Package ‘Linnorm’

March 22, 2017

Type Package

Title Linear model and normality based transformation method (Linnorm)

Version 1.2.11

Date 2016-03-10

Author Shun Hang Yip <shunyip@bu.edu>, Panwen Wang <pwwang@pwwang.com>,
Jean-Pierre Kocher <Kocher.JeanPierre@mayo.edu>, Pak Chung Sham <pcsham@hku.hk>,
Junwen Wang <junwen@uw.edu>

Maintainer Ken Shun Hang Yip <shunyip@bu.edu>

Description Please note that significant updates to Linnorm are available in version 1.99.x +, we strongly suggest using the newest version.
Linnorm is an R package for the analysis of RNA-seq, scRNA-seq, ChIP-seq count data or any large scale count data. It transforms such datasets for parametric tests.
In addition to the transformation function, the following pipelines are implemented:
1. Cell subpopulation analysis and visualization using PCA clustering,
2. Differential expression analysis or differential peak detection using limma,
3. Highly variable gene discovery and visualization,
4. Gene correlation network analysis and visualization,
5. Hierarchical clustering and plotting.
Linnorm can work with raw count, CPM, RPKM, FPKM and TPM. Additionally, Linnorm provides the RnaXSim function for the simulation of RNA-seq raw counts for the evaluation of differential expression analysis methods. RnaXSim can simulate RNA-seq dataset in Gamma, Log Normal, Negative Binomial or Poisson distributions.

Depends R(>= 3.3)

License MIT + file LICENSE

Imports Rcpp (>= 0.12.2), RcppArmadillo, fpc, vegan, mclust,
apcluster, ggplot2, ellipse, limma, utils, statmod, MASS,
igraph, grDevices, graphics, fastcluster, ggdendro, zoo, stats,
amap

LinkingTo Rcpp, RcppArmadillo

Suggests BiocStyle, knitr, rmarkdown, ggplot, RColorBrewer

VignetteBuilder knitr

biocViews Sequencing, ChIPSeq, RNASeq, DifferentialExpression,
GeneExpression, Genetics, Normalization, Software,
Transcription, BatchEffect, PeakDetection, Clustering, Network

NeedsCompilation yes

LazyData false
Islam2011

URL: http://www.jjwanglab.org/Linnorm/
RoxygenNote: 5.0.1

R topics documented:

Islam2011 ..................................................... 2
LIHC .......................................................... 3
Linnorm ........................................................ 3
Linnorm.Cor .................................................. 4
Linnorm.HClust .............................................. 6
Linnorm.HVar ............................................... 8
Linnorm.limma .............................................. 10
Linnorm.PCA ................................................. 11
RnaXSim ..................................................... 13
SEQC .......................................................... 14

Index 15

Islam2011  scRNA-seq data from Islam et al. 2011

Description

GEO accession GSE29087: 92 single cells (48 mouse embryonic stem cells, 44 mouse embryonic fibroblasts and 4 negative controls) were analyzed by single-cell tagged reverse transcription (STRT).

Usage

data(Islam2011)

Format

A matrix with 22936 rows (genes) and 96 columns (samples). The first 48 columns are ES cells, the following 44 columns are mouse embryonic fibroblasts and the remaining 4 columns and negative controls. Data is in raw counts format.

References

**LIHC**

Partial RNA-seq data from TCGA LIHC (Liver Hepatocellular Carcinoma)

**Description**

TPM Expression data

**Usage**

data(LIHC)

**Format**

A matrix with 25914 rows (genes) and 20 columns (samples). The first 10 columns are Tumor samples, the remaining 10 columns are adjacent Normal samples. They are paired samples from 10 individuals. Data is in TPM format.

**References**

https://tcga-data.nci.nih.gov/

**Linnorm**

Linnorm Transformation Function

**Description**

This function performs the Linear model and normality based transformation method (Linnorm) for RNA-seq expression data or large scale count data.

**Usage**

Linnorm(datamatrix, showinfo = FALSE, method = "default", 
perturbation = 10, minZeroPortion = 2/3, keepAll = TRUE)

**Arguments**

- **datamatrix**
  The matrix or data frame that contains your dataset. Each row is a feature (or Gene) and each column is a sample (or replicate). Raw Counts, CPM, RPKM, FPKM or TPM are supported. Undefined values such as NA are not supported. It is not compatible with log transformed datasets.

- **showinfo**
  Logical. Show lambda value calculated. Defaults to FALSE.

- **method**
  Character. "default" or "lambda" The program will output the transformed matrix if the method is "default". If the method is "lambda", the program will output a lambda value.
perturbation Integer >= 2. To search for an optimal minimal deviation parameter (please see the article), Linnorm uses the iterated local search algorithm which perturbs away from the initial local minimum. The range of the area searched in each perturbation is exponentially increased as the area get further away from the initial local minimum, which is determined by their index. This range is calculated by 10 ^ (perturbation ^ index).

minZeroPortion Double >= 0, <= 1. For example, setting minZeroPortion as 0.5 will remove genes with more than half data values being zero in the calculation of normalizing parameter. It is strongly suggested to change this to 0.5 for single cell RNA-seq data. Defaults to 2/3.

keepAll Logical. After applying minZeroPortion filtering, should Linnorm keep all genes in the results? Defaults to TRUE.

Details

If method is default, Linnorm outputs a transformed expression matrix. For users who wish to work with lambda instead, the output is a single lambda value. Please note that users with the lambda value can obtain a transformed Linnorm dataset by: log1p(lambda * datamatrix). There is no need to rerun the program if a lambda is already calculated.

Value

This function returns either a transformed data matrix or a lambda value.

Examples

# Obtain example matrix:
data(LIHC)
# Transformation:
transformedExp <- Linnorm(LIHC)
transformedExp <- Linnorm(LIHC, method = "lambda")

Linnorm.Cor  

Linnorm-gene correlation network analysis.

Description

This function first performs Linnorm transformation on the dataset. Then, it will perform correlation network analysis on the dataset.

Usage

Linnorm.Cor(datamatrix, input = "Raw", method = "pearson", showinfo = FALSE, perturbation = 10, minZeroPortion = 2/3, sig.q = 0.05, plotNetwork = TRUE, plotNumPairs = 5000, plotdegree = 0, plotname = "networkplot", plotformat = "png", plotVertexSize = 1, plotFontSize = 1, plot.Pos.cor.col = "red", plot.Neg.cor.col = "green", vertex.col = "cluster", plotlayout = "kk", clusterMethod = "cluster_edge_betweenness")
Arguments

**datamatrix**  The matrix or data frame that contains your dataset. Each row is a feature (or Gene) and each column is a sample (or replicate). Raw Counts, CPM, RPKM, FPKM or TPM are supported. Undefined values such as NA are not supported. It is not compatible with log transformed datasets. If a Linnorm transforemed dataset is being used, please set the "input" argument into "Linnorm".

**input**  Character. "Raw" or "Linnorm". In case you have already transformed your dataset with Linnorm, set input into "Linnorm" so that you can input the Linnorm transformed dataset into the "datamatrix" argument. Defaults to "Raw".

**method**  Character. "pearson", "kendall" or "spearman". Method for the calculation of correlation coefficients. Defaults to "pearson"

**showinfo**  Logical. Show lambda value calculated. Defaults to FALSE.

**perturbation**  Integer >=2. To search for an optimal minimal deviation parameter (please see the article), Linnorm uses the iterated local search algorithm which perturbs away from the initial local minimum. The range of the area searched in each perturbation is exponentially increased as the area get further away from the initial local minimum, which is determined by their index. This range is calculated by 10 * (perturbation ^ index).

**minZeroPortion**  Double >=0, <= 1. For example, setting minZeroPortion as 0.5 will remove genes with more than half data values being zero in the calculation of normalizing parameter. Since this test is based on correlation coefficient, which requires more non-zero values, it is suggested to set it to a larger value. Defaults to 2/3.

**sig.q**  Double >=0, <= 1. Only gene pairs with q values less than this threshold will be included in the "Results" data frame. Defaults to 0.05.

**plotNetwork**  Logical. Should the program output the network plot to a file? An "igraph" object will be included in the output regardless. Defaults to TRUE.

**plotNumPairs**  Integer >= 50. Number of gene pairs to be used in the network plot. Defaults to 5000.

**plotdegree**  Integer >= 0. In the network plot, genes (vertices) without at least this number of degree will be removed. Defaults to 0.

**plotname**  Character. Name of the network plot. File extension will be appended to it. Defaults to "networkplot".

**plotformat**  Character. "pdf" or "png". Network plot output format. Defaults to "png".

**plotVertexSize**  Double >0. Controls vertex Size in the network plot. Defaults to 1.

**plotFontSize**  Double >0. Controls font Size in the network plot. Defaults to 1.

**plot.Pos.cor.col**  Character. Color of the edges of positively correlated gene pairs. Defaults to "red".

**plot.Neg.cor.col**  Character. Color of the edges of negatively correlated gene pairs. Defaults to "green".

**vertex.col**  Character. "cluster" or a color. This controls the color of the vertices. Defaults to "cluster".

**plotlayout**  Character. "kk" or "fr". "kk" uses Kamada-Kawai algorithm in igraph to assign vertex and edges. It scales edge length with correlation strength. However, it can cause overlaps between vertices. "fr" uses Fruchterman-Reingold algorithm in igraph to assign vertex and edges. It prevents overlaps between vertices better than "kk", but edge lengths are not scaled to correlation strength. Defaults to "kk".

Linnorm.HClust

clusterMethod  Character. "cluster_edge_betweenness", "cluster_fast_greedy", "cluster_infomap", "cluster_label_prop", "cluster_leading_eigen", "cluster_louvain", "cluster_optimal", "cluster_spinglass" or "cluster_walktrap". These are clustering functions from the igraph package. Defaults to "cluster_edge_betweenness".

Details

This function performed gene correlated study in the dataset by using Linnorm transformation.

Value

This function will output a list with the following objects:

• Results: A data frame containing the results of the analysis, showing only the significant results determined by "sig.q" (see below).
• Cor.Matrix: The resulting correlation matrix between each gene.
• q.Matrix: A matrix of q values of each of the correlation coefficient from Cor.Matrix.
• Cluster: A data frame that shows which gene belongs to which cluster.
• igraph: The igraph object for users who want to draw the network plot manually.
• Linnorm: Linnorm transformed and filtered data matrix.

The "Results" data frame has the following columns:

• Gene1: Name of gene 1.
• Gene2: Name of gene 2.
• XPM1: Gene 1 average expression level in XPM. If input is raw counts or CPM, this column is in CPM unit. If input is RPKM, FPKM or TPM, this column is in the TPM unit.
• XPM2: Gene 2 average expression level in XPM. If input is raw counts or CPM, this column is in CPM unit. If input is RPKM, FPKM or TPM, this column is in the TPM unit.
• Cor: Correlation coefficient between the two genes.
• p.value: p value of the correlation coefficient.
• q.value: q value of the correlation coefficient.

Examples

data(Islam2011)
#Analysis on Islam2011 embryonic stem cells
results <- Linnorm.Cor(Islam2011[,1:48])

Linnorm.HClust  Linnorm-hierarchical clustering analysis.

Description

This function first performs Linnorm transformation on the dataset. Then, it will perform hierarchical clustering analysis.
### Linnorm.HClust

#### Usage

```r
Linnorm.HClust(datamatrix, showinfo = FALSE, input = "Raw",
perturbation = 10, minZeroPortion = 0, keepAll = TRUE,
method_hclust = "ward.D2", method_dist = "maximum", Group = NULL,
num_Clust = 4, ClustRect = TRUE, RectColor = "red", fontsize = 0.5,
linethickness = 0.5)
```

#### Arguments

- **datamatrix**: The matrix or data frame that contains your dataset. Each row is a feature (or Gene) and each column is a sample (or replicate). Raw Counts, CPM, RPKM, FPKM or TPM are supported. Undefined values such as NA are not supported. It is not compatible with log transformed datasets. If a Linnorm transfored dataset is being used, please set the "input" argument into "Linnorm".

- **showinfo**: Logical. Show information about the computing process. Defaults to FALSE.

- **input**: Character. "Raw" or "Linnorm". In case you have already transformed your dataset with Linnorm, set input into "Linnorm" so that you can input the Linnorm transformed dataset into the "datamatrix" argument. Defaults to "Raw".

- **perturbation**: Integer >=2. To search for an optimal minimal deviation parameter (please see the article), Linnorm uses the iterated local search algorithm which perturbs away from the initial local minimum. The range of the area searched in each perturbation is exponentially increased as the area get further away from the initial local minimum, which is determined by their index. This range is calculated by 10 * (perturbation ^ index).

- **minZeroPortion**: Double >=0, <= 1. For example, setting minZeroPortion as 0.5 will remove genes with more than half data values being zero in the calculation of normalizing parameter. Defaults to 0.

- **keepAll**: Logical. After applying minZeroPortion filtering, should Linnorm keep all genes in the results? Defaults to TRUE.

- **method_hclust**: Character. Method to be used in hierarchical clustering. (From hclust fastcluster: the agglomeration method to be used. This should be (an unambiguous abbreviation of) one of "ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median" or "centroid".) Defaults to "ward.D2".

- **method_dist**: Character. Method to be used in hierarchical clustering. (From Dist amap: the distance measure to be used. This must be one of "euclidean", "maximum", "manhattan", "canberra", "binary", "pearson", "correlation", "spearman" or "kendall". Any unambiguous substring can be given.) Defaults to "maximum".

- **Group**: Character vector with length equals to sample size. Each character in this vector corresponds to each of the columns (samples) in the datamatrix. If this is provided, sample names will be colored according to their group. Defaults to NULL.

- **num_Clust**: Integer >= 0. Number of clusters in hierarchical clustering. No cluster will be highlighted if this is set to 0. Defaults to 4.

- **ClustRect**: Logical. If num_Clust > 0, should a rectangle be used to highlight the clusters? Defaults to TRUE.

- **RectColor**: Character. If ClustRect is TRUE, this controls the color of the rectangle. Defaults to "red".

- **fontsize**: Numeric. Font size of the texts in the figure. Defaults to 0.5.

- **linethickness**: Numeric. Controls the thickness of the lines in the figure. Defaults to 0.5.
Details
This function performs PCA clustering using Linnorm transformation.

Value
It returns a list with the following objects:

- Results: If num_Clust > 0, this outputs a named vector that contains the cluster assignment information of each sample. Else, this outputs a number 0.
- plot: Plot of hierarchical clustering.
- Linnorm: Linnorm transformed and filtered data matrix.

Examples
# Obtain example matrix:
data(Islam2011)
# Example:
HClust.results <- Linnorm.HClust(Islam2011, Group=c(rep("ESC",48), rep("EF",44), rep("NegCtrl",4)), num_Clust=3, fontsize=2)

Linnorm.HVar
Linnorm-Hvar pipeline for highly variable gene discovery.

Description
This function first performs Linnorm transformation on the dataset. Then, it will perform highly variable gene discovery.

Usage
Linnorm.HVar(datamatrix, input = "Raw", method = "SD", spikein = NULL, showinfo = FALSE, perturbation = 10, minZeroPortion = 2/3, keepAll = FALSE, log.p = FALSE, sig.value = "p", sig = 0.05)

Arguments
- datamatrix: The matrix or data frame that contains your dataset. Each row is a feature (or Gene) and each column is a sample (or replicate). Raw Counts, CPM, RPKM, FPKM or TPM are supported. Undefined values such as NA are not supported. It is not compatible with log transformed datasets. If a Linnorm transfored dataset is being used, please set the "input" argument into "Linnorm".
- input: Character. "Raw" or "Linnorm". In case you have already transformed your dataset with Linnorm, set input into "Linnorm" so that you can input the Linnorm transformed dataset into the "datamatrix" argument. Defaults to "Raw".
- method: Character. "SE" or "SD". Use Standard Error (SE) or Standard Deviation (SD) to calculate p values. Defaults to SD.
- spikein: character vector. Row names of the spike-in genes in the datamatrix. If this is provided, test of significance will be performed against the spike in genes. Defaults to NULL.
- showinfo: Logical. Show lambda value calculated. Defaults to FALSE.
perturbation  Integer >=2. To search for an optimal minimal deviation parameter (please see the article), Linnorm uses the iterated local search algorithm which perturbs away from the initial local minimum. The range of the area searched in each perturbation is exponentially increased as the area get further away from the initial local minimum, which is determined by their index. This range is calculated by 10 * (perturbation ^ index).

minZeroPortion  Double >=0, <= 1. For example, setting minZeroPortion as 0.5 will remove genes with more than half data values being zero in the calculation of normalizing parameter. Since this test is based on variance, which requires more non-zero values, it is suggested to set it to a larger value. Defaults to 2/3.

keepAll  Logical. After applying minZeroPortion filtering, should Linnorm keep all genes in the results? Defualts to FALSE.

log.p  Logical. Output p/q values in log scale. Defaults to FALSE.

sig.value  Character. "p" or "q". Use p or q value for highlighting significant genes. Defaults to "p".

sig  Double >0, <= 1. Significant level of p or q value for plotting. Defaults to 0.05.

Details

This function discovers highly variable gene in the dataset using Linnorm transformation.

Value

This function will output a list with the following objects:

- Results: A matrix with the results.
- plot: Mean vs Standard Deviation Plot which highlights significant genes.
- Linnorm: Linnorm transformed and filtered data matrix.

The Results matrix has the following columns:

- XPM: Average non-zero expression level in XPM. If input is raw counts or CPM, this column is in CPM unit. If input is RPKM, FPKM or TPM, this column is in the TPM unit.
- XPM.SD: Standard deviation of average non-zero expression.
- Transformed.SD: Standard deviation of non-zero Linnorm transformed data.
- p.value: p value of the statistical test.
- q.value: q value/false discovery rate/adjusted p value of the statistical test.

Examples

data(Islam2011)
results <- Linnorm.HVar(Islam2011)
Linnorm.limma pipeline for Differentially Expression Analysis

Description

This function first performs Linnorm transformation on the dataset. Then, it will perform limma for DEG analysis. Please cite both Linnorm and limma when you use this function for publications.

Usage

Linnorm.limma(datamatrix, design = NULL, input = "Raw", output = "DEResults", noINF = TRUE, showinfo = FALSE, perturbation = 10, minZeroPortion = 2/3, keepAll = TRUE, robust = TRUE)

Arguments

datamatrix The matrix or data frame that contains your dataset. Each row is a feature (or Gene) and each column is a sample (or replicate). Raw Counts, CPM, RPKM, FPKM or TPM are supported. Undefined values such as NA are not supported. It is not compatible with log transformed datasets. If a Linnorm transfored dataset is being used, please set the "input" argument into "Linnorm".
design A design matrix required for limma. Please see limma’s documentation or our vignettes for more detail.
input Character. "Raw" or "Linnorm". In case you have already transformed your dataset with Linnorm, set input into "Linnorm" so that you can input the Linnorm transformed dataset into the "datamatrix" argument. Defaults to "Raw".
output Character. "DEResults" or "Both". Set to "DEResults" to output a matrix that contains Differential Expression Analysis Results. Set to "Both" to output a list that contains both Differential Expression Analysis Results and the transformed data matrix.
noINF Logical. Prevent generating INF in the fold change column by using Linnorm’s lambda and adding one. If it is set to FALSE, INF will be generated if one of the conditions has zero expression. Defaults to TRUE.
showinfo Logical. Show lambda value calculated. Defaults to FALSE.
perturbation Integer >=2. To search for an optimal minimal deviation parameter (please see the article), Linnorm uses the iterated local search algorithm which perturbs away from the initial local minimum. The range of the area searched in each perturbation is exponentially increased as the area get further away from the initial local minimum, which is determined by their index. This range is calculated by 10 * (perturbation ^ index).
minZeroPortion Double >=0, <= 1. For example, setting minZeroPortion as 0.5 will remove genes with more than half data values being zero in the calculation of normalizing parameter. It is strongly suggested to change this to 0.5 for single cell RNA-seq data. Defaults to 2/3.
keepAll Logical. After applying minZeroPortion filtering, should Linnorm keep all genes in the results? Defaults to TRUE.
robust Logical. In the eBayes function of Limma, run with robust setting with TRUE or FALSE. Defaults to TRUE.
Details
This function performs both Linnorm and limma for users who are interested in differential expression analysis. Please note that if you directly use a Linnorm Normalized dataset with limma, the output fold change and average expression with be wrong. (p values and adj.pvalues will be fine.) This is because the voom-limma pipeline assumes input to be in raw counts. This function is written to fix this problem.

Value
If output is set to "DEResults", this function will output a matrix with Differential Expression Analysis Results with the following columns:

- logFC: Log 2 Fold Change
- XPM: Average Expression. If input is raw count or CPM, this column has the CPM unit. If input is RPKM, FPKM or TPM, this column has the TPM unit.
- t: moderated t-statistic
- P.Value: p value
- adj.P.Val: Adjusted p value. This is also called False Discovery Rate or q value.
- B: log odds that the feature is differential

If output is set to Both, this function will output a list with the following objects:

- DEResults: Differential Expression Analysis Results as described above.
- Linnorm: Linnorm transformed and filtered data matrix.

Examples
#Obtain example matrix:
data(LIHC)
#Create limma design matrix (first 10 columns are tumor, last 10 columns are normal)
designmatrix <- c(rep(1,10),rep(2,10))
designmatrix <- model.matrix(~ 0+factor(designmatrix))
colnames(designmatrix) <- c("group1", "group2")
rownames(designmatrix) <- colnames(LIHC)
#DEG analysis
DEGResults <- Linnorm.limma(LIHC, designmatrix)

Linnorm.PCA

Linnorm-PCA Clustering pipeline for subpopulation Analysis

Description
This function first performs Linnorm transformation on the dataset. Then, it will perform Principal component analysis on the dataset and use k-means clustering to identify subpopulations of cells.

Usage
Linnorm.PCA(datamatrix, showinfo = FALSE, input = "Raw",
perturbation = 10, minZeroPortion = 0, keepAll = TRUE, num_PC = 2,
um_center = c(1:20), Group = NULL, Coloring = "Group",
pca.scale = FALSE, kmeans.iter = 2000)
### Arguments

**datamatrix**  The matrix or data frame that contains your dataset. Each row is a feature (or Gene) and each column is a sample (or replicate). Raw Counts, CPM, RPKM, FPKM or TPM are supported. Undefined values such as NA are not supported. It is not compatible with log transformed datasets. If a Linnorm transformed dataset is being used, please set the "input" argument into "Linnorm".

**showinfo**  Logical. Show information about the computing process. Defaults to FALSE.

**input**  Character. "Raw" or "Linnorm". In case you have already transformed your dataset with Linnorm, set input into "Linnorm" so that you can input the Linnorm transformed dataset into the "datamatrix" argument. Defaults to "Raw".

**perturbation**  Integer >=2. To search for an optimal minimal deviation parameter (please see the article), Linnorm uses the iterated local search algorithm which perturbs away from the initial local minimum. The range of the area searched in each perturbation is exponentially increased as the area get further away from the initial local minimum, which is determined by their index. This range is calculated by 10 * \( perturbation ^\text{index} \).

**minZeroPortion**  Double >=0, <= 1. For example, setting minZeroPortion as 0.5 will remove genes with more than half data values being zero in the calculation of normalizing parameter. Defaults to 0.

**keepAll**  Logical. After applying minZeroPortion filtering, should Linnorm keep all genes in the results? Defaults to TRUE.

**num_PC**  Integer >= 2. Number of principal components to be used in K-means clustering. Defaults to 3.

**num_center**  Numeric vector. Number of clusters to be tested for k-means clustering. fpc, vegan, mclust and apcluster packages are used to determine the number of clusters needed. If only one number is supplied, it will be used and this test will be skipped. Defaults to c(1:20).

**Group**  Character vector with length equals to sample size. Each character in this vector corresponds to each of the columns (samples) in the datamatrix. This is for plotting purposes only. In the plot, the shape of the points that represent each sample will be indicated by their group assignment. Defaults to NULL.

**Coloring**  Character. "kmeans" or "Group". If Group is not NA, coloring in the PCA plot will reflect each sample’s group. Otherwise, coloring will reflect k means clustering results. Defaults to "Group".

**pca.scale**  Logical. In the prcomp(for Principal component analysis) function, set the "scale." parameter. It signals the function to scale unit variances in the variables before the analysis takes place. Defaults to FALSE.


### Details

This function performs PCA clustering using Linnorm transformation.

### Value

It returns a list with the following objects:

- **k_means**: Output of kmeans(for K-means clustering) from the stat package. Note: It contains a "cluster" object that indicates each sample’s cluster assignment.
• PCA: Output of prcomp(for Principal component analysis) from the stat package.
• plot: Plot of PCA clustering.
• Linnorm: Linnorm transformed and filtered data matrix.

Examples

```r
# Obtain example matrix:
data(Islam2011)
# Example:
PCA.results <- Linnorm.PCA(Islam2011)
```

---

**RnaXSim**  
This function simulates a RNA-seq dataset based on a given distribution.

**Description**

This function simulates a RNA-seq dataset based on a given distribution.

**Usage**

```r
RnaXSim(datamatrix, distribution = "Poisson", NumRep = 3, NumDiff = 2000, 
NumFea = 20000, showinfo = FALSE, DEGlog2FC = "Auto", 
MaxLibSizeLog2FC = 0.5)
```

**Arguments**

- **datamatrix**  
  Matrix. The matrix or data frame that contains your dataset. Each row is a feature (or Gene) and each column is a sample (or replicate). Raw Counts, CPM, RPKM, FPKM or TPM are supported. Undefined values such as NA are not supported. It is not compatible with log transformed datasets. This program assumes that all columns are replicates of the same sample.

- **distribution**  
  Character: Defaults to "Poisson". This parameter controls the output distribution of the simulated RNA-seq dataset. It can be one of "Gamma" (Gamma distribution), "Poisson" (Poisson distribution), "LogNorm" (Log Normal distribution) or "NB" (Negative Binomial distribution).

- **NumRep**  
  Integer: The number of replicates. This is half of the number of output samples. Defaults to 3.

- **NumDiff**  
  Integer: The number of Differentially Changed Features. Defaults to 2000.

- **NumFea**  
  Integer: The number of Total Features. Defaults to 20000.

- **showinfo**  
  Logical: should we show data information on the console? Defaults to FALSE.

- **DEGlog2FC**  
  "Auto" or Double: log 2 fold change threshold that defines differentially expressed genes. If set to "Auto," DEGlog2FC is defined at the level where ANOVA can get a q value of 0.05 with the average expression, where the data values are log1p transformed. Defaults to "Auto".

- **MaxLibSizeLog2FC**  
  Double: The maximum library size difference from the mean that is allowed, in terms of log 2 fold change. Set to 0 to prevent program from generating library size differences. Defaults to 0.5.
Value

This function returns a list that contains a matrix of count data in integer raw count and a vector that shows which genes are differentially expressed. In the matrix, each row is a gene and each column is a replicate. The first NumRep (see parameter) of the columns belong to sample 1, and the last NumRep (see parameter) of the columns belong to sample 2. There will be NumFea (see parameter) number of rows. The top NumCorr of genes will be positively or negatively correlated with each other (randomly); and they are evenly separated into groups. Each group is not intended to be correlated to each other, but, by chance, it can happen.

Examples

# Obtain example matrix:
data(SEQC)
SampleA <- SEQC
# Extract a portion of the matrix for an example
expMatrix <- SampleA[,1:10]
# Example for Negative Binomial distribution
simulateddata <- RnaXSim(expMatrix, distribution="NB", NumRep=3, NumDiff = 200, NumFea = 2000)
# Example for Poisson distribution
simulateddata <- RnaXSim(expMatrix, distribution="Poisson", NumRep=3, NumDiff = 200, NumFea = 2000)
# Example for Log Normal distribution
simulateddata <- RnaXSim(expMatrix, distribution="LogNorm", NumRep=3, NumDiff = 200, NumFea = 2000)
# Example for Gamma distribution
simulateddata <- RnaXSim(expMatrix, distribution="Gamma", NumRep=3, NumDiff = 200, NumFea = 2000)

SEQC Partial RNA-seq data from SEQC/MAQC-III Sample A

Description

Raw Count data

Usage

data(SEQC)

Format

A matrix with 50227 rows (genes) and 10 columns (samples).

References

# Index

- **Topic Analysis**
  - Linnorm.PCA, 11
- **Topic CPM**
  - Linnorm, 3
  - Linnorm.Cor, 4
  - Linnorm.HClust, 6
  - Linnorm.HVar, 8
  - Linnorm.limma, 10
  - Linnorm.PCA, 11
- **Topic Clustering**
  - Linnorm.HClust, 6
  - Linnorm.PCA, 11
- **Topic Component**
  - Linnorm.PCA, 11
- **Topic Count**
  - Linnorm, 3
  - Linnorm.Cor, 4
  - Linnorm.HClust, 6
  - Linnorm.HVar, 8
  - Linnorm.limma, 10
  - Linnorm.PCA, 11
  - RnaXSim, 13
- **Topic Expression**
  - Linnorm, 3
  - Linnorm.Cor, 4
  - Linnorm.HClust, 6
  - Linnorm.HVar, 8
  - Linnorm.limma, 10
  - Linnorm.PCA, 11
  - RnaXSim, 13
- **Topic FPKM**
  - Linnorm, 3
  - Linnorm.Cor, 4
  - Linnorm.HClust, 6
  - Linnorm.HVar, 8
  - Linnorm.limma, 10
  - Linnorm.PCA, 11
- **Topic Gamma**
  - RnaXSim, 13
- **Topic K-means**
  - Linnorm.PCA, 11
- **Topic Linnorm**
  - Linnorm, 3
  - Linnorm.Cor, 4
  - Linnorm.HClust, 6
  - Linnorm.HVar, 8
  - Linnorm.limma, 10
  - Linnorm.PCA, 11
  - RnaXSim, 13
- **Topic Log**
  - RnaXSim, 13
- **Topic Negative**
  - RnaXSim, 13
- **Topic PCA**
  - Linnorm.PCA, 11
- **Topic Parametric**
  - Linnorm, 3
  - Linnorm.Cor, 4
  - Linnorm.HClust, 6
  - Linnorm.HVar, 8
  - Linnorm.limma, 10
  - Linnorm.PCA, 11
- **Topic Poisson**
  - RnaXSim, 13
- **Topic Principal**
  - Linnorm.PCA, 11
- **Topic RNA-seq**
  - Linnorm, 3
  - Linnorm.Cor, 4
  - Linnorm.HClust, 6
  - Linnorm.HVar, 8
  - Linnorm.limma, 10
  - Linnorm.PCA, 11
  - RnaXSim, 13
- **Topic RPKM**
  - Linnorm, 3
  - Linnorm.Cor, 4
  - Linnorm.HClust, 6
  - Linnorm.HVar, 8
  - Linnorm.limma, 10
  - Linnorm.PCA, 11
- **Topic Raw**
  - Linnorm, 3
  - Linnorm.Cor, 4
  - Linnorm.HClust, 6
  - Linnorm.HVar, 8
  - Linnorm.limma, 10
INDEX

Linnorm.PCA, 11
RnaXSim, 13
*Topic Simulate
RnaXSim, 13
*Topic Simulation
RnaXSim, 13
*Topic TPM
Linnorm, 3
Linnorm.Cor, 4
Linnorm.HClust, 6
Linnorm.HVar, 8
Linnorm.limma, 10
Linnorm.PCA, 11
*Topic coefficient
Linnorm.Cor, 4
*Topic correlation
Linnorm.Cor, 4
*Topic distribution
RnaXSim, 13
*Topic hierarchical
Linnorm.HClust, 6
*Topic highly
Linnorm.HVar, 8
*Topic k-means
Linnorm.PCA, 11
*Topic kendall
Linnorm.Cor, 4
*Topic kmeans
Linnorm.PCA, 11
*Topic limma
Linnorm.limma, 10
*Topic normalization
Linnorm, 3
Linnorm.Cor, 4
Linnorm.HClust, 6
Linnorm.HVar, 8
Linnorm.limma, 10
Linnorm.PCA, 11
*Topic pearson
Linnorm.Cor, 4
*Topic spearman
Linnorm.Cor, 4
*Topic transformation
Linnorm, 3
Linnorm.Cor, 4
Linnorm.HClust, 6
Linnorm.HVar, 8
Linnorm.limma, 10
Linnorm.PCA, 11
*Topic variable
Linnorm.HVar, 8
*Topic variance

Islam2011, 2
LIHC, 3
Linnorm, 3
Linnorm.Cor, 4
Linnorm.HClust, 6
Linnorm.HVar, 8
Linnorm.limma, 10
Linnorm.PCA, 11
RnaXSim, 13
SEQC, 14