Package 'GDCRNATools'

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Title GDCRNATools: an R/Bioconductor package for integrative analysis of lncRNA, mRNA, and miRNA data in GDC

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Description This is an easy-to-use package for downloading, organizing, and integrative analyzing RNA expression data in GDC with an emphasis on deciphering the lncRNA-mRNA related ceRNA regulatory network in cancer. Three databases of lncRNA-miRNA interactions including spongeScan, starBase, and miRcode, as well as three databases of mRNA-miRNA interactions including miRTarBase, starBase, and miRcode are incorporated into the package for ceRNAs network construction. limma, edgeR, and DESeq2 can be used to identify differentially expressed genes/miRNAs. Functional enrichment analyses including GO, KEGG, and DO can be performed based on the clusterProfiler and DO packages. Both univariate CoxPH and KM survival analyses of multiple genes can be implemented in the package. Besides some routine visualization functions such as volcano plot, bar plot, and KM plot, a few simply shiny apps are developed to facilitate visualization of results on a local webpage.

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`GDCRNATools-package` *This is an easy-to-use package for downloading, organizing, and integrative analyzing RNA expression data in GDC with an emphasis on deciphering the lncRNA-mRNA related ceRNA regulatory network in cancer.*

Description

This is an easy-to-use package for downloading, organizing, and integrative analyzing RNA expression data in GDC with an emphasis on deciphering the lncRNA-mRNA related ceRNA regulatory network in cancer.

`DEGAll` *Output of* `gdcDEAnalysis` *for downstream analysis*

Description

Output of `gdcDEAnalysis` for downstream analysis

`enrichOutput` *Output of* `gdcEnrichAnalysis` *for visualization*

Description

Output of `gdcEnrichAnalysis` for visualization

gdcBarPlot *Bar plot of differentially expressed genes/miRNAs*

Description

A bar plot showing the number of down-regulated and up-regulated DE genes/miRNAs of different biotypes

Usage

```
gdcBarPlot(deg, angle = 0, data.type)
```

Arguments

deg	a dataframe generated from gdcDEReport containing DE genes/miRNAs ids, logFC, etc.
angle	a numeric value specifying the angle of text on x-axis. Default is 0
data.type	one of 'RNAseq' and 'miRNAs'

Value

A bar plot

Author(s)

Ruidong Li and Han Qu

Examples

```
genes <- c('ENSG00000231806','ENSG00000261211','ENSG00000260920',
        'ENSG00000228594','ENSG00000125170','ENSG00000179909',
        'ENSG00000280012','ENSG00000134612','ENSG00000213071')
symbol <- c('PCAT7','AL031123.2','AL031985.3',
            'FNDC10','DOK4','ZNF154',
            'RPL23AP61','FOLH1B','LPAL2')
group <- rep(c('long_non_coding','protein_coding','pseudogene'), each=3)
logFC <- c(2.8,2.3,-1.1,1.9,-1.2,-1.6,1.5,2.1,-1.1)
FDR <- rep(c(0.1,0.00001,0.0002), each=3)
deg <- data.frame(symbol, group, logFC, FDR)
rownames(deg) <- genes
gdcBarPlot(deg, angle=45, data.type='RNAseq')
```

gdcCEAnalysis *Competing endogenous RNAs (ceRNAs) analysis*

Description

Identify ceRNAs by (1) number of shared miRNAs between lncRNA and mRNA; (2) expression correlation of lncRNA and mRNA; (3) regulation similarity of shared miRNAs on lncRNA and mRNA; (4) sensitivity correlation

Usage

```
gdcCEAnalysis(lnc, pc, deMIR = NULL, lnc.targets = "starBase",
  pc.targets = "starBase", rna.expr, mir.expr)
```

Arguments

`lnc`	a vector of Ensembl long non-coding gene ids
`pc`	a vector of Ensembl protein coding gene ids
`deMIR`	a vector of differentially expressed miRNAs. Default is `NULL`
`lnc.targets`	a character string specifying the database of miRNA-lncRNA interactions. Should be one of `'spongeScan'`, `'starBase'`, and `'miRcode'`. Default is `'starBase'`. Or a `list` of miRNA-lncRNA interactions generated by users
`pc.targets`	a character string specifying the database of miRNA-lncRNA interactions. Should be one of `'spongeScan'`, `'starBase'`, and `'miRcode'`. Default is `'starBase'`. Or a `list` of miRNA-lncRNA interactions generated by users
`rna.expr`	`voom` transformed gene expression data
`mir.expr`	`voom` transformed mature miRNA expression data

Value

A dataframe containing ceRNA pairs, expression correlation between lncRNA and mRNA, the number and hypergeometric significance of shared miRNAs, regulation similarity score, and the mean sensitity correlation (the difference between Pearson correlation and partial correlation) of multiple lncRNA-miRNA-mRNA triplets, etc.

Author(s)

Ruidong Li and Han Qu

References

Paci P, Colombo T, Farina L. Computational analysis identifies a sponge interaction network between long non-coding RNAs and messenger RNAs in human breast cancer. BMC systems biology. 2014 Jul 17;8(1):83.

Examples

```
####### ceRNA network analysis #######
deLNC <- c('ENSG00000260920','ENSG00000242125','ENSG00000261211')
dePC <- c('ENSG00000043355','ENSG00000109586','ENSG00000144355')
genes <- c(deLNC, dePC)
samples <- c('TCGA-2F-A9KO-01', 'TCGA-2F-A9KP-01',
             'TCGA-2F-A9KQ-01', 'TCGA-2F-A9KR-01',
             'TCGA-2F-A9KT-01', 'TCGA-2F-A9KW-01')
rnaExpr <- data.frame(matrix(c(2.7,7.0,4.9,6.9,4.6,2.5,
                    0.5,2.5,5.7,6.5,4.9,3.8,
                    2.1,2.9,5.9,5.7,4.5,3.5,
                    2.7,5.9,4.5,5.8,5.2,3.0,
                    2.5,2.2,5.3,4.4,4.4,2.9,
                    2.4,3.8,6.2,3.8,3.8,4.2),6,6),
                    stringsAsFactors=FALSE)
rownames(rnaExpr) <- genes
colnames(rnaExpr) <- samples

mirExpr <- data.frame(matrix(c(7.7,7.4,7.9,8.9,8.6,9.5,
                    5.1,4.4,5.5,8.5,4.4,3.5,
                    4.9,5.5,6.9,6.1,5.5,4.1,
                    12.4,13.5,15.1,15.4,13.0,12.8,
                    2.5,2.2,5.3,4.4,4.4,2.9,
                    2.4,2.7,6.2,1.5,4.4,4.2),6,6),
                    stringsAsFactors=FALSE)
colnames(mirExpr) <- samples
rownames(mirExpr) <- c('hsa-miR-340-5p','hsa-miR-181b-5p',
                    'hsa-miR-181a-5p', 'hsa-miR-181c-5p',
                    'hsa-miR-199b-5p','hsa-miR-182-5p')

ceOutput <- gdcCEAnalysis(lnc       = deLNC,
                          pc        = dePC,
                          lnc.targets = 'starBase',
                          pc.targets  = 'starBase',
                          rna.expr    = rnaExpr,
                          mir.expr    = mirExpr)
```

`gdcClinicalDownload` *Download clinical data in GDC*

Description

Download clinical data in GDC either by providing the manifest file or specifying the project id and data type

Usage

```
gdcClinicalDownload(manifest = NULL, project.id,
  directory = "Clinical", write.manifest = FALSE,
  method = "gdc-client")
```

Arguments

manifest	menifest file that is downloaded from the GDC cart. If provided, files whose UUIDs are in the manifest file will be downloaded via gdc-client, otherwise, project id argument should be provided to download data automatically. Default is NULL
project.id	project id in GDC
directory	the folder to save downloaded files. Default is 'Clinical'
write.manifest	logical, whether to write out the manifest file
method	method that is used to download data. Either 'GenomicDataCommons' which is a well established method developed in the **GenomicDataCommons'** package, or alternatively 'gdc-client' which uses the gdc-client tool developed by GDC. Default is 'gdc-client'.

Value

downloaded files in the specified directory

Author(s)

Ruidong Li and Han Qu

Examples

```
####### Download Clinical data by manifest file #######
manifest <- 'Clinical.manifest.txt'
## Not run: gdcClinicalDownload(manifest  = manifest,
                    directory = 'Clinical')
## End(Not run)

####### Download Clinical data by project id #######
project <- 'TCGA-PRAD'
## Not run: gdcClinicalDownload(project.id     = project,
                    write.manifest = TRUE,
                    directory      = 'Clinical')
## End(Not run)
```

---

gdcClinicalMerge *Merge clinical data*

Description

Merge clinical data in .xml files that are downloaded from GDC to a dataframe

Usage

```
gdcClinicalMerge(path, key.info = TRUE, organized = FALSE)
```

Arguments

path	path to downloaded files for merging
key.info	logical, whether to return the key clinical information only. If TRUE, only clinical information such as age, stage, grade, overall survial, etc. will be returned
organized	logical, whether the clinical data have already been organized into a single folder (eg., data downloaded by the 'GenomicDataCommons' method are already organized). Default is FALSE.

Value

A dataframe of clinical data with rows are patients and columns are clinical traits

Author(s)

Ruidong Li and Han Qu

Examples

```
####### Merge clinical data #######
path <- 'Clinical/'
## Not run: clinicalDa <- gdcClinicalMerge(path=path, key.info=TRUE)
```

---

gdcCorPlot *Correlation plot of two genes/miRNAs*

Description

Scatter plot showing the expression correlation between two genes/miRNAs

Usage

```
gdcCorPlot(gene1, gene2, rna.expr, metadata)
```

Arguments

gene1	an Ensembl gene id or miRBase v21 mature miRNA id
gene2	an Ensembl gene id or miRBase v21 mature miRNA id
rna.expr	voom transformed expression data
metadata	metadata parsed from gdcParseMetadata

Value

A scatter plot with line of best fit

Author(s)

Ruidong Li and Han Qu

Examples

```
genes <- c('ENSG00000000938','ENSG00000000971','ENSG00000001036',
        'ENSG00000001084','ENSG00000001167','ENSG00000001460')

samples <- c('TCGA-2F-A9KO-01', 'TCGA-2F-A9KP-01',
            'TCGA-2F-A9KQ-01', 'TCGA-2F-A9KR-11',
            'TCGA-2F-A9KT-11', 'TCGA-2F-A9KW-11')

metaMatrix <- data.frame(sample_type=rep(c('PrimaryTumor',
                        'SolidTissueNormal'),each=3),
                        sample=samples,
                        days_to_death=seq(100,600,100),
                        days_to_last_follow_up=rep(NA,6))

rnaExpr <- matrix(c(2.7,7.0,4.9,6.9,4.6,2.5,
                    0.5,2.5,5.7,6.5,4.9,3.8,
                    2.1,2.9,5.9,5.7,4.5,3.5,
                    2.7,5.9,4.5,5.8,5.2,3.0,
                    2.5,2.2,5.3,4.4,4.4,2.9,
                    2.4,3.8,6.2,3.8,3.8,4.2),6,6)
rownames(rnaExpr) <- genes
colnames(rnaExpr) <- samples
gdcCorPlot(gene1 = 'ENSG00000000938',
        gene2    = 'ENSG00000001084',
        rna.expr = rnaExpr,
        metadata = metaMatrix)
```

`gdcDEAnalysis` *Differential gene expression analysis*

Description

Performs differential gene expression analysis by **limma**, **edgeR**, and **DESeq2**

Usage

```
gdcDEAnalysis(counts, group, comparison, method = "limma",
  n.cores = NULL, filter = TRUE)
```

Arguments

`counts`	a dataframe or numeric matrix of raw counts data generated from `gdcRNAMerge`
`group`	a vector giving the group that each sample belongs to
`comparison`	a character string specifying the two groups being compared. Example: `comparison='PrimaryTumor-SolidTissueNormal'`
`method`	one of `'limma'`, `'edgeR'`, and `'DESeq2'`. Default is `'limma'` Note: It may takes long time for `method='DESeq2'` with a single core

n.cores	a numeric value of cores to be used for method='DESeq2' to accelate the analysis process. Default is NULL
filter	logical, whether to filter out low expression genes. If TRUE, only genes with cpm > 1 in more than half of the samples will be kept. Default is TRUE

Value

A dataframe containing Ensembl gene ids/miRBase v21 mature miRNA ids, gene symbols, biotypes, fold change on the log2 scale, p value, and FDR etc. of all genes/miRNAs of analysis.

Note

It may takes long time for method='DESeq2' with a single core. Please use multiple cores if possible

Author(s)

Ruidong Li and Han Qu

References

Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010 Jan 1;26(1):139-40.
Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic acids research. 2015 Jan 20; 43(7):e47-e47.
Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology. 2014 Dec 5; 15(12):550.

Examples

```
genes <- c('ENSG00000000938','ENSG00000000971','ENSG00000001036',
        'ENSG00000001084','ENSG00000001167','ENSG00000001460')

samples <- c('TCGA-2F-A9KO-01', 'TCGA-2F-A9KP-01',
            'TCGA-2F-A9KQ-01', 'TCGA-2F-A9KR-11',
            'TCGA-2F-A9KT-11', 'TCGA-2F-A9KW-11')

metaMatrix <- data.frame(sample_type=rep(c('PrimaryTumor',
                    'SolidTissueNormal'),each=3),
                    sample=samples,
                    days_to_death=seq(100,600,100),
                    days_to_last_follow_up=rep(NA,6))
rnaMatrix <- matrix(c(6092,11652,5426,4383,3334,2656,
                    8436,2547,7943,3741,6302,13976,
                    1506,6467,5324,3651,1566,2780,
                    834,4623,10275,5639,6183,4548,
                    24702,43,1987,269,3322,2410,
                    2815,2089,3804,230,883,5415), 6,6)
rownames(rnaMatrix) <- genes
colnames(rnaMatrix) <- samples
```

```
DEGAll <- gdcDEAnalysis(counts     = rnaMatrix,
                        group      = metaMatrix$sample_type,
                        comparison = 'PrimaryTumor-SolidTissueNormal',
                        method     = 'limma')
```

---

gdcDEReport *Report differentially expressed genes/miRNAs*

Description

Report genes/miRNAs that are differentially expressed satisfying a given threshold

Usage

```
gdcDEReport(deg, gene.type = "all", fc = 2, pval = 0.01)
```

Arguments

deg	A dataframe of DE analysis result from gdcDEAnalysis
gene.type	one of 'all', 'long_non_coding', 'protein_coding', and 'miRNAs'. Default is 'all'
fc	a numeric value specifying the threshold of fold change
pval	a nuemric value specifying the threshold of p value

Value

A dataframe or numeric matrix of differentially expressed genes/miRNAs

Author(s)

Ruidong Li and Han Qu

Examples

```
genes <- c('ENSG00000000938','ENSG00000000971','ENSG00000001036',
        'ENSG00000001084','ENSG00000001167','ENSG00000001460')

samples <- c('TCGA-2F-A9KO-01', 'TCGA-2F-A9KP-01',
            'TCGA-2F-A9KQ-01', 'TCGA-2F-A9KR-11',
             'TCGA-2F-A9KT-11', 'TCGA-2F-A9KW-11')

metaMatrix <- data.frame(sample_type=rep(c('PrimaryTumor',
                        'SolidTissueNormal'),each=3),
                        sample=samples,
                        days_to_death=seq(100,600,100),
                        days_to_last_follow_up=rep(NA,6))
rnaMatrix <- matrix(c(6092,11652,5426,4383,3334,2656,
                    8436,2547,7943,3741,6302,13976,
```

```
                    1506,6467,5324,3651,1566,2780,
                    834,4623,10275,5639,6183,4548,
                    24702,43,1987,269,3322,2410,
                    2815,2089,3804,230,883,5415), 6,6)
rownames(rnaMatrix) <- genes
colnames(rnaMatrix) <- samples
DEGAll <- gdcDEAnalysis(counts     = rnaMatrix,
                        group      = metaMatrix$sample_type,
                        comparison = 'PrimaryTumor-SolidTissueNormal',
                        method     = 'limma')
dePC <- gdcDEReport(deg=DEGAll)
```

---

gdcEnrichAnalysis *Functional enrichment analysis*

Description

Performs Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Disease Ontology (DO) enrichment analyses by **clusterProfiler** and **DOSE** packages

Usage

```
gdcEnrichAnalysis(gene, simplify = TRUE, level = 0)
```

Arguments

gene	a vector of Ensembl gene id
simplify	logical, specifying whether to remove redundant GO terms. Default simplify=TRUE
level	a numeric value, restrict the GO enrichment result at a specific GO level. Default is 0, which means all terms should be returned

Value

A dataframe of enrichment analysis result containing enriched terms, number of overlpped genes, p value of hypergeometric test, fdr, fold of enrichment, Ensembl gene ids, gene symbols, and functional categories, etc.

Author(s)

Ruidong Li and Han Qu

References

Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. Omics: a journal of integrative biology. 2012 May 1;16(5):284-7.
Yu G, Wang LG, Yan GR, He QY. DOSE: an R/Bioconductor package for disease ontology semantic and enrichment analysis. Bioinformatics. 2014 Oct 17;31(4):608-9.

Examples

```
####### GO, KEGG, DO enrichment analysis #######
deg <- c('ENSG00000000938','ENSG00000000971','ENSG00000001036',
        'ENSG00000001084','ENSG00000001167','ENSG00000001460')
## Not run: enrichOutput <- gdcEnrichAnalysis(gene=deg, simplify=TRUE)
```

---

gdcEnrichPlot *Plots for enrichment analysis*

Description

Bar plot and bubble plot for GO, KEGG, and DO functional enrichment analysis

Usage

```
gdcEnrichPlot(enrichment, type = "bar", category = "KEGG",
  num.terms = 10, bar.color = "black")
```

Arguments

enrichment	a dataframe generated from gdcEnrichAnalysis
type	type of the plot, should be one of 'bar' and 'bubble'
category	which category should be plotted. Possible values are 'KEGG', 'GO', 'GO_BP', 'GO_CC', 'GO_MF', and 'DO'. Default is 'KEGG'
num.terms	number of terms to be plotted. Default is 10
bar.color	color of the bar plot. Default is 'black'

Value

A bar plot or bubble plot of functional enrichment analysis

Author(s)

Ruidong Li and Han Qu

Examples

```
####### Enrichment plots #######
enrichOutput<-data.frame(Terms=c('hsa05414~Dilated cardiomyopathy (DCM)',
                                 'hsa04510~Focal adhesion',
                                 'hsa05205~Proteoglycans in cancer'),
                         Category=rep('KEGG',3),
                         FDR=c(0.001,0.002,0.003))
gdcEnrichPlot(enrichment=enrichOutput, type='bar', category='KEGG')
```

gdcExportNetwork *Export network for Cytoscape*

Description

Export nodes and edges of ce network for **Cytoscape** visualization

Usage

```
gdcExportNetwork(ceNetwork, net)
```

Arguments

ceNetwork	a dataframe generated from gdcCEAnalysis
net	one of 'nodes' and 'edges'

Value

A dataframe of nodes or edges

Author(s)

Ruidong Li and Han Qu

Examples

```
####### ceRNA network analysis #######
ceOutput <- data.frame(lncRNAs=c('ENSG00000242125','ENSG00000242125',
                                 'ENSG00000245532'),
                       Genes=c('ENSG00000043355','ENSG00000109586',
                                 'ENSG00000144355'),
                       miRNAs=c('hsa-miR-340-5p','hsa-miR-340-5p',
                              'hsa-miR-320b,hsa-miR-320d,
                              hsa-miR-320c,hsa-miR-320a'),
                       Counts=c(1,1,4), stringsAsFactors=FALSE)
####### Export edges #######
edges <- gdcExportNetwork(ceNetwork=ceOutput, net='edges')

####### Export nodes #######
## Not run: nodes <- gdcExportNetwork(ceNetwork=ceOutput, net='nodes')
```

gdcFilterDuplicate *Filter out duplicated samples*

Description

Filter out samples that are sequenced for two or more times

Usage

```
gdcFilterDuplicate(metadata)
```

Arguments

metadata	metadata parsed from gdcParseMetadata

Value

A filtered dataframe of metadata without duplicated samples

Author(s)

Ruidong Li and Han Qu

Examples

```
####### Parse metadata by project id and data type #######
metaMatrix <- gdcParseMetadata(project.id='TARGET-RT', data.type='RNAseq')
metaMatrix <- gdcFilterDuplicate(metadata=metaMatrix)
```

gdcFilterSampleType *Filter out other type of samples*

Description

Filter out samples that are neither *Solid Tissue Normal* nor *Primary Tumor*

Usage

```
gdcFilterSampleType(metadata)
```

Arguments

metadata	metadata parsed from gdcParseMetadata

Value

A filtered dataframe of metadata with *Solid Tissue Normal* and *Primary Tumor* samples only

Author(s)

Ruidong Li and Han Qu

Examples

```
####### Parse metadata by project id and data type #######
metaMatrix <- gdcParseMetadata(project.id='TARGET-RT', data.type='RNAseq')
metaMatrix <- gdcFilterSampleType(metadata=metaMatrix)
```

---

gdcHeatmap *Heatmap of differentially expressed genes/miRNAs*

Description

A heatmap showing unsupervised hierarchical clustering of DE genes/miRNAs by heatmap.2 in the **gplots** package

Usage

```
gdcHeatmap(deg.id, metadata, rna.expr)
```

Arguments

deg.id	a vector of Ensembl gene ids or miRBase v21 mature miRNA ids
metadata	metadata parsed from gdcParseMetadata
rna.expr	voom transformed expression data

Value

A heatmap with rows are DE genes/miRNAs and columns are samples. *Solid Tissue Normal* samples are labeled with blue and *Primary Tumor* samples are labeled with red

Author(s)

Ruidong Li and Han Qu

Examples

```
genes <- c('ENSG00000000938','ENSG00000000971','ENSG00000001036',
        'ENSG00000001084','ENSG00000001167','ENSG00000001460')

samples <- c('TCGA-2F-A9KO-01', 'TCGA-2F-A9KP-01',
            'TCGA-2F-A9KQ-01', 'TCGA-2F-A9KR-01',
             'TCGA-2F-A9KT-01', 'TCGA-2F-A9KW-01')

metaMatrix <- data.frame(sample_type=rep('PrimaryTumor',6),
                         sample=samples,
                         days_to_death=seq(100,600,100),
```

```
                        days_to_last_follow_up=rep(NA,6))
rnaExpr <- matrix(c(2.7,7.0,4.9,6.9,4.6,2.5,
                    0.5,2.5,5.7,6.5,4.9,3.8,
                    2.1,2.9,5.9,5.7,4.5,3.5,
                    2.7,5.9,4.5,5.8,5.2,3.0,
                    2.5,2.2,5.3,4.4,4.4,2.9,
                    2.4,3.8,6.2,3.8,3.8,4.2),6,6)
rownames(rnaExpr) <- genes
colnames(rnaExpr) <- samples
gdcHeatmap(deg.id=genes, metadata=metaMatrix, rna.expr=rnaExpr)
```

---

gdcKMPlot *Kaplan Meier plot*

Description

Plot Kaplan Meier survival curve

Usage

```
gdcKMPlot(gene, rna.expr, metadata, sep = "median")
```

Arguments

gene	an Ensembl gene id
rna.expr	voom transformed expression data
metadata	metadata parsed from gdcParseMetadata
sep	a character string specifying which point should be used to separate low-expression and high-expression groups. Possible values are '1stQu', 'mean', 'median', and '3rdQu'. Default is 'median'

Value

A plot of Kaplan Meier survival curve

Author(s)

Ruidong Li and Han Qu

Examples

```
####### KM plots #######
genes <- c('ENSG00000000938','ENSG00000000971','ENSG00000001036',
        'ENSG00000001084','ENSG00000001167','ENSG00000001460')

samples <- c('TCGA-2F-A9KO-01', 'TCGA-2F-A9KP-01',
            'TCGA-2F-A9KQ-01', 'TCGA-2F-A9KR-01',
            'TCGA-2F-A9KT-01', 'TCGA-2F-A9KW-01')
```

```
metaMatrix <- data.frame(sample_type=rep('PrimaryTumor',6),
                         sample=samples,
                         days_to_death=seq(100,600,100),
                         days_to_last_follow_up=rep(NA,6))
rnaExpr <- matrix(c(2.7,7.0,4.9,6.9,4.6,2.5,
                    0.5,2.5,5.7,6.5,4.9,3.8,
                    2.1,2.9,5.9,5.7,4.5,3.5,
                    2.7,5.9,4.5,5.8,5.2,3.0,
                    2.5,2.2,5.3,4.4,4.4,2.9,
                    2.4,3.8,6.2,3.8,3.8,4.2),6,6)
rownames(rnaExpr) <- genes
colnames(rnaExpr) <- samples
gdcKMPlot(gene='ENSG00000000938', rna.expr=rnaExpr,
    metadata=metaMatrix, sep='median')
```

`gdcMatchSamples` *Match samples in metadata and expression matrix*

Description

Check if samples in the metadata and expression data match

Usage

```
gdcMatchSamples(metadata, rna.expr)
```

Arguments

`metadata`	metadata parsed from `gdcParseMetadata`
`rna.expr`	`voom` transformed expression data

Value

A logical value. If `TRUE`, all the samples matched

Author(s)

Ruidong Li and Han Qu

Examples

```
genes <- c('ENSG00000000938','ENSG00000000971','ENSG00000001036',
        'ENSG00000001084','ENSG00000001167','ENSG00000001460')

samples <- c('TCGA-2F-A9KO-01', 'TCGA-2F-A9KP-01',
            'TCGA-2F-A9KQ-01', 'TCGA-2F-A9KR-01',
            'TCGA-2F-A9KT-01', 'TCGA-2F-A9KW-01')
```

```
metaMatrix <- data.frame(sample_type=rep('PrimaryTumor',6),
                         sample=samples,
                         days_to_death=seq(100,600,100),
                         days_to_last_follow_up=rep(NA,6))
rnaExpr <- matrix(c(2.7,7.0,4.9,6.9,4.6,2.5,
                    0.5,2.5,5.7,6.5,4.9,3.8,
                    2.1,2.9,5.9,5.7,4.5,3.5,
                    2.7,5.9,4.5,5.8,5.2,3.0,
                    2.5,2.2,5.3,4.4,4.4,2.9,
                    2.4,3.8,6.2,3.8,3.8,4.2),6,6)
rownames(rnaExpr) <- genes
colnames(rnaExpr) <- samples
gdcMatchSamples(metadata=metaMatrix, rna.expr=rnaExpr)
```

---

gdcParseMetadata *Parse metadata*

Description

Parse metadata either by providing the *.json* file that is downloaded from GDC cart or by parse metadata automatically by providing the projct id and data type

Usage

```
gdcParseMetadata(metafile = NULL, project.id, data.type,
  write.meta = FALSE)
```

Arguments

metafile	metadata file in .json format download from GDC cart. If provided, the metadata will be parsed from this file, otherwise, project and data.type arguments should be provided to retrieve metadata automatically. Default is NULL
project.id	project id in GDC
data.type	one of 'RNAseq' and 'miRNAs'
write.meta	logical, whether to write the metadata to a .json file

Value

A dataframe of metadata containing file_name, sample_id, etc. as well as some basic clinical data

Author(s)

Ruidong Li and Han Qu

Examples

```
####### Merge RNA expression data #######
metaMatrix <- gdcParseMetadata(project.id='TARGET-RT', data.type='RNAseq')
```

gdcRNADownload *Download RNA data in GDC*

Description

Download gene expression quantification and isoform expression quantification data from GDC either by providing the manifest file or by sepcifying the project id and data type

Usage

```
gdcRNADownload(manifest = NULL, project.id, data.type,
  directory = "Data", write.manifest = FALSE, method = "gdc-client")
```

Arguments

manifest	menifest file that is downloaded from the GDC cart. If provided, files whose UUIDs are in the manifest file will be downloaded via gdc-client, otherwise, project and data.type arguments should be provided to download data automatically. Default is NULL
project.id	project id in GDC
data.type	one of 'RNAseq' and 'miRNAs'
directory	the folder to save downloaded files. Default is 'Data'
write.manifest	logical, whether to write out the manifest file
method	method that is used to download data. Either 'GenomicDataCommons' which is a well established method developed in the **GenomicDataCommons'** package, or alternatively 'gdc-client' which uses the gdc-client tool developed by GDC. Default is 'gdc-client'.

Value

Downloaded files in the specified directory

Author(s)

Ruidong Li and Han Qu

Examples

```
####### Download RNA data by menifest file #######
manifest <- 'RNAseq.manifest.txt'
## Not run: gdcRNADownload(manifest=manifest)

####### Download RNA data by project id and data type #######
project <- 'TCGA-PRAD'
## Not run: gdcRNADownload(project.id=project, data.type='RNAseq')
```

gdcRNAMerge *Merge RNA/miRNAs raw counts data*

Description

Merge raw counts data that is downloaded from GDC to a single expression matrix

Usage

```
gdcRNAMerge(metadata, path, data.type, organized = FALSE)
```

Arguments

metadata	metadata parsed from gdcParseMetadata
path	path to downloaded files for merging
data.type	one of 'RNAseq' and 'miRNAs'
organized	logical, whether the raw counts data have already been organized into a single folder (eg., data downloaded by the 'GenomicDataCommons' method are already organized). Default is FALSE.

Value

A dataframe or numeric matrix of raw counts data with rows are genes or miRNAs and columns are samples

Author(s)

Ruidong Li and Han Qu

Examples

```
####### Merge RNA expression data #######
metaMatrix <- gdcParseMetadata(project.id='TARGET-RT',
    data.type='RNAseq')
## Not run: rnaExpr <- gdcRNAMerge(metadata=metaMatrix, path='RNAseq/',
    data.type='RNAseq')
## End(Not run)
```

`gdcSurvivalAnalysis` *Univariate survival analysis of multiple genes*

Description

Univariate Cox Proportional-Hazards and Kaplan Meier survival analysis of a vector of genes

Usage

```
gdcSurvivalAnalysis(gene, rna.expr, metadata, method = "coxph",
  sep = "median")
```

Arguments

`gene`	a vector of Ensembl gene ids
`rna.expr`	`voom` transformed expression data
`metadata`	metadata parsed from `gdcParseMetadata`
`method`	method for survival analysis. Possible values are `'coxph'` and `'KM'`. Default is `'coxph'`
`sep`	which point should be used to separate low-expression and high-expression groups for `method='KM'`. Possible values are `'1stQu'`, `'mean'`, `'median'`, and `'3rdQu'`. Default is `'median'`

Value

A dataframe or numeric matrix of hazard ratio, 95% confidence interval, p value, and FDR

Author(s)

Ruidong Li and Han Qu

References

Therneau TM, Lumley T. Package 'survival'.
Andersen PK, Gill RD. Cox's regression model for counting processes: a large sample study. The annals of statistics. 1982 Dec 1:1100-20.
Therneau TM, Grambsch PM. Extending the Cox model. Edited by P. Bickel, P. Diggle, S. Fienberg, K. Krickeberg. 2000:51.
Harrington DP, Fleming TR. A class of rank test procedures for censored survival data.Biometrika. 1982 Dec 1;69(3):553-66.

Examples

```
genes <- c('ENSG00000000938','ENSG00000000971','ENSG00000001036',
        'ENSG00000001084','ENSG00000001167','ENSG00000001460')

samples <- c('TCGA-2F-A9KO-01', 'TCGA-2F-A9KP-01',
            'TCGA-2F-A9KQ-01', 'TCGA-2F-A9KR-01',
            'TCGA-2F-A9KT-01', 'TCGA-2F-A9KW-01')

metaMatrix <- data.frame(sample_type=rep('PrimaryTumor',6),
                         sample=samples,
                         days_to_death=seq(100,600,100),
                         days_to_last_follow_up=rep(NA,6))
rnaExpr <- matrix(c(2.7,7.0,4.9,6.9,4.6,2.5,
                    0.5,2.5,5.7,6.5,4.9,3.8,
                    2.1,2.9,5.9,5.7,4.5,3.5,
                    2.7,5.9,4.5,5.8,5.2,3.0,
                    2.5,2.2,5.3,4.4,4.4,2.9,
                    2.4,3.8,6.2,3.8,3.8,4.2),6,6)
rownames(rnaExpr) <- genes
colnames(rnaExpr) <- samples
survOutput <- gdcSurvivalAnalysis(gene=genes,
    rna.expr=rnaExpr, metadata=metaMatrix)
```

`gdcVolcanoPlot` *Volcano plot of differentially expressed genes/miRNAs*

Description

A volcano plot showing differentially expressed genes/miRNAs

Usage

```
gdcVolcanoPlot(deg.all, fc = 2, pval = 0.01)
```

Arguments

`deg.all`	a dataframe generated from `gdcDEAnalysis` containing all genes of analysis no matter they are differentially expressed or not
`fc`	a numeric value specifying the threshold of fold change
`pval`	a nuemric value specifying the threshold of p value

Value

A volcano plot

Author(s)

Ruidong Li and Han Qu

Examples

```
genes <- c('ENSG00000231806','ENSG00000261211','ENSG00000260920',
        'ENSG00000228594','ENSG00000125170','ENSG00000179909',
        'ENSG00000280012','ENSG00000134612','ENSG00000213071')
symbol <- c('PCAT7','AL031123.2','AL031985.3',
            'FNDC10','DOK4','ZNF154',
            'RPL23AP61','FOLH1B','LPAL2')
group <- rep(c('long_non_coding','protein_coding','pseudogene'), each=3)
logFC <- c(2.8,2.3,-1.1,1.9,-1.2,-1.6,1.5,2.1,-1.1)
FDR <- rep(c(0.1,0.00001,0.0002), each=3)
deg <- data.frame(symbol, group, logFC, FDR)
rownames(deg) <- genes
gdcVolcanoPlot(deg.all=deg)
```

`gdcVoomNormalization` *TMM normalization and voom transformation*

Description

Normalize raw counts data by TMM implemented in **edgeR** and then transform it by `voom` in **limma**

Usage

```
gdcVoomNormalization(counts, filter = TRUE)
```

Arguments

`counts`	raw counts of RNA/miRNA expression data
`filter`	logical, whether to filter out low-expression genes. If `TRUE`, only genes with `cpm` > 1 in more than half of the samples will be kept. Default is `TRUE`

Value

A dataframe or numeric matrix of TMM normalized and `voom` transformed expression values on the log2 scale

Author(s)

Ruidong Li and Han Qu

References

Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010 Jan 1;26(1):139-40.
Law CW, Chen Y, Shi W, Smyth GK. Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome biology. 2014 Feb 3;15(2):R29.

Examples

```
####### Normalization #######
rnaMatrix <- matrix(sample(1:100,100), 4, 25)
rnaExpr <- gdcVoomNormalization(counts=rnaMatrix, filter=FALSE)
```

`lncTarget` *miRNA-lncRNA interactions*

Description

miRNA-lncRNA interactions

`mirCounts` *miRNA counts data of TCGA-CHOL*

Description

miRNA counts data of TCGA-CHOL

`pcTarget` *miRNA-mRNA interactions*

Description

miRNA-mRNA interactions

`rnaCounts` *RNAseq counts data of TCGA-CHOL*

Description

RNAseq counts data of TCGA-CHOL

shinyCorPlot *Shiny correlation plot*

Description

A simple **shiny** app to show scatter plot of correlations between two genes/miRNAs on local web browser

Usage

```
shinyCorPlot(gene1, gene2, rna.expr, metadata)
```

Arguments

gene1	a vector of Ensembl gene ids or miRBase v21 mature miRNA ids
gene2	a vector of Ensembl gene ids or miRBase v21 mature miRNA ids
rna.expr	voom transformed expression data
metadata	metadata parsed from gdcParseMetadata

Value

a local webpage for visualization of correlation plots

Author(s)

Ruidong Li and Han Qu

Examples

```
genes <- c('ENSG00000000938','ENSG00000000971','ENSG00000001036',
        'ENSG00000001084','ENSG00000001167','ENSG00000001460')

samples <- c('TCGA-2F-A9KO-01', 'TCGA-2F-A9KP-01',
            'TCGA-2F-A9KQ-01', 'TCGA-2F-A9KR-01',
            'TCGA-2F-A9KT-01', 'TCGA-2F-A9KW-01')

metaMatrix <- data.frame(sample_type=rep('PrimaryTumor',6),
                         sample=samples,
                         days_to_death=seq(100,600,100),
                         days_to_last_follow_up=rep(NA,6))
rnaExpr <- matrix(c(2.7,7.0,4.9,6.9,4.6,2.5,
                    0.5,2.5,5.7,6.5,4.9,3.8,
                    2.1,2.9,5.9,5.7,4.5,3.5,
                    2.7,5.9,4.5,5.8,5.2,3.0,
                    2.5,2.2,5.3,4.4,4.4,2.9,
                    2.4,3.8,6.2,3.8,3.8,4.2),6,6)
rownames(rnaExpr) <- genes
colnames(rnaExpr) <- samples
```

```
## Not run: shinyCorPlot(gene1=genes[1:3], gene2=genes[4:5], rna.expr=rnaExpr,
    metadata=metaMatrix)
## End(Not run)
```

---

shinyKMPlot *Shiny Kaplan Meier (KM) plot*

Description

A simple **shiny** app to show KM survival curves on local web browser

Usage

```
shinyKMPlot(gene, rna.expr, metadata)
```

Arguments

gene	a vector of Ensembl gene ids
rna.expr	voom transformed expression data
metadata	metadata parsed from gdcParseMetadata

Value

a local webpage for visualization of KM plots

Author(s)

Ruidong Li and Han Qu

Examples

```
genes <- c('ENSG00000000938','ENSG00000000971','ENSG00000001036',
        'ENSG00000001084','ENSG00000001167','ENSG00000001460')

samples <- c('TCGA-2F-A9KO-01', 'TCGA-2F-A9KP-01',
            'TCGA-2F-A9KQ-01', 'TCGA-2F-A9KR-01',
            'TCGA-2F-A9KT-01', 'TCGA-2F-A9KW-01')

metaMatrix <- data.frame(sample_type=rep('PrimaryTumor',6),
                         sample=samples,
                         days_to_death=seq(100,600,100),
                         days_to_last_follow_up=rep(NA,6))
rnaExpr <- matrix(c(2.7,7.0,4.9,6.9,4.6,2.5,
                    0.5,2.5,5.7,6.5,4.9,3.8,
                    2.1,2.9,5.9,5.7,4.5,3.5,
                    2.7,5.9,4.5,5.8,5.2,3.0,
                    2.5,2.2,5.3,4.4,4.4,2.9,
                    2.4,3.8,6.2,3.8,3.8,4.2),6,6)
rownames(rnaExpr) <- genes
```

```
colnames(rnaExpr) <- samples
## Not run: shinyKMPlot(gene=genes, rna.expr=rnaExpr,
    metadata=metaMatrix)
## End(Not run)
```

---

shinyPathview *Shiny pathview*

Description

A simple **shiny** app to show pathways genetrated by **pathview** package on local web browser

Usage

```
shinyPathview(gene, pathways, directory = ".")
```

Arguments

gene	a vector of numeric values (eg. fold change on log2 scale) with names are Ensembl gene ids
pathways	a vector of KEGG pathway ids
directory	the folder to save pathway figures. Default is the working directory

Value

a local webpage for visualization of KEGG maps

Author(s)

Ruidong Li and Han Qu

Examples

```
genes <- c('ENSG00000000938','ENSG00000000971','ENSG00000001036',
        'ENSG00000001084','ENSG00000001167','ENSG00000001460')
pathways <- c("hsa05414~Dilated cardiomyopathy (DCM)",
            "hsa05410~Hypertrophic cardiomyopathy (HCM)",
            "hsa05412~Arrhythmogenic right ventricular cardiomyopathy",
            "hsa04512~ECM-receptor interaction",
            "hsa04510~Focal adhesion",
            "hsa04360~Axon guidance",
            "hsa04270~Vascular smooth muscle contraction",
            "hsa05205~Proteoglycans in cancer",
            "hsa04022~cGMP-PKG signaling pathway",
            "hsa00480~Glutathione metabolism")
## Not run: shinyPathview(gene=genes, pathways=pathways)
```

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