Package 'CNVPanelizer'

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Type Package

Title Reliable CNV detection in targeted sequencing applications

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Description A method that allows for the use of a collection of non-matched normal tissue samples. Our approach uses a non-parametric bootstrap subsampling of the available reference samples to estimate the distribution of read counts from targeted sequencing. As inspired by random forest, this is combined with a procedure that subsamples the amplicons associated with each of the targeted genes. The obtained information allows us to reliably classify the copy number aberrations on the gene level.

Depends R (>= 3.2.0), GenomicRanges

Suggests knitr, RUnit, BiocGenerics

Imports S4Vectors, grDevices, stats, utils, NOISeq, IRanges, Rsamtools, exomeCopy, foreach, ggplot2, plyr, openxlsx

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Author Cristiano Oliveira [aut], Thomas Wolf [aut, cre], Albrecht Stenzinger [ctb], Volker Endris [ctb], Nicole Pfarr [ctb], Benedikt Brors [ths], Wilko Weichert [ths]

Maintainer Thomas Wolf <thomas_wolf71@gmx.de>

R topics documented:

`CNVPanelizer-package` *Reliable CNV detection in targeted sequencing applications*

Description

This package implements an algorithm that uses a collection of non-matched normal tissue samples as a reference set to detect CNV aberrations in data generated from amplicon based targeted sequencing.

Details

Our approach uses a non-parametric bootstrap subsampling of the available reference samples, to estimate the distribution

For a complete list of functions, use library(help = "CNVPanelizer").

Package:	CNVPanelizer
Type:	Package
License:	GPL-3

Author(s)

Thomas Wolf <thomas_wolf71f@gmx.de>
Cristiano Oliveira <cristiano.oliveira@med.uni-heidelberg.de>

`Background` *Background*

Description

Makes use of a subsampling approach to estimate the background noise when sequencing a gene with a specific number of amplicons. The 95 percent confidence interval is returned for each unique number of amplicons in the experiment.

Usage

```
Background(geneNames,
           samplesNormalizedReadCounts,
           referenceNormalizedReadCounts,
           bootList,
           replicates = 1000,
           significanceLevel = 0.05,
           robust = FALSE)
```

Arguments

geneNames	A vector of gene names, with one entry for each sequenced amplicon.
samplesNormalizedReadCounts	A matrix with the normalized read counts of the samples of interest
referenceNormalizedReadCounts	A matrix with the normalized reference read counts
bootList	A list as returned by BootList
replicates	an integer number of how many replicates should be performed
significanceLevel	The significance level for the calculated confidence interval
robust	If set to true the confidence interval is calculated replacing mean with median and sd with mad.

Value

Returns a list of data frames. One data frame for each sample of interest. The data frames report the 95 percent confidence interval of the background noise for each number of amplicons and sample combination.

Author(s)

Thomas Wolf, Cristiano Oliveira

Examples

```
data(sampleReadCounts)
data(referenceReadCounts)
## Gene names should be same size as row columns
geneNames <- row.names(referenceReadCounts)

ampliconNames <- NULL

normalizedReadCounts <- CombinedNormalizedCounts(sampleReadCounts,
                                                 referenceReadCounts,
                                                 ampliconNames = ampliconNames)

# After normalization data sets need to be splitted again to perform bootstrap
samplesNormalizedReadCounts = normalizedReadCounts["samples"][[1]]
referenceNormalizedReadCounts = normalizedReadCounts["reference"][[1]]

#Values above 10000 should be used
replicates <- 10
```

```
# Perform the bootstrap based analysis
bootList <- BootList(geneNames,
                     samplesNormalizedReadCounts,
                     referenceNormalizedReadCounts,
                     replicates = replicates)

background <- Background(geneNames,
                         samplesNormalizedReadCounts,
                         referenceNormalizedReadCounts,
                         bootList,
                         replicates = replicates,
                         significanceLevel = 0.1)
```

`BedToGenomicRanges` *BedToGenomicRanges*

Description

It generates a GenomicRanges object from a bed file. Needs to be passed the correct number of the gene name column. If the strings contain more information then just the gene name, a splitting character (split) has to be defined. I.e GeneName1;Amplicon2

Usage

```
BedToGenomicRanges(panelBedFilepath,
                   ampliconColumn,
                   split,
                   doReduce,
                   rangeExtend,
                   skip)
```

Arguments

`panelBedFilepath`	Filepath of the bed file.
`ampliconColumn`	Number of the column that identifies the gene name in the bed file passed through `panelBedFilepath`.
`split`	The character used as separator in the `ampliconColumn`. It is ";" by default.
`doReduce`	Should overlapping ranges be merged.
`rangeExtend`	Should the defined ranges be extended left and right by the given value. Affects the merging of overlapping regions and also read counting.
`skip`	How many lines should be skipped from the top of the bed file. The function assumes a bed file with column names. Thus default is skip = 1.

Value

A `GenomicRanges` object containing information about the amplicons described in the bed file.

Author(s)

Thomas Wolf, Cristiano Oliveira

Examples

```
bedFilepath <- file.path("someFile.bed")
ampliconColumn <- 4
genomicRangesFromBed <- BedToGenomicRanges(bedFilepath, ampliconColumn)
```

BootList *BootList*

Description

Performs a hybrid bootstrapping subsampling procedure similar to random forest. It bootstraps the reference samples and subsamples the amplicons associated with each gene. Returns a distribution of sample/reference ratios for each gene and sample of interest combination.

Usage

```
BootList(geneNames, sampleMatrix, refmat, replicates)
```

Arguments

geneNames	A vector of gene names, with one entry for each sequenced amplicon.
sampleMatrix	A vector or matrix of the read counts from the sample of interest. In the case of a matrix columns represent samples and rows amplicons.
refmat	A matrix of the read counts obtianed from the reference samples. Columns represent reference samples and rows amplicons.
replicates	How many bootstrap replicates should be performed.

Value

Returns a list of numeric matrices: For each matrix a row represent a gene while each column represents a bootstrapping/subsampling iteration.

Author(s)

Thomas Wolf, Cristiano Oliveira

Examples

```
data(sampleReadCounts)
data(referenceReadCounts)
## Gene names should be same size as row columns
geneNames <- row.names(referenceReadCounts)

ampliconNames <- NULL

normalizedReadCounts <- CombinedNormalizedCounts(sampleReadCounts,
                                                 referenceReadCounts,
                                                 ampliconNames = ampliconNames)
```

```
# After normalization data sets need to be splitted again to perform bootstrap
samplesNormalizedReadCounts = normalizedReadCounts["samples"][[1]]
referenceNormalizedReadCounts = normalizedReadCounts["reference"][[1]]

# Should be used values above 10000
replicates <- 10

# Perform the bootstrap based analysis
bootList <- BootList(geneNames,
         samplesNormalizedReadCounts,
         referenceNormalizedReadCounts,
         replicates = replicates)
```

`CombinedNormalizedCounts` *CombinedNormalizedCounts*

Description

This function makes use of NOISeq::tmm to normalize the read counts of all samples and references to the same median read count

Usage

```
CombinedNormalizedCounts(sampleCounts,
                         referenceCounts,
                         ampliconNames = NULL)
```

Arguments

`sampleCounts`	Matrix or vector with sample read counts (rows: amplicons, columns: samples)
`referenceCounts`	Matrix with reference read counts (rows: amplicons, columns: samples)
`ampliconNames`	A vector with amplicon defining names for the reference and sample matrices

Value

A list object with two matrices

`samples`	The samples matrix normalized
`reference`	The reference matrix normalized

Author(s)

Cristiano Oliveira, Thomas Wolf

Examples

```
data(sampleReadCounts)
data(referenceReadCounts)

normalizedReadCounts <- CombinedNormalizedCounts(sampleReadCounts,
                                                 referenceReadCounts)
```

IndexMultipleBams *IndexMultipleBams*

Description

Index a list of bam files if there is no index exists for the file entries in the list.

Usage

```
IndexMultipleBams(bams, index_type = ".bam.bai")
```

Arguments

bams	A character vector of bam files to be indexed
index_type	The index file type extension

Value

Not returning any value

Author(s)

Thomas Wolf, Cristiano Oliveira

Examples

```
files = c("file1.bam","file2.bam","file3.bam")
IndexMultipleBams(bams = files)
```

PlotBootstrapDistributions *PlotBootstrapDistributions*

Description

Plots the generated bootstrap distribution as violin plots. Genes showing significant values are marked in a different color.

Usage

```
PlotBootstrapDistributions(bootList,
                           reportTables,
                           outputFolder = getwd(),
                           sampleNames = NULL,
                           save = FALSE,
                           scale = 7)
```

Arguments

bootList	List of bootstrapped read counts for each sample data
reportTables	List of report tables for each sample data
outputFolder	Path to the folder where the data plots will be created
sampleNames	List with sample names
save	Boolean to save the plots to the output folder
scale	Numeric scale factor

Value

A list with ggplot2 objects.

Author(s)

Thomas Wolf, Cristiano Oliveira

Examples

```
data(sampleReadCounts)
data(referenceReadCounts)
## Gene names should be same size as row columns
geneNames <- row.names(referenceReadCounts)

ampliconNames <- NULL

normalizedReadCounts <- CombinedNormalizedCounts(sampleReadCounts,
                                                 referenceReadCounts,
                                                 ampliconNames = ampliconNames)

# After normalization data sets need to be splitted again to perform bootstrap
samplesNormalizedReadCounts = normalizedReadCounts["samples"][[1]]
referenceNormalizedReadCounts = normalizedReadCounts["reference"][[1]]

# Should be used values above 10000
replicates <- 10

# Perform the bootstrap based analysis
bootList <- BootList(geneNames,
                     samplesNormalizedReadCounts,
                     referenceNormalizedReadCounts,
                     replicates = replicates)

backgroundNoise <- Background(geneNames,
           samplesNormalizedReadCounts,
           referenceNormalizedReadCounts,
           bootList,
           replicates = replicates)

reportTables <- ReportTables(geneNames,
             samplesNormalizedReadCounts,
             referenceNormalizedReadCounts,
             bootList,
             backgroundNoise)
```

```
PlotBootstrapDistributions(bootList, reportTables, save = FALSE)
```

ReadCountsFromBam *ReadCountsFromBam*

Description

Returns a matrix with the read counts from a set of bam files.

Usage

```
ReadCountsFromBam(bamFilenames,
                  sampleNames,
                  gr,
                  ampliconNames,
                  removeDup = FALSE)
```

Arguments

bamFilenames	Vector of bamfile filepaths
sampleNames	Vector of sample names to be used as colums names instead of bam filepaths
gr	Genomic Range object as created by BedToGenomicRanges
ampliconNames	List of amplicon defining names
removeDup	Boolean value to remove duplicates. For reads with the same start site, end site and orientation only one is kept. For IonTorrent data this can be used to as an additional quality control. For Illumina data too many reads are being removed.

Value

A matrix with read counts where the rows represents the Amplicons and the columns represents the samples.

Author(s)

Thomas Wolf, Cristiano Oliveira

Examples

```
ReadCountsFromBam(bamFilenames,
                  sampleNames,
                  gr,
                  ampliconNames,
                  removeDup)
```

ReadXLSXToList *ReadXLSXToList*

Description

Reads a list of read count matrices from a xlsx as generated by `WriteReadCountsToXLSX`

Usage

```
ReadXLSXToList(filepath, rowNames = TRUE, colNames = TRUE)
```

Arguments

`filepath`	filepath
`rowNames`	if row names should be included
`colNames`	if col names should be included

Value

A list of read count matrices

Author(s)

Thomas Wolf, Cristiano Oliveira

Examples

```
ReadXLSXToList(filepath)
```

referenceReadCounts *Reference sample data*

Description

Synthetic reference data set of simulated read counts. Only to be used for code examples.

Usage

```
referenceSamples
```

Format

A matrix with columns identifying the sample names and columns the gene names

Value

A matrix with columns identifying the sample names and columns the gene names

Source

Artificially generated data

`ReportTables` *ReportTables*

Description

This function generates the final report of the CNV detection procedure. One data frame is generated for each sample of interest.

Usage

```
ReportTables(geneNames,
             samplesNormalizedReadCounts,
             referenceNormalizedReadCounts,
             bootList,
             backgroundNoise)
```

Arguments

`geneNames`	Describe `geneNames` here
`samplesNormalizedReadCounts`	Describe `samplesNormalizedReadCounts` here
`referenceNormalizedReadCounts`	Describe `referenceNormalizedReadCounts` here
`bootList`	A list as returned by the `BootList` function
`backgroundNoise`	A list of background noise as returned by the `Background` function

Value

Returns a list of tables, one for each sample of interest. Each of these tables contains numerical information of the aberration status of each gene. For a detailed description see the Vignette.

Author(s)

Thomas Wolf, Cristiano Oliveira

Examples

```
data(sampleReadCounts)
data(referenceReadCounts)
## Gene names should be same size as row columns
geneNames <- row.names(referenceReadCounts)

ampliconNames <- NULL

normalizedReadCounts <- CombinedNormalizedCounts(sampleReadCounts,
```

```
                                                referenceReadCounts,
                                                ampliconNames = ampliconNames)

# After normalization data sets need to be splitted again to perform bootstrap
samplesNormalizedReadCounts = normalizedReadCounts["samples"][[1]]
referenceNormalizedReadCounts = normalizedReadCounts["reference"][[1]]

# Should be used values above 10000
replicates <- 10

# Perform the bootstrap based analysis
bootList <- BootList(geneNames,
                     samplesNormalizedReadCounts,
                     referenceNormalizedReadCounts,
                     replicates = replicates)

backgroundNoise = Background(geneNames,
                             samplesNormalizedReadCounts,
                             referenceNormalizedReadCounts,
                             bootList,
                             replicates = replicates)

reportTables <- ReportTables(geneNames,
             samplesNormalizedReadCounts,
             referenceNormalizedReadCounts,
             bootList,
             backgroundNoise)
```

`sampleReadCounts` *Test sample data*

Description

Synthetic data set of simulated read counts. Only to be used for running the code examples.

Usage

```
testSamples
```

Format

A matrix with columns identifying the sample names and columns the gene names

Value

A matrix with columns identifying the sample names and columns the gene names

Source

Artificially generated data

WriteListToXLSX *WriteListToXLSX*

Description

Writes list of data frames to an xlsx file

Usage

```
WriteListToXLSX(listOfDataFrames, filepath = "list.xlsx")
```

Arguments

listOfDataFrames	list of dataframes
filepath	filepath

Value

Not returning any value

Author(s)

Thomas Wolf, Cristiano Oliveira

Examples

```
WriteListToXLSX(listOfDataFrames = exampleList, filepath = "list.xlsx")
```

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