Package 'ramwas'

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Type Package

Title Fast Methylome-Wide Association Study Pipeline for Enrichment Platforms

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Description 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 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 methlyation and genotype data.

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

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

License LGPL-3

LazyLoad yes

NeedsCompilation yes

Depends R (>= 3.3.0), methods, filematrix

VignetteBuilder knitr

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Imports graphics, stats, utils, digest, glmnet, KernSmooth, grDevices, GenomicAlignments, Rsamtools, parallel, biomaRt, Biostrings, BiocGenerics

biocViews DNAMethylation, Sequencing, QualityControl, Coverage, Preprocessing, Normalization, BatchEffect, PrincipalComponent, DifferentialMethylation, Visualization

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R topics documented:

`ramwas-package` *Fast Methylome-wide Association Study Pipeline for Enrichment Platforms*

Description

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 correctingfor 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 Shabalin <ashabalin@vcu.edu>

Maintainer: Andrey Shabalin <ashabalin@vcu.edu>

See Also

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

`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 instanteneously.

Usage

```
cachedRDSload(rdsfilename)
```

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 Shabalin <ashabalin@vcu.edu>

Examples

```
### Change filename to hg19 CpGset

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

```
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 <ashabalin@vcu.edu>

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 ))
```

`getCpGset` *Construct CpG set for a Reference Genome*

Description

Finds all CpGs in a reference genome.

Usage

```
getCpGsetCG(genome)
getCpGsetALL(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 <ashabalin@vcu.edu>

Examples

```
### Using a BSGenome input

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

genome = list(
    chr1 = "AGCGTTTTCATTCTGACTGCAACGGGCYR",
    chr2 = "AAAAAACGCCTTAGTAAGTGATTTTCGYR")
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)
```

`getDataByLocation` *Extract Data (Coverage) Matrix Columns by Genomic Location*

Description

Extract data (coverage) matrix columns by genomic location.

Usage

```
getDataByLocation(x, chr, position)
```

Arguments

`x`	Parameter list or directory with coverage matrix.
`chr`	Chromosome name or number.
`position`	Position(s) on the chromosome.

Details

The function returns only data for locations that were tested. Other locations are ignored.

Value

Returns a list with 3 entries

`chr`	Chromosome
`position`	position
`mat`	Data matrix for selected genomic locations

Author(s)

Andrey A Shabalin <ashabalin@vcu.edu>

See Also

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

Examples

```
## Not run:
# Extract one location
getDataByLocation(param, 1, 123321)

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

# Extract multiple locations
# on the same chromosome
getDataByLocation(param, 1, c(123123,123321))

# on different chromosomes
getDataByLocation(param, c("chr1","chr2"), c(123123,123321))

## End(Not run)
```

`getTestsByLocation` *Extract MWAS Test Results by Genomic Location*

Description

Extract MWAS test results by genomic location.

Usage

```
getTestsByLocation(x, chr, position)
```

Arguments

`x`	Parameter list or MWAS directory.
`chr`	Chromosome name or number.
`position`	Position(s) on the chromosome.

Details

The function returns only records for locations that were tested. Other locations are ignored.

Value

Returns a data frame with

`chr`	Chromosome
`position`	position
`cor`	coverage - phenotype correlation
`t.test`	t-statistic
`p.value`	p-value
`q.value`	q-value (FDR)

If the outcome variable was categorical, columns `R.squared` and `F-test` are present in place of `cor` and `t.test`.

Author(s)

Andrey A Shabalin <ashabalin@vcu.edu>

See Also

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

Examples

```
## Not run:
# Extract one location
getTestsByLocation(param, 1, 123321)

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

# Extract multiple locations
# on the same chromosome
getTestsByLocation(param, 1, c(123123,123321))

# on different chromosomes
getTestsByLocation(param, c("chr1","chr2"), c(123123,123321))

## End(Not run)
```

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

Description

Injects SNPs from a VCF count file into a DNA sequence.

Usage

```
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.

Author(s)

Andrey A Shabalin <ashabalin@vcu.edu>

See Also

See injectSNPs for the standard analog function without MAF filtering.

Examples

```
gensequence1 = "AAAACAAAA"
frqcount = c(
    "CHROM\tPOS\tN_ALLELES\tN_CHR\t{ALLELE:COUNT}",
    "1\t6\t2\t1000\tA:400\tG:600",
    "1\t7\t2\t1000\tA:800\tC:200",
    "1\t9\t2\t1000\tA:900\tG:100")
MAF = 0.01

gensequence2 = injectSNPsMAF(gensequence1, frqcount, MAF)

### No CpGs without SNPs
show(gensequence1)
getCpGsetCG(gensequence1)

### SNPs create 1 CpG
show(gensequence2)
getCpGsetALL(gensequence2)
```

---

insilicoFASTQ *Construct FASTQ File for In-silico Alignment Experiment*

Description

Creates a FASTQ file with all fragments of fraglength bp long.

Usage

```
insilicoFASTQ(con, gensequence, fraglength)
```

Arguments

con	A 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.
gensequence	A string or DNAString of the DNA sequence.
fraglength	Fragment length.

Details

The function a FASTQ file with all fragments of fraglength bp long from the forward strand of the DNA sequence.

Value

Returns a list with CpG coordinates for each genome sequence.

Author(s)

Andrey A Shabalin <ashabalin@vcu.edu>

Examples

```
## 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

```
isAbsolutePath(path)
```

Arguments

path	Path to be tested.

Details

The function is designed to word 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 <ashabalin@vcu.edu>

See Also

See also makefullpath.

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 <ashabalin@vcu.edu>

See Also

See also isAbsolutePath.

Examples

```
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"
```

`orthonormalizeCovariates` *Orthonormalize Covariates*

Description

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

Usage

```
orthonormalizeCovariates(cvrt, modelhasconstant)
```

Arguments

`cvrt`	A matrix or data frame with covariates (one column per covariate).
`modelhasconstant`	Set to `TRUE` to add a constant covariate into the set before normalization.

Details

Factor variables are split into dummy variables before orthonormalization.
The operation is performed via QR decomposition (qr).

Value

Returns a matrix with orthogonal columns with unit length, whose columns spans the same space as the covariates plus a constant (if `modelhasconstant` is `TRUE`).

Note

This function is used in several parts of the pipeline.

Author(s)

Andrey A Shabalin <ashabalin@vcu.edu>

Examples

```
# Sample matrix of covariates
covariates = data.frame(a = 1:12, b = 12:1)

# Orthonormalizing Covariates
cvrtqr = orthonormalizeCovariates(covariates, modelhasconstant = TRUE)

# Checking the results (round to ignore rounding errors)
print( round(crossprod(cvrtqr),15) )

# Stop if not orthonormal
stopifnot(all.equal( crossprod(cvrtqr), diag(ncol(cvrtqr)) ))

# Example with a factor variable
groups = data.frame(gr = c("a","a","a","b","b","b","c","c","c"))
orthonormalizeCovariates(groups)
```

parameterDump *Save Parameters in a Text File*

Description

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

Usage

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

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.
`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 <ashabalin@vcu.edu>

See Also

See vignettes: `browseVignettes("ramwas")`

Examples

```
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.

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 processed 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 <ashabalin@vcu.edu>

See Also

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

Examples

```
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

```
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 <ashabalin@vcu.edu>

See Also

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

Examples

```
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

```
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 <ashabalin@vcu.edu>

See Also

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

Examples

```
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)
```

plotROC *Plot ROC Curve.*

Description

Plot ROC Curve for a binary outcome and its predictor.

Usage

```
plotROC(outcome, forecast)
```

Arguments

outcome	Outcome vector taking two distinct values
forecast	Numeric predictor vector. Larger values of forecast should correspond to more likely chance of the larger outcome.

Details

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

Value

This function plots a ROC curve and returns nothing.

Note

The input vectors must be of equal length and have matching values.

Author(s)

Andrey A Shabalin <ashabalin@vcu.edu>

See Also

See vignettes: browseVignettes("ramwas").

Examples

```
# Generate binary outcome and continuous predictor
# for 1000 observations
n = 1000;
outcome = sample(0:1, size = n, replace = TRUE)
forecast = outcome + rnorm(n);

# Make ROC plot
plotROC(outcome, forecast)
```

processCommandLine *Scan Parameters From Command Line*

Description

The pipeline parameters can be provided via command line.

For example:
`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 <ashabalin@vcu.edu>

See Also

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

Examples

```
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

```
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`.

Author(s)

Andrey A Shabalin <ashabalin@vcu.edu>

Examples

```
pv = runif(20)^2

qv = pvalue2qvalue(pv)
```

QCs *Quality Control Measures*

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 ploting options run
`vignette("RW3_BAM_QCs")`

Usage

```
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)

## S3 method for class 'qcHistScore'
plot(x, samplename="", xstep = 25, ...)
## S3 method for class 'qcHistScoreBF'
plot(x, samplename="", xstep = 25, ...)
## S3 method for class 'qcEditDist'
plot(x, samplename="", xstep = 5, ...)
## S3 method for class 'qcEditDistBF'
plot(x, samplename="", xstep = 5, ...)
## S3 method for class 'qcLengthMatched'
plot(x, samplename="", xstep = 25, ...)
## S3 method for class 'qcLengthMatchedBF'
```

```
plot(x, samplename="", xstep = 25, ...)
## S3 method for class 'qcIsoDist'
plot(x, samplename="", xstep = 25, ...)
## S3 method for class 'qcCoverageByDensity'
plot(x, samplename="", ...)
```

Arguments

x	The QC object. See the examples below.
samplename	Name of the sample for plot title.
xstep	The distance between x axis ticks.
...	Parameters passed to the underlying plot or barplot function.

Value

Function qcmean returns one value summary of most QC measures. Run vignette("RW3_BAM_QCs") for description of values returned by it.

Author(s)

Andrey A Shabalin <ashabalin@vcu.edu>

See Also

See vignettes: browseVignettes("ramwas").

Examples

```
# Load QC data from a sample project
filename = system.file("extdata", "bigQC.rds", package = "ramwas")
qc = readRDS(filename)$qc


## The number of BAM files
cat("N BAMs:", qc$nbams)

## Total number of reads in the BAM file(s)
cat("Reads total:", qc$reads.total)

## Number of reads aligned to the reference genome
cat("Reads aligned:", qc$reads.aligned, "\n")
cat("This is ", qc$reads.aligned / qc$reads.total * 100,
    "% of all reads", sep="")

## Number of reads that passed minimum score filter and are recorded
cat("Reads recorded:",qc$reads.recorded,"\n")
cat("This is ", qc$reads.recorded / qc$reads.aligned * 100,
    "% of aligned reads", sep="")

## Number of recorded reads aligned to
## the forward and reverse strands respectively
cat("Reads on forward strand:", qc$frwrev[1],"\n")
cat("Reads on reverse strand:", qc$frwrev[2],"\n")
cat("Fraction of reads on forward strand:", qcmean(qc$frwrev), "\n")
```

```
## Distribution of the read scores
cat("Average alignment score:", qcmean(qc$hist.score1), "\n")
cat("Average alignment score, no filter:", qcmean(qc$bf.hist.score1), "\n")
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$hist.length.matched), "\n")
cat("Average aligned length, no filter:",
    qcmean(qc$bf.hist.length.matched), "\n")
par(mfrow = c(1,2))
plot(qc$hist.length.matched)
plot(qc$bf.hist.length.matched)

## Distribution of edit distance between
## the aligned part of the read and the reference genome
cat("Average edit distance:", qcmean(qc$hist.edit.dist1), "\n")
cat("Average edit distance, no filter:", qcmean(qc$bf.hist.edit.dist1), "\n")
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$reads.recorded.no.repeats, "\n")
cat("This is ", qc$reads.recorded.no.repeats / qc$reads.recorded * 100,
    "% of aligned reads", "\n", sep="")
cat("Fraction of reads on forward strand (with    duplicates):",
    qcmean(qc$frwrev), "\n")
cat("Fraction of reads on forward strand (without duplicates):",
    qcmean(qc$frwrev.no.repeats), "\n")

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

## Average coverage of CpGs and non-CpGs
cat("Summed across", qc$nbams, "bams", "\n")
cat("Average     CpG coverage:", qc$avg.cpg.coverage,   "\n")
cat("Average non-CpG coverage:", qc$avg.noncpg.coverage,"\n")
cat("Enrichment ratio:", qc$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$chrX.count[1], ", which is ",
    qcmean(qc$chrX.count)*100, "% of total", sep="", "\n")
cat("ChrX reads: ", qc$chrY.count[1], ", which is ",
    qcmean(qc$chrY.count)*100, "% of total", sep="", "\n")

## 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)
```

qqPlotFast *Fast QQ-plot for Large Number of P-values*

Description

Plots a QQ-plot with a confidence band and an estimate of inflation factor lambda. Works quickly even for tens of millions of p-values.

Usage

```
qqPlotFast(pvalues, ntests = NULL, ci.level = 0.05)
```

Arguments

pvalues	Vector of p-values, preferably sorted.
ntests	If only significant p-values are provided, the total number of tests performed. By default equal to the length of pvalues.
ci.level	Significance level of the confidence band.

Details

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

Value

This function plots a QQ-plot and returns nothing.

Note

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

Author(s)

Andrey A Shabalin <ashabalin@vcu.edu>

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)
```

`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

```
ramwas0createArtificialData(dir,
                            nsamples = 20,
                            nreads = 1e6,
                            ncpgs = 500e3,
                            randseed = 18090212,
                            verbose = TRUE)
```

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.
`verbose`	If TRUE, produce a message for each BAM file.

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 <ashabalin@vcu.edu>

See Also

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

Examples

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

ramwas0createArtificialData(
    dr,
    nsamples = 4,
    nreads = 10e3,
    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`	Vector 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 <ashabalin@vcu.edu>

See Also

See vignettes: browseVignettes("ramwas")

Examples

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

param = ramwasParameters(
    bihost = bihost,
    bimart = bimart,
    bidataset = bidataset,
    biattributes = biattributes,
    bifilters = bifilters,
    biflank = biflank);

# Test a location
chr = "chr1"
pos =  15975530

ramwasAnnotateLocations(param, chr, pos)
```

---

ramwasParameters *Function for Convenient Filling of the RaMWAS Parameter List.*

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

```
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,
    bimart,
    bidataset,
    biattributes,
    bifilters,
    biflank,
    fileSNPs,
    dirSNPs,
    ...)
```

Arguments

`dirproject`	The project directory. Default is currect 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", there "MAPQ" is the BAM field used for filtering and "4" is the thredhold.
`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".

`dirrqc` 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 differenciate 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".

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

`bam2sample` Allowes multiple BAMs contain information about common sample.
Not required if "filebam2sample" is specified.
Must be a list like `list(sample1 = c("bam1","bam2","bam3"), sample2 = "bam2")`

`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`.

`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.

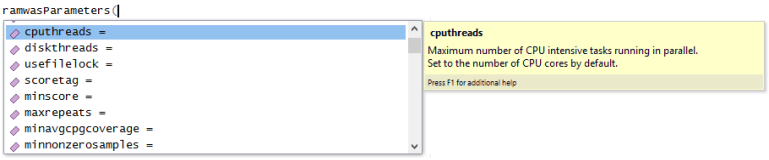
`dirSNPs` Directory where RaMWAS saves the results of joint methylation-genotype analysis.

`...` 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:



Value

List with provided parameters.

Author(s)

Andrey A Shabalin <ashabalin@vcu.edu>

See Also

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

Examples

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

rowcolSumSq *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)

Andrey A Shabalin <ashabalin@vcu.edu>

See Also

See `rowSums` and `colSums` for simple (not squared) row/column sums.

Examples

```
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) ))
```

testPhenotype *Test the Phenotype of Interest for Association with Methylation Coverage.*

Description

An internal, low-level function for fast association testing. It tests the phenotype of interest for association with methylation coverage.

Usage

```
testPhenotype(phenotype, data, cvrtqr)
```

Arguments

phenotype	Vector with phenotype. Can be numerical or character/factor variable.
data	Matrix with normalized coverage, one CpG per column.
cvrtqr	Orthonormalized covariates (transposed). See orthonormalizeCovariates.

Details

The testing is performed in a fast way, using 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)

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

Note

This function is used in several parts of the pipeline.

Author(s)

Andrey A Shabalin <ashabalin@vcu.edu>

See Also

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

Also check `orthonormalizeCovariates`.

Examples

```
# Random data with signal in the first column
data = matrix( runif(30*3), 30, 3)
data[,1] = data[,1] + rep(0:2, each = 10)

# Random covariate
cvrtqr = orthonormalizeCovariates(matrix(runif(30),ncol=1))


# Categorical, 3 group phenotype
phenotype = rep(c("Normal","Sick","Dead"), each = 10)

# Test for association
output = testPhenotype(phenotype, data, t(cvrtqr))

print(output)

# Numerical, 3 value phenotype
phenotype = rep(1:3, each = 10)

# Test for association
output = testPhenotype(phenotype, data, t(cvrtqr))

print(output)
```

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