TRUE on success

Author(s)

Paul Pyl

Examples

```
prepareTallyFile( file.path( tempdir(), "test.tally.hfs5" ), "SomeStudy", "ChromosomeB", 1e6, 20 )
```

---

tallyBAM *tallyBAM*

Description

Function for creating tallies from bam files.

Usage

```
tallyBAM(file, chr, start, stop, q=25, ncycles = 0, max.depth=1000000, verbose=FALSE, reference = NULL)
```

Arguments

file	filename of the BAM file that should be tallies
chr	Chromosome in which to tally
start	First position of the tally
stop	Last position of the tally
q	quality cut-off for considering a base call
ncycles	number of sequencing cycles form the front and back of the read that should be considered unreliable
max.depth	only tally a position if there are less than this many reads overlapping it - can prevent long runtimes in unreliable regions
verbose	should additional information be printed
reference	DNAString object holding the reference sequence of the region being tallies, if this is NULL (the default) the raw tally is returned, otherwise prepareForHDF5 is called with the raw tally and the reference and the prepared tally is returned instead

Details

This function tallies nucleotides and deletion counts in the specified region of a given BAM file. The results can be processed with the `prepareForHDF5` function.

This function was adapted from the `bam2R` function provided by the `deepSNV` package.

Value

An array object with dimensions `[stop - start + 1, 18, 2]` which represent positions times nucleotides (4 bases + deletions + insertions times three for early, middle and late sequencing cycles) times strands.

Author(s)

Paul Pyl

Examples

```
library(h5vc)
files <- c("NRAS.AML.bam","NRAS.Control.bam")
bamFiles <- file.path( system.file("extdata", package = "h5vcData"), files)
chrom = "1"
startpos <- 115247090
endpos <- 115259515
theData <- lapply( bamFiles, function(bamf){
  tallyBAM( file = bamf, chr = chrom, start = startpos, stop = endpos, ncycles = 10 )
})
str(theData)
print(theData[[1]][,,,9491]) #position 9491 of the pileup
```

`tallyRanges` *Tallying function with a* `GRanges` *interface.*

Description

Functions for tallying bam files in genomic intervals provided as `GRanges` objects, special version of the function for direct writing or computation on a cluster exist.

Usage

```
tallyRanges(bamfiles, ranges, reference, q = 25, ncycles = 10, max.depth = 1e+06)
tallyRangesToFile(tallyFile, study, bamfiles, ranges, reference, samples = NULL, q = 25, ncycles = 0, ma
tallyRangesBatch(tallyFile, study, bamfiles, ranges, reference, q = 25, ncycles = 10, max.depth=1e6, re
```

Arguments

bamfiles	Character vector giving the locations of the bam files to be tallied
ranges	A GRanges object describing the ranges that tallies shalle be generated in, e.g. the result of a call to binGenome or a set of exon or gene annotations provided by a TxDB object.
reference	BSgenome object describing the reference genome that the alignments were made against.
samples	The indices (within the HDF5 datasets) corresponding to the samples that the data represents. You can use this option to write sub-sets of samples from a cohort.
q	Read alignment quality cut-off.
ncycles	Number of cycles from the front and back of the reads that should be considered unreliable for mismatch detection
max.depth	Maximum depth of coverage to consider
tallyFile	Filename of the HDF5 tally file that the data shall be written to
study	The location within the HDF5 file that corresponds to the HDF5-group representing the study we are working on.
regID	Identifier for a BatchJobs registry which will be used to store and organise the cluster jobs used for parallelisation of the work.
res	Resource list specifying the compute resources to be requested for each of the cluster jobs.
written	Numerical vector indicating the Job IDs of jobs whose results have already been written to the tally file, this can be used to resume writing after a crash.
wrfile	Filename for a file to store the IDs of already written jobs in, can be used to resume writing after a crash.
waitTime	How long shall the function wait on cluster jobst to finish, before giving up. Default is wait forever.

Details

tallyRanges returns the tallies corresponding to the specifed ranges, tallyToFile performs the same task but writes the results to the tally file directly. tallyRangesBatch uses the BatchJobs package to set up cluster jobs for tallying and collects and writes the results of those jobs to the tally file. It is important to have a properly configured cluster (inlcuding a .BatchJobs.R as well as a template file). See the documentation of BatchJobs for that information.

Value

For tallyRanges the return value is a list of lists, where the top level corresponds to the ranges provided as an input to the function and each element is a list of the datasets in compatible format, that can directly be written to an HDF5 file using the writeToTallyFile function. The other two function perform the writing directly and return

Author(s)

Paul Theodor Pyl

Examples

```
suppressPackageStartupMessages(library("h5vc"))
suppressPackageStartupMessages(library("rhdf5"))
files <- list.files( system.file("extdata", package = "h5vcData"), "Pt.*bam$" )
bamFiles <- file.path( system.file("extdata", package = "h5vcData"), files)
suppressPackageStartupMessages(require(BSgenome.Hsapiens.NCBI.GRCh38))
suppressPackageStartupMessages(require(GenomicRanges))
dnmt3a <- read.table(system.file("extdata", "dnmt3a.txt", package = "h5vcData"), header=TRUE, stringsAsFactors = F
dnmt3a <- with( dnmt3a, GRanges(seqname, ranges = IRanges(start = start, end = end)))
dnmt3a <- reduce(dnmt3a)
require(BiocParallel)
register(MulticoreParam())
theData <- tallyRanges( bamFiles, ranges = dnmt3a[1:3], reference = Hsapiens )
str(theData)
```

---

writeReference *Filling the Reference dataset in a tally file from a DNAString*

Description

Function to fill the Reference dataset of a tally file from a DNAString object

Usage

```
writeReference( tallyFile, group, dnastring, blocksize = 1000000, verbose = TRUE )
```

Arguments

tallyFile	filename of a tally file matching the variant calls
group	The group that the Reference dataset is located in
dnastring	A DNAString object containing the new reference sequence
blocksize	The size of blocks in which to process the reference (higher values imply higher memory consumption)
verbose	Boolean flag to specify if diagnostic messages should be printed

Details

This function takes a tally file, a location within it (the group argument) and a reference sequence as a DNAString object, encodes the reference in the appropriate way and writes it to the location in the tally file in blocks of size specified in blocksize. The reference will be written to a dataset with the path paste(group, "Reference", sep = "/") within the tally file. The dataset itself must exists and have the correct dimensions to hold the sequence specified in dnastring.

Value

Returns TRUE on success.

Author(s)

Paul Pyl

Examples

```
library(h5vc)
library(rhdf5)
library(Biostrings)
filename = file.path(tempdir(), "write.ref.test.hfs5")
prepareTallyFile(filename=filename,study="SomeStudy",chrom="Foo",chromlength=8,nsamples=1)
writeReference(filename, group = "/SomeStudy/Foo", dnastring = DNAString("GATTACCA"))
h5dump(filename)$SomeStudy$Foo$Reference
```

`writeToTallyFile` *Writing data to an HDF5 tally file*

Description

This function is used to write the results of a call to `tallyRanges` to an HDF5 tally file.

Usage

```
writeToTallyFile( theData, file, study, ranges, samples = NULL )
```

Arguments

`theData`	A `list` of `lists` of datasets as returned by a call to e.g. `tallyRanges`
`file`	The target filename
`study`	The location of the Group (within the HDF5 file) representing the study the data belongs to.
`ranges`	A `GRanges` object defining the ranges that the elements of `theData` correspond to
`samples`	The indexes of the samples that the data corresponds to, this can be extracted from the 'Column'-field in the sample metadata and is used to write data corresponding to subsets of the cohort samples. The default (`NULL`) indicates that all samples are present and will be written.

Author(s)

Paul Theodor Pyl

Examples

```
suppressPackageStartupMessages(library("h5vc"))
suppressPackageStartupMessages(library("rhdf5"))
files <- list.files( system.file("extdata", package = "h5vcData"), "Pt.*bam$" )
bamFiles <- file.path( system.file("extdata", package = "h5vcData"), files)
suppressPackageStartupMessages(require(BSgenome.Hsapiens.NCBI.GRCh38))
suppressPackageStartupMessages(require(GenomicRanges))
dnmt3a <- read.table(system.file("extdata", "dnmt3a.txt", package = "h5vcData"), header=TRUE, stringsAsFactors = F
dnmt3a <- with( dnmt3a, GRanges(seqname, ranges = IRanges(start = start, end = end)))
dnmt3a <- reduce(dnmt3a)
require(BiocParallel)
register(MulticoreParam())
theData <- tallyRanges( bamFiles, ranges = dnmt3a[1:3], reference = Hsapiens )
chrom <- "2"
chromlength <- 250e6
study <- "/DNMT3A"
tallyFile <- file.path( tempdir(), "DNMT3A.tally.hfs5" )
if( file.exists(tallyFile) ){
  file.remove(tallyFile)
}
if( prepareTallyFile( tallyFile, study, chrom, chromlength, nsamples = length(files) ) ){
  h5ls(tallyFile)
}else{
  message( paste( "Preparation of:", tallyFile, "failed" ) )
}
writeToTallyFile(theData, tallyFile, study = "/DNMT3A", ranges = dnmt3a[1:3])
```

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