

Package ‘TCGAbiolinks’

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Type Package

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Description The aim of TCGAbiolinks is : i) facilitate the TCGA open-access data retrieval, ii) prepare the data using the appropriate pre-processing strategies, iii) provide the means to carry out different standard analyses and iv) allow the user to download a specific version of the data and thus to easily reproduce earlier research results. In more detail, the package

provides multiple methods for analysis (e.g., differential expression analysis, identifying differentially methylated regions) and methods for visualization (e.g., survival plots, volcano plots, starburst plots) in order to easily develop complete analysis pipelines.

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biocViews DNAMethylation, DifferentialMethylation, GeneRegulation, GeneExpression, MethylationArray, DifferentialExpression, Pathways, Network, Sequencing, Survival

Suggests testthat, png, BiocStyle, rmarkdown, devtools

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URL <https://github.com/BioinformaticsFMRP/TCGAbiolinks>

BugReports <https://github.com/BioinformaticsFMRP/TCGAbiolinks/issues>

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GDCdownload	<i>Download GDC data</i>
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Description

Uses GDC API or GDC transfer tool to download gdc data The user can use query argument The data from query will be save in a folder: project/data.category

Usage

```
GDCdownload(query, token.file, method = "api", directory = "GDCdata",
  chunks.per.download = NULL)
```

Arguments

query	A query for GDCquery function
token.file	Token file to download controled data (only for method = "client")
method	Uses the API (POST method) or gdc client tool. Options "api", "client". API is faster, but the data might get corrupted in the download, and it might need to be executed again
directory	Directory/Folder where the data was downloaded. Default: GDCdata
chunks.per.download	This will make the API method only download n (chunks.per.download) files at a time. This may reduce the download problems when the data size is too large. Expected a integer number (example chunks.per.download = 6)

Value

Shows the output from the GDC transfer tools

Examples

```
query <- GDCquery(project = "TCGA-ACC",
  data.category = "Copy number variation",
  legacy = TRUE,
  file.type = "hg19.seg",
  barcode = c("TCGA-OR-A5LR-01A-11D-A29H-01", "TCGA-OR-A5LJ-10A-01D-A29K-01"))
# data will be saved in GDCdata/TCGA-ACC/legacy/Copy_number_variation/Copy_number_segmentation
GDCdownload(query, method = "api")
```

```

query <- GDCquery(project = "TARGET-AML",
                 data.category = "Transcriptome Profiling",
                 data.type = "miRNA Expression Quantification",
                 workflow.type = "BCGSC miRNA Profiling",
                 barcode = c("TARGET-20-PARUDL-03A-01R", "TARGET-20-PASRRB-03A-01R"))
# data will be saved in:
# example_data_dir/TARGET-AML/harmonized/Transcriptome_Profiling/miRNA_Expression_Quantification
GDCdownload(query, method = "client", directory = "example_data_dir")
query <- GDCquery(project = "TCGA-COAD", data.category = "Clinical")
GDCdownload(query, chunks.per.download = 200)
## Not run:
  acc.gbm <- GDCquery(project = c("TCGA-ACC", "TCGA-GBM"),
                    data.category = "Transcriptome Profiling",
                    data.type = "Gene Expression Quantification",
                    workflow.type = "HTSeq - Counts")
  GDCdownload(acc.gbm, method = "api", directory = "example", chunks.per.download = 50)

## End(Not run)

```

GDCprepare

Prepare GDC data

Description

Reads the data downloaded and prepare it into an R object

Usage

```

GDCprepare(query, save = FALSE, save.filename, directory = "GDCdata",
           summarizedExperiment = TRUE, remove.files.prepared = FALSE,
           add.gistic2.mut = NULL, mut.pipeline = "mutect2")

```

Arguments

query	A query for GDCquery function
save	Save result as RData object?
save.filename	Name of the file to be save if empty an automatic will be created
directory	Directory/Folder where the data was downloaded. Default: GDCdata
summarizedExperiment	Create a summarizedExperiment? Default TRUE (if possible)
remove.files.prepared	Remove the files read? Default: FALSE This argument will be considered only if save argument is set to true
add.gistic2.mut	If a list of genes (gene symbol) is given, columns with gistic2 results from GDAC firehose (hg19) and a column indicating if there is or not mutation in that gene (hg38) (TRUE or FALSE - use the MAF file for more information) will be added to the sample matrix in the summarized Experiment object.
mut.pipeline	If add.gistic2.mut is not NULL this field will be taken in consideration. Four separate variant calling pipelines are implemented for GDC data harmonization. Options: muse, varscan2, somaticsniiper, MuTect2. For more information: https://gdc-docs.nci.nih.gov/Data/Bioinformatics_Pipelines/DNA_Seq_Variant_Calling_Pipeline/

Value

A summarizedExperiment or a data.frame

Examples

```

query <- GDCquery(project = "TCGA-KIRP",
                  data.category = "Simple Nucleotide Variation",
                  data.type = "Masked Somatic Mutation",
                  workflow.type = "MuSE Variant Aggregation and Masking")
GDCdownload(query, method = "api", directory = "maf")
maf <- GDCprepare(query, directory = "maf")

query <- GDCquery(project = "TCGA-ACC",
                  data.category = "Copy number variation",
                  legacy = TRUE,
                  file.type = "hg19.seg",
                  barcode = c("TCGA-OR-A5LR-01A-11D-A29H-01", "TCGA-OR-A5LJ-10A-01D-A29K-01"))
# data will be saved in GDCdata/TCGA-ACC/legacy/Copy_number_variation/Copy_number_segmentation
GDCdownload(query, method = "api")
acc.cnv <- GDCprepare(query)
## Not run:
query <- GDCquery(project = "TCGA-GBM",
                  legacy = TRUE,
                  data.category = "Gene expression",
                  data.type = "Gene expression quantification",
                  platform = "Illumina HiSeq",
                  file.type = "normalized_results",
                  experimental.strategy = "RNA-Seq")
GDCdownload(query, method = "api")
data <- GDCprepare(query, add.gistic2.mut = c("PTEN", "FOXJ1"))

## End(Not run)

```

GDCprepare_clinic *Parsing clinical xml files*

Description

This function receives the query argument and parses the clinical xml files based on the desired information

Usage

```
GDCprepare_clinic(query, clinical.info, directory = "GDCdata")
```

Arguments

query	Result from GDCquery, with data.category set to Clinical
clinical.info	Which information should be retrieved. Options Clinical: drug, admin, follow_up, radiation, patient, stage_event or new_tumor_event Options Biospecimen: protocol, admin, aliquot, analyte, bio_patient, sample, portion, slide
directory	Directory/Folder where the data was downloaded. Default: GDCdata

Examples

```

query <- GDCquery(project = "TCGA-COAD",
                  data.category = "Clinical",
                  barcode = c("TCGA-RU-A8FL", "TCGA-AA-3972"))
GDCdownload(query)
clinical <- GDCprepare_clinic(query, "patient")
clinical.drug <- GDCprepare_clinic(query, "drug")
clinical.radiation <- GDCprepare_clinic(query, "radiation")
clinical.admin <- GDCprepare_clinic(query, "admin")
query <- GDCquery(project = "TCGA-COAD",
                  data.category = "Biospecimen",
                  barcode = c("TCGA-RU-A8FL", "TCGA-AA-3972"))
GDCdownload(query)
clinical <- GDCprepare_clinic(query, "admin")
clinical.drug <- GDCprepare_clinic(query, "sample")
clinical.radiation <- GDCprepare_clinic(query, "portion")
clinical.admin <- GDCprepare_clinic(query, "slide")

```

GDCquery

*Query GDC data***Description**

Uses GDC API to search for search, it searches for both controlled and open-access data. For GDC data arguments project, data.category, data.type and workflow.type should be used For the legacy data arguments project, data.category, platform and/or file.extension should be used. Please, see the vignette for a table with the possibilities.

Usage

```

GDCquery(project, data.category, data.type, workflow.type, legacy = FALSE,
         access, platform, file.type, barcode, experimental.strategy, sample.type)

```

Arguments

project	A list of valid project (see list with <code>TCGAbiolinks::getGDCprojects()\$project_id</code>]
data.category	A valid project (see list with <code>TCGAbiolinks::getProjectSummary(project)</code>)
data.type	A data type to filter the files to download
workflow.type	GDC workflow type
legacy	Search in the legacy repository
access	Filter by access type. Possible values: controlled, open
platform	Example:

CGH- 1x1M_G4447A	IlluminaGA_RNASeqV2
AgilentG4502A_07	IlluminaGA_mRNA_DGE
HumanIMDuo	HumanMethylation450
HG-CGH-415K_G4124A	IlluminaGA_miRNASeq
HumanHap550	IlluminaHiSeq_miRNASeq
ABI	H-miRNA_8x15K
HG-CGH-244A	SOLiD_DNASeq

ILLUMINADNAMETHYLATION_OMA003_CPI	ILLUMINAGA_DNASeq_automated
ILLUMINADNAMETHYLATION_OMA002_CPI	HG-U133_Plus_2
HuEx-1_0-st-v2	Mixed_DNASeq
H-miRNA_8x15Kv2	ILLUMINAGA_DNASeq_curated
MDA_RPPA_Core	ILLUMINAHiSeq_TotalRNASeqV2
HT_HG-U133A	ILLUMINAHiSeq_DNASeq_automated
diagnostic_images	microsat_i
ILLUMINAHiSeq_RNASeq	SOLiD_DNASeq_curated
ILLUMINAHiSeq_DNASeqC	Mixed_DNASeq_curated
ILLUMINAGA_RNASeq	ILLUMINAGA_DNASeq_Cont_automated
ILLUMINAGA_DNASeq	ILLUMINAHiSeq_WGBS
pathology_reports	ILLUMINAHiSeq_DNASeq_Cont_automated
Genome_Wide_SNP_6	bio
tissue_images	Mixed_DNASeq_automated
HumanMethylation27	Mixed_DNASeq_Cont_curated
ILLUMINAHiSeq_RNASeqV2	Mixed_DNASeq_Cont

file.type	To be used in the legacy database for some platforms, to define which file types to be used.
barcode	A list of barcodes to filter the files to download
experimental.strategy	Filter to experimental strategy. Harmonized: WXS, RNA-Seq, miRNA-Seq, Genotyping Array. Legacy: WXS, RNA-Seq, miRNA-Seq, Genotyping Array, DNA-Seq, Methylation array, Protein expression array, WXS,CGH array, VALIDATION, Gene expression array,WGS, MSI-Mono-Dinucleotide Assay, miRNA expression array, Mixed strategies, AMPLICON, Exon array, Total RNA-Seq, Capillary sequencing, Bisulfite-Seq
sample.type	A sample type to filter the files to download

Value

A data frame with the results and the parameters used

Examples

```

query <- GDCquery(project = "TCGA-ACC",
  data.category = "Copy Number Variation",
  data.type = "Copy Number Segment")
query.met <- GDCquery(project = c("TCGA-GBM","TCGA-LGG"),
  legacy = TRUE,
  data.category = "DNA methylation",
  platform = "Illumina Human Methylation 450")
query <- GDCquery(project = "TARGET-AML",
  data.category = "Transcriptome Profiling",
  data.type = "miRNA Expression Quantification",
  workflow.type = "BCGSC miRNA Profiling",
  barcode = c("TARGET-20-PARUDL-03A-01R","TARGET-20-PASRRB-03A-01R"))
query <- GDCquery(project = "TCGA-ACC",
  data.category = "Copy Number Variation",
  data.type = "Masked Copy Number Segment",
  sample.type = c("Primary solid Tumor"))
query <- GDCquery(project = "TARGET-AML",
  data.category = "Transcriptome Profiling",
  data.type = "Gene Expression Quantification",

```

```

        workflow.type = "HTSeq - Counts",
        barcode = c("TARGET-20-PADZCG-04A-01R","TARGET-20-PARJCR-09A-01R"))
query <- GDCquery(project = "TCGA-ACC",
                 data.category = "Copy number variation",
                 legacy = TRUE,
                 file.type = "hg19.seg",
                 barcode = c("TCGA-OR-A5LR-01A-11D-A29H-01"))

```

GDCquery_clinic *Get GDC clinical data*

Description

GDCquery_clinic will download all clinical information from the API as the one with using the button from each project

Usage

```
GDCquery_clinic(project, type = "clinical", save.csv = FALSE)
```

Arguments

project	A valid project (see list with <code>getGDCprojects()\$project_id</code>]
type	A valid type. Options "clinical", "Biospecimen" (see list with <code>getGDCprojects()\$project_id</code>]
save.csv	Write clinical information into a csv document

Value

A data frame with the clinical information

Examples

```

clin <- GDCquery_clinic("TCGA-ACC", type = "clinical", save.csv = TRUE)
clin <- GDCquery_clinic("TCGA-ACC", type = "biospecimen", save.csv = TRUE)

```

GDCquery_Maf *Retrieve open access maf files from GDC server*

Description

GDCquery_Maf uses the following guide to download maf files https://gdc-docs.nci.nih.gov/Data/Release_Notes/Data_R

Usage

```
GDCquery_Maf(tumor, save.csv = FALSE, directory = "GDCdata",
             pipelines = NULL)
```


Arguments

tumor	a valid tumor
save.csv	Write maf file into a csv document
directory	Directory/Folder where the data will downloaded. Default: GDCdata
pipelines	Four separate variant calling pipelines are implemented for GDC data harmonization. Options: muse, varscan2, somaticsniiper, mutect2. For more information: https://gdc-docs.nci.nih.gov/Data/Bioinformatics_Pipelines/DNA_Seq_Variant_Calling_Pipeline/

Value

A data frame with the maf file information

Examples

```
acc.muse.maf <- GDCquery_Maf("ACC", pipelines = "muse")
## Not run:
acc.varscan2.maf <- GDCquery_Maf("ACC", pipelines = "varscan2")
acc.somaticsniper.maf <- GDCquery_Maf("ACC", pipelines = "somaticsniper")
acc.mutect.maf <- GDCquery_Maf("ACC", pipelines = "mutect2")

## End(Not run)
```

getGDCprojects	<i>Retrieve all GDC projects</i>
----------------	----------------------------------

Description

getGDCprojects uses the following api to get projects <https://gdc-api.nci.nih.gov/projects>

Usage

```
getGDCprojects()
```

Value

A data frame with last GDC projects

Examples

```
projects <- getGDCprojects()
```

getResults	<i>Get the results table from query</i>
------------	---

Description

Get the results table from query, it can select columns with cols argument and return a number of rows using rows argument.

Usage

```
getResults(query, rows, cols)
```

Arguments

query	A object from GDCquery
rows	Rows identifiers (row numbers)
cols	Columns identifiers (col names)

Examples

```
query <- GDCquery(project = "TCGA-GBM",
  data.category = "Transcriptome Profiling",
  data.type = "Gene Expression Quantification",
  workflow.type = "HTSeq - Counts",
  barcode = c("TCGA-14-0736-02A-01R-2005-01", "TCGA-06-0211-02A-02R-2005-01"))
results <- getResults(query)
```

isServeOK	<i>Check GDC server status</i>
-----------	--------------------------------

Description

Check GDC server status using the api <https://gdc-api.nci.nih.gov/status>

Usage

```
isServeOK()
```

Value

Return true if status is ok

Examples

```
status <- isServeOK()
```

 TCGAanalyze_analyseGRN

Generate network

Description

TCGAanalyze_analyseGRN perform gene regulatory network.

Usage

```
TCGAanalyze_analyseGRN(TFs, normCounts, kNum)
```

Arguments

TFs	a vector of genes.
normCounts	is a matrix of gene expression with genes in rows and samples in columns.
kNum	the number of nearest neighbors to consider to estimate the mutual information. Must be less than the number of columns of normCounts.

Value

an adjacent matrix

TCGAanalyze_Clustering

Hierarchical cluster analysis

Description

Hierarchical cluster analysis using several methods such as ward.D", "ward.D2", "single", "complete", "average" (= UPGMA), "mcquitty" (= WPGMA), "median" (= WPGMC) or "centroid" (= UPGMC).

Usage

```
TCGAanalyze_Clustering(tabDF, method, methodHC = "ward.D2")
```

Arguments

tabDF	is a dataframe or numeric matrix, each row represents a gene, each column represents a sample come from TCGAPrepare.
method	is method to be used for generic cluster such as 'hclust' or 'consensus'
methodHC	is method to be used for Hierarchical cluster.

Value

object of class hclust if method selected is 'hclust'. If method selected is 'Consensus' returns a list of length maxK (maximum cluster number to evaluate.). Each element is a list containing consensus-Matrix (numerical matrix), consensusTree (hclust), consensusClass (consensus class assignments). ConsensusClusterPlus also produces images.

TCGAanalyze_DEA

*Differentially expression analysis (DEA) using edgeR package.***Description**

TCGAanalyze_DEA allows user to perform Differentially expression analysis (DEA), using edgeR package to identify differentially expressed genes (DEGs). It is possible to do a two-class analysis.

TCGAanalyze_DEA performs DEA using following functions from edgeR:

1. edgeR::DGEList converts the count matrix into an edgeR object.
2. edgeR::estimateCommonDisp each gene gets assigned the same dispersion estimate.
3. edgeR::exactTest performs pair-wise tests for differential expression between two groups.
4. edgeR::topTags takes the output from exactTest(), adjusts the raw p-values using the False Discovery Rate (FDR) correction, and returns the top differentially expressed genes.

Usage

```
TCGAanalyze_DEA(mat1, mat2, Cond1type, Cond2type, method = "exactTest",
  fdr.cut = 1, logFC.cut = 0, elementsRatio = 30000)
```

Arguments

mat1	numeric matrix, each row represents a gene, each column represents a sample with Cond1type
mat2	numeric matrix, each row represents a gene, each column represents a sample with Cond2type
Cond1type	a string containing the class label of the samples in mat1 (e.g., control group)
Cond2type	a string containing the class label of the samples in mat2 (e.g., case group)
method	is 'glmLRT' (1) or 'exactTest' (2). (1) Fit a negative binomial generalized log-linear model to the read counts for each gene (2) Compute genewise exact tests for differences in the means between two groups of negative-binomially distributed counts.
fdr.cut	is a threshold to filter DEGs according their p-value corrected
logFC.cut	is a threshold to filter DEGs according their logFC
elementsRatio	is number of elements processed for second for time consumption estimation

Value

table with DEGs containing for each gene logFC, logCPM, pValue, and FDR

Examples

```
dataNorm <- TCGAbiolinks::TCGAanalyze_Normalization(dataBRCA, geneInfo)
dataFilt <- TCGAanalyze_Filtering(tabDF = dataBRCA, method = "quantile", qnt.cut = 0.25)
samplesNT <- TCGAquery_SampleTypes(colnames(dataFilt), typesample = c("NT"))
samplesTP <- TCGAquery_SampleTypes(colnames(dataFilt), typesample = c("TP"))
dataDEGs <- TCGAanalyze_DEA(dataFilt[,samplesNT],
  dataFilt[,samplesTP], "Normal", "Tumor")
```

TCGAanalyze_DEA_Affy *Differentially expression analysis (DEA) using limma package.*

Description

Differentially expression analysis (DEA) using limma package.

Usage

```
TCGAanalyze_DEA_Affy(AffySet, FC.cut = 0.01)
```

Arguments

AffySet	A matrix-like data object containing log-ratios or log-expression values for a series of arrays, with rows corresponding to genes and columns to samples
FC.cut	write

Value

List of list with tables in 2 by 2 comparison of the top-ranked genes from a linear model fitted by DEA's limma

Examples

```
## Not run:
to add example

## End(Not run)
```

TCGAanalyze_DMR *Differentially methylated regions Analysis*

Description

This function will search for differentially methylated CpG sites, which are regarded as possible functional regions involved in gene transcriptional regulation.

In order to find these regions we use the beta-values (methylation values ranging from 0.0 to 1.0) to compare two groups.

Firstly, it calculates the difference between the mean methylation of each group for each probes. Secondly, it calculates the p-value using the wilcoxon test using the Benjamini-Hochberg adjustment method. The default parameters will require a minimum absolute beta values delta of 0.2 and a false discovery rate (FDR)-adjusted Wilcoxon rank-sum P-value of < 0.01 for the difference.

After these analysis, we save a volcano plot (x-axis:diff mean methylation, y-axis: significance) that will help the user identify the differentially methylated CpG sites and return the object with the calculus in the rowRanges.

If the calculus already exists in the object it will not recalculated. You should set overwrite parameter to TRUE to force it, or remove the collumns with the results from the object.

Usage

```
TCGAanalyze_DMR(data, groupCol = NULL, group1 = NULL, group2 = NULL,
  calculate.pvalues.probes = "all",
  plot.filename = "methylation_volcano.pdf",
  ylab = expression(paste(-Log[10], " (FDR corrected -P values)")),
  xlab = expression(paste("DNA Methylation difference (", beta, "-values)")),
  title = NULL, legend = "Legend", color = c("black", "red", "darkgreen"),
  label = NULL, xlim = NULL, ylim = NULL, p.cut = 0.01,
  probe.names = FALSE, diffmean.cut = 0.2, paired = FALSE,
  adj.method = "BH", overwrite = FALSE, cores = 1, save = TRUE,
  save.directory = ".", filename = NULL)
```

Arguments

data	SummarizedExperiment obtained from the TCGAPrepare
groupCol	Columns with the groups inside the SummarizedExperiment object. (This will be obtained by the function colData(data))
group1	In case our object has more than 2 groups, you should set the name of the group
group2	In case our object has more than 2 groups, you should set the name of the group
calculate.pvalues.probes	In order to get the probes faster the user can select to calculate the pvalues only for the probes with a difference in DNA methylation. The default is to calculate to all probes. Possible values: "all", "differential". Default "all"
plot.filename	Filename. Default: volcano.pdf, volcano.svg, volcano.png. If set to FALSE, there will be no plot.
ylab	y axis text
xlab	x axis text
title	main title. If not specified it will be "Volcano plot (group1 vs group2)"
legend	Legend title
color	vector of colors to be used in graph
label	vector of labels to be used in the figure. Example: c("Not Significant", "Hypermethylated in group1", "Hypomethylated in group1")
xlim	x limits to cut image
ylim	y limits to cut image
p.cut	p values threshold. Default: 0.01
probe.names	is probe.names
diffmean.cut	diffmean threshold. Default: 0.2
paired	Wilcoxon paired parameter. Default: FALSE
adj.method	Adjusted method for the p-value calculation
overwrite	Overwrite the pvalues and diffmean values if already in the object for both groups? Default: FALSE
cores	Number of cores to be used in the non-parametric test Default = groupCol.group1.group2.rda
save	Save object with results? Default: TRUE
save.directory	Directory to save the files. Default: working directory
filename	Name of the file to save the object.

Value

Volcano plot saved and the given data with the results (`diffmean.group1.group2`, `p.value.group1.group2`, `p.value.adj.group1.group2`, `status.group1.group2`) in the `rowRanges` where `group1` and `group2` are the names of the groups

Examples

```
nrows <- 200; ncols <- 20
counts <- matrix(runif(nrows * ncols, 1, 1e4), nrows)
rowRanges <- GenomicRanges::GRanges(rep(c("chr1", "chr2"), c(50, 150)),
  IRanges::IRanges(floor(runif(200, 1e5, 1e6)), width=100),
  strand=sample(c("+", "-"), 200, TRUE),
  feature_id=sprintf("ID%03d", 1:200))
colData <- S4Vectors::DataFrame(Treatment=rep(c("ChIP", "Input"), 5),
  row.names=LETTERS[1:20],
  group=rep(c("group1", "group2"), c(10, 10)))
data <- SummarizedExperiment::SummarizedExperiment(
  assays=S4Vectors::SimpleList(counts=counts),
  rowRanges=rowRanges,
  colData=colData)
SummarizedExperiment::colData(data)$group <- c(rep("group 1", ncol(data)/2),
  rep("group 2", ncol(data)/2))
hypo.hyper <- TCGAanalyze_DMR(data, p.cut = 0.85, "group", "group 1", "group 2")
SummarizedExperiment::colData(data)$group2 <- c(rep("group_1", ncol(data)/2),
  rep("group_2", ncol(data)/2))
hypo.hyper <- TCGAanalyze_DMR(data, p.cut = 0.85, "group2", "group_1", "group_2")
```

TCGAanalyze_EA

Enrichment analysis of a gene-set with GO [BP, MF, CC] and pathways.

Description

The rationale behind an enrichment analysis (gene-set, pathway etc) is to compute statistics of whether the overlap between the focus list (signature) and the gene-set is significant, i.e. the confidence that overlap between the list is not due to chance. The Gene Ontology project describes genes (gene products) using terms from three structured vocabularies: biological process, cellular component and molecular function. The Gene Ontology Enrichment component, also referred to as the "GO Terms" component, allows the genes in any such "changed-gene" list to be characterized using the Gene Ontology terms annotated to them. It asks, whether for any particular GO term, the fraction of genes assigned to it in the "changed-gene" list is higher than expected by chance (is over-represented), relative to the fraction of genes assigned to that term in the reference set. In statistical terms it performs the analysis tests the null hypothesis that, for any particular ontology term, there is no difference in the proportion of genes annotated to it in the reference list and the proportion annotated to it in the test list. We adopted a Fisher Exact Test to perform the EA.

Usage

```
TCGAanalyze_EA(GeneName, RegulonList, TableEnrichment, EAGenes, GOtype,
  FDRThresh = 0.01)
```

Arguments

GeneName	is the name of gene signatures list
RegulonList	is a gene signature (list of genes) in which perform EA.
TableEnrichment	is a table related to annotations of gene symbols such as GO[BP,MF,CC] and Pathways. It was created from DAVID gene ontology on-line.
EAGenes	is a table with informations about genes such as ID, Gene, Description, Location and Family.
GOtype	is type of gene ontology Biological process (BP), Molecular Function (MF), Cellular component (CC)
FDRThresh	pvalue corrected (FDR) as threshold to selected significant BP, MF,CC, or pathways. (default FDR < 0.01)

Value

Table with enriched GO or pathways by selected gene signature.

Examples

```
## Not run:
EAGenes <- get("EAGenes")
RegulonList <- rownames(dataDEGsFiltLevel)
ResBP <- TCGAanalyze_EA(GeneName="DEA genes Normal Vs Tumor",
                        RegulonList,DAVID_BP_matrix,
                        EAGenes,GOtype = "DavidBP")

## End(Not run)
```

TCGAanalyze_EAcomplete

Enrichment analysis for Gene Ontology (GO) [BP,MF,CC] and Pathways

Description

Researchers, in order to better understand the underlying biological processes, often want to retrieve a functional profile of a set of genes that might have an important role. This can be done by performing an enrichment analysis.

We will perform an enrichment analysis on gene sets using the TCGAanalyze_EAcomplete function. Given a set of genes that are up-regulated under certain conditions, an enrichment analysis will find identify classes of genes or proteins that are # over-represented using annotations for that gene set.

Usage

```
TCGAanalyze_EAcomplete(TFname, RegulonList)
```

Arguments

TFname	is the name of the list of genes or TF's regulon.
RegulonList	List of genes such as TF's regulon or DEGs where to find enrichment.

Value

Enrichment analysis GO[BP,MF,CC] and Pathways complete table enriched by genelist.

Examples

```
Genelist <- c("FN1","COL1A1")
ansEA <- TCGAanalyze_EAcomplete(TFname="DEA genes Normal Vs Tumor",Genelist)
## Not run:
Genelist <- rownames(dataDEGsFiltLevel)
system.time(ansEA <- TCGAanalyze_EAcomplete(TFname="DEA genes Normal Vs Tumor",Genelist))

## End(Not run)
```

TCGAanalyze_Filtering *Filtering mRNA transcripts and miRNA selecting a threshold.*

Description

TCGAanalyze_Filtering allows user to filter mRNA transcripts and miRNA, selecting a threshold. For instance returns all mRNA or miRNA with mean across all samples, higher than the threshold defined quantile mean across all samples.

Usage

```
TCGAanalyze_Filtering(tabDF, method, qnt.cut = 0.25, var.func = IQR,
  var.cutoff = 0.75, eta = 0.05, foldChange = 1)
```

Arguments

tabDF	is a dataframe or numeric matrix, each row represents a gene, each column represents a sample come from TCGAPrepare
method	is method of filtering such as 'quantile', 'varFilter', 'filter1', 'filter2'
qnt.cut	is threshold selected as mean for filtering
var.func	is function used as the per-feature filtering statistic. See genefilter documentation
var.cutoff	is a numeric value. See genefilter documentation
eta	is a parameter for filter1. default eta = 0.05.
foldChange	is a parameter for filter2. default foldChange = 1.

Value

A filtered dataframe or numeric matrix where each row represents a gene, each column represents a sample

Examples

```
dataNorm <- TCGAbiolinks::TCGAanalyze_Normalization(dataBRCA, geneInfo)
dataNorm <- TCGAanalyze_Normalization(tabDF = dataBRCA,
  geneInfo = geneInfo,
  method = "geneLength")
dataFilt <- TCGAanalyze_Filtering(tabDF = dataNorm, method = "quantile", qnt.cut = 0.25)
```

TCGAanalyze_LevelTab *Adding information related to DEGs genes from DEA as mean values in two conditions.*

Description

TCGAanalyze_LevelTab allows user to add information related to DEGs genes from Differentially expression analysis (DEA) such as mean values and in two conditions.

Usage

```
TCGAanalyze_LevelTab(FC_FDR_table_mRNA, typeCond1, typeCond2, TableCond1,
  TableCond2, typeOrder = TRUE)
```

Arguments

FC_FDR_table_mRNA	Output of dataDEGs filter by $\text{abs}(\text{LogFC}) \geq 1$
typeCond1	a string containing the class label of the samples in TableCond1 (e.g., control group)
typeCond2	a string containing the class label of the samples in TableCond2 (e.g., case group)
TableCond1	numeric matrix, each row represents a gene, each column represents a sample with Cond1type
TableCond2	numeric matrix, each row represents a gene, each column represents a sample with Cond2type
typeOrder	typeOrder

Value

table with DEGs, log Fold Change (FC), false discovery rate (FDR), the gene expression level for samples in Cond1type, and Cond2type, and Delta value (the difference of gene expression between the two conditions multiplied logFC)

Examples

```
dataNorm <- TCGAbiolinks::TCGAanalyze_Normalization(dataBRCA, geneInfo)
dataFilt <- TCGAanalyze_Filtering(tabDF = dataBRCA, method = "quantile", qnt.cut = 0.25)
samplesNT <- TCGAquery_SampleTypes(colnames(dataFilt), typesample = c("NT"))
samplesTP <- TCGAquery_SampleTypes(colnames(dataFilt), typesample = c("TP"))
dataDEGs <- TCGAanalyze_DEA(dataFilt[,samplesNT], dataFilt[,samplesTP],
  "Normal", "Tumor")
dataDEGsFilt <- dataDEGs[abs(dataDEGs$logFC) >= 1,]
dataTP <- dataFilt[,samplesTP]
dataTN <- dataFilt[,samplesNT]
dataDEGsFiltLevel <- TCGAanalyze_LevelTab(dataDEGsFilt,"Tumor","Normal",
  dataTP,dataTN)
```

TCGAanalyze_networkInference
infer gene regulatory networks

Description

TCGAanalyze_networkInference taking expression data as input, this will return an adjacency matrix of interactions

Usage

```
TCGAanalyze_networkInference(data, optionMethod = "clr")
```

Arguments

data expression data, genes in columns, samples in rows
optionMethod inference method, chose from aracne, c3net, clr and mrnet

Value

an adjacent matrix

TCGAanalyze_Normalization
normalization mRNA transcripts and miRNA using EDASeq package.

Description

TCGAanalyze_Normalization allows user to normalize mRNA transcripts and miRNA, using EDASeq package.

Normalization for RNA-Seq Numerical and graphical summaries of RNA-Seq read data. Within-lane normalization procedures to adjust for GC-content effect (or other gene-level effects) on read counts: loess robust local regression, global-scaling, and full-quantile normalization (Risso et al., 2011). Between-lane normalization procedures to adjust for distributional differences between lanes (e.g., sequencing depth): global-scaling and full-quantile normalization (Bullard et al., 2010).

For instance returns all mRNA or miRNA with mean across all samples, higher than the threshold defined quantile mean across all samples.

TCGAanalyze_Normalization performs normalization using following functions from EDASeq

1. EDASeq::newSeqExpressionSet
2. EDASeq::withinLaneNormalization
3. EDASeq::betweenLaneNormalization
4. EDASeq::counts

Usage

```
TCGAanalyze_Normalization(tabDF, geneInfo, method = "geneLength")
```

Arguments

tabDF	Rnaseq numeric matrix, each row represents a gene, each column represents a sample
geneInfo	Information matrix of 20531 genes about geneLength and gcContent. Two objects are provided: TCGAAbiolinks::geneInfoHT,TCGAAbiolinks::geneInfo
method	is method of normalization such as 'gcContent' or 'geneLength'

Value

Rnaseq matrix normalized with counts slot holds the count data as a matrix of non-negative integer count values, one row for each observational unit (gene or the like), and one column for each sample.

Examples

```
dataNorm <- TCGAAbiolinks::TCGAanalyze_Normalization(dataBRCA, geneInfo)
```

TCGAanalyze_Pathview *Generate pathview graph*

Description

TCGAanalyze_Pathview pathway based data integration and visualization.

Usage

```
TCGAanalyze_Pathview(dataDEGs, pathwayKEGG = "hsa05200")
```

Arguments

dataDEGs	dataDEGs
pathwayKEGG	pathwayKEGG

Value

an adjacent matrix

TCGAanalyze_Preprocessing
Array Array Intensity correlation (AAIC) and correlation boxplot to define outlier

Description

TCGAanalyze_Preprocessing perform Array Array Intensity correlation (AAIC). It defines a square symmetric matrix of pearson correlation among samples. According this matrix and boxplot of correlation samples by samples it is possible to find samples with low correlation that can be identified as possible outliers.

Usage

```
TCGAanalyze_Preprocessing(object, cor.cut = 0, filename = NULL,
  width = 500, height = 500, datatype = names(assays(object))[1])
```

Arguments

object	of gene expression of class RangedSummarizedExperiment from TCGAprepare
cor.cut	is a threshold to filter samples according their spearman correlation in samples by samples. default cor.cut is 0
filename	Filename of the image file
width	Image width
height	Image height
datatype	is a string from RangedSummarizedExperiment assay

Value

Plot with array array intensity correlation and boxplot of correlation samples by samples

TCGAanalyze_survival *Creates survival analysis*

Description

Creates a survival plot from TCGA patient clinical data using survival library. It uses the fields days_to_death and vital, plus a columns for groups.

Usage

```
TCGAanalyze_survival(data, clusterCol = NULL, legend = "Legend",
  labels = NULL, risk.table = TRUE, xlim = NULL,
  main = "Kaplan-Meier Overall Survival Curves",
  ylab = "Probability of survival", xlab = "Time since diagnosis (days)",
  filename = "survival.pdf", color = NULL, height = 8, width = 12,
  dpi = 300, pvalue = TRUE, conf.int = TRUE, ...)
```

Arguments

data	TCGA Clinical patient with the information days_to_death
clusterCol	Column with groups to plot. This is a mandatory field, the caption will be based in this column
legend	Legend title of the figure
labels	labels of the plot
risk.table	show or not the risk table
xlim	x axis limits e.g. xlim = c(0, 1000). Present narrower X axis, but not affect survival estimates.
main	main title of the plot
ylab	y axis text of the plot

xlab	x axis text of the plot
filename	The name of the pdf file.
color	Define the colors/Pallete for lines.
height	Image height
width	Image width
dpi	Figure quality
pvalue	show p-value of log-rank test
conf.int	show confidence intervals for point estimaes of survival curves.
...	Further arguments passed to ggsurvplot .

Value

Survival plot

Examples

```
clin <- GDCquery_clinic("TCGA-LGG", type = "clinical", save.csv = FALSE)
TCGAanalyze_survival(clin, clusterCol="gender")
TCGAanalyze_survival(clin, clusterCol="gender", xlim = 1000)
TCGAanalyze_survival(clin,
  clusterCol="gender",
  risk.table = FALSE,
  conf.int = FALSE,
  color = c("pink", "blue"))
TCGAanalyze_survival(clin,
  clusterCol="gender",
  risk.table = FALSE,
  xlim = c(100,1000),
  conf.int = FALSE,
  color = c("Dark2"))
```

TCGAanalyze_SurvivalKM

survival analysis (SA) univariate with Kaplan-Meier (KM) method.

Description

TCGAanalyze_SurvivalKM perform an univariate Kaplan-Meier (KM) survival analysis (SA). It performed Kaplan-Meier survival univariate using complte follow up with all days taking one gene a time from Genelist of gene symbols. For each gene according its level of mean expression in cancer samples, defining two thresholds for quantile expression of that gene in all samples (default ThreshTop=0.67,ThreshDown=0.33) it is possible to define a threshold of intensity of gene expression to divide the samples in 3 groups (High, intermediate, low). TCGAanalyze_SurvivalKM performs SA between High and low groups using following functions from survival package

1. survival::Surv
2. survival::survdiff
3. survival::survfit

Usage

```
TCGAanalyze_SurvivalKM(clinical_patient, dataGE, Genelist, Survresult,
  ThreshTop = 0.67, ThreshDown = 0.33, p.cut = 0.05, group1, group2)
```

Arguments

clinical_patient	is a data.frame using function 'clinic' with information related to barcode / samples such as bcr_patient_barcode, days_to_death , days_to_last_follow_up , vital_status, etc
dataGE	is a matrix of Gene expression (genes in rows, samples in cols) from TCGAprepare
Genelist	is a list of gene symbols where perform survival KM.
Survresult	is a parameter (default = FALSE) if is TRUE will show KM plot and results.
ThreshTop	is a quantile threshold to identify samples with high expression of a gene
ThreshDown	is a quantile threshold to identify samples with low expression of a gene
p.cut	p.values threshold. Default: 0.05
group1	a string containing the barcode list of the samples in in control group
group2	a string containing the barcode list of the samples in in disease group

Value

table with survival genes pvalues from KM.

Examples

```
clinical_patient_Cancer <- GDCquery_clinic("TCGA-BRCA","clinical")
dataBRCAcomplete <- log2(BRCA_rnaseqv2)
# Selecting only 100 genes for example
dataBRCAcomplete <- dataBRCAcomplete[1:100,]
dataGE <- dataBRCAcomplete
group1 <- TCGAquery_SampleTypes(colnames(dataGE), typesample = c("NT"))
group2 <- TCGAquery_SampleTypes(colnames(dataGE), typesample = c("TP"))

tabSurvKM<-TCGAanalyze_SurvivalKM(clinical_patient_Cancer,dataBRCAcomplete,
  Genelist = rownames(dataBRCAcomplete), Survresult = FALSE,ThreshTop=0.67,ThreshDown=0.33)
group1 <- TCGAquery_SampleTypes(colnames(dataGE), typesample = c("NT"))
group2 <- TCGAquery_SampleTypes(colnames(dataGE), typesample = c("TP"))
```

TCGAbiolinks

The aim of TCGAbiolinks is : i) facilitate the TCGA open-access data retrieval, ii) prepare the data using the appropriate pre-processing strategies, iii) provide the means to carry out different standard analyses and iv) allow the user to download a specific version of the data and thus to easily reproduce earlier research results. In more detail, the package provides multiple methods for analysis (e.g., differential expression analysis, identifying differentially methylated regions) and methods for visualization (e.g., survival plots, volcano plots, starburst plots) in order to easily develop complete analysis pipelines.

Description

The functions you're likely to need from **TCGAbiolinks** is [GDCdownload](#), [GDCquery](#). Otherwise refer to the vignettes to see how to format the documentation.

TCGAPrepare_Affy *Prepare CEL files into an AffyBatch.*

Description

Prepare CEL files into an AffyBatch.

Usage

```
TCGAPrepare_Affy(ClinData, PathFolder, TabCel)
```

Arguments

ClinData	write
PathFolder	write
TabCel	write

Value

Normalized Expression data from Affy eSets

Examples

```
## Not run:
to add example

## End(Not run)
```

TCGAPrepare_elmer *Prepare the data for ELEMN package*

Description

Prepare the data for ELEMN package

Usage

```
TCGAPrepare_elmer(data, platform, met.na.cut = 0.2, save = FALSE)
```

Arguments

data	A data frame or summarized experiment from TCGAPrepare
platform	platform of the data. Example: "HumanMethylation450", "IlluminaHiSeq_RNASeqV2"
met.na.cut	Define the percentage of NA that the line should have to remove the probes for humanmethylation platforms.
save	Save object? Default: FALSE. Names of the files will be: "Exp_elmer.rda" (object Exp) and "Met_elmer.rda" (object Met)

Value

Matrix prepared for fetch.mee function

Examples

```
df <- data.frame(runif(200, 1e5, 1e6),runif(200, 1e5, 1e6))
rownames(df) <- sprintf("%03d", 1:200)
df <- TCGAprepare_elmer(df,platform="IlluminaHiSeq_RNASeqV2")
```

TCGAquery_MatchedCoupledSampleTypes

Retrieve multiple tissue types from the same patients.

Description

TCGAquery_MatchedCoupledSampleTypes

Usage

```
TCGAquery_MatchedCoupledSampleTypes(barcode, typesample)
```

Arguments

barcode	barcode
typesample	typesample

Value

a list of samples / barcode filtered by type sample selected

Examples

```
TCGAquery_MatchedCoupledSampleTypes(c("TCGA-B0-4698-01Z-00-DX1",
                                       "TCGA-B0-4698-02Z-00-DX1"),
                                       c("TP", "TR"))
```

TCGAquery_SampleTypes *Retrieve multiple tissue types not from the same patients.*

Description

TCGAquery_SampleTypes for a given list of samples and types, return the union of samples that are from these type.

Usage

```
TCGAquery_SampleTypes(barcode, typesample)
```

Arguments

barcode	is a list of samples as TCGA barcodes
typesample	a character vector indicating tissue type to query. Example:

TP	PRIMARY SOLID TUMOR
TR	RECURRENT SOLID TUMOR
TB	Primary Blood Derived Cancer-Peripheral Blood
TRBM	Recurrent Blood Derived Cancer-Bone Marrow
TAP	Additional-New Primary
TM	Metastatic
TAM	Additional Metastatic
THOC	Human Tumor Original Cells
TBM	Primary Blood Derived Cancer-Bone Marrow
NB	Blood Derived Normal
NT	Solid Tissue Normal
NBC	Buccal Cell Normal
NEBV	EBV Immortalized Normal
NBM	Bone Marrow Normal

Value

a list of samples / barcode filtered by type sample selected

Examples

```
# selection of normal samples "NT"
barcode <- c("TCGA-B0-4698-01Z-00-DX1", "TCGA-CZ-4863-02Z-00-DX1")
# Returns the second barcode
TCGAquery_SampleTypes(barcode, "TR")
# Returns both barcode
TCGAquery_SampleTypes(barcode, c("TR", "TP"))
```

TCGAquery_subtype *Retrieve molecular subtypes for a given tumor*

Description

TCGAquery_subtype Retrieve molecular subtypes for a given tumor

Usage

```
TCGAquery_subtype(tumor)
```

Arguments

tumor is a cancer Examples:

```
                          lgg    gbm    luad    stad    brca
                          coad   read
```

Value

a data.frame with barcode and molecular subtypes

Examples

```
dataSubt <- TCGAquery_subtype(tumor = "lgg")
```

TCGAvsualize_BarPlot *Barplot of subtypes and clinical info in groups of gene expression clustered.*

Description

Barplot of subtypes and clinical info in groups of gene expression clustered.

Usage

```
TCGAvsualize_BarPlot(DFfilt, DFclin, DFsubt, data_Hc2, Subtype, cbPalette,
  filename, width, height, dpi)
```

Arguments

DFfilt	write
DFclin	write
DFsubt	write
data_Hc2	write
Subtype	write
cbPalette	Define the colors of the bar.
filename	The name of the pdf file
width	Image width
height	Image height
dpi	Image dpi

Value

barplot image in pdf or png file

TCGAvsualize_EAbarplot
barPlot for a complete Enrichment Analysis

Description

The figure shows canonical pathways significantly overrepresented (enriched) by the DEGs (differentially expressed genes). The most statistically significant canonical pathways identified in DEGs list are listed according to their p value corrected FDR (-Log) (colored bars) and the ratio of list genes found in each pathway over the total number of genes in that pathway (Ratio, red line).

Usage

```
TCGAVisualize_EAbarplot(tf, GOMFTab, GOBPTab, GOCCTab, PathTab, nBar, nRGTAB,
  filename = "TCGAVisualize_EAbarplot_Output.pdf", text.size = 1,
  mfrow = c(2, 2), xlim = NULL, color = c("orange", "cyan", "green",
  "yellow"))
```

Arguments

tf	is a list of gene symbols
GOMFTab	is results from TCGAanalyze_EAcomplete related to Molecular Function (MF)
GOBPTab	is results from TCGAanalyze_EAcomplete related to Biological Process (BP)
GOCCTab	is results from TCGAanalyze_EAcomplete related to Cellular Component (CC)
PathTab	is results from TCGAanalyze_EAcomplete related to Pathways EA
nBar	is the number of bar histogram selected to show (default = 10)
nRGTAB	is the gene signature list with gene symbols.
filename	Name for the pdf. If null it will return the plot.
text.size	Text size
mfrow	Vector with number of rows/columns of the plot. Default 2 rows/2 columns "c(2,2)"
xlim	Upper limit of the x-axis.
color	A vector of colors for each barplot. Deafult: c("orange", "cyan", "green", "yellow")

Value

Complete barPlot from Enrichment Analysis showing significant (default FDR < 0.01) BP,CC,MF and pathways enriched by list of genes.

Examples

```
Genelist <- c("FN1", "COL1A1")
ansEA <- TCGAanalyze_EAcomplete(TFname="DEA genes Normal Vs Tumor", Genelist)
TCGAVisualize_EAbarplot(tf = rownames(ansEA$ResBP),
  GOBPTab = ansEA$ResBP,
  GOCCTab = ansEA$ResCC,
  GOMFTab = ansEA$ResMF,
  PathTab = ansEA$ResPat,
  nRGTAB = Genelist,
  nBar = 10,
  filename="a.pdf")
while (!(is.null(dev.list()["RStudioGD"]))) {dev.off()}
## Not run:
Genelist <- rownames(dataDEGsFiltLevel)
system.time(ansEA <- TCGAanalyze_EAcomplete(TFname="DEA genes Normal Vs Tumor", Genelist))
# Enrichment Analysis EA (TCGAVisualize)
# Gene Ontology (GO) and Pathway enrichment barPlot
TCGAVisualize_EAbarplot(tf = rownames(ansEA$ResBP),
  GOBPTab = ansEA$ResBP,
  GOCCTab = ansEA$ResCC,
  GOMFTab = ansEA$ResMF,
  PathTab = ansEA$ResPat,
  nRGTAB = Genelist,
```

```

        nBar = 10)

## End(Not run)

```

TCGAvsualize_Heatmap *Heatmap with more sensible behavior using heatmap.plus*

Description

Heatmap with more sensible behavior using heatmap.plus

Usage

```

TCGAvsualize_Heatmap(data, col.metadata, row.metadata, col.colors = NULL,
  row.colors = NULL, show_column_names = FALSE, show_row_names = FALSE,
  cluster_rows = FALSE, cluster_columns = FALSE, sortCol, extremis = NULL,
  rownames.size = 12, title = NULL, color.levels = NULL,
  values.label = NULL, filename = "heatmap.pdf", width = 10,
  height = 10, type = "expression", scale = "none",
  heatmap.legend.color.bar = "continuous")

```

Arguments

data	The object to with the heatmap data (expression, methylation)
col.metadata	Metadata for the columns (samples). It should have on of the following columns: barcode (28 characters) column to match with the samples. It will also work with "bcr_patient_barcode"(12 chars),"patient"(12 chars),"sample"(16 chars) columns but as one patient might have more than one sample, this coul lead to errors in the annotation. The code will throw a warning in case two samples are from the same patient.
row.metadata	Metadata for the rows genes (expression) or probes (methylation)
col.colors	A list of names colors
row.colors	A list of named colors
show_column_names	Show column names names? Dafault: FALSE
show_row_names	Show row names? Dafault: FALSE
cluster_rows	Cluster rows ? Dafault: FALSE
cluster_columns	Cluster columns ? Dafault: FALSE
sortCol	Name of the column to be used to sort the columns
extremis	Extremis of colors (vector of 3 values)
rownames.size	Rownames size
title	Title of the plot
color.levels	A vector with the colors (low level, middle level, high level)
values.label	Text of the levels in the heatmap
filename	Filename to save the heatmap. Default: heatmap.png
width	figure width

height	figure height
type	Select the colors of the heatmap values. Possible values are "expression" (default), "methylation"
scale	Use z-score to make the heatmap? If we want to show differences between genes, it is good to make Z-score by samples (force each sample to have zero mean and standard deviation=1). If we want to show differences between samples, it is good to make Z-score by genes (force each gene to have zero mean and standard deviation=1). Possibilities: "row", "col". Default "none"
heatmap.legend.color.bar	Heatmap legends values type. Options: "continuous", "discrete"

Value

Heatmap plotted in the device

Examples

```

row.mdat <- matrix(c("FALSE", "FALSE",
                    "TRUE", "TRUE",
                    "FALSE", "FALSE",
                    "TRUE", "FALSE",
                    "FALSE", "TRUE"
                    ),
                  nrow = 5, ncol = 2, byrow = TRUE,
                  dimnames = list(
                    c("probe1", "probe2", "probe3", "probe4", "probe5"),
                    c("duplicated", "Enhancer region")))
dat <- matrix(c(0.3, 0.2, 0.3, 1, 1, 0.1, 1, 1, 0, 0.8, 1, 0.7, 0.7, 0.3, 1),
             nrow = 5, ncol = 3, byrow = TRUE,
             dimnames = list(
               c("probe1", "probe2", "probe3", "probe4", "probe5"),
               c("TCGA-DU-6410",
                 "TCGA-DU-A5TS",
                 "TCGA-HT-7688")))

mdat <- data.frame(patient=c("TCGA-DU-6410", "TCGA-DU-A5TS", "TCGA-HT-7688"),
                  Sex=c("Male", "Female", "Male"),
                  COCcluster=c("coc1", "coc1", "coc1"),
                  IDHtype=c("IDHwt", "IDHMut-cod", "IDHMut-noncod"))

TCGAvisualize_Heatmap(dat,
                      col.metadata = mdat,
                      row.metadata = row.mdat,
                      row.colors = list(duplicated = c("FALSE" = "pink",
                                                       "TRUE" = "green"),
                                       "Enhancer region" = c("FALSE" = "purple",
                                                            "TRUE" = "grey")),
                      col.colors = list(Sex = c("Male" = "blue", "Female" = "red"),
                                       COCcluster = c("coc1" = "grey"),
                                       IDHtype = c("IDHwt" = "cyan",
                                                  "IDHMut-cod" = "tomato",
                                                  "IDHMut-noncod" = "gold")),
                      type = "methylation",
                      show_row_names = TRUE)
if (!is.null(dev.list()["RStudioGD"])){dev.off()}

```

 TCGAvsualize_meanMethylation

Mean methylation boxplot

Description

Creates a mean methylation boxplot for groups (groupCol), subgroups will be highlighted as shapes if the subgroupCol was set.

Observation: Data is a summarizedExperiment.

Usage

```
TCGAvsualize_meanMethylation(data, groupCol = NULL, subgroupCol = NULL,
  shapes = NULL, print.pvalue = FALSE, plot.jitter = TRUE,
  jitter.size = 3, filename = "groupMeanMet.pdf",
  ylab = expression(paste("Mean DNA methylation (", beta, "-values)")),
  xlab = NULL, title = "Mean DNA methylation", labels = NULL,
  group.legend = NULL, subgroup.legend = NULL, color = NULL,
  y.limits = NULL, sort, order, legend.position = "top",
  legend.title.position = "top", legend.ncols = 3, add.axis.x.text = TRUE,
  width = 10, height = 10, dpi = 600, axis.text.x.angle = 90)
```

Arguments

data	SummarizedExperiment object obtained from TCGAPrepare
groupCol	Columns in colData(data) that defines the groups. If no columns defined a columns called "Patients" will be used
subgroupCol	Columns in colData(data) that defines the subgroups.
shapes	Shape vector of the subgroups. It must have the size of the levels of the subgroups. Example: shapes = c(21,23) if for two levels
print.pvalue	Print p-value for two groups
plot.jitter	Plot jitter? Default TRUE
jitter.size	Plot jitter size? Default 3
filename	The name of the pdf that will be saved
ylab	y axis text in the plot
xlab	x axis text in the plot
title	main title in the plot
labels	Labels of the groups
group.legend	Name of the group legend. DEFAULT: groupCol
subgroup.legend	Name of the subgroup legend. DEFAULT: subgroupCol
color	vector of colors to be used in graph
y.limits	Change lower/upper y-axis limit
sort	Sort boxplot by mean or median. Possible values: mean.asc, mean.desc, median.asc, median.desc

order Order of the boxplots
 legend.position Legend position ("top", "right","left","bottom")
 legend.title.position Legend title position ("top", "right","left","bottom")
 legend.ncols Number of columns of the legend
 add.axis.x.text Add text to x-axis? Default: FALSE
 width Plot width default:10
 height Plot height default:10
 dpi Pdf dpi default:600
 axis.text.x.angle Angle of text in the x axis

Value

Save the pdf survival plot

Examples

```

nrows <- 200; ncols <- 21
counts <- matrix(runif(nrows * ncols, 0, 1), nrows)
rowRanges <- GenomicRanges::GRanges(rep(c("chr1", "chr2"), c(50, 150)),
  IRanges::IRanges(floor(runif(200, 1e5, 1e6)), width=100),
  strand=sample(c("+", "-"), 200, TRUE),
  feature_id=sprintf("ID%03d", 1:200))
colData <- S4Vectors::DataFrame(Treatment=rep(c("ChIP", "Input", "Other"), 7),
  row.names=LETTERS[1:21],
  group=rep(c("group1", "group2", "group3"), c(7,7,7)),
  subgroup=rep(c("subgroup1", "subgroup2", "subgroup3"), 7))
data <- SummarizedExperiment::SummarizedExperiment(
  assays=S4Vectors::SimpleList(counts=counts),
  rowRanges=rowRanges,
  colData=colData)
TCGAvisualize_meanMethylation(data, groupCol = "group")
# change lower/upper y-axis limit
TCGAvisualize_meanMethylation(data, groupCol = "group", y.limits = c(0,1))
# change lower y-axis limit
TCGAvisualize_meanMethylation(data, groupCol = "group", y.limits = 0)
TCGAvisualize_meanMethylation(data, groupCol = "group", subgroupCol="subgroup")
TCGAvisualize_meanMethylation(data, groupCol = "group")
TCGAvisualize_meanMethylation(data, groupCol = "group", sort="mean.desc", filename="meandesc.pdf")
TCGAvisualize_meanMethylation(data, groupCol = "group", sort="mean.asc", filename="meanasc.pdf")
TCGAvisualize_meanMethylation(data, groupCol = "group", sort="median.asc", filename="medianasc.pdf")
TCGAvisualize_meanMethylation(data, groupCol = "group", sort="median.desc", filename="mediandesc.pdf")
if (!(is.null(dev.list()["RStudioGD"]))) {dev.off()}

```

 TCGAvizualize_oncoprint

Creating a oncoprint

Description

Creating a oncoprint

Usage

```
TCGAvizualize_oncoprint(mut, genes, filename, color,
  annotation.position = "bottom", annotation, height, width = 10,
  rm.empty.columns = FALSE, show.column.names = FALSE,
  show.row.barplot = TRUE, label.title = "Mutation",
  column.names.size = 8, label.font.size = 16, rows.font.size = 16,
  dist.col = 0.5, dist.row = 0.5, information = "Variant_Type",
  row.order = TRUE, col.order = TRUE, heatmap.legend.side = "bottom",
  annotation.legend.side = "bottom")
```

Arguments

mut	A dataframe from the mutation annotation file (see TCGAquery_maf from TC- GAbiolinks)
genes	Gene list
filename	name of the pdf
color	named vector for the plot
annotation.position	Position of the annotation "bottom" or "top"
annotation	Matrix or data frame with the annotation. Should have a column bcr_patient_barcode with the same ID of the mutation object
height	pdf height
width	pdf width
rm.empty.columns	If there is no alteration in that sample, whether remove it on the oncoprint
show.column.names	Show column names? Default: FALSE
show.row.barplot	Show barplot annotation on rows?
label.title	Title of the label
column.names.size	Size of the fonts of the columns names
label.font.size	Size of the fonts
rows.font.size	Size of the fonts
dist.col	distance between columns in the plot
dist.row	distance between rows in the plot

information	Which column to use as information from MAF. Options: 1) "Variant_Classification" (The information will be "Frame_Shift_Del", "Frame_Shift_Ins", "In_Frame_Del", "In_Frame_Ins", "Missense_Mutation", "Nonsense_Mutation", "Nonstop_Mutation", "RNA", "Silent", "Splice_Site", "Targeted_Region", "Translation_Start_Site") 2) "Variant_Type" (The information will be INS,DEL,SNP)
row.order	Order the genes (rows) Default:TRUE. Genes with more mutations will be in the first rows
col.order	Order columns. Default:TRUE.
heatmap.legend.side	Position of the heatmap legend
annotation.legend.side	Position of the annotation legend

Value

A oncoprint plot

Examples

```
mut <- GDCquery_Maf(tumor = "ACC", pipelines = "mutect")
TCGAvisualize_oncoprint(mut = mut, genes = mut$Hugo_Symbol[1:10], rm.empty.columns = TRUE)
TCGAvisualize_oncoprint(mut = mut, genes = mut$Hugo_Symbol[1:10],
  filename = "onco.pdf",
  color=c("background"="#CCCCCC", "DEL"="purple", "INS"="yellow", "SNP"="brown"))
clin <- GDCquery_clinic("TCGA-ACC", "clinical")
clin <- clin[,c("bcr_patient_barcode", "disease", "gender", "tumor_stage", "race", "vital_status")]
TCGAvisualize_oncoprint(mut = mut, genes = mut$Hugo_Symbol[1:20],
  filename = "onco.pdf",
  annotation = clin,
  color=c("background"="#CCCCCC", "DEL"="purple", "INS"="yellow", "SNP"="brown"),
  rows.font.size=10,
  heatmap.legend.side = "right",
  dist.col = 0,
  label.font.size = 10)
```

TCGAvisualize_PCA *Principal components analysis (PCA) plot*

Description

TCGAvisualize_PCA performs a principal components analysis (PCA) on the given data matrix and returns the results as an object of class `prcomp`, and shows results in PCA level.

Usage

```
TCGAvisualize_PCA(dataFile, dataDEGsFileLevel, ntopgenes, group1, group2)
```

Arguments

<code>dataFilt</code>	A filtered dataframe or numeric matrix where each row represents a gene, each column represents a sample from function <code>TCGAanalyze_Filtering</code>
<code>dataDEGsFiltLevel</code>	table with DEGs, log Fold Change (FC), false discovery rate (FDR), the gene expression level, etc, from function <code>TCGAanalyze_LevelTab</code> .
<code>ntopgenes</code>	number of DEGs genes to plot in PCA
<code>group1</code>	a string containing the barcode list of the samples in in control group
<code>group2</code>	a string containing the barcode list of the samples in in disease group the name of the group

Value

principal components analysis (PCA) plot of PC1 and PC2

Examples

```
# normalization of genes
dataNorm <- TCGAanalyze_Normalization(tabDF = dataBRCA, geneInfo = geneInfo,
method = "geneLength")
# quantile filter of genes
dataFilt <- TCGAanalyze_Filtering(tabDF = dataBRCA, method = "quantile", qnt.cut = 0.25)
# Principal Component Analysis plot for ntop selected DEGs
  # selection of normal samples "NT"
  group1 <- TCGAquery_SampleTypes(colnames(dataFilt), typesample = c("NT"))
  # selection of normal samples "TP"
  group2 <- TCGAquery_SampleTypes(colnames(dataFilt), typesample = c("TP"))
pca <- TCGAvsualize_PCA(dataFilt,dataDEGsFiltLevel, ntopgenes = 200, group1, group2)
if (!is.null(dev.list()["RStudioGD"])){dev.off()}
```

TCGAvsualize_starburst

Create starburst plot

Description

Create Starburst plot for comparison of DNA methylation and gene expression. The log₁₀ (FDR-corrected P value) is plotted for beta value for DNA methylation (x axis) and gene expression (y axis) for each gene.

The black dashed line shows the FDR-adjusted P value of 0.01.

You can set names to TRUE to get the names of the significant genes.

Candidate biologically significant genes will be circled in the plot.

Candidate biologically significant are the genes that respect the expression (`logFC.cut`), DNA methylation (`diffmean.cut`) and significance thresholds (`exp.p.cut`, `met.p.cut`)

Usage

```
TCGAVisualize_starburst(met, exp, group1 = NULL, group2 = NULL,
  exp.p.cut = 0.01, met.p.cut = 0.01, diffmean.cut = 0, logFC.cut = 0,
  names = FALSE, names.fill = TRUE, circle = TRUE,
  filename = "starburst.pdf", return.plot = FALSE,
  ylab = expression(atop("Gene Expression", paste(Log[10],
  " (FDR corrected P values)"))), xlab = expression(atop("DNA Methylation",
  paste(Log[10], " (FDR corrected P values)"))), title = "Starburst Plot",
  legend = "DNA Methylation/Expression Relation", color = NULL,
  label = c("Not Significant", "Up regulated & Hypo methylated",
  "Down regulated & Hypo methylated", "hypo methylated", "hyper methylated",
  "Up regulated", "Down regulated", "Up regulated & Hyper methylated",
  "Down regulated & Hyper methylated"), xlim = NULL, ylim = NULL,
  height = 10, width = 20, dpi = 600)
```

Arguments

met	A SummarizedExperiment with methylation data obtained from the TCGAPrepare or Data frame from DMR_results file. Expected colData columns: diffmean, p.value.adj and p.value Execute volcanoPlot function in order to obtain these values for the object.
exp	Object obtained by DEArnaSEQ function
group1	The name of the group 1 Obs: Column p.value.adj.group1.group2 should exist
group2	The name of the group 2. Obs: Column p.value.adj.group1.group2 should exist
exp.p.cut	expression p value cut-off
met.p.cut	methylation p value cut-off
diffmean.cut	If set, the probes with diffmean higher than methylation cut-off will be highlighted in the plot. And the data frame return will be subseted.
logFC.cut	If set, the probes with expression fold change higher than methylation cut-off will be highlighted in the plot. And the data frame return will be subseted.
names	Add the names of the significant genes? Default: FALSE
names.fill	Names should be filled in a color box? Default: TRUE
circle	Circle pair gene/probe that respects diffmean.cut and logFC.cut Default: TRUE
filename	The filename of the file (it can be pdf, svg, png, etc)
return.plot	If true only plot object will be returned (pdf will not be created)
ylab	y axis text
xlab	x axis text
title	main title
legend	legend title
color	vector of colors to be used in graph
label	vector of labels to be used in graph
xlim	x limits to cut image
ylim	y limits to cut image
height	Figure height
width	Figure width
dpi	Figure dpi

Details

Input: data with gene expression/methylation expression Output: starburst plot

Value

Save a starburst plot

Examples

```
library(SummarizedExperiment)
nrows <- 20000; ncols <- 20
counts <- matrix(runif(nrows * ncols, 1, 1e4), nrows)
ranges <- GenomicRanges::GRanges(rep(c("chr1", "chr2"), c(5000, 15000)),
  IRanges::IRanges(floor(runif(20000, 1e5, 1e6)), width=100),
  strand=sample(c("+", "-"), 20000, TRUE),
  probeID=sprintf("ID%03d", 1:20000),
  Gene_Symbol=sprintf("ID%03d", 1:20000))
colData <- S4Vectors::DataFrame(Treatment=rep(c("ChIP", "Input"), 5),
  row.names=LETTERS[1:20],
  group=rep(c("group1", "group2"),c(10,10)))
data <- SummarizedExperiment::SummarizedExperiment(
  assays=S4Vectors::SimpleList(counts=counts),
  rowRanges=ranges,
  colData=colData)
met <- data
exp <- data.frame(row.names=sprintf("ID%03d", 1:20000),
  logFC=runif(20000, -5, 5),
  FDR=runif(20000, 0.01, 1))
rowRanges(met)$diffmean.g1.g2 <- c(runif(20000, -0.1, 0.1))
rowRanges(met)$diffmean.g2.g1 <- -1*(rowRanges(met)$diffmean.g1.g2)
rowRanges(met)$p.value.g1.g2 <- c(runif(20000, 0, 1))
rowRanges(met)$p.value.adj.g1.g2 <- c(runif(20000, 0, 1))
result <- TCGAvsualize_starburst(met,exp,
  exp.p.cut = 0.05, met.p.cut = 0.05,
  group1="g1",group2="g2",
  diffmean.cut=0.0,
  names=TRUE, circle = FALSE)
result <- TCGAvsualize_starburst(SummarizedExperiment::values(met),
  exp,
  exp.p.cut = 0.05, met.p.cut = 0.05,
  group1="g1",group2="g2",
  diffmean.cut=0.0,
  names=TRUE, circle = FALSE)
```

TCGAvsualize_SurvivalCoxNET

Survival analysis with univariate Cox regression package (dnet)

Description

TCGAvsualize_SurvivalCoxNET can help an user to identify a group of survival genes that are significant from univariate Kaplan Meier Analysis and also for Cox Regression. It shows in the end a network build with community of genes with similar range of pvalues from Cox regression (same

color) and that interaction among those genes is already validated in literatures using the STRING database (version 9.1). TCGAvsualize_SurvivalCoxNET perform survival analysis with univariate Cox regression and package (dnet) using following functions wrapping from these packages:

1. survival::coxph
2. igraph::subgraph.edges
3. igraph::layout.fruchterman.reingold
4. igraph::spinglass.community
5. igraph::communities
6. dnet::dRDataLoader
7. dnet::dNetInduce
8. dnet::dNetPipeline
9. dnet::visNet
10. dnet::dCommSignif

Usage

```
TCGAvsualize_SurvivalCoxNET(clinical_patient, dataGE, Genelist, org.Hs.string,
  scoreConfidence = 700, titlePlot = "TCGAvsualize_SurvivalCoxNET Example")
```

Arguments

clinical_patient	is a data.frame using function 'clinic' with information related to barcode / samples such as bcr_patient_barcode, days_to_death , days_to_last_followup , vital_status, etc
dataGE	is a matrix of Gene expression (genes in rows, samples in cols) from TCGAprepare
Genelist	is a list of gene symbols where perform survival KM.
org.Hs.string	an igraph object that contains a functional protein association network in human. The network is extracted from the STRING database (version 10).
scoreConfidence	restrict to those edges with high confidence (eg. score>=700)
titlePlot	is the title to show in the final plot.

Details

TCGAvsualize_SurvivalCoxNET allow user to perform the complete workflow using coxph and dnet package related to survival analysis with an identification of gene-active networks from high-throughput omics data using gene expression and clinical data.

1. Cox regression survival analysis to obtain hazard ratio (HR) and pvalues
2. fit a Cox proportional hazards model and ANOVA (Chisq test)
3. Network comunitas
4. An igraph object that contains a functional protein association network in human. The network is extracted from the STRING database (version 9.1). Only those associations with medium confidence (score>=400) are retained.
5. restrict to those edges with high confidence (score>=700)

6. extract network that only contains genes in pvals
7. Identification of gene-active network
8. visualisation of the gene-active network itself
9. the layout of the network visualisation (fixed in different visuals)
10. color nodes according to communities (identified via a spin-glass model and simulated annealing)
11. node sizes according to degrees
12. highlight different communities
13. visualise the subnetwork

Value

net IGRAPH with related Cox survival genes in community (same pval and color) and with interactions from STRING database.

TCGAvsualize_Tables *Visaulize results in format of latex tables.*

Description

Visaulize results in format of latex tables.

Usage

```
TCGAvsualize_Tables(Table, rowsForPage, TableTitle, LabelTitle, withrows, size)
```

Arguments

Table	write
rowsForPage	write
TableTitle	write
LabelTitle	write
withrows	write
size	size selected for font, 'small', 'tiny'

Value

table in latex format to use in beamer presentation or sweave files

Examples

```
library(stringr)
tabDEGsTFPubmed$PMID <- str_sub(tabDEGsTFPubmed$PMID,0,30)
TCGAvsualize_Tables(Table = tabDEGsTFPubmed,
rowsForPage = 5,
TableTitle = "pip",
LabelTitle = "pip2",
withrows = FALSE,
size = "small")
```

TCGAVisualize_volcano *Creates a volcano plot for DNA methylation or expression*

Description

Creates a volcano plot from the expression and methylation analysis.

Usage

```
TCGAVisualize_volcano(x, y, filename = "volcano.pdf",
  ylab = expression(paste(-Log[10], " (FDR corrected -P values)")),
  xlab = NULL, title = "Volcano plot", legend = NULL, label = NULL,
  xlim = NULL, ylim = NULL, color = c("black", "red", "green"),
  names = NULL, names.fill = TRUE, show.names = "significant",
  x.cut = 0, y.cut = 0.01, height = 5, width = 10, highlight = NULL,
  highlight.color = "orange", names.size = 4, dpi = 300)
```

Arguments

x	x-axis data
y	y-axis data
filename	Filename. Default: volcano.pdf, volcano.svg, volcano.png
ylab	y axis text
xlab	x axis text
title	main title. If not specified it will be "Volcano plot (group1 vs group2)
legend	Legend title
label	vector of labels to be used in the figure. Example: c("Not Significant", "Hypermethylated in group1", "Hypomethylated in group1"))#'
xlim	x limits to cut image
ylim	y limits to cut image
color	vector of colors to be used in graph
names	Names to be plotted if significant. Should be the same size of x and y
names.fill	Names should be filled in a color box? Default: TRUE
show.names	What names will be showd? Possibilities: "both", "significant", "highlighted"
x.cut	x-axis threshold. Default: 0.0 If you give only one number (e.g. 0.2) the cut-offs will be -0.2 and 0.2. Or you can give diffenrent cutt-ofs as a vector (e.g. c(-0.3,0.4))
y.cut	p-values threshold.
height	Figure height
width	Figure width
highlight	List of genes/probes to be highlighted. It should be in the names argument.
highlight.color	Color of the points highlighted
names.size	Size of the names text
dpi	Figure dpi

Details

Creates a volcano plot from the expression and methylation analysis. Please see the vignette for more information Observation: This function automatically is called by TCGAanalyse_DMR

Value

Saves the volcano plot in the current folder

Examples

```
x <- runif(200, -1, 1)
y <- runif(200, 0.01, 1)
TCGAVisualize_volcano(x,y)
TCGAVisualize_volcano(x,y,filename = NULL,y.cut = 10000000,x.cut=0.8,
                      names = rep("AAAA",length(x)), legend = "Status",
                      names.fill = FALSE)
TCGAVisualize_volcano(x,y,filename = NULL,y.cut = 10000000,x.cut=0.8,
                      names = as.character(1:length(x)), legend = "Status",
                      names.fill = TRUE, highlight = c("1","2"),show="both")
TCGAVisualize_volcano(x,y,filename = NULL,y.cut = 10000000,x.cut=c(-0.3,0.8),
                      names = as.character(1:length(x)), legend = "Status",
                      names.fill = TRUE, highlight = c("1","2"),show="both")
while (!(is.null(dev.list()["RStudioGD"]))) {dev.off() }
```

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