Package ‘lfproQC’

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Type Package

Title Quality Control for Label-Free Proteomics Expression Data

Version 0.1.0

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Description Label-free bottom-up proteomics expression data is often affected by data heterogeneity and missing values. Normalization and missing value imputation are commonly used techniques to address these issues and make the dataset suitable for further downstream analysis. This package provides an optimal combination of normalization and imputation methods for the dataset. The package utilizes three normalization methods and three imputation methods. The statistical evaluation measures named pooled coefficient of variance, pooled estimate of variance and pooled median absolute deviation are used for selecting the best combination of normalization and imputation method for the given dataset. The user can also visualize the results by using various plots available in this package. The user can also perform the differential expression analysis between two sample groups with the function included in this package. The chosen three normalization methods, three imputation methods and three evaluation measures were chosen for this study based on the research papers published by Välikangas et al. (2016) <doi:10.1093/bib/bbw095>, Jin et al. (2021) <doi:10.1038/s41598-021-81279-4> and Srivastava et al. (2023) <doi:10.2174/1574893618666230223150253>.

Imports VIM, dplyr, limma, matrixStats, pcaMethods, vsn, reshape2, laeken, ggplot2, Hmisc, reshape, stats, tidyr, magrittr, plotly, MASS, tidyselect

Suggests knitr, plyr, rmarkdown, testthat (>= 3.0.0), tibble

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Best combination

Description

This function will provide the best combinations of normalization and imputation methods for the user given dataset based on the intragroup variation evaluation parameters called PCV, PEV and PMAD.

Usage

best_combination(data_input, groups)

Arguments

data_input: Label-free proteomics expression data as a dataframe

groups: Group information about the input data
Label-free LC-MS proteomics expression data is often affected by heterogeneity and missing values. Normalization and missing value imputation are the commonly used techniques to solve these issues and make the dataset suitable for further downstream analysis. This function provides the best combination of normalization and imputation methods for the dataset, choosing from the three normalization methods (vsn, loess, and rlr) and three imputation methods (knn, lls, svd). The intra-group variation evaluation measures named pooled co-efficient of variance (PCV), pooled estimate of variance (PEV) and pooled median absolute deviation (PMAD) are used for selecting the best combination of normalization and imputation method for the given dataset. It will return the best combinations based on each evaluation parameters of PCV, PEV, and PMAD.

Along with this, the user can get all three normalized datasets, nine combinations of normalized and missing values imputed datasets, and the PCV, PEV, and PMAD result values.

This function gives the list which consist of following results.

‘Best Combinations’ The best combinations based on each PCV, PEV and PMAD for the given dataset.

‘PCV Result’ Values of groupwise PCV, overall PCV, PCV mean, PCV median and PCV standard deviation for all combinations.

‘PEV Result’ Values of groupwise PEV, overall PEV, PEV mean, PEV median and PEV standard deviation for all combinations.

‘PMAD Result’ Values of groupwise PMAD, overall PMAD, PMAD mean, PMAD median and PMAD standard deviation for all combinations.

‘vsn_data’ The ‘vsn’ normalized dataset

‘loess_data’ The ‘loess’ normalized dataset

‘rlr_data’ The ‘rlr’ normalized dataset

‘knn_vsn_data’ The dataset normalized by ‘vsn’ method and missing values imputed by ‘knn’ method.

‘knn_loess_data’ The dataset normalized by ‘loess’ method and missing values imputed by ‘knn’ method.

‘knn_rlr_data’ The dataset normalized by ‘rlr’ method and missing values imputed by ‘knn’ method.

‘lls_vsn_data’ The dataset normalized by ‘vsn’ method and missing values imputed by ‘lls’ method.

‘lls_loess_data’ The dataset normalized by ‘loess’ method and missing values imputed by ‘lls’ method.

‘lls_rlr_data’ The dataset normalized by ‘rlr’ method and missing values imputed by ‘lls’ method.

‘svd_vsn_data’ The dataset normalized by ‘vsn’ method and missing values imputed by ‘svd’ method.

‘svd_loess_data’ The dataset normalized by ‘loess’ method and missing values imputed by ‘svd’ method.

‘svd_rlr_data’ The dataset normalized by ‘rlr’ method and missing values imputed by ‘svd’ method.
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Examples

```r
result <- best_combination(yeast_data, yeast_groups)
result$`Best combinations`
result$`PCV Result`
result$`PMAD Result`
result$`knn_rlr_data`
```

Description
The box and whiskers plot displays the distribution of a continuous variable. It visualises five summary statistics (the median, two hinges and two whiskers), and all "outlying" points individually. The ‘ggplot2‘ package is used here for creating the boxplot.

Usage

Boxplot_data(data)

Arguments

data Proteomics expression dataset (original or normalized dataset)

Details
This can also be used for comparing the original dataset with the normalized dataset.

Value
Interactive box and whiskers plot

See Also
‘geom_boxplot()’

Examples

Boxplot_data(yeast_data)
Boxplot_data(knn_rlr_yeast_data)"
**Corrplot_data**  
*Creating Correlation matrix plot for a dataset*

**Description**  
A graphical display of a correlation matrix.

**Usage**  
Corrplot_data(data)

**Arguments**  
- **data**  
  Proteomics expression dataset (original or normalized dataset) along with the protein information

**Details**  
This can also be used for comparing the original dataset with the normalized dataset.

**Value**  
Interactive correlation matrix plot

**Examples**

```r
Corrplot_data(yeast_data)  
Corrplot_data(knn_rlr_yeast_data)
```

---

**Densityplot_data**  
*Creating Density plot for a dataset*

**Description**  
Computes and draws kernel density estimate, which is a smoothed version of the histogram. This is a useful alternative to the histogram for continuous data that comes from an underlying smooth distribution. The ‘ggplot2’ package is used here for creating the boxplot.

**Usage**  
Densityplot_data(data)

**Arguments**  
- **data**  
  Proteomics expression dataset (original or normalized dataset) along with the protein information
knr_rlr_yeast_data

**Details**

This can also be used for comparing the original dataset with the normalized dataset.

**Value**

Interactive column-wise density plot

**See Also**

`geom_density()`

**Examples**

```
Densityplot_data(yeast_data)
Densityplot_data(knr_rlr_yeast_data)
```

---

**knr_rlr_yeast_data**

*Normalized and imputed complete yeast lysate - UPS1 benchmark dataset*

**Description**

This is the groupwise normalized and missing values imputed dataset of the complete yeast lysate - UPS1 benchmark dataset. Normalization has been done by RLR normalization method and missing values imputation has been done by KNN imputation method.

**Usage**

`knr_rlr_yeast_data`

**Format**

A data frame with 954 rows and 6 variables:

- **Majority protein IDs**
  - Protein ID information
  - **A1** 1st sample group, 1st technical replicate
  - **A2** 1st sample group, 2nd technical replicate
  - **A3** 1st sample group, 3rd technical replicate
  - **B1** 2nd sample group, 1st technical replicate
  - **B2** 2nd sample group, 2nd technical replicate
  - **B3** 2nd sample group, 3rd technical replicate
MAplot_DE_fn

Find out the Up and Down regulated proteins from MA plot

Description

MA plot is used for visualizing the differentially expressed proteins by plotting the log mean intensity data in x axis and log fold change values in y axis.

This function can be used for visualizing the up regulated, down regulated, and non-significant proteins along with their information.

Usage

MAplot_DE_fn(top_table, x1 = NULL, x2 = NULL, p = NULL)

Arguments

top_table  Top table information
x1         Cut-off limit for down-regulated proteins
x2         Cut-off limit for up-regulated proteins
p          Cut-off limit for p-values

Value

‘Result’ Top table along with up, down, significant and non-significant protein information.
‘MA plot’ Interactive MA plot with the details of up and down regulated proteins
‘Up-regulated’ Up-regulated protein information
‘Down-regulated’ Down-regulated protein information
‘Non-significant’ Non-significant protein information

Examples

result <- MAplot_DE_fn(yeast_top_table, -1, 1, 0.05)
result$'MA Plot'
result$'Result'
result$'Up-regulated'
result$'Down-regulated'
result$'Non-significant'
**MDSplot_data**  
*Creating MDS plot for a dataset*

**Description**
Multi-dimensional scaling (MDS) plots showing a 2-dimensional projection of distances between the dataset samples.

**Usage**

```r
MDSplot_data(data)
```

**Arguments**

- `data`  
  Normalized and imputed Proteomics expression dataset along with the protein information

**Value**

MDS plot

**See Also**

- `mdsPlot`

**Examples**

```r
MDSplot_data(knn_rlr_yeast_data)
```

---

**QQplot_data**  
*Creating QQ-Plot for a dataset*

**Description**
A Q–Q plot (quantile-quantile plot) is a plot of the quantiles of two distributions against each other, or a plot based on estimates of the quantiles. The normality of the data can be understand by this plot.

**Usage**

```r
QQplot_data(data)
```

**Arguments**

- `data`  
  Proteomics expression dataset (original or normalized dataset)
Details
This can be used for comparing the original dataset with the normalized dataset.

Value
Interactive column-wise QQ-plot

Examples
```r
qqplot <- QQplot_data(knn_rlr_yeast_data)
```

---

**top_table_fn**

Creating the top table

Description
Top table can be used for identifying the pairwise differential abundance analysis of proteins in the dataset.

Usage
```r
top_table_fn(data, groups, ch_gr1, ch_gr2)
```

Arguments
- **data**: Normalized and missing values imputed expression dataset containing protein information
- **groups**: Group information about the input data
- **ch_gr1**: Group number of the dataset for pairwise comparison with the another group
- **ch_gr2**: Group number of the dataset to be compared with the chosen group

Value
Top table consists of following values
- 'logFC' - Log fold change values,
- 'AveExpr' - Average intensity values,
- 't' - t-statistic values,
- 'P.Value' - P-values,
- 'adj.P.Val' - Adjusted P-values,
- 'B' - B-statistic values

See Also
- 'limma::topTable'
Examples

    top_table <- top_table_fn(knn_rlr_yeast_data, yeast_groups, 2, 1)
    top_table

volcanoplot_DE_fn

Find out the Up and Down regulated proteins from volcano plot

Description

Volcano plot is used for visualizing the differentially expressed proteins by plotting the log fold change values in x axis and (-log10 p-values) in y axis.
This function can be used for visualizing the up regulated, down regulated, and non-significant proteins along with their information.

Usage

volcanoplot_DE_fn(top_table, x1 = NULL, x2 = NULL, p = NULL)

Arguments

top_table
x1
x2
p

Value

‘Result’ Top table along with up, down, significant and non-significant protein information.
‘Volcano plot’ Interactive MA plot with the details of up and down regulated proteins
‘Up-regulated’ Up-regulated protein information
‘Down-regulated’ Down-regulated protein information
‘Non-significant’ Non-significant protein information

Examples

    result <- volcanoplot_DE_fn(yeast_top_table, -1, 1, 0.05)
    result$`Volcano Plot`
    result$`Result`
    result$`Up-regulated`
    result$`Down-regulated`
    result$`Non-significant`
Description

This dataset was given by Ramus et al., (2016). It is based on a highly complex sample (yeast lysate) spiked with different spiked amounts of the UPS1 standard mixture of 48 recombinant proteins. The original dataset contains 2644 rows of proteins and 2 groups of samples with three replicates each. The total number of missing values present in the sample is 579 (around 3.6

Usage

yeast_data

Format

A data frame with 1000 rows and 7 variables:

Majority protein IDs  Protein ID information
A1  1st condition, 1st technical replicate
A2  1st condition, 2nd technical replicate
A3  1st condition, 3rd technical replicate
B1  2nd condition, 1st technical replicate
B2  2nd condition, 2nd technical replicate
B3  2nd condition, 3rd technical replicate

Details

This standard proteomic dataset is suitable for benchmarking and comparing software for label-free quantification. And can also be applied to the evaluation of post-processing steps such as normalization, imputation of missing values, and statistical methods.

Here only the portion of the dataset is taken for running the functions.

Source

doi:10.1016/j.dib.2015.11.063
**Description**

The standard benchmark yeast lysate - UPS1 dataset contains the variables of 6 columns, 2 conditions and 3 technical replicates.

**Usage**

`yeast_groups`

**Format**

A data frame with 6 rows and 2 variables:

- **Samples**: Technical replicate details - Two kinds of technical replicates (A1, A2, A3) and (B1, B2, B3)
- **Groups**: Condition details - Two conditions (GrA) and (GrB)

**Details**

The above information is required for further analysis of the dataset.

**Source**

doi:10.1016/j.dib.2015.11.063

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**Description**

Top table can be used for identifying the differential abundance analysis of proteins in the dataset.

**Usage**

`yeast_top_table`
Format

A data frame with 954 rows and 6 variables along with 'Fasta headers':

- logFC  Log fold change values
- AveExpr Average intensity values
- t  t-statistic values
- P.Value  P-values
- adj.P.Val  Adjusted P-values
- B  B-statistic values
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