Package ‘ramwas’

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Type Package

Title Fast Methylome-Wide Association Study Pipeline for Enrichment Platforms

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Description A complete toolset for methylome-wide association studies (MWAS).
It is specifically designed for data from enrichment based methylation assays,
but can be applied to other data as well.
The analysis pipeline includes seven steps:
(1) scanning aligned reads from BAM files,
(2) calculation of quality control measures,
(3) creation of methylation score (coverage) matrix,
(4) principal component analysis for capturing batch effects and detection of outliers,
(5) association analysis with respect to phenotypes of interest while correcting for top PCs and known covariates,
(6) annotation of significant findings, and
(7) multi-marker analysis (methylation risk score) using elastic net.
Additionally, RaMWAS include tools for joint analysis of methylation and genotype data.
This work is published in Bioinformatics, Shabalin et al. (2018) <doi:10.1093/bioinformatics/bty069>.

URL https://bioconductor.org/packages/ramwas/

BugReports https://github.com/andreyshabalin/ramwas/issues

License LGPL-3

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NeedsCompilation yes

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**biocViews**  DNA Methylation, Sequencing, Quality Control, Coverage, Preprocessing, Normalization, Batch Effect, Principal Component, Differential Methylation, Visualization  

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RaMWAS provides a complete toolset for methylome-wide association studies (MWAS). It is specifically designed for data from enrichment based methylation assays, but can be applied to other methylomic data as well. The analysis pipeline includes seven steps: (1) scanning aligned reads from BAM files, (2) calculation of quality control measures, (3) creation of methylation score (coverage) matrix, (4) principal component analysis for capturing batch effects and detection of outliers, (5) association analysis with respect to phenotypes of interest while correcting for top PCs and known covariates, (6) annotation of significant findings, and (7) multi-marker analysis (methylation risk score) using elastic net.

Details

Package: ramwas
Type: Package
License: LGPL-3
LazyLoad: yes
Depends: methods

Author(s)

Andrey A Shabalin <andrey.shabalin@gmail.com>
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See Also

See vignettes: browseVignettes("ramwas").

Description

cachedRDSload

Cached Loading of RDS Files

Description

Loads an .rds file rdsfilename using readRDS and returns the loaded object. The object is also saved in a cache so that repeated calls of the function with the same filename return the same object instantaneously.

Usage

cachedRDSload(rdsfilename)
findBestNpvs

Arguments

  rdsfilename Name of the RDS file.

Details

  The cached object is stored in a private package environment.

Value

  Returns the object loaded with `readRDS` from `rdsfilename` at this or a previous call of the function.

Author(s)

  Andrey A Shabalin <andrey.shabalin@gmail.com>

Examples

### Change filename to hg19 CpGset

```r
filename = system.file("extdata", "qc_sample.rds", package = "ramwas")

time1 = system.time( {obj1 = cachedRDSload(filename)} )
time2 = system.time( {obj1 = cachedRDSload(filename)} )

cat("First loading time:",time1[3]," seconds","\n")
cat("Second loading time:",time2[3]," seconds","\n")
```

---

findBestNpvs  Quickly Find N Smallest P-values in a Long Vector

Description

Finding top, say, 100 p-values out of millions can be slow. This function does it much faster than the usual application of `order(pv)[1:N]`.

Usage

```r
findBestNpvs(pv, n)
```

Arguments

  pv Vector of p-values.
  n Number of best p-values to select.

Details

  The function is a faster analog of `sort(order(pv)[1:N])`

Value

  Return a vector of positions of the smallest N p-values in pv.
Author(s)
Andrey A Shabalin <andrey.shabalin@gmail.com>

See Also
See order.

Examples
pv = runif(1000)^10
n = 100

# Faster version
topSites1 = findBestNpvs(pv, n)

# Slow alternative
topSites2 = sort(order(pv)[1:n])

# The results must match
stopifnot(all( topSites1 == topSites2 ))

get Functions for Access to Data, MWAS Results, and Location Information

Description
Functions for access to data, MWAS results, and location information.
Function getLocations obtains the location information for all variables (CpGs).
Function getMWASandLocations obtains both MWAS results and location information in a single
data frame.
Functions getDataByLocation and getMWASrange return the data (coverage) and MWAS results
for the selected set of variables (CpGs).

Usage
getLocations(x)
getMWAS(x)
getMWASandLocations(x)
getMWASrange(x, chr, start, end)
getDataByLocation(x, chr, start, end)

Arguments
x Name of directory or list of RaMWAS parameters as described in the "RW6_param.Rmd"
vignette.
Try: vignette("RW6_param","ramwas").

If a directory name is provided, it must point to
• Data (coverage) directory (parameter dircoveragenorm) for getDataByLocation
and getLocations
• MWAS directory (parameter dirMwas) for getMWAS, getMWASandLocations, and getMWASrange

<table>
<thead>
<tr>
<th>chr</th>
<th>Chromosome name or number.</th>
</tr>
</thead>
<tbody>
<tr>
<td>start</td>
<td>Start position of the genomic region of interest.</td>
</tr>
<tr>
<td>end</td>
<td>End position of the genomic region of interest.</td>
</tr>
</tbody>
</table>

**Details**

The functions return the MWAS results and/or locations.

**Value**

Function getLocations returns a data frame with

<table>
<thead>
<tr>
<th>chr</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>start</td>
<td>Start position</td>
</tr>
<tr>
<td>end</td>
<td>End position</td>
</tr>
</tbody>
</table>

Function getMWAS returns a data frame with

<table>
<thead>
<tr>
<th>cor</th>
<th>coverage - phenotype correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>t.test</td>
<td>t-statistic</td>
</tr>
<tr>
<td>p.value</td>
<td>p-value</td>
</tr>
<tr>
<td>q.value</td>
<td>q-value (FDR)</td>
</tr>
</tbody>
</table>

If the outcome variable was categorical, columns cor and t.test are replaced with R.squared and F-test.

Functions getMWASandLocations and getMWASrange return a data frame with elements of output of both getLocations and getMWAS

Function getDataByLocation returns a list with

<table>
<thead>
<tr>
<th>locations</th>
<th>Chromosomal location information for located variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>matrix</td>
<td>Data (coverage) matrix for the selected locations</td>
</tr>
</tbody>
</table>

**Author(s)**

Andrey A Shabalin <andrey.shabalin@gmail.com>

**See Also**

See vignettes: browseVignettes("ramwas").

**Examples**

```R
# Not run:
# Extract locations using parameter vector
getLocations(param)

# Extract locations using directory name
getLocations("/data/myMWAS")

# Extract MWAS using parameter vector
```

```R
```
getWAS(param)

# Extract MWAS using directory name
getWAS("/data/myMWAS")

# Extract MWAS using parameter vector
getMWASandLocations(param)

# Extract MWAS using directory name
getMWASandLocations("/data/myMWAS")

# Extract MWAS for a region
getMWASrange(param, 1, 123321, 223321)

# Chromosome can be character
getMWASrange(param, "chr1", 123321, 223321)

# Extract data for a region
getDataByLocation(param, 1, 123321, 223321)

# Chromosome can be character
getDataByLocation(param, "chr1", 123321, 223321)

## End(Not run)

---

**getCpGset**

*Construct CpG set for a Reference Genome*

**Description**

Finds all CpGs in a reference genome.

**Usage**

```r
c CpGsetCG(genome)
c CpGsetALL(genome)
```

**Arguments**

- **genome**: A `BSgenome` object or a character vector with genome sequences.

**Details**

The `getCpGsetCG` function searches for all CG pairs in the genome.

The `getCpGsetALL` function also works for genomes with injected SNPs.

**Value**

Returns a list with CpG coordinates for each genome sequence.
Author(s)
Andrey A Shabalin <andrey.shabalin@gmail.com>

Examples
### Using a BSGenome input
```r
library(BSgenome.Ecoli.NCBI.20080805)
cpgset = getCpGsetCG(BSgenome.Ecoli.NCBI.20080805)
print("First 10 CpGs in NC_008253:")
print(cpgset$NC_008253[1:10])
```

### Using a character vector input
```r
genome = list(
    chr1 = "AGCGTTTTCATTCTGACTGCAACGGGCYR",
    chr2 = "AAAAACGCTTAGTAAGTGATTTTCGYR")
cpgset1 = getCpGsetCG(genome)
cpgset2 = getCpGsetALL(genome)
print("Pure CG coordinates in the toy genome:")
print(cpgset1)
print("CG coordinates in the toy genome possible with SNPs:")
print(cpgset2)
```

---

`injectSNPsMAF`  *Inject SNPs from VCF Count File into a DNA Sequence*

**Description**
Infects SNPs from a VCF count file into a DNA sequence.

**Usage**

```r
injectSNPsMAF(gensequence, frqcount, MAF = 0.01)
```

**Arguments**
- `gensequence`: A string or `DNAString` of the DNA sequence.
- `frqcount`: File name of the allele count file produced by `vcftools` with `--counts` parameter. Alternatively, the file content can be provided as a character vector (see `readLines`).
- `MAF`: SNPs with minor allele frequency at or above `MAF` are injected.

**Value**
Returns a string with the genome sequence with SNPs injected.
Construct FASTQ File for In-silico Alignment Experiment

Creates a FASTQ file with all fragments of \texttt{fraglength} bp long.

\section*{Usage}
\begin{verbatim}
insilicoFASTQ(con, gensequence, fraglength)
\end{verbatim}

\section*{Arguments}
\begin{itemize}
  \item \texttt{con} \hspace{1cm} A \texttt{connection} object or a character string naming the output file. If the name ends with ".gz", a compressed file is created. An empty string can be used to output to the console.
  \item \texttt{gensequence} \hspace{1cm} A string or \texttt{DNAString} of the DNA sequence.
  \item \texttt{fraglength} \hspace{1cm} Fragment length.
\end{itemize}

\section*{Details}
The function a FASTQ file with all fragments of \texttt{fraglength} bp long from the forward strand of the DNA sequence.
isAbsolutePath

Value

Returns a list with CpG coordinates for each genome sequence.

Author(s)

Andrey A Shabalin <andrey.shabalin@gmail.com>

Examples

```r
## There are four 4 bp fragments in a 7 basepair sequence:
insilicoFASTQ(con="", gensequence = "ABCDEFG", fraglength=4)
```

---

isAbsolutePath Check if Path is Absolute.

Description

Check whether a path is relative or absolute.

Usage

```r
isAbsolutePath(path)
```

Arguments

- `path` Path to be tested.

Details

The function is designed to work with both Windows and Unix paths.

Value

TRUE if the path is absolute, FALSE otherwise.

Note

This function improves upon the analog function in R.utils package. For instance, "~hi" is not an absolute path.

Author(s)

Andrey A Shabalin <andrey.shabalin@gmail.com>

See Also

See also `makefullpath`. 
madeBED

Export MWAS results in BED format.

Description

Functions for exporting MWAS results in BED format files.
Function madeBED saves MWAS findings in BED format for all variables (CpGs), while madeBEDrange selects only variables on a given chromosome between given locations.
Functions madeBEDgraph and madeBEDgraphRange do the same, but create a file in BedGraph format.

Usage

madeBED(x, filename)
madeBEDrange(x, filename, chr, start, end)
madeBEDgraph(x, filename)
madeBEDgraphRange(x, filename, chr, start, end)

Arguments

x Name of MWAS directory (parameter dirmwas) or list of RaMWAS parameters as described in the "RW6_param.Rmd" vignette.
Try: vignette("RW6_param","ramwas").
filename Name of the BED file to create. If file exists, it’s overwritten.
chr Chromosome name or number.
start Start position of the genomic region of interest.
end End position of the genomic region of interest.

Details

The function returns the MWAS results with locations.

Value

Returns a data.frame with BED file content:

 chrom Chromosome
 chromStart Start position
 chromEnd End position
 name Empty name column. BED format only
 score p-value

Examples

isAbsolutePath( "C:/123" ) # TRUE
isAbsolutePath( "~123" ) # FALSE
isAbsolutePath( "~/123" ) # TRUE
isAbsolutePath( "/123" ) # TRUE
isAbsolutePath( "\\123" ) # TRUE
isAbsolutePath( "asd\\123" ) # FALSE
isAbsolutePath( "a\\123" ) # FALSE
makefullpath

Combine Path and Filename into Filename with Path

Description

Combine a path with a filename into filename with path.

Usage

makefullpath(path, filename)

Arguments

path
Path, relative to which the filename is expected to be. Can be absolute, relative, or NULL.

filename
Can be just filename, include relative path, or include absolute path.

Details

Function returns filename if it includes absolute path or if path is NULL.

Value

Filename with the path included.

Author(s)

Andrey A Shabalin <andrey.shabalin@gmail.com>

See Also

See also isAbsolutePath.
Examples

```r
makefullpath("dir1/dir2", "file.txt")
# "dir1/dir2/file.txt"

makefullpath("dir1/dir2", "dir3/file.txt")
# "dir1/dir2/dir3/file.txt"

# Path is ignored if the filename already includes absolute path
makefullpath("dir1/dir2", "/file.txt")
# "/file.txt"

makefullpath("dir1/dir2", "C:/file.txt")
# "C:/file.txt"
```

---

**Description**

The function `manPlotFast` creates a Manhattan plot. The function `manPlotPrepare` extracts necessary information from a vector of p-values sufficient for creating a Manhattan plot. It optimized to work quickly even for tens of millions of p-values.

**Usage**

```r
manPlotPrepare(
  pvalues,
  chr,
  pos,
  ismlog10 = FALSE,
  chrmargins = 5e6)

manPlotFast(
  man,
  ylim = NULL,
  colorSet = c('steelblue4','#2C82D1','#4CB2D1'),
  yaxmax = NULL,
  lwd = 3,
  axistep = 2,
  cex = 1)
```

**Arguments**

- `pvalues` Vector of p-values. As is (if `ismlog10 = FALSE`) or minus log10 transformed (if `ismlog10 = TRUE`).
- `chr, pos` Vectors indicating the chromosomes and genomic positions (in basepairs) for each p-value in `pvalues`.
- `ismlog10` Specifies whether the provides p-values (`pvalues` parameter) are minus log10 transformed (\(-\log_{10}(pv)\))
The plot margins at the ends of chromosomes (in basepairs).

Object returned by manPlotPrepare.

Numeric vectors of length 2, giving the y coordinate range. Exactly as in Plotting Parameters.

Colors of points, rotating over chromosomes. Points for first chromosome have color colorSet[1], next colorSet[2], etc. Once the colors are exhausted, the colors are reused from the beginning.

Maximum reach of the y axis.

The line width. As in Graphics Parameters.

Distance between axis label ticks for y axis.

The size of Manhattan plot points. As in Graphics Parameters.

The function manPlotFast creates Manhattan plot. It requires the use of the function manPlotPrepare which extracts the necessary information from a vector of p-values sufficient for creating Manhattan plot. The resulting object is many times smaller than the vector of p-values.

This function manPlotPrepare returns an object with information for creating Manhattan plot.

The plot has no title. To add a title use title.

Andrey A Shabalin <andrey.shabalin@gmail.com>

See vignettes: browseVignettes("ramwas").

Examples

# Simulate data (9 chromosomes, million tests each)
chr = rep(paste0('chr', 1:9), each = 1e6)
pos = rep(1:1e6, 9)
pv = runif(9e6)^1.1

# Extract the Manhattan plot info
man = manPlotPrepare(pv, chr, pos, chrmargins = 1000)

# Create Manhattan plot
manPlotFast(man)
title("Manhattan plot")

# Size of p-values before extraction of Manhattan plot info
object.size(list(pv, chr, pos))
# Size of the Manhattan plot info object

```r
object.size(man)
```

---

**mat2cols**

### Split a Matrix into Column Vectors

**Description**

Internal function for splitting a matrix into column vectors.

**Usage**

```r
mat2cols(x)
```

**Arguments**

- `x` A matrix.

**Value**

List of matrix columns.

**Author(s)**

Andrey A Shabalin <andrey.shabalin@gmail.com>

**See Also**

See vignettes: `browseVignettes("ramwas")`.

**Examples**

```r
# Sample data
data = matrix(1:12, nrow = 3)

# Split it
mat2cols(data)
```

---

**orthonormalizeCovariates**

### Orthonormalize Covariates

**Description**

Takes a matrix of data frame with covariates, adds a constant covariate (optional), and orthonormalizes the set.

**Usage**

```r
orthonormalizeCovariates(cvrt, modelhasconstant)
```
**parameterDump**

Save Parameters in a Text File

**Description**

Saves parameters in a text file, prioritizing those listed in toplines.

**Usage**

```r
parameterDump(dir, param, toplines = NULL)
```
**parameterDump**

**Arguments**

- **dir**
  - Directory to save the parameters to. The file is named "UsedSettings.txt".

- **param**
  - A list with RaMWAS parameters. Or any list in general.
  - For detailed description of all available parameters run: browseVignettes("ramwas").

- **toplines**
  - Names of the elements in param to save first (top of the file).

**Details**

- This function is used internally by multiple RaMWAS functions to record parameters used to run the analysis.

**Value**

- The function creates a file and returns nothing.

**Note**

- This function is not intended to be run by the user.

**Author(s)**

- Andrey A Shabalin <andrey.shabalin@gmail.com>

**See Also**

- See vignettes: browseVignettes("ramwas")

**Examples**

```r
param = ramwasParameters(
  number = 123123,
  integer = 312L,
  textline = "Hi there",
  characterVector = c("Hi","Hi again","Bye"),
  dataframe = data.frame(a = 1:12, b = 12:1)
)

thedir = tempdir()

parameterDump(thedir, param, c("integer","characterVector"))

cat( readlines( paste0(thedir,"/UsedSettings.txt") ), sep = "\n")

file.remove( paste0(thedir,"/UsedSettings.txt") )
```
parameterPreprocess Preprocess Pipeline Parameter List.

Description
Fill in missing parameters with default values, read supporting data files, make relative directory path parameters absolute.

Usage
parameterPreprocess(param)

Arguments
param List with RaMWAS parameters.
For detailed description of all available parameters run:
browseVignettes(“ramwas”).

Details
A number of common preprocessing steps necessary for parameters of multiple pipeline parts are combined in this function. The actions include

• Fill in default values for all missing parameters.
• Set bamnames parameter to the content filebamlist file (if bamnames was not set).
• Set bam2sample parameter to the content of filebam2sample file (if bam2sample was not set).
• Set covariates parameter to the data frame from filecovariates file (if covariates was not set).
• Check parameters for consistency, i.e. that modelcovariates include only names of columns in covariates.
• Check that files filecpgset and filenoncpgset exist if the parameters are set.

Value
Returns preprocessed list of parameters.

Note
This function is not intended to be run by the user.

Author(s)
Andrey A Shabalin <andrey.shabalin@gmail.com>

See Also
See vignettes: browseVignettes(“ramwas”).
parametersFromFile

**Examples**

```r
param = ramwasParameters(
    dirproject = "."
)

param2 = parameterPreprocess(param)

print(param2)
```

**parametersFromFile**  
*Scan Parameters From a R Code File*

**Description**

The pipeline parameters can be stored in a simple file, formatted as R code. The `parametersFromFile` function transforms them into a parameter list used by RaMWAS steps.

**Usage**

```r
parametersFromFile(.parameterfile)
```

**Arguments**

- `.parameterfile`  Name of the file with the parameters set as R variables. See the example below.

**Details**

Variables with names starting with period (.) are ignored.

**Value**

Returns the list with all the variables set in the file.

**Note**

The file `.parameterfile` is executed as R code, so use only trusted parameter files.

**Author(s)**

Andrey A Shabalin <andrey.shabalin@gmail.com>

**See Also**

See vignettes: `browseVignettes("ramwas")`. 
Examples

```r
filename = tempfile()

# Create a file with lines
# dirproject = "."
# modelcovariates = c("Age","Sex")
writeLines(
  con = filename,
  text = c(
    "dirproject = ".",
    "modelcovariates = c("Age","Sex")"
  )
)

# Scan the file into a list
param = parametersFromFile(filename)

# Show the list
print(param)

file.remove(filename)
```

---

**pipeline**  

**RaMWAS: High Level Pipeline Functions**

### Description

These functions provide a simple way to run all steps of RaMWAS pipeline.

### Usage

```r
ramwas1scanBams(param)
pipelineProcessBam(bamname, param)
ramwas2collectqc(param)
ramwas3normalizedCoverage(param)
ramwas4PCA(param)
ramwas5MWAS(param)
ramwas6annotateTopFindings(param)
ramwas7ArunMWASes(param)
ramwas7BrunElasticNet(param)
ramwas7CplotByNCpGs(param)
ramwas7riskScoreCV(param)
ramwasSNPs(param)
```

### Arguments

- **param**  
  List with RaMWAS parameters.  
  For detailed description of all available parameters run:  
  `browseVignettes("ramwas")`.

- **bamname**  
  Name of the BAM file to process. Can be absolute or relative to `dirbam` parameter (in `param` list).
Details

See vignettes for details: browseVignettes("ramwas").

Value

Function pipelineProcessBam returns "OK. <bamname>" if no error occurred. Otherwise, returns text with error. Other functions return nothing.

Author(s)

Andrey A Shabalin <andrey.shabalin@gmail.com>

See Also

See vignettes: browseVignettes("ramwas").

Examples

```r
param = ramwasParameters(
  dirbam = "/project/bams",
  dirproject = "/project",
  filebamlist = "000_list_of_files.txt",
  scoretag = "AS",
  minscore = 100,
  cputhreads = 4,
  filecpgset = "/RamWAS/hg19_1kG_MAF_0.01_chr1-22_bowtie2_75bp.rds",
  filenoncpgset = "/RamWAS/hg19_1kG_MAF_0.01_chr1-22_bowtie2_75bp_nonCpG.rds",
  maxrepeats = 3,
  maxfragmentsize = 250,
  minfragmentsize = 75,
  filebam2sample = "000_list_of_files.txt",
  filecovariates = "Covariates.txt",
  modelcovariates = c("Age","Sex"),
  modeloutcome = "CellType",
  modelPCs = 1,
  cvnfolds = 10,
  mmncpgs = 1000,
  mmalpha = 0
)
## Not run:
ramwas1scanBams(param)
ramwas2collectqc(param)
ramwas3normalizedCoverage(param)
ramwas4PCA(param)
ramwas5MWAS(param)
ramwas6annotateTopFindings(param)
ramwas7riskScoreCV(param)
## End(Not run)
```
plotCV

Plotting Functions used in Cross Validation Analysis (Methylation Risk Score).

Description

The function plotPrediction plots cross validation predictions of a phenotype against true values of the phenotype with multiple summary stats in the title.

The function plotCVcors plots the predictive power (correlations) across predictions using various numbers of markers.

The function plotROC plots an ROC (Receiver operating characteristic) curve for predictions of a binary outcome.

Usage

plotPrediction(param, outcome, forecast, cpgs2use, main, dfFull = NULL)

plotCVcors(cl, param)

plotROC(outcome, forecast)

Arguments

param List of parameters as described in the "RW6_param.Rmd" vignette. Try: vignette("RW6_param","ramwas"). Only modeloutcome, cvnfolds and mmalpha elements are used.

outcome Values of a phenotype. Must be binary for plotROC.

forecast Predictions for the phenotype.

cpgs2use Number of variables used for prediction (for the legend).

main Part of the title (summary stats are added beneath).

dfFull Number of degrees of freedom for the significance testing. Default is: length(forecast) - 2

cl List with three elements:

- x - vector with the number of variables used for prediction
- corp - Pearson correlations between the predictions and the true value of the phenotype.
- cors - Spearman correlations between the predictions and the true value of the phenotype.

Details

The plotROC and plot has no title.
To add a title use title.
The `plotROC` returns the area under the curve (AUC) for the ROC. The `plotPrediction` function returns the list of calculated statistics printed in the title. The `plotCVcors` returns nothing (NULL).

**Author(s)**

Andrey A Shabalin <andrey.shabalin@gmail.com>

**See Also**

See vignettes: `browseVignettes("ramwas")`.

**Examples**

```r
# Sample data
n = 300
param = list(modeloutcome = "Age", malpha = 0, cvnfolds = 5)
outcome = rnorm(n, mean = 50, sd = 20)
forecast = outcome + rnorm(n, mean = 0, sd = 20)
cpgs2use = 1000
main = "Prediction success (simulated data)"

# Plot phenotype-prediction plot
plotPrediction(
  param,
  outcome,
  forecast,
  cpgs2use,
  main)

# Artificial data for plotCVcors()
cl = list(
  x = c(50, 100, 200, 500, 1000),
  corp = c(0.1, 0.6, 0.7, 0.85, 0.8),
  cors = c(0.1, 0.6, 0.7, 0.85, 0.8) + rnorm(5)*0.1)

# Plot prediction performance by the number of markers
plotCVcors(cl, param)

# Make the outcome binary for ROC plot
outcome = (outcome > 50)

# Plot ROC curve and calculate the AUC
plotROC(outcome, forecast)
```

---

**plotFragmentSizeDistributionEstimate**

Estimate and plot Fragment Size Distribution.

**Description**

RaMWAS functions for estimation and plotting of the fragment size distribution.
Usage

```r
estimateFragmentSizeDistribution(frdata, seqLength)
plotFragmentSizeDistributionEstimate(
  frdata, estimate, col1 = "blue", col2 = "red")
```

Arguments

- `frdata`: Distribution of distances from the starts of isolated reads to the respective CpGs.
- `seqLength`: The length of sequenced part of the fragments. The fragments are assumed to not be smaller than `seqLength`.
- `estimate`: Fragment size distribution estimate.
- `col1`: Color of `frdata` points.
- `col2`: Color of `estimate` curve.

Value

The function `estimateFragmentSizeDistribution` returns the estimate of the fragment size distribution.

Note

If the length of `frdata` is equal to `seqLength`, the fragments are assumed to all be of length `seqLength`.

Author(s)

Andrey A Shabalin <andrey.shabalin@gmail.com>

See Also

See vignettes: `browseVignettes("ramwas")`.

Examples

```r
# Simulate data
x = 0:250
truemean = 1 - pnorm(x, mean = 150, sd = 50)
frdata = rpois(n = length(x), lambda = truemean*300)

# Estimate fragment size distribution
estimate = estimateFragmentSizeDistribution(frdata, seqLength = 50)

# Plot fragment size distribution estimate
plotFragmentSizeDistributionEstimate(frdata, estimate)
```
Description

The function `plotPCvalues` plots PC values (variation explained). The function `plotPCvectors` plots PC vectors (loadings).

Usage

```
plotPCvalues(values, n = 40, ylim = NULL, col = "blue")
plotPCvectors(eigenvector, i, col = "blue")
```

Arguments

- `values`: Vector of PC values.
- `n`: Number of top PCs to plot.
- `ylim`: Numeric vectors of length 2, giving the y coordinate range. Exactly as in Plotting Parameters.
- `col`: Color of the plotted points.
- `eigenvector`: The i-th eigenvector. See `eigen`.
- `i`: Indicates loadings of which PC to plot.

Value

This function creates a PC plot and returns nothing (NULL).

Author(s)

Andrey A Shabalin <andrey.shabalin@gmail.com>

See Also

See vignettes: `browseVignettes("ramwas")`.

Examples

```
# Sample data
# for 1000 observations and 10 samples
m = 1000
n = 10
data = matrix(rnorm(n*m), nrow = m)

covmat = crossprod(data)
e = eigen(covmat)

# Plot PC values
plotPCvalues(e$values)
```
# Plot PC vectors
plotPCvectors(e$vectors[,1], 1)
plotPCvectors(e$vectors[,2], 2)

---

**processCommandLine**  **Scan Parameters From Command Line**

### Description
The pipeline parameters can be provided via command line.

For example:

```r
R pipeline.r dirproject="/project" maxrepeats=0 modeloutcome="Age"
```

Each command line argument is treated as an R statement.

All variables defined this way are collected in a list which is returned.

### Usage
```
processCommandLine(.arg = NULL)
```

### Arguments
- `.arg` Vector of command line parameters. Obtained from `commandArgs` if omitted.

### Details
If a command line argument defines variable "fileparam", it is assumed to be a filename, and the file with this name is scanned for extra pipeline parameters, as by `parametersFromFile`.

### Value
Returns the list with all the variables set by the statement in the command line.

### Note
Variables with names starting with period (.) are ignored.

### Author(s)
Andrey A Shabalin <andrey.shabalin@gmail.com>

### See Also
See vignettes: `browseVignettes("ramwas")`. 
Examples

```r
filename = tempfile()

# Assume command line with two components:
# dirproject="."  
# modelcovariates=c("Age","Sex")

arg = c(
    "dirproject = ".","",
    "modelcovariates = c("Age","Sex")")

# Process the command line
param = processCommandLine(arg)

# Show the list
print(param)
```

---

**pvalue2qvalue**

*Calculate Benjamini-Hochberg q-values*

Description

Calculate Benjamini-Hochberg q-values for a vector of p-values.

Usage

```r
pvalue2qvalue(pv, n = length(pv))
```

Arguments

- **pv**: Vector of p-values.
- **n**: If `pv` has only top p-values from a bigger set, `n` should indicate the number of tests performed.

Details

The q-values can be slightly conservative compared to other popular q-value calculation methods.

Value

Return a vector of q-values matching p-values in `pv`.

Note

The function runs faster if the vector `pv` is sorted.

Author(s)

Andrey A Shabalin <andrey.shabalin@gmail.com>
Examples

```r
pv = runif(20)^2
qv = pvalue2qvalue(pv)
```

---

### Description

RaMWas calculates a number of QC measures for each BAM and saves them in R .rds files.

For full description of the QC measures and the plotting options run `vignette("RW3_BAM_QCs")`

### Usage

```r
qcmean(x)
## S3 method for class 'NULL'
qcmean(x)
## S3 method for class 'qcChrX'
qcmean(x)
## S3 method for class 'qcChrY'
qcmean(x)
## S3 method for class 'qcCoverageByDensity'
qcmean(x)
## S3 method for class 'qcEditDist'
qcmean(x)
## S3 method for class 'qcEditDistBF'
qcmean(x)
## S3 method for class 'qcFrwrev'
qcmean(x)
## S3 method for class 'qcHistScore'
qcmean(x)
## S3 method for class 'qcHistScoreBF'
qcmean(x)
## S3 method for class 'qcIsoDist'
qcmean(x)
## S3 method for class 'qcLengthMatched'
qcmean(x)
## S3 method for class 'qcLengthMatchedBF'
qcmean(x)
## S3 method for class 'qcNonCpGreads'
qcmean(x)
```

```r
plot(x, samplename="", xstep = 25, ...)
## S3 method for class 'qcHistScore'
plot(x, samplename="", xstep = 25, ...)
## S3 method for class 'qcHistScoreBF'
plot(x, samplename="", xstep = 25, ...)
```

---

### Quality Control Measures

<table>
<thead>
<tr>
<th>QC Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>RaMWAS calculates a number of QC measures for each BAM and saves them in R .rds files.</td>
</tr>
<tr>
<td>For full description of the QC measures and the plotting options run <code>vignette(&quot;RW3_BAM_QCs&quot;)</code></td>
</tr>
</tbody>
</table>
## S3 method for class 'quotesingle.Var'
cqEditDistBF
plot(x, samplename="", xstep = 5, ...)
## S3 method for class 'quotesingle.Var'
cqLengthMatched
plot(x, samplename="", xstep = 25, ...)
## S3 method for class 'quotesingle.Var'
cqLengthMatchedBF
plot(x, samplename="", xstep = 25, ...)
## S3 method for class 'quotesingle.Var'
cqIsoDist
plot(x, samplename="", xstep = 25, ...)
## S3 method for class 'quotesingle.Var'
cqCoverageByDensity
plot(x, samplename="", ...)
## Number of recorded reads aligned to
## the forward and reverse strands respectively

cat("Reads on forward strand: ", qc$frwrev[1], 
"
")
cat("Reads on reverse strand: ", qc$frwrev[2], 
"
")
cat("Fraction of reads on forward strand: ", qcmean(qc$frwrev), 
"
")

## Distribution of the read scores

cat("Average alignment score: ", qcmean(qc$hist.score1), 
"
")
cat("Average alignment score, no filter: ", qcmean(qc$bf.hist.score1), 
"
")
par(mfrow=c(1,2))
plot(qc$hist.score1)
plot(qc$bf.hist.score1)

## Distribution of the length of the aligned part of the reads

cat("Average aligned length: ", qcmean(qc$hist.length.matched), 
"
")
cat("Average aligned length, no filter: ", 


cat("Average aligned length, no filter: ", 


cat("Average aligned length, no filter: ", 


## Distribution of edit distance between
## the aligned part of the read and the reference genome

cat("Average edit distance: ", qcmean(qc$hist.edit.dist1), 
"
")
cat("Average edit distance, no filter: ", qcmean(qc$bf.hist.edit.dist1), 
"
")
par(mfrow = c(1,2))
plot(qc$hist.edit.dist1)
plot(qc$bf.hist.edit.dist1)

## Number of reads after removal of duplicate reads

cat("Reads without duplicates: ", qc$reads.recorded.no.repeats, 
"
")
cat("This is ", qc$reads.recorded.no.repeats / qc$reads.recorded * 100, 
"% of aligned reads", 
"
", sep="")
cat("Fraction of reads on forward strand (with duplicates): ", 


cat("Fraction of reads on forward strand (without duplicates): ", 


## Number of reads away from CpGs

cat("Non-CpG reads: ", qc$cnt.nonCpG.reads[1], 
"
")
cat("This is ", qcmean(qc$cnt.nonCpG.reads)*100, 
"% of recorded reads", 
"
", sep="")

## Average coverage of CpGs and non-CpGs

cat("Summed across ", qc$nbams, " bams", 
"
")
cat("Average CpG coverage: ", qc$avg.cpg.coverage, 
"
")
cat("Average non-CpG coverage: ", qc$avg.noncpg.coverage, 
"
")
cat("Enrichment ratio: ", qc$avg.cpg.coverage / qc$avg.noncpg.coverage)

## Coverage around isolated CpGs

plot(qc$hist.isolated.dist1)

## Fraction of reads from chrX and chrY

cat("chrX reads: ", qc$chrX.count[1], 
"
", 


cat("chrX reads: ", qc$chrX.count[1], 
"
", 


## Coverage vs. CpG density

cat("Highest coverage is observed at CpG density of",
    qcmean(qc$avg.coverage.by.density)^2)
plot(qc$avg.coverage.by.density)

### Description

Function `qqPlotFast` creates a QQ-plot with a confidence band and an estimate of inflation factor lambda. It optimized to work quickly even for tens of millions of p-values.

#### Usage

```r
qqPlotPrepare(
  pvalues,
  ntests = NULL,
  ismlog10 = FALSE)

qqPlotFast(
  x,
  ntests = NULL,
  ismlog10 = FALSE,
  ci.level = 0.05,
  ylim = NULL,
  newplot = TRUE,
  col = "#D94D4C",
  cex = 0.5,
  yaxmax = NULL,
  lwd = 3,
  axistep = 2,
  col.band = "#ECA538",
  makelegend = TRUE,
  xlab = expression(-log10(P), null),
  ylab = expression(-log10(P), observed))
```

#### Arguments

- **pvalues**: Vector of p-values. As is (if `ismlog10 = FALSE`) or minus log10 transformed (if `ismlog10 = TRUE`).
- **ntests**: If only significant p-values are provided, the total number of tests performed. By default `ntests` is equal to the length of `pvalues`.
- **ismlog10**: Specifies whether the provided p-values (pvalues parameter) are minus log10 transformed (~ log10(pv)).
- **x**: Either a vector of p-values, as in `qqPlotPrepare`, or the object returned by `qqPlotPrepare`.
- **ci.level**: Significance level of the confidence band. Set to `NULL` avoid plotting the confidence band.
qqPlotFast

ylim Numeric vectors of length 2, giving the y coordinate range. Exactly as in Plotting Parameters.
newplot If TRUE, the function creates a new plot window.
col The QQ-plot curve color.
col.band Confidence band curve color.
cex The size of QQ-plot points. As in Graphics Parameters.
lwd The line width. As in Graphics Parameters.
axistep Distance between axis label ticks for both axis.
yamax Maximum reach of the y axis.
makelegend If true, add legend to the plot.
xlab, ylab Axis labels. As in plot function.

Details

The function qqPlotFast creates a QQ-plot.
The function qqPlotPrepare extracts the necessary information from a vector of p-values sufficient for creating QQ-plot.
The resulting object is many times smaller than the vector of p-values.

Value

The function qqPlotPrepare returns an object with the necessary information from a vector of p-values sufficient for creating QQ-plot.

Note

The plot has no title. To add a title use title.

Note

The function works faster if the p-values are sorted.

Author(s)

Andrey A Shabalin <andrey.shabalin@gmail.com>

See Also

See vignettes: browseVignettes("ramwas").

Examples

# Million p-values
n = 1e6

# Null p-values
pv = runif(n)

# QQ-plot should be nearly diagonal
qqPlotFast(pv)
title("QQ-plot")
```
# Size of p-values before extraction of QQ-plot info
object.size(pv)

# Extract the QQ-plot info
qq = qqPlotPrepare(pv)

# Size of the QQ-plot info object
object.size(qq)

# Create QQ-plot, it is the same
qqPlotFast(qq)

# Create QQ-plot with plotting parameters
qqPlotFast(qq, ylim = c(0,10), yaxmax = 9, axistep = 3, lwd = 3, cex = 1)
```

---

**ramwas0createArtificialData**

*Create Artificial Data Set*

**Description**

Creates a set of artificial BAM files and supplementary files which can be used to test run the pipeline. The BAMs contain reads aligned only to one human chromosome, with methylation effects embedded for simulated age and case-control status.

**Usage**

```r
test <- ramwas0createArtificialData(dir, 
  nsamples = 20, 
  nreads = 1e6, 
  ncpgs = 500e3, 
  randseed = 18090212, 
  threads = 1)
```

**Arguments**

- `dir`  
  Directory for generated RaMWAS project files and BAMs.
- `nsamples`  
  Number of samples/BAMs to create.
- `nreads`  
  Number of reads in each BAM file.
- `ncpgs`  
  Number of CpGs in the generated genome (with a single chromosome).
- `randseed`  
  Random number generator seed for consistency of the output.
- `threads`  
  Number of CPU cores to use for data generation.

**Details**

The function generates a number of files within `dir` directory.

1. `bam_list.txt` - list of created BAM files. To be used in `filebamlist` and `filebam2sample` parameters in the pipeline.
2. `covariates.txt` - table with age and sex status covariates. For use in `filecovariates` parameter in the pipeline.

3. `Single_chromosome.rds` - CpG location file with the selected chromosome only.

4. `bams` - directory with all the BAM files.

The generated BAMs have 600 CpGs affected by `sex`, namely fully methylated or not methylated at all, depending on `sex`. The methylation level of 1% of all CpGs is affected by age. The methylation of those CpGs is equal to `age/100` or `1-age/100`. The age is generated randomly in the range from 20 to 80.

**Value**

The function creates multiple files but returns no value.

**Author(s)**

Andrey A Shabalin <andrey.shabalin@gmail.com>

**See Also**

See vignettes: `browseVignettes("ramwas")`.

**Examples**

```r
### Location for the artificial project
dr = paste0(tempdir(), "/simulated_project")

ramwas0createArtificialData(
  dr,
  nsamples = 4,
  nreads = 1e3,
  ncpgs = 1e3)

# Artificial project files created in:
dr
# The generated files are:
as.matrix(list.files(dr, recursive=TRUE))

### Clean up
unlink(paste0(dr,"/"), recursive=TRUE)
```

---

**ramwasAnnotateLocations**

*Extract Biomart Annotation for a Vector of Locations.*

**Description**

Calls biomart annotation database for a vector of locations and assignes the tracks to the locations.

**Usage**

`ramwasAnnotateLocations(param, chr, pos)`
Arguments

param List of parameters as described in the "RW6_param.Rmd" vignette. Try: vignette("RW6_param","ramwas").

chr A vector of chromosome names or numbers.

pos A vector of genomic locations on the chromosomes.

Details

This function is used internally by RaMWAS annotation step.

Value

An annotation table, on line per supplied location.

Author(s)

Andrey A Shabalin <andrey.shabalin@gmail.com>

See Also

See vignettes: browseVignettes("ramwas")

Examples

bihost = "grch37.ensembl.org"
bimart = "ENSEMBL_MART_ENSEMBL"
bidataset = "hsapiens_gene_ensembl"
biaattributes = c("hgnc_symbol", "entrezgene", "strand")
bifilters = list(with_hgnc_trans_name = TRUE)
biflank = 0

param = ramwasParameters(
  bihost = bihost,
  bimart = bimart,
  bidataset = bidataset,
  biaattributes = biaattributes,
  bifilters = bifilters,
  biflank = biflank)

# Test a location
chr = "chr1"
pos = 15975530
## Not run:
ramwasAnnotateLocations(param, chr, pos)
## End(Not run)
ramwasParameters

Description

RaMWAS parameter vector which is used by major functions of the pipeline is a regular R list and setting it does not require a special function. However, using this function makes it much simpler in RStudio as the names and role of every parameter is showed in the RStudio IDE.

Usage

```r
ramwasParameters(
  dirproject,
  dirfilter,
  dirrbam,
  dirrqc,
  dirqc,
  dircoveragenorm,
  dirtemp,
  dirpca,
  dirmwas,
  dircv,
  dirbam,
  filebamlist,
  bamnames,
  filebam2sample,
  bam2sample,
  filecpgset,
  filenoncpgset,
  filecovariates,
  covariates,
  cputhreads,
  diskthreads,
  usefilelock,
  scoretag,
  minscore,
  maxrepeats,
  minavgcpgcoverage,
  minnonzerosamples,
 buffersize,
  doublesize,
  modelcovariates,
  modeloutcome,
  modelPCs,
  modelhasconstant,
  qqplottitle,
  toppvthreshold,
  mmncpgs,
  mmalpha,
  cvnfolds,
  bihost,
)```
Arguments

**dirproject**
The project directory. Default is current directory.
Files specified by "file" parameters are looked for here, unless they have full path specified.

**dirfilter**
By default, the same as "dirproject".
All files created by RaMWAS are created within this directory.
If the user wants to test different read filtering rules, they can dirfilter to TRUE.
This will set it to something like "Filter_MAPQ_4", where "MAPQ" is the BAM field used for filtering and "4" is the threshold.

**dirrbam**
Directory where RaMWAS saves RaMWAS raw data files (read start locations) after scanning BAMs.
It is "rds_rbam" by default and located in "dirfilter".

**drrqc**
Directory where RaMWAS saves QC files in R format after scanning BAMs.
It is "rds_qc" by default and located in "dirfilter".

**dirqc**
Directory where RaMWAS saves QC plots and text files (BAM QC info) after scanning BAMs.
It is "qc" by default and located in "dirfilter".

**dircoveragenorm**
Directory where RaMWAS saves coverage matrix at Step 3 of the pipeline.
It is "coverage_norm_123" by default (123 is the number of samples) and located in "dirfilter".

**dirtemp**
Directory where RaMWAS stores temporary files during construction of coverage matrix at Step 3 of the pipeline.
It is "temp" by default and located in "dircoveragenorm".
For better performance it can be set to a location on a different hard drive than "dircoveragenorm".

**dirpca**
Directory where RaMWAS saves results of PCA analysis at Step 4 of the pipeline.
It is "PCA_12_cvrts_0b0a0c" by default and located in "dircoveragenorm", where
12 is the number of covariates regressed out and "0b0a0c" is a unique code to
differentiate different sets of 12 covariates.

**dirmwas**
Directory where RaMWAS saves results of MWAS analysis at Step 5 of the pipeline.
It is "Testing_age_7_PCs" by default and located in "dirpca", where "age" is the phenotype being tested and "7" is number of top PCs included in the model.

**dircv**
Directory where RaMWAS saves results of Methylation Risk Score analysis at Step 7 of the pipeline.
It is "CV_10_folds" by default and located in "dirmwas", where 10 is number of folds in N-fold cross validation.

**dirbam**
Location of BAM files.
If not absolute, it is considered to be relative to "dirproject".
ramwasParameters

filebamlist If defined, must point to a text file with one BAM file name per line. BAM file names may include path, relative to "dirbam" or absolute.

bamnames A character vector with BAM file names. Not required if "filebamlist" is specified. BAM file names may include path, relative to "dirbam" or absolute.

filebam2sample Allows multiple BAMs contain information about common sample. Must point to a file with lines like "sample1=bam1,bam2,bam3".

bam2sample Allows multiple BAMs contain information about common sample. Not required if "filebam2sample" is specified.

filecpgset Name of the file storing a set of CpGs.

filenoncpgset If defined, must point to a file storing vetted locations away from any CpGs.

filecovariates Name of the file containing phenotype and covariates for the available samples. If the file has extension ".csv", it is assumed to be comma separated, otherwise - tab separated.

covariates Data frame with phenotype and covariates for the available samples. Not required if "filecovariates" is specified.

cputhreads Maximum number of CPU intensive tasks running in parallel. Set to the number of CPU cores by default.

diskthreads Maximum number of disk intensive tasks running in parallel. Set to 2 by default.

usefilelock If TRUE, parallel jobs are prevented from simultaneous access to file matrices. Can improve performance on some systems.

scoretag Reads from BAM files are filtered by this tag. The "minscore" parameter defines the minimum admissible score.

minscore Reads from BAM files with score "scoretag" below this are excluded.

maxrepeats Duplicate reads (reads with the same start position and direction) in excess of this limit are removed.

minavgcpgcoverage CpGs with average coverage below this threshold are removed.

minnonzerosamples CpGs with fraction of samples with non-zero coverage below this threshold are removed.

buffersize Coverage matrix transposition is performed using buffers of this size. Larger "buffersize" improves speed of Step 3 of the pipeline, but requires more memory. Default is 1e9, i.e. 1 GB.

doublesize The coverage matrix is stored with this number of bytes per value. Set to 8 for full (double) precision. Set to 4 to use single precision and create 50% smaller coverage filematrix.

modelcovariates Names of covariates included in PCA and MWAS.

modeloutcome Name of the outcome variable for MWAS.

modelPCs Number of principal components accounted for in MWAS.

modelhasconstant By default, the tested linear model includes a constant. To exclude it, set "modelhasconstant" parameter to FALSE.
**ramwasParameters**

- **qqplottitle**: The title of the QQ-plot produced by MWAS (step 4 of the pipeline).
- **toppvthreshold**: Determines the number of top MWAS results saved in text file. If it is 1 or smaller, it defines the p-value threshold. If larger than 1, it defines the exact number of top results.
- **mmncpgs**: Parameter for multi-marker elastic net cross validation (MRS). Defines the number of top CpGs on which to train the elastic net. Can be set of a vector of multiple values, each is tested separately.
- **mmalpha**: Parameter for multi-marker elastic net cross validation (MRS). Elastic net mixing parameter alpha. Set to 0 by default.
- **cvnfolds**: Parameter for multi-marker elastic net cross validation (MRS). The number of folds in the N-fold cross validation.
- **bihost**: Parameter for BiomaRt annotation (Step 6 of the pipeline). BioMart host site. Set to "grch37.ensembl.org" by default.
- **bimart**: Parameter for BiomaRt annotation (Step 6 of the pipeline). BioMart database name, see `listMarts`. Set to "ENSEMBL_MART_ENSEMBL" by default.
- **bidataset**: Parameter for BiomaRt annotation (Step 6 of the pipeline). BioMart data set, see `listDatasets`. Set to "hsapiens_gene_ensembl" by default.
- **biattributes**: Parameter for BiomaRt annotation (Step 6 of the pipeline). BioMart attributes of interest, see `listAttributes`. Set to c("hgnc_symbol","entrezgene","strand") by default.
- **bifilters**: Parameter for BiomaRt annotation (Step 6 of the pipeline). BioMart filters (if any), see `listFilters`. Set to `list(with_hgnc_transcript_name=TRUE)` by default ignore genes without names.
- **biflank**: Parameter for BiomaRt annotation (Step 6 of the pipeline). Allowed distance between CpGs and genes or other annotation track elements. Set to 0 by default, requiring direct overlap.
- **fileSNPs**: Name of the filematrix with genotype (SNP) data. The filematrix dimensions must match the coverage matrix.
- **dirSNPs**: Directory where RaMWAS saves the results of joint methylation-genotype analysis.
- **...**: Any other named parameters can be added here.

**Details**

The function simply collects all the parameters in a list. The main benefit of the function is that the user does not need to memorize the names of RaMWAS parameters.

Here is how it helps in RStudio:
rowcolSumSq

Value
List with provided parameters.

Author(s)
Andrey A Shabalin <andrey.shabalin@gmail.com>

See Also
See vignettes: browseVignettes("ramwas").

Examples
ramwasParameters(dirproject = ".", cputhreads = 4)

---

Form Row and Column Sums of Squares

Description
Form row and column sums of squares for numeric matrices. The functions are introduced as faster analogs of rowSums(x^2) and colSums(x^2) calls.

Usage
rowSumsSq(x)
colSumsSq(x)

Arguments
x Numeric matrix.

Details
The function is implemented in C for better performance.

Value
Return a vector of sums of values in each row/column for matrix x (rowSumsSq/colSumsSq).

Author(s)
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See Also
See rowSums and colSums for simple (not squared) row/column sums.
Examples

```r
x = matrix( 1:99, 9, 11)

# Calculate sums of squared elements in each row
rsum2 = rowSumsSq(x)

# Compare with alternative calculation
stopifnot( all.equal( rsum2, rowSums(x^2) ))

# Calculate sums of squared elements in each column
csum2 = colSumsSq(x)

# Compare with alternative calculation
stopifnot( all.equal( csum2, colSums(x^2) ))
```

**rwDataClass-class**

*Class for Accessing Data (Coverage) Matrix*

**Description**

This class is a wrapper for accessing the data (coverage) matrix. It automatically subsets the samples to those listed in the covariates. Data access function imputes missing values and can residualize the variables.

**Extends**

rwDataClass is a reference classes (see envRefClass).

**Fields**

- `fmdata`: Filematrix object for the data matrix. Not intended to be accessed directly.
- `samplenames`: Vector of sample names.
- `nsamples`: Number of samples
- `ncpgs`: Number of variables (CpG sites) in the data matrix.
- `ndatarows`: Number of variables in the data matrix (may be bigger than the number of samples).
- `rowsubset`: Indices of samples in the data matrix.
- `cvrtqr`: Matrix of orthonormalized covariates.

**Methods**

- `initialize(param = NULL, getPCs = TRUE, lockfile = NULL)`: Create the data access class. 'param' should contain the RaMWAS parameter vector. 'getPCs' indicates if the covariate set should include Principal components. 'lockfile' is the 'lockfile' parameter used in accessing the data filematrix.
- `open(param = NULL, getPCs = TRUE, lockfile = NULL)`: The same as 'initialize' method, but for already created object.
- `close()`: Clears the object. Closes the filematrix.
- `getDataRez(colset, resid = TRUE)`: Extracts data for variables indexed by 'colset'. The data is residualized unless `resid = FALSE`. 
subsetData

Subset a data matrix and locations

Description

Subset a data (coverage) matrix and corresponding matrix of locations to a specified set of locations.

Usage

subsetCoverageDirByLocation(x, chr, start, targetdir)

Arguments

x Name of data (coverage) directory or list of RaMWAS parameters as described in the "RW6_param.Rmd" vignette. Try: vignette("RW6_param","ramwas").
chr Vector of chromosome names or numbers.
start Start positions of the CpGs of interest.
targetdir Directory name for the new (subset) data matrix and locations.

Value

The function returns nothing.

Author(s)

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testPhenotype

See Also
See vignettes: browseVignettes("ramwas").

Examples

x = "/data/myCoverageMatrix"
chr = c("chr1", "chr2", "chr3")
start = c(12345, 123, 12)
targetdir = "/data/subsetCoverageMatrix"

## Not run:
subsetCoverageDirByLocation(x, chr, start, targetdir)
## End(Not run)

testPhenotype

Test the Phenotype of Interest for Association with Methylation Coverage.

Description
An internal, function for fast association testing. It tests the phenotype of interest for association with methylation coverage (columns of the data parameter).

Usage
testPhenotype(phenotype, data1, cvrtqr)

Arguments

- **phenotype** Vector with phenotype. Can be numerical, character, or factor vector.
- **data1** Matrix with data (normalized coverage), one variable (CpG) per column.
- **cvrtqr** Orthonormalized covariates, one covariate per column. See orthonormalizeCovariates.

Details
The testing is performed using matrix operations and C/C++ code, employing an approach similar to that in MatrixEQTL.

Value
If the phenotype is numerical, the output is a list with

- **correlation** Correlations between residualized phenotype and data columns.
- **tstat** Corresponding T-statistics
- **pvalue** Corresponding P-values
- **nVarTested** Always 1
- **dfFull** Number of degrees of freedom of the T-test

If the phenotype is a factor (or character)
testPhenotype

R-squared  R-squared for the residualized ANOVA F-test.
Fstat      Corresponding F-test
pvalue     Corresponding P-values
nVarTested First number of degrees of freedom for the F-test. Equal to the number of factor levels reduced by 1
dffull     Second number of degrees of freedom for the F-test.

Note
This function is used in several parts of the pipeline.

Author(s)
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See Also
See vignettes: browseVignettes("ramwas").
Also check orthonormalizeCovariates.

Examples

### Generate data inputs
# Random data matrix with signal in the first column
data = matrix(runif(30*5), nrow = 30, ncol = 5)
data[,1] = data[,1] + rep(0:2, each = 10)
# Two random covariates
cvrt = matrix(runif(2*30), nrow = 30, ncol = 2)
cvrtqr = orthonormalizeCovariates(cvrt)

### First, illustrate with numerical phenotype
# Numerical, 3 value phenotype
phenotype = rep(1:3, each = 10)
# Test for association
output = testPhenotype(phenotype, data, cvrtqr)
# Show the results
print(output)
# Comparing with standard R code for the first variable
summary(lm( data[,1] ~ phenotype + cvrt ))

### First, illustrate with numerical phenotype
# Categorical, 3 group phenotype
phenotype = rep(c("Normal", "Sick", "Dead"), each = 10)
# Test for association
output = testPhenotype(phenotype, data, cvrtqr)
# Show the results
print(output)

# Comparing with standard R code for the first variable
anova(lm( data[,1] ~ cvrt + phenotype ))
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