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

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`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 of re

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

Package:	CNVPanelizer
Type:	Package
License:	GPL-3

Author(s)

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`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,
                   dropChromossomes,
                   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.
dropChromossomes	Drop chromossomes.
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)
```

CNVPanelizerFromReadCounts

CNVPanelizerFromReadCounts

Description

Performs the workflow analysis with CNVPanelizer from the read counts and splitting the batch of samples analyzed

Usage

```
CNVPanelizerFromReadCounts(sampleReadCounts,
                           referenceReadCounts,
                           genomicRangesFromBed,
                           numberOfBootstrapReplicates = 10000,
                           normalizationMethod = "tmm",
                           robust = TRUE,
                           backgroundSignificanceLevel = 0.05,
                           outputDir = file.path(getwd(), "CNVPanelizer"))
```

Arguments

sampleReadCounts	samples read counts matrix
referenceReadCounts	reference read counts matrix
genomicRangesFromBed	genomic ranges from bed
numberOfBootstrapReplicates	number of bootstrap replicates
normalizationMethod	Normalization method ("tmm" or "tss")
robust	if TRUE, the median is used instead of mean
backgroundSignificanceLevel	The background Significance Level
outputDir	Output directory

Value

Returns a list with the results of each samples analyzed

Author(s)

Cristiano Oliveira

Examples

```
CNVPanelizerFromReadCounts(sampleReadCounts,
                           referenceReadCounts,
                           genomicRangesFromBed,
                           numberOfBootstrapReplicates = 10000,
                           normalizationMethod = "tmm",
                           robust = TRUE,
                           backgroundSignificanceLevel = 0.05,
                           outputDir = file.path(getwd(), "CNVPanelizer"))
```

`CNVPanelizerFromReadCountsHELPER`
CNVPanelizerFromReadCountsHELPER

Description

Helper to performs the workflow analysis with CNVPanelizer from the read counts and splitting the batch of samples analyzed

Usage

```
CNVPanelizerFromReadCountsHELPER(sampleReadCounts,
                                 referenceReadCounts,
                                 genomicRangesFromBed,
                                 numberOfBootstrapReplicates = 10000,
                                 normalizationMethod = "tmm",
                                 robust = TRUE,
                                 backgroundSignificanceLevel = 0.05,
                                 outputDir = file.path(getwd(), "CNVPanelizer"),
                                 splitSize = 5)
```

Arguments

`sampleReadCounts`
: samples read counts matrix

`referenceReadCounts`
: reference read counts matrix

genomicRangesFromBed
: genomic ranges from bed

numberOfBootstrapReplicates
: number of bootstrap replicates

normalizationMethod
: Normalization method ("tmm" or "tss")

robust
: if TRUE, the median is used instead of mean

backgroundSignificanceLevel
: The background Significance Level

outputDir
: Output directory

splitSize
: Split size of the batches analyzed

Value

Returns a list with the results of each samples analyzed

Author(s)

Cristiano Oliveira

Examples

```
CNVPanelizerFromReadCountsHELPER(sampleReadCounts,
                                 referenceReadCounts,
                                 genomicRangesFromBed,
                                 numberOfBootstrapReplicates = 10000,
                                 normalizationMethod = "tmm",
                                 robust = TRUE,
                                 backgroundSignificanceLevel = 0.05,
                               outputDir = file.path(getwd(), "CNVPanelizer"),
                                 splitSize = 5)
```

CollectColumnFromAllReportTables

CollectColumnFromAllReportTables

Description

Collect a single column from all report tables at the list

Usage

```
CollectColumnFromAllReportTables(reportTables, columnName)
```

Arguments

reportTables	A list of report tables
columnName	The column name

Value

Returns a data frame with where the columns were collected from the entire list of report tables

Author(s)

Cristiano Oliveira

Examples

```
CollectColumnFromAllReportTables(reportTables, columnName)
```

CombinedNormalizedCounts

CombinedNormalizedCounts

Description

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

Usage

```
CombinedNormalizedCounts(sampleCounts,
                         referenceCounts,
                         method,
                         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)
method	either "tmm" (trimmed mean of m values) or "tss"(total sum scaling)
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)
```

NormalizeCounts *NormalizeCounts*

Description

This function normalize counts use of Total sum scaling or NOISeq::tmm to normalize the read counts

Usage

```
NormalizeCounts(allCounts,
                method)
```

Arguments

allCounts	Matrix or vector with sample read counts (rows: amplicons, columns: samples)
method	either "tmm" (trimmed mean of m values) or "tss"(total sum scaling)

Value

A matrice

samples	The samples matrix normalized

Author(s)

Cristiano Oliveira, Thomas Wolf

Examples

```
data(sampleReadCounts)

normalizedReadCounts <- NormalizeCounts(sampleReadCounts)
```

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 = 10)
```

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,
                  minimumMappingQuality,
                  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
minimumMappingQuality	Minimum mapping quality
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)
```

RunCNVPanelizerShiny *RunCNVPanelizerShiny*

Description

Run CNVPanelizer as a shiny app

Usage

```
RunCNVPanelizerShiny(port = 8100)
```

Arguments

port	Port where the app will be listening

Value

Not returning any value

Author(s)

Thomas Wolf, Cristiano Oliveira

Examples

```
RunCNVPanelizerShiny(port=8080)
```

---

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

`SelectReferenceSetByInterquartileRange` *SelectReferenceSetByInterquartileRange*

Description

Select a reference set using a factor of the Interquartile Range

Usage

```
SelectReferenceSetByInterquartileRange(allSamplesReadCounts,
                                       normalizationMethod = "tmm",
                                            iqrFactor = 1)
```

Arguments

`allSamplesReadCounts`	All samples read counts matrix
`normalizationMethod`	tmm (trimmed mean of m values) or tss (total sum scaling)
`iqrFactor`	Interquantile range factor

Value

Returns a list of sample identifiers to be used as reference

Author(s)

Cristiano Oliveira

Examples

```
SelectReferenceSetByPercentil(allSamplesReadCounts,
                              normalizationMethod = "tmm",
                              iqrFactor = 1)
```

SelectReferenceSetByKmeans
SelectReferenceSetByKmeans

Description

Select a reference set using Kmeans

Usage

```
SelectReferenceSetByKmeans(allSamplesReadCounts,
      normalizationMethod = "tmm",
      referenceNumberOfElements)
```

Arguments

allSamplesReadCounts	All samples read counts matrix
normalizationMethod	tmm (trimmed mean of m values) or tss (total sum scaling)
referenceNumberOfElements	Number of elements to select for the reference set

Value

Returns a list of sample identifiers to be used as reference

Author(s)

Cristiano Oliveira

Examples

```
SelectReferenceSetByKmeans(allSamplesReadCounts,
                           normalizationMethod = "tmm",
                           referenceNumberOfElements)
```

SelectReferenceSetByPercentil
SelectReferenceSetByPercentil

Description

Select a reference set using percentiles

Usage

```
SelectReferenceSetByPercentil(allSamplesReadCounts,
                              normalizationMethod = "tmm",
                              lowerBoundPercentage = 1,
                              upperBoundPercentage = 99)
```

Arguments

allSamplesReadCounts	All samples read counts matrix
normalizationMethod	tmm (trimmed mean of m values) or tss (total sum scaling)
lowerBoundPercentage	Lower bound percentage
upperBoundPercentage	Upper bound percentage

Value

Returns a list of sample identifiers to be used as reference

Author(s)

Cristiano Oliveira

Examples

```
SelectReferenceSetByPercentil(allSamplesReadCounts,
                              normalizationMethod = "tmm",
                              lowerBoundPercentage = 1,
                              upperBoundPercentage = 99)
```

SelectReferenceSetFromReadCounts
SelectReferenceSetFromReadCounts

Description

Select a reference set from read counts

Usage

```
SelectReferenceSetFromReadCounts(allSamplesReadCounts,
                                 normalizationMethod = "tmm",
                                 referenceMaximumNumberOfElements = 30,
                                 referenceSelectionMethod = "kmeans",
                                 lowerBoundPercentage = 1,
                                 upperBoundPercentage = 99)
```

Arguments

`allSamplesReadCounts`
: All samples read counts matrix

`normalizationMethod`
: tmm (trimmed mean of m values) or tss (total sum scaling)

`referenceMaximumNumberOfElements`
: Maximum number of elements to consider as reference (only to be used in case interquantile reference selection method)

`referenceSelectionMethod`
: Reference selection method ("kmeans", ...)

`lowerBoundPercentage`
: Lower bound percentage (only to be used in case interquantile reference selection method)

`upperBoundPercentage`
: Upper bound percentage (only to be used in case interquantile reference selection method)

Value

Returns a list of sample identifiers to be used as reference

Author(s)

Cristiano Oliveira

Examples

```
SelectReferenceSetFromReadCounts(allSamplesReadCounts,
                                 normalizationMethod = "tmm",
                                 referenceMaximumNumberOfElements = 30,
                                 referenceSelectionMethod = "kmeans")
```

StatusHeatmap *StatusHeatmap*

Description

Generates a status heapmap for all samples analyzed

Usage

```
StatusHeatmap(dfData,
              statusColors = c("Deletion" = "blue",
                               "Normal" = "green",
                               "Amplification" = "red"),
              header = "Status Heatmap",
              filepath = "CNVPanelizerHeatMap.png")
```

Arguments

dfData	data frame with the "Amplification", "Deletion" and "Normal" status
statusColors	A named vector with the colors associated with each level
header	Header text at the plot
filepath	Filepath where the generated heatmap is saved

Value

Returns the filepath of the saved Heatmap

Author(s)

Cristiano Oliveira

Examples

```
StatusHeatmap(dfData,
              statusColors = c("Deletion" = "blue",
                               "Normal" = "green",
                               "Amplification" = "red"),
              header = "Status Heatmap",
              filepath = "CNVPanelizerHeatMap.png")
```

`WriteListToXLSX` *WriteListToXLSX*

Description

Writes list of data frames to an xlsx file

Usage

```
WriteListToXLSX(listOfDataFrames,
                multipleFiles = FALSE,
                outputFolder = file.path(getwd(), "xlsx"),
                filepath = "list.xlsx")
```

Arguments

`listOfDataFrames`	list of dataframes
`multipleFiles`	If should be generated on single file with all results or multiple files
`outputFolder`	Output folder
`filepath`	filepath

Value

Not returning any value

Author(s)

Thomas Wolf, Cristiano Oliveira

Examples

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

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