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

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

```
referenceNormalizedReadCounts,
bootList,
replicates = 1000,
significanceLevel = 0.05,
robust = FALSE)
```

Arguments

geneNames	A vector of gene names, with one entry for each sequenced amplicon.	
samplesNormal:	izedReadCounts	
	A matrix with the normalized read counts of the samples of interest	
referenceNorma	alizedReadCounts	
	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	
significanceLe	evel	
	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)</pre>
ampliconNames <- NULL</pre>
normalizedReadCounts <- CombinedNormalizedCounts(sampleReadCounts,</pre>
                                                   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,</pre>
                      samplesNormalizedReadCounts,
                      referenceNormalizedReadCounts,
                      replicates = replicates)
background <- Background(geneNames,</pre>
                         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

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BootList

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)</pre>
```

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)</pre>
ampliconNames <- NULL</pre>
normalizedReadCounts <- CombinedNormalizedCounts(sampleReadCounts,</pre>
                                                   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,</pre>
         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)
```

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

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NormalizeCounts

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)</pre>

PlotBootstrapDistributions

PlotBootstrapDistributions

Description

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

Usage

```
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)</pre>
```

```
ampliconNames <- NULL
```

```
# 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,</pre>
                      samplesNormalizedReadCounts,
                      referenceNormalizedReadCounts,
                      replicates = replicates)
backgroundNoise <- Background(geneNames,</pre>
           samplesNormalizedReadCounts,
           referenceNormalizedReadCounts,
           bootList,
           replicates = replicates)
reportTables <- ReportTables(geneNames,</pre>
             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

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)

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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 herehemend arise as returned by the Deslamented for
```

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)</pre>
ampliconNames <- NULL</pre>
normalizedReadCounts <- CombinedNormalizedCounts(sampleReadCounts,</pre>
                                                   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,</pre>
                      samplesNormalizedReadCounts,
                      referenceNormalizedReadCounts,
                      replicates = replicates)
backgroundNoise = Background(geneNames,
                              samplesNormalizedReadCounts,
                              referenceNormalizedReadCounts,
                              bootList,
                              replicates = replicates)
reportTables <- ReportTables(geneNames,</pre>
             samplesNormalizedReadCounts,
             referenceNormalizedReadCounts,
             bootList,
             backgroundNoise)
```

RunCNVPanelizerShiny RunCNVPanelizerShiny

Description

Run CNVPanelizer as a shiny app

sampleReadCounts

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

Arguments

allSamplesRead	Counts
	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

SelectReferenceSetByKmeans

SelectReferenceSetByKmeans

Description

Select a reference set using Kmeans

Usage

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

SelectReferenceSetByPercentil SelectReferenceSetByPercentil

Description

Select a reference set using percentiles

Usage

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
```

Returns a list of sample identifiers to be used as reference

Author(s)

Cristiano Oliveira

Examples

 $Select Reference Set From Read Counts \\ Select Reference Set From Read Counts$

Description

Select a reference set from read counts

Usage

referenceMaximumNumberOfElements = 30, referenceSelectionMethod = "kmeans", lowerBoundPercentage = 1, upperBoundPercentage = 99)

Arguments

guments
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)

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StatusHeatmap

Value

Returns a list of sample identifiers to be used as reference

Author(s)

Cristiano Oliveira

Examples

StatusHeatmap StatusHeatmap

Description

Generates a status heapmap for all samples analyzed

Usage

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

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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