Package 'TRAPR'

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Title Statistical analysis and visualization of RNA-seq data

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Description High-throughput transcriptome sequencing, also known as RNA-Seq, is a standard technology for measuring gene expression at an unprecedented accuracy. Numerous Bioconductor packages have been developed for statistical analysis of RNA-Seq derived expression data. However, those tools focus on specific aspects of data analysis pipeline and lack visualization methods to confirm data integrity and process. Here, we present an R based RNA-Seq analysis pipeline, TRAPR, an integrated tool including statistical analysis and visualization of RNA-Seq expression data. TRAPR provides various functions for data management, filtering for low quality data, normalization, transformation, statistical analysis, data visualization, and result visualization that researchers can constitute customized pipeline.

Depends R (>= 2.15.0), edgeR, ggplot2, gplots, gridExtra,preprocessCore, reshape2, vsn

License GPL-2

R topics documented:

TRAPR-package *Statistical analysis and visualization of RNA-seq data*

Description

High-throughput transcriptome sequencing, also known as RNA-Seq, is a standard technology for measuring gene expression at an unprecedented accuracy. Numerous Bioconductor packages have been developed for statistical analysis of RNA-Seq derived expression data. However, those tools focus on specific aspects of data analysis pipeline and lack visualization methods to confirm data integrity and process. Here, we present an R based RNA-Seq analysis pipeline, TRAPR, an integrated tool including statistical analysis and visualization of RNA-Seq expression data. TRAPR provides various functions for data management, filtering for low quality data, normalization, transformation, statistical analysis, data visualization, and result visualization that researchers can constitute customized pipeline.

Details

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Date:	2012-10-10
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Data <- TRAPR.Data.ReadExpressionTable('TRAPR_Sample.txt', sep='\t', Exp1, Exp2) Data <- TRAPR.Filter.ZeroValues(Data) Data <- TRAPR.Normalize.UpperQuartile(Data) Data <- TRAPR.StatisticalTest(Data, Method, FDRControl, Pvalue) TRAPR.ResultVisualization(Data, 'ALL')

Author(s)

Jae Hyun Lim, Soo Youn Lee, Ju Han Kim

Maintainer: juhan@snu.ac.kr

References

Anders, S. and Huber, W. (2010) Differential expression analysis for sequence count data, Genome biology, 11, R106. Auer, P.L., Srivastava, S. and Doerge, R.W. (2012) Differential expression– the next generation and beyond, Briefings in functional genomics, 11, 57-62. Bullard, J.H., et al. (2010) Evaluation of statistical methods for normalization and differential expression in mRNA-Seq experiments, BMC bioinformatics, 11, 94. Calza, S., et al. (2007) Filtering genes to improve sensitivity in oligonucleotide microarray data analysis, Nucleic acids research, 35, e102. Garber, M., et al. (2011) Computational methods for transcriptome annotation and quantification using RNA-seq, Nature methods, 8, 469-477. Gentleman, R.C., et al. (2004) Bioconductor: open software development for computational biology and bioinformatics, Genome biology, 5, R80. Hardcastle, T.J. and Kelly, K.A. (2010) baySeq: empirical Bayesian methods for identifying differential expression in sequence count data, BMC bioinformatics, 11, 422. Huber, W., et al. (2002) Variance stabilization applied to microarray data calibration and to the quantification of differential expression, Bioinformatics, 18 Suppl 1, S96-104. Kadota, K., Nishiyama, T. and Shimizu, K. (2012) A normalization strategy for comparing tag count data, Algorithms for molecular biology : AMB, 7, 5. Robinson, M.D., McCarthy, D.J. and Smyth, G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data, Bioinformatics, 26, 139-140. Sultan, M., et al. (2008) A global view of gene activity and alternative splicing by deep sequencing of the human transcriptome, Science, 321, 956-960. Tarazona, S., et al. (2011) Differential expression in RNA-seq: a matter of depth, Genome research, 21, 2213-2223. Wang, L., et al. (2010) DEGseq: an R package for identifying differentially expressed genes from RNA-seq data, Bioinformatics, 26, 136-138. Wang, Z., Gerstein, M. and Snyder, M. (2009) RNA-Seq: a revolutionary tool for transcriptomics, Nature reviews. Genetics, 10, 57-63.

`TRAPR.Data.ChangeExp` *TRAPR Functions for label change*

Description

TRAPR Functions for experiment label change. In TRAPR data type, columns indicate biological conditions. If you have misunderstood biological conditions for each columns, you can fix it by this function.

Usage

```
TRAPR.Data.ChangeExp(Data, Exp1, Exp2)
```

Arguments

`Data`	TRAPR type data.
`Exp1`	Column numbers which belongs biological condition 1.
`Exp2`	Column numbers which belongs biological condition 2.

Value

TRAPR type data

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
str(TRAPRExample)
TRAPRExample <- TRAPR.Data.ChangeExp(TRAPRExample, Exp1 = c(1:8), Exp2 = c(9:19))
str(TRAPRExample)
```

`TRAPR.Data.DEGNameListtoFile`

TRAPR Functions to save list of differentially expressed genes as files

---

Description

TRAPR Functions to save list of differentially expressed genes as files.

Usage

```
TRAPR.Data.DEGNameListtoFile(Data, FileNamePrefix = "DEGList")
```

Arguments

`Data`	TRAPR type data.
`FileNamePrefix`	Prefix for name of output files

Value

Two files containing list of DEGs.

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPRExample <- TRAPR.StatisticalTest(TRAPRExample)
TRAPR.Data.DEGNameListtoFile(TRAPRExample)
```

`TRAPR.Data.DEGResulttoFile`

TRAPR Functions to save analysis results

Description

TRAPR Functions to save analysis results, which includes p-values, q-values, FC, gene list, and relative expression level.

Usage

```
TRAPR.Data.DEGResulttoFile(Data, FileName = "Result.txt")
```

Arguments

Data	TRAPR type data.
FileName	Name for result file.

Value

A file containing analysis result.

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPRExample <- TRAPR.StatisticalTest(TRAPRExample)
TRAPR.Data.DEGResulttoFile(TRAPRExample)
```

TRAPR.Data.ExpressionMatrixtoFile

TRAPR Function to save processed expression matrix

Description

TRAPR Function to save processed expression matrix which is able to apply other tools.

Usage

```
TRAPR.Data.ExpressionMatrixtoFile(Data, FileName = "output.txt")
```

Arguments

Data	TRAPR type data
FileName	Name of output files

Value

A file containing processed(normalized, transformed, or filtered) expression matrix.

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPRExample <- TRAPR.Normalize(TRAPRExample, Method = 'UpperQuartile')
TRAPR.Data.ExpressionMatrixtoFile
```

TRAPR.Data.ReadExpressionTable

TRAPR Function to read files

Description

TRAPR Functions to read files containing expression tables, whose columns are samples, rows are features, and cells are expression levels.

Usage

```
TRAPR.Data.ReadExpressionTable(File, sep = "\t", Exp1, Exp2, Tag = c("Exp1", "Exp2"))
```

Arguments

File	the name of the file which the data are to be read from.
sep	the field separator charactor.
Exp1	column numberes which belong biological condition 1.
Exp2	column numbers which belong biological condition 2.
Tag	Names for each biological condition. must be vector of two character variables.

Value

TRAPR Data type

Author(s)

Jae Hyun Lim

TRAPR.Data.ReadGeneList

TRAPR Functions to read gene list

Description

TRAPR Functions to read list of genes.

Usage

```
TRAPR.Data.ReadGeneList(File)
```

Arguments

File	the name of the file which the gene list are to be read from

Value

A vector containing list of genes

Author(s)

Jae Hyun Lim

`TRAPR.DataVisualization` *TRAPR Functions for pre-analysis data(filtering + normalization) visualization*

Description

visualization. Data visualization function include 5 kinds of plots(MA plot, Mean' and 'Variance plot, Scatter plot, density plot).'ALL' is plotting all kinds plots together.

Usage

```
TRAPR.DataVisualization(Data, type_of_plot, logged = TRUE)
```

Arguments

`Data`	gene matirx of after filtering and normalization
`type_of_plot`	Type of plot for result visulaization.Data visualization function include 5 kinds of plots(MA plot, box plot, mean and variance plot, scatter plot, density plot). MA plot : 'MA' box plot : 'box' Mean and variance plot : 'MV' Scatter plot : 'SE' Density plot : 'DS'
`logged`	whether the data has been log transformed or not.

Value

`box`	boxplot
`MA`	MA plot
`MV`	Mean and Variance plot
`SE`	Scatter plot
`DS`	Density plot
`ALL`	Plotting all kinds plots together

Author(s)

Soo Yeon Lee, Jae Hyun Lim

References

ggplot2 : http://had.co.nz/ggplot2/

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', logged = FALSE)
TRAPRExample <- TRAPR.Normalize(TRAPRExample, Method = 'UpperQuartile')
TRAPR.DataVisualization(TRAPRExample, 'ALL', logged = FALSE)
TRAPR.DataVisualization(TRAPRExample, 'ALL', logged = TRUE)
TRAPRExample <- TRAPR.Transformation.log2(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', logged = TRUE)
```

TRAPR.Filter.GeneDeletion *TRAPR Function for filtering genes.*

Description

This function is TRAPR Function for filtering genes.

Usage

```
TRAPR.Filter.GeneDeletion(Data, Outlier)
```

Arguments

Data	TRAPR type data
Outlier	Row numbers of genes need to be filtered

Value

TRAPR type data which does not include genes that filtered

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
TRAPRExample <- TRAPR.Filter.GeneDeletion(TRAPRExample, 1)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
```

TRAPR.Filter.GeneList *TRAPR Function for filtering genes.*

Description

This function is TRAPR Functions for filtering genes.

Usage

```
TRAPR.Filter.GeneList(Data, GeneList)
```

Arguments

Data	TRAPR type data
GeneList	List of genes of concerns.

Value

TRAPR type data which only includes genes of concerns.

Author(s)

Jae Hyun Lim

`TRAPR.Filter.LowExpression` *TRAPR Function for filtering low expressed genes.*

Description

This function is TRAPR Function for filtering low expressed genes. In typical RNA-seq experiments, genes which have low expression level normally does not have enough read for valid statistical analysis.

Usage

```
TRAPR.Filter.LowExpression(Data, Method = "mean", Thre = 0.01)
```

Arguments

`Data`	TRAPR type data
`Method`	Criteria for distinguishing low expressed genes. mean : mean of expression levels of gene. min : minimum of expression levels of gene. max : maximum of expression levels of gene. median : median of expression levels of gene.
`Thre`	Threshold for distinguishing low expressed genes.

Value

TRAPR type data which does not include low expressed genes.

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
TRAPRExample <- TRAPR.Filter.LowExpression(TRAPRExample, Method = 'mean', Thre = 0.01)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
```

TRAPR.Filter.LowVariance

TRAPR Function for filtering genes which have low variance

Description

This function is TRAPR Function for filtering genes which have low variance.

Usage

```
TRAPR.Filter.LowVariance(Data, Thre = 0.1)
```

Arguments

Data	TRAPR type data
Thre	Threshold for distinguishing genes which have low variance.

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
TRAPRExample <- TRAPR.Filter.LowVariance(TRAPRExample, Thre = 0.1)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
```

TRAPR.Filter.SampleDeletion

TRAPR Function for fitering samples.

Description

This function is TRAPR Function for filtering outliers.

Usage

```
TRAPR.Filter.SampleDeletion(Data, Outlier)
```

Arguments

Data	TRAPR type data
Outlier	Column numbers of outliers.

Value

TRAPR type data which does not include outliers.

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
TRAPRExample <- TRAPR.Filter.SampleDeletion(TRAPRExample, c(1:5))
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
```

TRAPR.Filter.ZeroValue

TRAPR Function for filtering genes which have zero expression levels.

Description

This function is TRAPR function for filtering genes which have zero expression levels in whole columns or each biological condition. It might interrupt further analysis procedures as log2 transformation or statistical test like t-test.

Usage

```
TRAPR.Filter.ZeroValue(Data)
```

Arguments

Data	TRAPR type data

Value

TRAPR type data which does not include genes that have zero expression levels. Filtered genes are saved in 'NonExpressedGenes', 'Exp1OnlyGene', 'Exp2OnlyGene'

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
TRAPRExample <- TRAPR.Filter.ZeroValue(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
```

TRAPR.Normalize *TRAPR Function for Normalization*

Description

This is TRAPR function for normalization. TRAPR provides 4 methods to normalize; mean, median, quantile, and upperquartile normalization.

Usage

```
TRAPR.Normalize(Data, Method = "UpperQuartile")
```

Arguments

Data	TRAPR type data
Method	Normalization method; 'UpperQuartile', 'Quantile', 'Median', 'Mean' default and recommended method is UpperQuartile Normalization.

Value

Normalized TRAPR data

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
TRAPRExample <- TRAPR.Normalize(TRAPRExample, Method = 'UpperQuartile')
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
```

TRAPR.Normalize.Mean *TRAPR Function for mean normalization*

Description

This is TRAPR function for normalization by mean

Usage

```
TRAPR.Normalize.Mean(Data)
```

Arguments

Data	TRAPR type data

Value

Normalized TRAPR type data

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
TRAPRExample <- TRAPR.Normalize.Mean(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
```

TRAPR.Normalize.Median

TRAPR Function for median normalization

Description

This is TRAPR function for normalization by median

Usage

```
TRAPR.Normalize.Median(Data)
```

Arguments

Data	TRAPR type data

Value

Normalized TRAPR type data

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
TRAPRExample <- TRAPR.Normalize.Median(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
```

TRAPR.Normalize.Quantile

TRAPR Function for quantile normalization

Description

This is TRAPR function for quantile normalization, by preprocessCore package.

Usage

```
TRAPR.Normalize.Quantile(Data)
```

Arguments

Data	TRAPR type data

Value

Normalized TRAPR type data

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
TRAPRExample <- TRAPR.Normalize.Quantile(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
```

TRAPR.Normalize.UpperQuartile

TRAPR Function for Upper quartile normalization

Description

This is TRAPR function for Upper quanrile normalization.

Usage

```
TRAPR.Normalize.UpperQuartile(Data)
```

Arguments

Data	TRAPR type data

Value

Normalized TRAPR type data

Author(s)

Jae Hyun Lim

References

Bullard, J.H., et al. (2010) Evaluation of statistical methods for normalization and differential expression in mRNA-Seq experiments, BMC bioinformatics, 11, 94.

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
TRAPRExample <- TRAPR.Normalize.UpperQuartile(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL', FALSE)
```

TRAPR.ResultVisualization

TRAPR Functions for Result Visualization

Description

This function is used to result data of statistical testing visualization. This function provide 2 kinds of plots. The one plot is Heatmap and the other is volcano plot.

Usage

```
TRAPR.ResultVisualization(Data, type_of_plot)
```

Arguments

Data	TRAPR data type
type_of_plot	Type of plot for result visulaization. TRAPR provide 2 kind of plots(heatmap, volcano plot). 'ALL' is plotting all kinds plots together. type_of_plot='ALL', 'HM','VO'

Value

Result plot

Author(s)

Soo Yeon Lee, Jae Hyun Lim

References

ggplot2 : http://had.co.nz/ggplot2/

Examples

```
data(TRAPRExample)
TRAPRExample <- TRAPR.StatisticalTest(TRAPRExample)
TRAPR.ResultVisualization(TRAPRExample, type_of_plot = 'ALL')
```

TRAPR.StatisticalTest *TRAPR Function for statistical test*

Description

This is TRAPR function for statistical test. Currently, TRAPR provides 4 testing methods; t-test, wilcoxon, edgeR based method, Fold Change.

Usage

```
TRAPR.StatisticalTest(Data, Method = "ttest", FDRControl = "none", PvalueThre = 0.01, FCThre = 0
```

Arguments

Data	TRAPR type data
Method	Statistical method you want. 'ttest', 'wilcoxon', 'edgeR', 'FC'
FDRControl	FDR Control method. "holm", "hochberg", "hommel", "bonferroni", "BH", "BY","fdr", "none"
PvalueThre	Threshold for p or qvalue
FCThre	Threshold for Fold Change

Value

TRAPR type data which includes results of statistical test

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPRExample <- TRAPR.StatisticalTest(TRAPRExample, Method = 'ttest', FDRControl = 'none', PvalueThre = 0.0
TRAPR.ResultVisualization(TRAPRExample, 'ALL')
```

TRAPR.StatisticalTest.EdgeR *TRAPR Function for statistical test by EdgeR based method*

Description

TRAPR Function for statistical test by EdgeR based method

Usage

```
TRAPR.StatisticalTest.EdgeR(Data)
```

Arguments

Data	TRAPR type data

Value

TRAPR type data which includes results of statistical test

Author(s)

Jae Hyun Lim

References

Robinson, M.D., McCarthy, D.J. and Smyth, G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data, Bioinformatics, 26, 139-140.

`TRAPR.StatisticalTest.FC`
TRAPR Function for Fold Change

Description

TRAPR Function for Fold Change

Usage

```
TRAPR.StatisticalTest.FC(Data)
```

Arguments

`Data`	TRAPR type data

Value

TRAPR type data which includes results of statistical test

Author(s)

Jae Hyun Lim

`TRAPR.StatisticalTest.ReThreshold`
TRAPR Function for rethreshold

Description

TRAPR Function for re-adjust p-values and FC for statistical test.

Usage

```
TRAPR.StatisticalTest.ReThreshold(Data, PvalueThre = 0.01, FCThre = 0)
```

Arguments

Data	TRAPR type data
PvalueThre	threshold for P-value
FCThre	threshold for FC

Value

TRAPR type data which includes results of statistical test

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPRExample <- TRAPR.StatisticalTest(TRAPRExample)
TRAPR.ResultVisualization(TRAPRExample, 'ALL')
TRAPRExample <- TRAPR.StatisticalTest.ReThreshold(TRAPRExample, PvalueThre = 0.1, FCThre = 0.5)
TRAPR.ResultVisualization(TRAPRExample, 'ALL')
```

TRAPR.StatisticalTest.ttest

TRAPR Function for statistical test by t-test

Description

TRAPR Function for statistical test by t-test

Usage

```
TRAPR.StatisticalTest.ttest(Data)
```

Arguments

Data	TRAPR type data

Value

TRAPR type data which includes results of statistical test

Author(s)

Jae Hyun Lim

TRAPR.StatisticalTest.Wilcoxon

TRAPR Function for statistical test by wilcoxon-ranksum test

Description

TRAPR Function for statistical test by wilcoxon-ranksum test

Usage

```
TRAPR.StatisticalTest.Wilcoxon(Data)
```

Arguments

Data	TRAPR type data

Value

TRAPR type data which includes results of statistical test

Author(s)

Jae Hyun Lim

TRAPR.Transformation.log2

TRAPR Function for log2 transformation

Description

TRAPR Function for log2 transformation

Usage

```
TRAPR.Transformation.log2(Data)
```

Arguments

Data	TRAPR type data

Value

TRAPR type data which contains log2 transformed expression matrix

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL')
TRAPRExample <- TRAPR.Transformation.log2(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL')
```

`TRAPR.Transformation.VSN` *TRAPR Function for VSN transformation*

Description

TRAPR Function for VSN transformation

Usage

```
TRAPR.Transformation.VSN(Data)
```

Arguments

`Data`	TRAPR type data

Value

TRAPR type data which contains VSN transformed expression matrix

Author(s)

Jae Hyun Lim

Examples

```
data(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL')
TRAPRExample <- TRAPR.Transformation.VSN(TRAPRExample)
TRAPR.DataVisualization(TRAPRExample, 'ALL')
```

`TRAPRExample` *TRAPR Example data from TCGA breast cancer RNA-seq data.*

Description

TRAPR Example data from TCGA breast cancer RNA-seq data.

Usage

```
data(TRAPRExample)
```

Format

The format is: List of 17 $ Exp1 : int [1:9] 1 2 3 4 5 6 7 8 9 $ Exp2 : int [1:10] 10 11 12 13 14 15 16 17 18 19 $ Tag : chr [1:2] "Exp1" "Exp2" $ SampleTag : chr [1:19] "Cancer1" "Cancer2" "Cancer3" "Cancer4" ... $ GeneTag : chr [1:1000] "ARHGEF10L" "HIF3A" "RNF17" "RNF10" ... $ RawMatrix : num [1:1000, 1:19] 7.302 0.337 0 42.578 42.499 ... $ CurrentMatrix : num [1:1000, 1:19] 7.302 0.337 0 42.578 42.499 ... $ CurrentSample : chr [1:19] "Cancer1" "Cancer2" "Cancer3" "Cancer4" ... $ CurrentGene : chr [1:1000] "ARHGEF10L" "HIF3A" "RNF17" "RNF10" ... $ Exp1OnlyGene : chr "NA" $ Exp2OnlyGene : chr "NA" $ NonExpressedGene: chr "NA" $ pvalues : chr "NA" $ qvalues : chr "NA" $ DEGName : chr "NA" $ DEGIndex : chr "NA" $ FC : chr "NA" - attr(*, "class")= chr "TRAPR"

References

http://cancergenome.nih.gov/

Examples

```
data(TRAPRExample)
str(TRAPRExample)
```

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