# Package 'TCGAbiolinks'

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Type Package

**Title** TCGAbiolinks: An R/Bioconductor package for integrative analysis with TCGA data

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GenomicRanges, XML, Biobase, affy, xtable, data.table, EDASeq
(>= 2.0.0), RCurl, curl, edgeR (>= 3.0.0), jsonlite, plyr,
knitr, biomaRt, coin, gplots, ggplot2, ggthemes, survival,
stringr (>= 1.0.0), IRanges, scales, rvest (>= 0.3.0), stats,
utils, dnet, igraph, supraHex, S4Vectors, ComplexHeatmap,
R.utils, SummarizedExperiment, BiocGenerics, GenomicFeatures,
TxDb.Hsapiens.UCSC.hg19.knownGene, limma, genefilter,
ConsensusClusterPlus, readr, RColorBrewer, doParallel, dplyr,
parallel, xml2, sjPlot (>= 2.0), magrittr, cowplot, reshape2,

gtable, httr, sjmisc, matlab, circlize, ggrepel

**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.

License GPL (>= 3)

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

Suggests testthat, png, BiocStyle, rmarkdown, devtools

VignetteBuilder knitr

LazyData true

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

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

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# **R** topics documented:

GDCdownload
GDCprepare
GDCprepare_clinic
GDCquery
GDCquery_clinic
GDCquery_Maf
getGDCprojects
isServeOK
TCGAanalyze_Clustering
TCGAanalyze_DEA
TCGAanalyze_DEA_Affy
TCGAanalyze_DMR
TCGAanalyze_EA
TCGAanalyze_EAcomplete
TCGAanalyze_Filtering
TCGAanalyze_LevelTab
TCGAanalyze_Normalization
TCGAanalyze_Preprocessing
TCGAanalyze_survival
TCGAanalyze_SurvivalKM
TCGAbiolinks
TCGAdownload
TCGAprepare 22

GDCdownload 3

GDCd	ownload Download GDC data	
Index		49
	1. Contributing the contribution of the contri	Γ/
	TCGAVisualize_volcano	
	TCGAvisualize_Tables	
	TCGAvisualize_SurvivalCoxNET	
	TCGAvisualize_starburst	
	TCGAvisualize_profilePlot	
	TCGAvisualize_PCA	
	TCGAvisualize_nicoprint	
	TCGAvisualize_Heatmap	
	TCGAvisualize_EAbarplot	
	TCGAvigueliae FAborelet	
	TCGAquery_subtype	
	TCGA groups arbitrary	
	TCGAquery_MatchedCoupledSampleTypes	
	TCGAquery_maf	
	TCGAquery_Investigate	
	TCGAquery_clinicFilt	
	TCGAquery_clinic	
	TCGAquery	25
	TCGAprepare_elmer	24
	TCGAprepare_Affy	24

# 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")
```

# Arguments

query A query for GDCquery function

token.file Token file to download controlled data (only for method = "client)

method Use api method or gdc client tool (API is faster)

### Value

Shows the output from the GDC transfer tools

4 GDCprepare\_clinic

**GDCprepare** 

Prepare GDC data

#### **Description**

Reads the data downloaded and prepare it into an R object

#### Usage

```
GDCprepare(query, save = FALSE, save.filename, summarizedExperiment = TRUE)
```

### **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

summarizedExperiment

Create a summarizedExperiment? Default TRUE (if possible)

#### Value

A summarizedExperiment or a data.frame

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)
```

# **Arguments**

query Result from GDCquery, with data.category set to Clinical

clinical.info Which information should be retrieved. Options: drug, admin, follow\_up,radiation,

patient, stage\_event or new\_tumor event

GDC query 5

#### **Examples**

GDCquery

Query GDC data

#### **Description**

Uses GDC API to search for search, it searches for both controlled and open-acess 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.

### Usage

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

#### **Arguments**

project A valid project (see list with TCGAbiolinks:::getGDCprojects()\$project\_id)]
data.category A valid project (see list with TCGAbiolinks:::getProjectSummary(project))

data.type A data type to filter the files to download

workflow.type GDC workflow type

legacy Search in the legacy repository

platform Example:

CGH- 1x1M\_G4447A IlluminaGA\_RNASeqV2
AgilentG4502A\_07 IlluminaGA\_mRNA\_DGE
Human1MDuo 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

H-miRNA\_8x15Kv2 IlluminaGA\_DNASeq\_curated MDA\_RPPA\_Core IlluminaHiSeq\_TotalRNASeqV2 HT\_HG-U133A IlluminaHiSeq\_DNASeq\_automated

6 GDCquery\_clinic

diagnostic\_images microsat\_i

IlluminaHiSeq\_RNASeq SOLiD\_DNASeq\_curated IlluminaHiSeq\_DNASeqC Mixed\_DNASeq\_curated

IlluminaGA\_RNASeq IlluminaGA\_DNASeq\_Cont\_automated

IlluminaGA\_DNASeq IlluminaHiSeq\_WGBS

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 sample.type A sample type to filter the files to download

#### Value

A data frame with the results and the parameters used

GDCquery\_clinic Get DDC 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 getGDCprojects()\$project\_id)]

type A valid type. Options "clinical", "Biospecimen" (see list with getGDCpro-

jects()\$project\_id)]

save.csv Write clinical information into a csv document

#### Value

A data frame with the clinical information

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

GDCquery\_Maf 7

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\_Release\_Notes/

# Usage

```
GDCquery_Maf(tumor, save.csv = FALSE)
```

### **Arguments**

tumor

a valid tumor

save.csv

Write maf file into a csv document

#### Value

A data frame with the maf file information

# **Examples**

```
acc.maf <- GDCquery_Maf("ACC")</pre>
```

getGDCprojects

Retrieve all GDC projects

# 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

```
projects <- getGDCprojects()</pre>
```

isServeOK

Check GDC server status

### 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()</pre>
```

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 rep-

resents 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 helust if method selected is 'helust'. 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 (helust), consensusClass (consensus class asssignments). ConsensusClusterPlus also produces images.

TCGAanalyze\_DEA 9

TCGAanalyze_DEA	Differentially expression analysis (DEA) using edgeR package.
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 dis- tributed 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 consumation estimation

### Value

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

### **Examples**

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

```
## Not run:
to add example
## End(Not run)
```

TCGAanalyze\_DMR 11

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,
    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,
    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
plot.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

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 = group Col. group 1. group 2. rda
save	Save object with results? Default: TRUE
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

```
nrows <- 200; ncols <- 20
counts <- matrix(runif(nrows * ncols, 1, 1e4), nrows)</pre>
rowRanges <- GenomicRanges::GRanges(rep(c("chr1", "chr2"), c(50, 150)),</pre>
                    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),</pre>
                     row.names=LETTERS[1:20],
                     group=rep(c("group1", "group2"), c(10,10)))
data <- SummarizedExperiment::SummarizedExperiment(</pre>
         assays=S4Vectors::SimpleList(counts=counts),
         rowRanges=rowRanges,
         colData=colData)
SummarizedExperiment::colData(data)$group <- c(rep("group1",ncol(data)/2),</pre>
                          rep("group2",ncol(data)/2))
hypo.hyper <- TCGAanalyze_DMR(data, p.cut = 0.85, "group", "group1", "group2")</pre>
```

TCGAanalyze\_EA

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

### **Description**

The rational behind a 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. ie 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 peform 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 (lisf 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 componet (CC)

FDRThresh pvalue corrected (FDR) as threshold to selected significant BP, MF,CC, or path-

ways. (default FDR < 0.01)

#### Value

Table with enriched GO or pathways by selected gene signature.

#### **Examples**

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.

```
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)</pre>
```

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 istance 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 paramter for filter 1. default eta = $0.05$ .
foldChange	is a paramter for filter2. default foldChange = 1.

### Value

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

```
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)</pre>
```

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 abs(LogFC) >=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)

```
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)</pre>
```

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. Withinlane 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 istance 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 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.

```
dataNorm <- TCGAbiolinks::TCGAanalyze_Normalization(dataBRCA, geneInfo)</pre>
```

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)
```

### **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

### 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, cutoff = 0,
  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, legend.position = "inside", legend.title.position = "top",
  legend.ncols = 1, add.legend = TRUE, print.value = TRUE,
  add.points = 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	
cutoff	xlim This parameter will be a limit in the x-axis. That means, that patients with days_to_deth > cutoff will be set to Alive.	
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 of the lines.	
height	Image height	
width	Image width	
dpi	Figure quality	
legend.position		
	Legend position ("top", "right", "left", "bottom")	
legend.title.p	osition	
	Legend title position ("top", "right", "left", "bottom")	
legend.ncols	Number of columns of the legend	
add.legend	If true, legend is created. Otherwise names will be added to the last point in the lines.	
print.value	Print pvalue in the plot? Default: TRUE	

### Value

Survival plot

add.points

### **Examples**

```
clin <- GDCquery_clinic("TCGA-LGG", type = "clinical", save.csv = FALSE)
TCGAanalyze_survival(clin, clusterCol="gender")</pre>
```

If true, shows each death at the line of survival curves

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)
```

#### 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 , vi-  $\$ 

tal\_status, etc

dataGE is a matrix of Gene expression (genes in rows, samples in cols) from TCGApre-

pare

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

#### Value

table with survival genes pvalues from KM.

TCGAbiolinks 21

### **Examples**

```
## Not run:
    clinical_patient_Cancer <- TCGAquery_clinic("brca","clinical_patient")
    dataBRCAcomplete <- log2(BRCA_rnaseqv2)
# Selecting only 10 genes for example
    dataBRCAcomplete <- dataBRCAcomplete[1:10,]
    tabSurvKM<-TCGAanalyze_SurvivalKM(clinical_patient_Cancer,dataBRCAcomplete,
    Genelist = rownames(dataBRCAcomplete), Survresult = FALSE,ThreshTop=0.67,ThreshDown=0.33)
## End(Not run)</pre>
```

TCGAbiolinks

Download data of samples from TCGA

### **Description**

TCGAbiolinks allows you to Download data of samples from TCGA

#### **Details**

The functions you're likely to need from **TCGAbiolinks** is TCGAdownload, TCGAquery. Otherwise refer to the vignettes to see how to format the documentation.

TCGAdownload	Download the data from TCGA using as reference the output from
	TCGAquery

#### **Description**

The TCGAdownload function will download the data using as reference the the lines of the TCGAquery search result.

There is an option to download the entire tar.gz folder or download specific files using the *type* parameter or the *samples* parameter

The outpufiles will be saved into the path parameters. If this path does not exists the package will try to create the directories.

By default, if a sample was already downloaded the function will not download again, unless the force parameter is set to TRUE

### Usage

```
TCGAdownload(data = NULL, path = ".", type = NULL, samples = NULL,
force = FALSE)
```

22 TCGAprepare

#### **Arguments**

data	The TCGAquery output
path	Directory to save the downloaded data
type	Filter the files that will be downloaded by type. Example: "rsem.genes.results"
samples	List of samples to download data
force	Download files even if it was already downladed? Default: FALSE

#### Value

Download TCGA data into the given path

### See Also

Other data.functions: TCGAquery

### **Examples**

TCGAprepare

Read the data from level 3 the experiments and prepare it for downstream analysis into a SummarizedExperiment object.

# Description

This function has been replaced by GDCprepare

List of accepted platforms:

- AgilentG4502A\_07\_1/AgilentG4502A\_07\_2/AgilentG4502A\_07\_3
- Genome\_Wide\_SNP\_6
- H-miRNA\_8x15K/H-miRNA\_8x15Kv2
- HG-U133\_Plus\_2
- HT\_HG-U133A
- HumanMethylation27
- HumanMethylation450

TCGAprepare 23

- IlluminaDNAMethylation\_OMA002\_CPI
- IlluminaDNAMethylation\_OMA003\_CPI
- IlluminaGA\_RNASeq
- IlluminaGA\_RNASeqV2
- IlluminaHiSeq\_RNASeq
- IlluminaHiSeq\_RNASeqV2
- IlluminaHiSeq\_TotalRNASeqV2

**Return**The default output is a SummarizedExperiment object.

# Usage

```
TCGAprepare(query = NULL, dir = NULL, samples = NULL, type = NULL,
save = FALSE, filename = NULL, summarizedExperiment = TRUE)
```

### Arguments

query	TCGAquery output	
dir	Directory with the files downloaded by TCGAdownload	
samples	List of samples to prepare the data	
type	Filter the files to prepare.	
save	Save a rda object with the prepared object? Default: FALSE	
filename	Name of the saved file	
summarizedExperiment		
	Output as SummarizedExperiment? Default: FALSE	

### Value

A SummarizedExperiment object (If SummarizedExperiment = FALSE, a data.frame)

```
## Not run:
sample <- "TCGA-06-0939-01A-01D-1228-05"
query <- TCGAquery(tumor = "GBM",samples = sample, level = 3)
TCGAdownload(query,path = "exampleData",samples = sample)
data <- TCGAprepare(query, dir="exampleData")
## End(Not run)</pre>
```

24 TCGAprepare\_elmer

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

Normalizd Expression data from Affy eSets

### **Examples**

```
## Not run:
to add example
## End(Not run)
```

TCGAprepare\_elmer

Prepare the data for ELEMR package

### Description

Prepare the data for ELEMR package

### Usage

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

### **Arguments**

		om TCGAPrepare
data		

 $platform \hspace{0.5cm} platform \hspace{0.5cm} of \hspace{0.5cm} the \hspace{0.5cm} data. \hspace{0.5cm} Example: "Human Methylation 450", "Illumina Hi Seq\_RNA Seq V2"$ 

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)

TCGAquery 25

#### 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")</pre>
```

**TCGAquery** 

Searches TCGA open-access data providing also latest version of the files.

### **Description**

This function has been replace by GDCquery

### Usage

```
TCGAquery(tumor = NULL, platform = NULL, samples = NULL, center = NULL,
level = NULL, version = NULL)
```

#### **Arguments**

tumor Disease Examples:

OV	BRCA	CESC	<b>ESCA</b>	PCPG
LUSC	LGG	SKCM	KICH	CHOL
GBM	UCEC	PRAD	PAAD	THYM
KIRC	THCA	SARC	LAML	TGCT
COAD	KIRP	HNSC	ACC	UVM
READ	BLCA	DLBC	UCS	FPPP
LUAD	LIHC	STAD	<b>MESO</b>	CNTL

platform Example:

CGH- 1x1M\_G4447A Illun
AgilentG4502A\_07 Illun
Human1MDuo Hum
HG-CGH-415K\_G4124A Illun
HumanHap550 Illun
ABI H-m
HG-CGH-244A SOL
IlluminaDNAMethylation\_OMA003\_CPI Illun
IlluminaDNAMethylation\_OMA002\_CPI HGHuEx- 1\_0-st-v2 Mixe
H-miRNA\_8x15Kv2 Illun

IlluminaGA\_RNASeqV2 IlluminaGA\_mRNA\_DGE HumanMethylation450 IlluminaGA\_miRNASeq IlluminaHiSeq\_miRNASeq H-miRNA\_8x15K SOLiD\_DNASeq IlluminaGA\_DNASeq\_automated

HG-U133\_Plus\_2 Mixed\_DNASeq

IlluminaGA\_DNASeq\_curated

26 TCGAquery\_clinic

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

Genome\_Wide\_SNP\_6 bio

IlluminaHiSeq\_RNASeqV2 Mixed\_DNASeq\_Cont

samples List of samples. Ex:c('TCGA-04-06','TCGA-61-1743-01A-01D-0649-04')

center center name level '1' '2' '3'

version List of vector with tumor/plaform/version to get old samples,

#### Value

A dataframe with the results of the query (lastest version of the files)

#### See Also

Other data.functions: TCGAdownload

# **Examples**

```
## Not run:
query <- TCGAquery(tumor = "gbm")
## End(Not run)
```

TCGAquery\_clinic

Get the clinical information

# Description

This function has been replaced. Use GDCquery\_clinic

### Usage

```
TCGAquery_clinic(tumor, clinical_data_type, samples, path = getwd())
```

#### Arguments

tumor a character vector indicating cancer type Examples:

TCGAquery\_clinic 27

OV	BRCA	CESC	<b>ESCA</b>	PCPG
LUSC	LGG	SKCM	KICH	CHOL
GBM	UCEC	PRAD	PAAD	THYM
KIRC	THCA	SARC	LAML	TGCT
COAD	KIRP	HNSC	ACC	UVM
<b>READ</b>	BLCA	DLBC	UCS	FPPP
LUAD	LIHC	STAD	MESO	CNTL

For information about cancer types: https://tcga-data.nci.nih.gov/tcga/

#### clinical\_data\_type

a character vector indicating the types of clinical data. Besides TCGA data, we created the clinical\_patient\_updated, which is the clinical\_patient file with the last follow up information from the last follow up file. Example:

biospecimen aliquot biospecimen\_analyte biospecimen\_cqcf biospecimen\_diagnostic\_slides biospecimen\_normal\_control biospecimen\_portion biospecimen\_protocol biospecimen\_sample biospecimen\_shipment\_portion biospecimen\_slide biospecimen\_tumor\_sample clinical\_cqcf clinical\_follow\_up\_v1.0 clinical\_follow\_up\_v1.5 clinical\_follow\_up\_v2.0 clinical\_follow\_up\_v2.1 clinical\_follow\_up\_v4.0 clinical\_follow\_up\_v4.0\_nte clinical\_nte clinical\_omf\_v4.0 clinical\_radiation clinical\_patient ssf\_tumor\_samples ssf normal controls clinical\_follow\_up\_v1.0\_nte clinical\_patient\_updated (TCGAbiolinks only)

samples List of barcodes to get the clinical data

path Directory to save the downloaded data default getwd()

### Value

clinic data

```
## Not run:
data <- TCGAquery_clinic("LGG","clinical_drug")
## End(Not run)</pre>
```

TCGAquery\_clinicFilt Filter samples using clinical data

### **Description**

This function will return the samples that matches all filters. Filters available: HER, ER, gender, PR, stage.

#### **Usage**

```
TCGAquery_clinicFilt(barcode, clinical_patient_data, HER = NULL, ER = NULL,
gender = NULL, PR = NULL, stage = NULL)
```

#### **Arguments**

barcode

PR Progesterone receptor status: "Positive" or "Negative"

stage Pathologic Stage: "stage\_IX", "stage\_I", "stage\_IA", "stage\_IB", "stage\_IIX", "stage\_IIX",

"stage\_IIA", "stage\_IIB", "stage\_IIIX", "stage\_IIIA", "stage\_IIIB", "stage\_IIIC",

"stage\_IV" -

List of barcodes

#### Value

List of samples that matches the filters

```
"TCGA-A0-A0J5-05A-11R-1789-07", "TCGA-BH-A0B4-11A-12R-1789-07",
"TCGA-B6-A1KN-60A-13R-1789-07", "TCGA-A0-A0J5-01A-11R-1789-07",
"TCGA-A0-A0J5-01A-11R-1789-07", "TCGA-G9-6336-11A-11R-1789-07",
"TCGA-G9-6380-11A-11R-1789-07", "TCGA-G9-6380-01A-11R-1789-07",
"TCGA-G9-6340-01A-11R-1789-07", "TCGA-G9-6340-11A-11R-1789-07")

TCGAquery_clinicFilt(c("TCGA-3C-AALK", "TCGA-A2-A04Q", "TCGA-A4-A04Q"), clin,
HER="Positive", gender="FEMALE", ER="Positive")
```

TCGAquery\_Investigate Find most studied TF in pubmed related to a specific cancer, disease, or tissue

### **Description**

Find most studied TF in pubmed related to a specific cancer, disease, or tissue

#### Usage

```
TCGAquery_Investigate(tumor, dataDEGsFiltLevelTF, topgenes)
```

### **Arguments**

#### Value

table with number of pubmed's publications related to tfs and disease selected

```
## Not run:
TFs <- EAGenes[EAGenes$Family =="transcription regulator",]
TFs_inDEGs <- intersect(TFs$Gene, dataDEGsFiltLevel$mRNA )
dataDEGsFiltLevelTFs <- dataDEGsFiltLevel[TFs_inDEGs,]
dataDEGsFiltLevelTFs <- dataDEGsFiltLevelTFs[order(dataDEGsFiltLevelTFs$Delta,decreasing = TRUE),]
# Find Pubmed of TF studied related to cancer
tabDEGsTFPubmed <- TCGAquery_Investigate("breast", dataDEGsFiltLevelTFs, topgenes = 1)
## End(Not run)</pre>
```

TCGAquery\_maf

Get last maf file for the tumor

### **Description**

This function has been replaced. Use GDCquery\_maf

# Usage

```
TCGAquery_maf(tumor = NULL, center = NULL, archive.name = NULL)
```

### **Arguments**

tumor type to filter the search
center Center name to filter the search
archive.name Archive name to filter the search

#### Value

list of samples for a tumor

### **Examples**

```
## Not run:
   query <- TCGAquery_maf(tumor = 'lgg')
## End(Not run)</pre>
```

 ${\tt TCGAquery\_MatchedCoupledSampleTypes}$ 

Retrieve multiple tissue types from the same patients.

### **Description**

 $TCGA query\_Matched Coupled Sample Types$ 

# Usage

TCGAquery\_MatchedCoupledSampleTypes(barcode, typesample)

### **Arguments**

barcode barcode
typesample typesample

#### Value

a list of samples / barcode filtered by type sample selected

### **Examples**

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 theses 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"))</pre>
```

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")</pre>
```

TCGAvisualize\_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

```
TCGAvisualize_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

TCGAvisualize\_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_EA complete related to Molecular Function (MF) $$
GOBPTab	is results from TCGAanalyze_EAcomplete related to Biological Process (BP)
GOCCTab	is results from TCGAanalyze_EA complete 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")</pre>
ansEA <- TCGAanalyze_EAcomplete(TFname="DEA genes Normal Vs Tumor",Genelist)</pre>
TCGAvisualize_EAbarplot(tf = rownames(ansEA$ResBP),
         GOBPTab = ansEA$ResBP,
         GOCCTab = ansEA$ResCC,
         GOMFTab = ansEA$ResMF,
        PathTab = ansEA$ResPat,
         nRGTab = Genelist,
         nBar = 10,
         filename="a.pdf")
while (!(is.null(dev.list()["RStudioGD"]))){dev.off()}
## Not run:
Genelist <- rownames(dataDEGsFiltLevel)</pre>
system.time(ansEA <- TCGAanalyze_EAcomplete(TFname="DEA genes Normal Vs Tumor",Genelist))</pre>
# Enrichment Analysis EA (TCGAVisualize)
# Gene Ontology (GO) and Pathway enrichment barPlot
TCGAvisualize_EAbarplot(tf = rownames(ansEA$ResBP),
         GOBPTab = ansEA$ResBP,
         GOCCTab = ansEA$ResCC,
         GOMFTab = ansEA$ResMF,
        PathTab = ansEA$ResPat,
         nRGTab = Genelist,
         nBar = 10)
## End(Not run)
```

### Description

Heatmap with more sensible behavior using heatmap.plus

#### Usage

```
TCGAvisualize_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, title,
  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

title Title of the plot

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" (de-

fault), "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", "disctrete

#### Value

Heatmap plotted in the device

#### **Examples**

```
row.mdat <- matrix(c("FALSE", "FALSE",</pre>
                     "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"),</pre>
                   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()}
```

 ${\tt TCGAvisualize\_meanMethylation}$ 

Mean methylation boxplot

#### **Description**

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

Observation: Data is a summarizedExperiment.

# Usage

```
TCGAvisualize_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 = FALSE, 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.legen	d	
	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, meadian.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)</pre>
rowRanges <- GenomicRanges::GRanges(rep(c("chr1", "chr2"), c(50, 150)),</pre>
                   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),</pre>
                    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(</pre>
         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()}
```

TCGAvisualize\_oncoprint

Creating a oncoprint

# **Description**

Creating a oncoprint

## Usage

```
TCGAvisualize_oncoprint(mut, genes, filename, color,
   annotation.position = "bottom", annotation, height,
   rm.empty.columns = FALSE, show.column.names = FALSE,
   show.row.barplot = TRUE, label.title = "Mutation", label.font.size = 16,
   rows.font.size = 16, dist.col = 0.5, dist.row = 0.5,
   row.order = FALSE, 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

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

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

row.order Order the genes (rows). Genes with more mutations will be in the first rows

heatmap.legend.side

Position of the heatmap legend

 $\verb"annotation.legend.side"$ 

Position of the annotation legend

# Value

A oncoprint plot

40 TCGAvisualize\_PCA

## **Examples**

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(dataFilt, dataDEGsFiltLevel, ntopgenes)
```

# **Arguments**

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

column represents a sample from function TCGAanalyze\_Filtering

dataDEGsFiltLevel

table with DEGs, log Fold Change (FC), false discovery rate (FDR), the gene expression level, etc, from function TCGAanalyze\_LevelTab.

ntopgenes number of DEGs genes to plot in PCA

## Value

principal components analysis (PCA) plot of PC1 and PC2

## **Examples**

```
# normalization of genes
dataNorm <- TCGAbiolinks::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
pca <- TCGAvisualize_PCA(dataFilt,dataDEGsFiltLevel, ntopgenes = 200)
if (!(is.null(dev.list()["RStudioGD"]))){dev.off()}</pre>
```

TCGAvisualize\_profilePlot

Profile plot

## **Description**

Displaty the association between cancer subtypes and any kind of clustering.

## Usage

```
TCGAvisualize_profilePlot(data = NULL, groupCol = NULL, subtypeCol = NULL,
  colors = NULL, filename = NULL, na.rm.groups = FALSE,
  na.rm.subtypes = FALSE, plot.margin = c(-2.5, -2.5, -0.5, 2),
  axis.title.size = 1.5, axis.textsize = 1.3, legend.size = 1.5,
  legend.title.size = 1.5, geom.label.size = 6,
  geom.label.color = "black")
```

# **Arguments**

A data frame with the cluters and subytpe of cancers data Names of tre columns with the cluster information groupCol subtypeCol Name of the column with the subtype information colors Vector of colors to be used in the bars filename Name of the file to save the plot, can be pdf, png, svg etc.. na.rm.groups Remove NA groups? Default = FALSE Remove NA subtypes? Default = FALSE na.rm.subtypes plot.margin Plot margin for cluster distribution. This can control the size of the bar if the output is not aligned axis.title.size axis.title.size axis.textsize axis.textsize Size of the legend legend.size legend.title.size Size of the legend title

```
geom.label.size
Size of percentage in the left barplot
geom.label.color
Color of percentage in the left barplot
```

#### Value

A plot

# **Examples**

```
while (!(is.null(dev.list()["RStudioGD"]))){dev.off()}
cluster <- c(rep("cluster1",30),</pre>
              rep("cluster2",30),
              rep("cluster3",30))
subtype <- rep(c(rep("subtype1",10),</pre>
            rep("subtype2",10),
            rep("subtype3",10)),3)
df <- data.frame(cluster, subtype)</pre>
TCGAvisualize_profilePlot(data = df, groupCol = "cluster", subtypeCol = "subtype",
                            plot.margin=c(-4.2, -2.5, -0.0, 2))
while (!(is.null(dev.list()["RStudioGD"]))){dev.off()}
cluster <- c(rep("cluster1",10),</pre>
              rep("cluster2",20),
              rep("cluster3",30),
              rep("cluster4",40))
subtype <- rep(c(rep("subtype1",5),</pre>
            rep("subtype2",10),
            rep("subtype3",10)),4)
df <- data.frame(cluster, subtype)</pre>
plot <- TCGAvisualize_profilePlot(data = df, groupCol = "cluster", subtypeCol = "subtype",</pre>
                            plot.margin=c(-4.2, -2.5, -0.5, 2))
```

TCGAvisualize\_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",
        "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**

```
nrows <- 20000; ncols <- 20
counts <- matrix(runif(nrows * ncols, 1, 1e4), nrows)</pre>
ranges <- GenomicRanges::GRanges(rep(c("chr1", "chr2"), c(5000, 15000)),</pre>
                    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),</pre>
                     row.names=LETTERS[1:20],
                     group=rep(c("group1", "group2"), c(10,10)))
data <- SummarizedExperiment::SummarizedExperiment(</pre>
         assays=S4Vectors::SimpleList(counts=counts),
         rowRanges=ranges,
         colData=colData)
met <- data
exp <- data.frame(row.names=sprintf("ID%03d", 1:20000),</pre>
                   logFC=runif(20000, -5, 5),
                   FDR=runif(20000, 0.01, 1))
SummarizedExperiment::rowRanges(met)$diffmean.g1.g2 <- c(runif(20000, -0.1, 0.1))
SummarizedExperiment::rowRanges(met)$diffmean.g2.g1 <- -1*(SummarizedExperiment::rowRanges(met)$diffmean.g1.g2
SummarizedExperiment::rowRanges(met)$p.value.g1.g2 <- c(runif(20000, 0, 1))</pre>
SummarizedExperiment::rowRanges(met)$p.value.adj.g1.g2 <- c(runif(20000, 0, 1))
result <- TCGAvisualize_starburst(met,exp,</pre>
                                   exp.p.cut = 0.05, met.p.cut = 0.05,
                                   group1="g1",group2="g2",
                                   diffmean.cut=0.0,
                                   names=TRUE, circle = FALSE)
result <- TCGAvisualize_starburst(SummarizedExperiment::values(met),</pre>
                                   exp.p.cut = 0.05, met.p.cut = 0.05,
                                   group1="g1",group2="g2",
                                   diffmean.cut=0.0,
                                   names=TRUE, circle = FALSE)
```

TCGAvisualize\_SurvivalCoxNET

Survival analysis with univariate Cox regression package (dnet)

## **Description**

TCGAvisualize\_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). TCGAvisualize\_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

TCGAvisualize\_SurvivalCoxNET(clinical\_patient, dataGE, Genelist, org.Hs.string, scoreConfidence = 700, titlePlot = "TCGAvisualize\_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 , vi-  $\$ 

tal\_status, etc

dataGE is a matrix of Gene expression (genes in rows, samples in cols) from TCGApre-

pare

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**

TCGAvisualize\_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 pvaules
- 2. fit a Cox proportional hazards model and ANOVA (Chisq test)
- 3. Network comunites
- 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. query <- TCGAquery(tumor = "lgg")

TCGAvisualize\_Tables Visaulize results in format of latex tables.

# **Description**

Visaulize results in format of latex tables.

## Usage

TCGAvisualize\_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)
TCGAvisualize_Tables(Table = tabDEGsTFPubmed,
rowsForPage = 5,
TableTitle = "pip",
LabelTitle = "pip2",
withrows = FALSE,
size = "small")</pre>
```

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 = NULL, 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**

```
Х
                   x-axis data
                   y-axis data
У
filename
                   Filename. Default: volcano.pdf, volcano.svg, volcano.png
ylab
                   y axis text
xlab
                   x axis text
                   main title. If not specified it will be "Volcano plot (group1 vs group2)
title
                   Legend title
legend
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 y.cut p-values threshold. Default: 0.01

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**

# **Index**

```
GDCdownload, 3
GDCprepare, 4
GDCprepare_clinic, 4
GDCquery, 5
GDCquery_clinic, 6
GDCquery_Maf, 7
getGDCprojects, 7
isServeOK, 8
TCGAanalyze_Clustering, 8
TCGAanalyze_DEA, 9
TCGAanalyze_DEA_Affy, 10
TCGAanalyze_DMR, 11
TCGAanalyze_EA, 13
TCGAanalyze_EAcomplete, 14
TCGAanalyze_Filtering, 15
TCGAanalyze_LevelTab, 16
TCGAanalyze_Normalization, 17
TCGAanalyze_Preprocessing, 18
TCGAanalyze_survival, 18
TCGAanalyze_SurvivalKM, 20
TCGAbiolinks, 21
TCGAbiolinks-package (TCGAbiolinks), 21
TCGAdownload, 21, 21, 26
TCGAprepare, 22
TCGAprepare_Affy, 24
TCGAprepare_elmer, 24
TCGAquery, 21, 22, 25
TCGAquery_clinic, 26
TCGAquery_clinicFilt, 28
TCGAquery_Investigate, 29
TCGAquery_maf, 30
TCGAquery_MatchedCoupledSampleTypes,
        30
TCGAquery_SampleTypes, 31
TCGAquery_subtype, 32
TCGAvisualize_BarPlot, 32
TCGAvisualize_EAbarplot, 33
TCGAvisualize_Heatmap, 34
```

TCGAvisualize\_meanMethylation, 36
TCGAvisualize\_oncoprint, 38
TCGAvisualize\_PCA, 40
TCGAvisualize\_profilePlot, 41
TCGAvisualize\_starburst, 42
TCGAvisualize\_SurvivalCoxNET, 45
TCGAvisualize\_Tables, 46
TCGAVisualize\_volcano, 47