

Package ‘CNVrd2’

October 15, 2025

Type Package

Title CNVrd2: a read depth-based method to detect and genotype complex common copy number variants from next generation sequencing data.

Version 1.46.0

Date 2014-10-04

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Depends R (>= 3.0.0), methods, VariantAnnotation, parallel, rjags, ggplot2, gridExtra

VignetteBuilder knitr

Suggests knitr

Maintainer Hoang Tan Nguyen <hoangtannguyenvn@gmail.com>

Description CNVrd2 uses next-generation sequencing data to measure human gene copy number for multiple samples, identify SNPs tagging copy number variants and detect copy number polymorphic genomic regions.

License GPL-2

Imports DNACopy, IRanges, Rsamtools

biocViews CopyNumberVariation, SNP, Sequencing, Software, Coverage, LinkageDisequilibrium, Clustering.

Collate AllClasses.R AllGenerics.R countReadInWindow.R
segmentSamples.R segmentSamplesUsingPopInformation.R
identifyPolymorphicRegion.R plotPolymorphicRegion.R
emnormalCNV.R groupCNVs.R searchGroupCNVs.R groupBayesianCNVs.R
plotCNVrd2.R calculateLDSNPandCNV.R

URL <https://github.com/hoangtn/CNVrd2>

git_url <https://git.bioconductor.org/packages/CNVrd2>

git_branch RELEASE_3_21

git_last_commit a9e11c4

git_last_commit_date 2025-04-15

Repository Bioconductor 3.21
Date/Publication 2025-10-15

Contents

CNVrd2-package	2
calculateLDSNPandCNV	3
ccl3l1 data	5
clusteringCNVs-class	5
CNVrd2-class	6
countReadInWindow	7
countReadInWindow-methods	8
emnormalCNV	9
emnormalCNV-methods	10
fcgr3bMXL	10
groupBayesianCNVs	11
groupCNVs	14
groupCNVs-methods	15
identifyPolymorphicRegion	15
identifyPolymorphicRegion-methods	17
numericOrNULL-class	17
plotCNVrd2	18
plotCNVrd2-methods	19
plotPolymorphicRegion	19
plotPolymorphicRegion-methods	20
searchGroupCNVs	20
searchGroupCNVs-methods	21
segmentSamples	21
segmentSamples-methods	22
segmentSamplesUsingPopInformation	23
segmentSamplesUsingPopInformation-methods	24
vectorORfactor-class	24
Index	25

CNVrd2-package	<i>CNVrd2</i>
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Description

CNVrd2 uses next-generation sequencing data to measure human gene copy number for multiple samples and indentify SNPs/INDELs which are in linkage disequilibrium with the gene copy number variation.

Details

Package: CNVrd2
 Type: Package
 Version: 1.0
 Date: 2013-03-26
 License: GPL-2
 Depends: methods

Author(s)

Maintainer: Hoang Tan Nguyen <hoangtannguyenvn@gmail.com>

calculateLDSNPandCNV *calculateLDSNPandCNV*

Description

Identifying SNPs/INDELs being in linkage disequilibrium with CNV.

Usage

```
calculateLDSNPandCNV(sampleCNV = NULL, vcfFile = NULL,
  matrixGenotype = NULL, cnvColumn = NULL, popColumn = NULL,
  population = NULL, chr = NULL, hg = "hg19",
  st = NULL, en = NULL, nChunkForVcf = 10,
  codeSNP = c("Two", "Three"), codeCNV = c("CN", "ThreeGroup"),
  typeTest = c("All", "Dup", "Del"), parallel = FALSE)
```

Arguments

sampleCNV	A data frame with no missing data; The first column is samples, the other columns are copy number, population names. This object can be obtained from the clustering step (allGroups).
vcfFile	Name of zipped vcf file including SNPs/INDELs.
matrixGenotype	A matrix of SNPs/INDELs coded: 0 for 0/0 or 0/0; 1 for 0/1, 1/0, 0/1, 1/0; 2 for 1/1, 1/1. Rows are SNPs/INDELs and columns are samples. If users use this argument then the argument vcfFile is not necessary.
cnvColumn	A number indicating the column of CNV.
popColumn	A number indicating the column of a population being calculated LD values.
population	A character() vector indicating names of populations.
chr	A character string indicating a chromosome of genes/SNPs/INDELs.
hg	A character string indicating the version of a reference genome (default: hg19).

<code>st</code>	A number indicating a starting coordinate to read the vcf file.
<code>en</code>	A number indicating an ending coordinate to read the vcf file.
<code>nChunkForVcf</code>	A number indicating how many chunks users would like to divide the vcf file to read into R. It depends on users' computers. We usually use 10 to 50 for this argument.
<code>codeSNP</code>	A character string indicating a way to code unphased/phased SNPs/INDELs into numeric values (Two: 0, 1 or Three: 0, 1, 2).
<code>codeCNV</code>	A character string partial matching to one of: "All" will test copy-number counts and SNPs/INDELs, "ThreeGroup" will divide samples into three groups: deletion, normality and duplication.
<code>typeTest</code>	A character string partial matching to one of: "All" will test all copy-number status, "Dup/Del" will test for only duplicated/deleted versus normal.
<code>parallel</code>	A logical value indicating whether multicores are used or NOT (default: FALSE).

Value

`r2Andpvalues`: A data frame (or a list) with each row for a SNP/INDEL: all information includes p-values adjusted by the method of Benjamini and Hocheberg (1995) and `r2` values between the SNP/INDEL and copy-number status.

Note

`st` and `en` must not be outside the coordinates of the VCF file.

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

References

Benjamini, Y., and Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, 57, 289-300.

See Also

[fcgr3bMXL](#)

Examples

```
##Load data: fcgr3bMXL in CNVrd2 package#####
data(fcgr3bMXL)
##Name a vcf file (vcfFile)
vcfFile <- system.file(package="CNVrd2", "extdata",
                        "chr1.161600000.161611000.vcf.gz")
##Make a data fame named sampleCNV including samples, CNs, population names

sampleCNV <- data.frame(copynumberGroups$allGroups[, c(1,2) ],rep("MXL", 58))
```

```

rownames(sampleCNV) <- substr(sampleCNV[, 1], 1, 7)
sampleCNV[, 1] <- rownames(sampleCNV)
##The first column must be the sample names
tagSNPandINDELofMXL <- calculateLDSNPandCNV(sampleCNV = sampleCNV,
                                             vcfFile = vcfFile, cnvColumn = 2,
                                             population = "MXL", popColumn = 3,
                                             nChunkForVcf = 5, chr = "1",
                                             st = 161600000, en = 161611000,
                                             codeSNP= "Three", codeCNV = "ThreeGroup")

tagSNPandINDELofMXL[1:3,]

```

ccl3l1data

Data of CCL3L1 gene (The 1000 Genomes Project)

Description

This data set includes: segmentation results, population information and CCL3L1 CN.

Usage

```
data(ccl3l1data)
```

Format

This is a data frame including four columns: *Name* (names of samples), *Pop* (names of populations), *SS* (segmentation scores of samples at the gene) and *CN* (CCL3L1 CN obtained by using a Bayesian clustering approach with European-ancestry as prior information).

References

www.1000genomes.org

clusteringCNVs-class

Class "clusteringCNVs"

Description

This class is used to cluster segmentation scores into copy-number groups.

Objects from the Class

Objects can be created by calls of the form `new("clusteringCNVs", ...)`.

Slots

x: Object of class "numeric".
k: Object of class "numeric" indicating a number of groups.
p: Object of class "numericOrNULL" indicating groups' proportions.
m: Object of class "numericOrNULL" indicating groups' means.
sigma: Object of class "numericOrNULL" indicating groups' standard deviations.
small: Object of class "numeric" indicating the value to stop the iteration process of the EM algorithm.
nMax: Object of class "numeric" indicating a maximum number of iterations.
EV: Object of class "logical" indicating whether all groups having equal variances or not (default).
eee: Object of class "numeric" indicating a pseudo value of 0.
nmaxInit: Object of class "numeric" indicating a number of iterations to obtain initial values.
nChangeVariance: Object of class "numeric" indicating a number of times to change from unequal variances to equal variances (*"this option is used to avoid the EM algorithm being broken down if there is one (or a few) sample in a group"*).
verbose: Object of class "logical" indicating whether printing out all loops.
groupDistance: Object of class "numericOrNULL" indicating the distance between groups.

Methods

emnormalCNV signature(Object = "clusteringCNVs"): run the EM algorithm.
groupCNVs signature(Object = "clusteringCNVs"): cluster segmentation scores into groups.
searchGroupCNVs signature(Object = "clusteringCNVs"): identify a number of groups.

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

Examples

```
showClass("clusteringCNVs")
```

CNVrd2-class	Class "CNVrd2"
--------------	----------------

Description

A class of reading BAM files into R and grouping read-count windows into similar segments.

Objects from the Class

Objects can be created by calls of the form `new("CNVrd2", ...)`.

Slots

windows: Object of class "numeric" indicating a window size.

chr: Object of class "character" indicating the chromosome of the region.

st: Object of class "numeric" indicating the starting coordinate of the region.

en: Object of class "numeric" indicating the ending coordinate of the region.

dirBamFile: Object of class "character" indicating a directory of BAM files.

dirCoordinate: Object of class "character" indicating a directory where all the positions of mapped reads will be written out to prepare for the segmentation process.

genes: Object of class "numeric" indicating gene coordinates.

geneNames: Object of class "character" indicating names of genes.

Methods

countReadInWindow signature(Object = "CNVrd2"): Count reads in windows.

plotCNVrd2 signature(Object = "CNVrd2"): Plot traces of samples.

segmentSamples signature(Object = "CNVrd2"): Cluster windows of read counts into regions having similar signal values.

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

Examples

```
showClass("CNVrd2")
```

countReadInWindow	<i>Obtain read counts in constant windows.</i>
-------------------	--

Description

Counting, transferring and standardizing read counts for all windows of samples. If correctGC = TRUE then all read-count windows will be corrected by the method of Yoon et al. (2009).

Usage

```
countReadInWindow(Object, ...)
```

Arguments

Object	An object of class CNVrd2.
...	Further arguments.

Value

readCountMatrix: a matrix of read counts for all samples (rows).

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

References

Yoon, S., Xuan, Z., Makarov, V., Ye, K., Sebat, J., 2009. Sensitive and accurate detection of copy number variants using read depth of coverage. *Genome research* 19 (9), 1586-1592.

Examples

```
## Not run:
data(fcgr3bMXL)
bamFiles <- dir("Bam", pattern = ".bam$")
objectCNVrd2 <- new("CNVrd2", windows = 1000, chr = "chr1",
                   st = 161100001, en = 162100000,
                   dirBamFile = "Bam",
                   genes = c(161592986, 161601753),
                   geneNames = "3B")

readCountMatrix <- countReadInWindow(Object = objectCNVrd2, correctGC = TRUE)
readCountMatrix[1:3, 1:3]

## End(Not run)
```

countReadInWindow-methods

Method countReadInWindow

Description

Method to count reads in windows

Methods

signature(Object = "CNVrd2") Count, transfer and standardize read count in windows of samples

See Also

[countReadInWindow](#)

Examples

```
##data(fcgr3bMXL)
##readCountMatrix <- countReadInWindow(Object = objectCNVrd2, correctGC = TRUE)
##readCountMatrix[1:3, 1:3]
```

emnormalCNV

*Implement the EM algorithm***Description**

This function is used to obtain the maximization likelihood estimation of normal mixture model by using the EM algorithm (Demster et al., 1977).

Usage

```
emnormalCNV(Object, ...)
```

Arguments

Object	An object of class clusteringCNVs.
...	Optional arguments

Value

loglk	Value of the likelihood function.
p	Proportions of groups.
m	Means of groups.
sigma	Standard deviations of groups.
count	A number of iteration to obtain convergence stage.
bic	See searchGroupCNVs .
z	Data frame of proportions of data in mixture components.

Note

In the package, the distance between two initial means of the two nearest neighbor groups was set `groupDistance = 0.25` as a default value to obtain initial values (using the `kmeans` function in R).

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

References

Dempster, A. P., Laird, N. M., Rubin, D. B., 1977. Maximum likelihood from incomplete data via the em algorithm. Journal of the Royal Statistical Society. Series B (Methodological), 1-38.

See Also

[searchGroupCNVs](#), [groupCNVs](#)

Examples

```
data(fcgr3bMXL)

sS <- resultSegment$segmentationScores
#####Histogram#####
###View segmentation scores#####
hist(sS[, 1], 100)
#####
###Number of components#####
###Make an object of clusteringCNVs class#####
objectCluster <- new("clusteringCNVs",
                     x = sS[, 1], k = 4, EV = TRUE)

set.seed(123)
copynumberGroups <- groupCNVs(Object = objectCluster)
```

emnormalCNV-methods	<i>Method</i> emnormalCNV
---------------------	---------------------------

Description

Implement the Expectation Maximization

Methods

```
signature(Object = "clusteringCNVs")
```

fcgr3bMXL	<i>MXL population data (The 1000 Genomes Project)</i>
-----------	---

Description

This data set includes: segmentation results “resultSegment”, information copy number “copynumberGroups” of *FCGR3B* gene.

Usage

```
data(fcgr3bMXL)
```

Format

All results analysed by the CNVrd2 package.

References

www.1000genomes.org

groupBayesianCNVs	<i>groupBayesianCNVs</i>
-------------------	--------------------------

Description

Cluster segmentation scores into different groups by using prior information from one population.

Usage

```
groupBayesianCNVs(xData, nGroups, lambda0, sd0, alpha0, distanceBetweenGroups, inits = NULL,
  precisionOfGroupMeans = 3000, sdOftau = NULL, n.adapt = 100,
  nUpdate = 1000, n.iter = 20000, thin = 5, n.chains = 1,
  heidel.diag = FALSE, leftLimit = NULL, rightLimit = NULL)
```

Arguments

xData	a numeric vector of observations (segmentation scores).
nGroups	an integer indicating a number of groups.
lambda0	Prior means of groups.
sd0	Prior standard deviations of groups.
alpha0	Prior parameters for mixing proportions.
distanceBetweenGroups	Prior value for the distance between groups.
inits	A list of initial values of parameters.
precisionOfGroupMeans	Prior parameter of group means (default = 3000).
sdOftau	Prior parameter of the standard deviations of group precisions.
n.adapt	the number of iterations for adaptation (rjags's parameter)
nUpdate	the number of iterations for burn-in process.
n.iter	the number of iterations for sampling (rjags's parameter)
thin	thinning interval for monitors (rjags's parameter)
n.chains	the number of parallel chains for the model (rjags's parameter, default = 1)
heidel.diag	If heidel.diag = TRUE then Heidelberg and Welch's convergence diagnostic is used.
leftLimit	Values which are less than this value will be allocated to the smallest group.
rightLimit	Values which are larger than this value will be allocated to the largest group.

Details

This function assumes that users already know the information of groups' means, standard deviations; the distances between groups.

Value

mcmcChains	A list of marray objects for means, standard deviations, proportions
m1	Means of groups
s1	Standard deviations of groups
p1	Proportions of groups
allGroups	A data.frame includes samples and their corresponding groups
hTest	Results of Heidelberger and Welch's convergence diagnostic

Note

#####

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

References

Martyn Plummer (2013). rjags: Bayesian graphical models using MCMC. R package version 3-10. <http://CRAN.R-project.org/package=rjags>.
 Lunn, David J., et al. WinBUGS-a Bayesian modelling framework: concepts, structure, and extensibility. Statistics and Computing 10.4 (2000): 325-337.

See Also

[groupCNVs](#)

Examples

```
## Not run:
data(ccl3l1data)

xyEuro <- ccl3l1data[grepl("CEU|TSI|IBS|GBR|FIN", ccl3l1data[, 2]), ]

names(yEuro) <- rownames(xyEuro)

##Clustering European segmentation scores into group: 5 groups were chosen

objectClusterEuroCCL3L1 <- new("clusteringCNVs", x = yEuro, k = 5)

europeanCCL3L1Groups <- groupCNVs(Object = objectClusterEuroCCL3L1)

##Obtain prior information
#Means
```

[illegible]

groupCNVs	<i>Cluster segmentation scores into groups.</i>
-----------	---

Description

Use the EM algorithm (Dempster et al., 1977) to cluster segmentation scores into various groups.

Usage

```
groupCNVs(Object, ...)
```

Arguments

Object	An object of class clusteringCNVs.
...	Further arguments.

Details

Users can set limits of segmentation scores: values being smaller than the left limit will be assigned to the smallest group and values being larger than right limit will be assigned to the largest group.

Value

allGroups	Samples and their corresponding groups
means	Means of groups.
sigma	Variances of groups.
p	Proportions of groups in all data set.
loglk	Value of loglikelihood function.

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

References

Dempster, A. P., Laird, N. M., Rubin, D. B., 1977. Maximum likelihood from incomplete data via the em algorithm. Journal of the Royal Statistical Society. Series B (Methodological), 1-38.

See Also

[emnormalCNV](#), [searchGroupCNVs](#)

Examples

```
data("fcgr3bMXL")
#resultSegment <- segmentSamples(Object = objectCNVrd2, stdCntMatrix = readCountMatrix)
objectCluster <- new("clusteringCNVs",
                     x = resultSegment$segmentationScores[, 1], k = 4, EV = TRUE)

#searchGroupCNVs(Object = objectCluster)
copynumberGroups <- groupCNVs(Object = objectCluster)
```

groupCNVs-methods	<i>Method</i> groupCNVs
-------------------	-------------------------

Description

Method groupCNVs

Methods

```
signature(Object = "clusteringCNVs")
```

identifyPolymorphicRegion	<i>Identity polymorphic regions.</i>
---------------------------	--------------------------------------

Description

Using quantile values to identify polymorphic regions.

Usage

```
identifyPolymorphicRegion(Object, ...)
```

Arguments

Object	An object of class CNVrd2
...	polymorphicRegionObjectAn object obtained from the process of identifying poplymorphic regions. Optional arguments.

identifyPolymorphicRegion-methods

Methods for Function identifyPolymorphicRegion

Description

Methods for function identifyPolymorphicRegion

Methods

signature(Object = "CNVrd2")

numericOrNULL-class *Class "numericOrNULL"*

Description

Auxiliary classes; may contain either a numeric vector or NULL [or a call / data.frame or NULL, respectively].

Objects from the Class

A virtual Class: No objects may be created from it.

Methods

No methods defined with class "numericOrNULL" in the signature.

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

plotCNVrd2	<i>Plot traces of samples.</i>
------------	--------------------------------

Description

Plot traces of samples.

Usage

```
plotCNVrd2(Object, ...)
```

Arguments

Object	An object of class CNVrd2.
...	Optional arguments.

Value

Plot

Note

Users can plot multiple samples simultaneously.

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

Examples

```
data(fcgr3bMXL)
##Obtain all information of CNVs
allGroups <- copynumberGroups$allGroups
###Obtain names of duplicate samples
duplicatedSamples <- rownames(allGroups[allGroups[, 2] > 2,])
###Plot the first duplicate samples
par(mfrow = c(3, 2))
for (ii in duplicatedSamples[1:6])
  plotCNVrd2(Object = objectCNVrd2,
             segmentObject = resultSegment,
             sampleName = ii)
```

plotCNVrd2-methods	<i>Method</i> plotCNVrd2
--------------------	--------------------------

Description

Method plotCNVrd2

Methods

signature(Object = "CNVrd2")

plotPolymorphicRegion	<i>Plot polymorphic regions.</i>
-----------------------	----------------------------------

Description

Plot polymorphic regions based on coordinates set by users.

Usage

```
plotPolymorphicRegion(Object, ...)
```

Arguments

Object	An object of class CNVrd2
...	polymorphicRegionObjectAn object obtained from the process of identifying polymorphic regions. Optional arguments.

Value

putativeBoundary	Putative boundaries of polymorphic regions based on quantile values.
------------------	--

Note

Users can choose various quantile values and adjust different thresholds to obtain polymorphic regions.

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

See Also

[identifyPolymorphicRegion](#)

Examples

```
## Not run:

plotPolymorphicRegion(Object = objectCNVrd2, polymorphicRegionObject = fcgr3PolymorphicRegion,
  xlim = c(161300000, 161800000), drawThresholds = TRUE,
  thresholdForPolymorphicRegions = c(0.75, 0.25))

##Change thresholds
plotPolymorphicRegion(Object = objectCNVrd2, polymorphicRegionObject = fcgr3PolymorphicRegion,
  xlim = c(161300000, 161800000), drawThresholds = TRUE,
  thresholdForPolymorphicRegions = c(0.9, 0.1))

##Plot standard deviation

plotPolymorphicRegion(Object = objectCNVrd2, polymorphicRegionObject = fcgr3PolymorphicRegion,
  xlim = c(161300000, 161800000), typePlot = "SD",
  thresholdForPolymorphicRegions = c(0.75, 0.25))

## End(Not run)
```

plotPolymorphicRegion-methods

Methods for Function plotPolymorphicRegion

Description

Methods for function plotPolymorphicRegion

Methods

signature(Object = "CNVrd2")

searchGroupCNVs

Choose a number of CN groups

Description

Choose a number of CN groups by using Bayesian Information Criterion (Schwarz, 1978).

Usage

```
searchGroupCNVs(Object, ...)
```

Arguments

Object	An object of class <i>clusteringCNVs</i> .
...	Optional arguments.

Value

Groups	General information.
nComponents	A suitable number of components.

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

References

Schwarz, G. , 1978. Estimating the dimension of a model. The Annals of Statistics 6(2), 461-464.

```
searchGroupCNVs-methods
```

```
Method searchGroupCNVs
```

Description

Method searchGroupCNVs

Methods

```
signature(Object = "clusteringCNVs")
```

```
segmentSamples
```

```
Implement the segmentation process
```

Description

Segment read-count windows into region having similar signal values by using the DNACopy package (Venkatraman and Olshen, 2007) and refine this process to obtain segmentation scores at genes.

Usage

```
segmentSamples(Object, ...)
```

Arguments

Object An object of class CNVrd2.
... Optional arguments.

Value

segmentResults All results of the segmentation process.
segmentationScores Segmentation scores of the gene(s) being measured.
observedReadCountRatios Observed read-count ratios of genes. This value is a matrix of observed read-count ratios at genes if (only inputBamFile = TRUE).
stdCntMatrix Matrix of read counts (standardized).

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

References

Venkatraman, E., Olshen, A. B., 2007. A faster circular binary segmentation algorithm for the analysis of array chg data. *Bioinformatics* 23 (6), 657-663.

See Also

[countReadInWindow](#), DNACopy

Examples

```
data(fcgr3bMXL)
## Not run: resultSegment <- segmentSamples(Object = objectCNVrd2, stdCntMatrix = readCountMatrix)
```

segmentSamples-methods

Method segmentSamples

Description

Method segmentSamples

Methods

signature(Object = "CNVrd2")

`segmentSamplesUsingPopInformation`*Implement the segmentation process for multiple populations*

Description

Segment read-count windows into region having similar signal values by using the DNACopy package (Venkatraman and Olshen, 2007) and refine this process to obtain segmentation scores at genes. Then, the function adjusts segmentation scores for multiple populations using a linear regression model.

Usage

```
segmentSamplesUsingPopInformation(Object, ...)
```

Arguments

Object	An object of class CNVrd2.
...	Optional arguments.

Value

segmentationScores	All adjusted results of the segmentation process.
segmentationScoresFromSinglePops	Segmentation scores of single populations.
segmentResults	Un-adjusted segmentation scores
observedReadCountRatios	Observed read-count ratios of genes. This value is a matrix of observed read-count ratios at genes if (only inputBamFile = TRUE).
stdCntMatrix	Matrix of read counts (standardized).

Author(s)

Hoang Tan Nguyen, Tony R Merriman and MA Black. <hoangtannguyenvn@gmail.com>

References

Venkatraman, E., Olshen, A. B., 2007. A faster circular binary segmentation algorithm for the analysis of array chg data. *Bioinformatics* 23 (6), 657-663.

See Also

[countReadInWindow](#), DNACopy

segmentSamplesUsingPopInformation-methods
Method segmentSamplesUsingPopInformation

Description

Method segmentSamplesUsingPopInformation

Methods

signature(Object = "CNVrd2")

vectorORfactor-class *Class* "vectorORfactor"

Description

Auxiliary class

Objects from the Class

A virtual Class: No objects may be created from it.

Methods

No methods defined with class "vectorORfactor" in the signature.

Author(s)

Hoang Tan Nguyen, Tony Merriman and Mik Black. <hoangtannguyenvn@gmail.com>

Index

- * **classes**
 - clusteringCNVs-class, 5
 - CNVrd2-class, 6
 - numericOrNULL-class, 17
 - vectorOrFactor-class, 24
- * **datasets, CCL3L1 CN**
 - cc13l1data, 5
- * **datasets**
 - fcgr3bMXL, 10
- * **methods**
 - countReadInWindow, 7
 - countReadInWindow-methods, 8
 - emnormalCNV-methods, 10
 - groupBayesianCNVs, 11
 - groupCNVs, 14
 - groupCNVs-methods, 15
 - identifyPolymorphicRegion, 15
 - identifyPolymorphicRegion-methods, 17
 - plotCNVrd2, 18
 - plotCNVrd2-methods, 19
 - plotPolymorphicRegion-methods, 20
 - searchGroupCNVs, 20
 - searchGroupCNVs-methods, 21
 - segmentSamples, 21
 - segmentSamples-methods, 22
 - segmentSamplesUsingPopInformation, 23
 - segmentSamplesUsingPopInformation-methods, 24
- * **package**
 - CNVrd2-package, 2
- * **plots**
 - plotCNVrd2, 18
- * **plot**
 - plotPolymorphicRegion, 19
- .Random.seed (fcgr3bMXL), 10
- allGroups (fcgr3bMXL), 10
- bamFiles (fcgr3bMXL), 10
- calculateLDSNPandCNV, 3
- cc13l1data, 5
- clusteringCNVs-class, 5
- CNVrd2-class, 6
- CNVrd2-package, 2
- copynumberGroups (fcgr3bMXL), 10
- countReadInWindow, 7, 8, 22, 23
- countReadInWindow, CNVrd2-method (CNVrd2-class), 6
- countReadInWindow-methods, 8
- emnormalCNV, 9, 14
- emnormalCNV, clusteringCNVs-method (clusteringCNVs-class), 5
- emnormalCNV-methods, 10
- fcgr3bMXL, 4, 10
- groupBayesianCNVs, 11
- groupCNVs, 10, 12, 14
- groupCNVs, clusteringCNVs-method (clusteringCNVs-class), 5
- groupCNVs-methods, 15
- identifyPolymorphicRegion, 15, 19
- identifyPolymorphicRegion, CNVrd2-method (identifyPolymorphicRegion-methods), 17
- identifyPolymorphicRegion-methods, 17
- numericOrNULL-class, 17
- objectCluster (clusteringCNVs-class), 5
- objectCNVrd2 (fcgr3bMXL), 10
- plotCNVrd2, 18
- plotCNVrd2, CNVrd2-method (CNVrd2-class), 6
- plotCNVrd2-methods, 19

plotPolymorphicRegion, [16](#), [19](#)
plotPolymorphicRegion, CNVrd2-method
 (plotPolymorphicRegion-methods),
 [20](#)
plotPolymorphicRegion-methods, [20](#)

readCountMatrix (fcgr3bMXL), [10](#)
resultSegment (fcgr3bMXL), [10](#)

searchGroupCNVs, [9](#), [10](#), [14](#), [20](#)
searchGroupCNVs, clusteringCNVs-method
 (clusteringCNVs-class), [5](#)
searchGroupCNVs-methods, [21](#)
segmentSamples, [21](#)
segmentSamples, CNVrd2-method
 (segmentSamples-methods), [22](#)
segmentSamples-methods, [22](#)
segmentSamplesUsingPopInformation, [23](#)
segmentSamplesUsingPopInformation, CNVrd2-method
 (segmentSamplesUsingPopInformation-methods),
 [24](#)
segmentSamplesUsingPopInformation-methods,
 [24](#)

vectorORfactor-class, [24](#)