

# Package ‘NormalyzerDE’

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**Title** Evaluation of normalization methods and calculation of differential expression analysis statistics

**Version** 1.14.0

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**Description** NormalyzerDE provides screening of normalization methods for LC-MS based expression data. It calculates a range of normalized matrices using both existing approaches and a novel time-segmented approach, calculates performance measures and generates an evaluation report. Furthermore, it provides an easy utility for Limma- or ANOVA- based differential expression analysis.

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**R topics documented:**

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---

analyzeNormalizations *Calculate measures for normalization results*

---

**Description**

This function prepares an `NormalizerEvaluationResults` object containing the evaluation measures CV (coefficient of variance), MAD (median absolute deviation), average variance, significance measures (ANOVA between condition groups) and correlation between replicates.

**Usage**

```
analyzeNormalizations(nr, categoricalAnova = FALSE)
```

**Arguments**

`nr` Normalyzer results object with calculated results.  
`categoricalAnova` Whether categorical or numerical (ordered) ANOVA should be calculated.

**Value**

Normalyzer results with attached evaluation results object.

**Examples**

```
data(example_summarized_experiment)
normObj <- getVerifiedNormalyzerObject("job_name", example_summarized_experiment)
normResults <- normMethods(normObj)
normResultsWithEval <- analyzeNormalizations(normResults)
```

---

|                                 |   |
|---------------------------------|---|
| <code>calculateContrasts</code> | <i>Performs statistical comparisons between the supplied conditions. It uses the design matrix and data matrix in the supplied Normalyzer-Statistics object. A column is supplied specifying which of the columns in the design matrix that is used for deciding the sample groups. The comparisons vector specifies which pairwise comparisons between condition levels that are to be calculated.</i> |
|---------------------------------|---|

---

**Description**

Optionally, a batch column can be specified allowing compensation for covariate variation in the statistical model. This is only compatible with a Limma-based statistical analysis.

**Usage**

```
calculateContrasts(
  nst,
  comparisons,
  condCol,
  batchCol = NULL,
  splitter = "-",
  type = "limma",
  leastRepCount = 1
)

## S4 method for signature 'NormalyzerStatistics'
calculateContrasts(
  nst,
  comparisons,
  condCol,
  batchCol = NULL,
```

```

    splitter = "-",
    type = "limma",
    leastRepCount = 1
  )

```

### Arguments

|               |   |
|---------------|---|
| nst           | Results evaluation object.  |
| comparisons   | String with comparisons for contrasts.                                  |
| condCol       | Column name in design matrix containing condition information.          |
| batchCol      | Column name in design matrix containing batch information.              |
| splitter      | Character dividing contrast conditions.                                 |
| type          | Type of statistical test (Limma or welch).                              |
| leastRepCount | Least replicates in each group to be retained for contrast calculations |

### Value

nst Statistics object with statistical measures calculated

### Examples

```

data(example_stat_summarized_experiment)
nst <- NormalizerStatistics(example_stat_summarized_experiment)
results <- calculateContrasts(nst, c("1-2", "2-3"), "group")
resultsBatch <- calculateContrasts(nst, c("1-2", "2-3"), "group", batchCol="batch")

```

---

generateAnnotatedMatrix

*Generate an annotated data frame from statistics object*

---

### Description

Extracts key values (p-value, adjusted p-value, log2-fold change and average expression values) from an NormalizerStatistics instance and appends these to the annotation- and data-matrices

### Usage

```
generateAnnotatedMatrix(nst, prefixSep = "_", compLabels = NULL)
```

### Arguments

|            |   |
|------------|---|
| nst        | NormalizerDE statistics object.   |
| prefixSep  | Character string for separating the prefix names from the statistics suffix |
| compLabels | Vector containing strings to use as prefix for statistical comparisons      |

**Value**

outDf Annotated statistics matrix

**Examples**

```
data(example_stat_summarized_experiment)
statObj <- NormalyzerStatistics(example_stat_summarized_experiment)
statObj <- calculateContrasts(statObj, comparisons=c("1-2", "2-3"), condCol="group", type="limma")
annotDf <- generateAnnotatedMatrix(statObj)
```

---

|               |   |
|---------------|---|
| generatePlots | <i>Generates a number of visualizations for the performance measures calculated for the normalized matrices. These contain both general measures and direct comparisons for different normalization approaches.</i> |
|---------------|---|

---

**Description**

They include:

**Usage**

```
generatePlots(nr, jobdir, plotRows = 3, plotCols = 4, writeAsPngs = FALSE)
```

**Arguments**

|             |  |
|-------------|--|
| nr          | Normalyzer results object.                             |
| jobdir      | Path to output directory for run.                      |
| plotRows    | Number of plot rows.                                   |
| plotCols    | Number of plot columns.                                |
| writeAsPngs | Output the report as PNG-plots instead of a single PDF |

**Details**

"Total intensity" Barplot showing the summed intensity in each sample for thelog2-transformed data

"Total missing" Barplot showing the number of missing values found in each sample for the log2-transformed data

Log2-MDS plot: MDS plot where data is reduced to two dimensions allowing inspection of the main global changes in the data

PCV - Intragroup: Mean of intragroup CV of all replicate groups

PMAD - Intragroup: Mean of intragroup median absolute deviation across replicate groups

PEV - Intragroup: Mean of intragroup pooled estimate of variance across the replicate groups

Relative PCV, PMAD and PEV compared to log2: The results from PCV, PMAD and PEV from all normalized data compared to the log2 data

Stable variables plot: 5 analysis of log2 transformed data. Thereafter, global CV of these variables is estimated from different normalized datasets. A plot of global CV of the stable variables from all datasets on the y-axis and PCV-compared to log2 on the x-axis is generated.

CV vs Raw Intensity plots: For the first replicate group in each of the normalized dataset, a plot of PCV of each variable compared to the average intensity of the variable in the replicate group is plotted.

MA plots: Plotted using the plotMA function of the limma package. The first sample in each dataset is plotted against the average of the replicate group that sample belong to.

Scatterplots: The first two samples from each dataset are plotted.

Q-Q plots: QQ-plots are plotted for the first sample in each normalized dataset.

Boxplots: Boxplots for all samples are plotted and colored according to the replicate grouping.

Relative Log Expression (RLE) plots: Relative log expression value plots. Ratio between the expression of the variable and the median expression of this variable across all samples. The samples should be aligned around zero. Any deviation would indicate discrepancies in the data.

Density plots: Density distributions for each sample using the density function. Can capture outliers (if single densities lies far from the others) and see if there is batch effects in the dataset (if for instance there is two clear collections of lines in the data).

MDS plots Multidimensional scaling plot using the cmdscale() function from the stats package. Is often able to show whether replicates group together, and whether there are any clear outliers in the data.

MeanSDplots Displays the standard deviation values against values ordered according to mean. If no dependency on mean is present (as is desired) a flat red line is shown.

Pearson and Spearman correlation Mean of intragroup Pearson and Spearman correlation values for each method.

Dendograms Generated using the hclust function. Data is centered and scaled prior to analysis. Coloring of replicates is done using as.phylo from the ape package.

P-value histograms Histogram plots of p-values after calculating an ANOVA between different condition groups. If no effect is present in the data a flat distribution is expected. If an effect is present a flat distribution is still expected, but with a sharp peak close to zero. If other effects are present it might indicate that the data doesn't support the assumptions of ANOVA, for instance if there are batch effects present in the data.

## Value

None

## Examples

```
data(example_summarized_experiment)
normObj <- getVerifiedNormalizerObject("job_name", example_summarized_experiment)
normResults <- normMethods(normObj)
normResultsWithEval <- analyzeNormalizations(normResults)
outputDir <- tempdir()
generatePlots(normResultsWithEval, outputDir)
```

---

|                     |   |
|---------------------|---|
| generateStatsReport | <i>Generate full output report plot document. Plots p-value histograms for each contrast in the NormalyzerStatistics instance and writes these to a PDF report.</i> |
|---------------------|---|

---

## Description

Generate full output report plot document. Plots p-value histograms for each contrast in the NormalyzerStatistics instance and writes these to a PDF report.

## Usage

```
generateStatsReport(  
  nst,  
  jobName,  
  jobDir,  
  sigThres = 0.1,  
  sigThresType = "fdr",  
  log2FoldThres = 0,  
  plotRows = 3,  
  plotCols = 4,  
  writeAsPngs = FALSE  
)
```

## Arguments

|               |  |
|---------------|--|
| nst           | NormalyzerDE statistics object.                                      |
| jobName       | Name of processing run.  |
| jobDir        | Path to output directory.  |
| sigThres      | Significance threshold for indicating as significant                 |
| sigThresType  | Type of significance threshold (FDR or p)                            |
| log2FoldThres | log2 fold-change required for being counted as significant           |
| plotRows      | Number of plot rows.   |
| plotCols      | Number of plot columns.  |
| writeAsPngs   | Output the report as separate PNG files instead of a single PDF file |

## Value

None

**Examples**

```

data(example_stat_summarized_experiment)
statObj <- NormalyzerStatistics(example_stat_summarized_experiment)
statObj <- calculateContrasts(statObj, comparisons=c("1-2", "2-3"),
  condCol="group", type="limma")
outputDir <- tempdir()
generateStatsReport(statObj, "jobName", outputDir)

```

---

getRTNormalizedMatrix *Perform RT-segmented normalization by performing the supplied normalization over retention-time sliced data*

---

**Description**

The function orders the retention times and steps through them using the supplied step size (in minutes). If smaller than a fixed lower boundary the window is expanded to ensure a minimum amount of data in each normalization step. An offset can be specified which can be used to perform multiple RT-segmentations with partial overlapping windows.

**Usage**

```

getRTNormalizedMatrix(
  rawMatrix,
  retentionTimes,
  normMethod,
  stepSizeMinutes = 1,
  windowMinCount = 100,
  offset = 0,
  noLogTransform = FALSE
)

```

**Arguments**

|                 |   |
|-----------------|---|
| rawMatrix       | Target matrix to be normalized                          |
| retentionTimes  | Vector of retention times corresponding to rawMatrix    |
| normMethod      | The normalization method to apply to the time windows   |
| stepSizeMinutes | Size of windows to be normalized                        |
| windowMinCount  | Minimum number of values for window to not be expanded. |
| offset          | Whether time window should shifted half step size       |
| noLogTransform  | Don't log-transform the data                            |

**Value**

Normalized matrix



**Examples**

```

data(example_data_small)
data(example_design_small)
data(example_data_only_values)
dataMat <- example_data_only_values
retentionTimes <- as.numeric(example_data[, "Average.RT"])
performCyclicLoessNormalization <- function(rawMatrix) {
  log2Matrix <- log2(rawMatrix)
  normMatrix <- limma::normalizeCyclicLoess(log2Matrix, method="fast")
  colnames(normMatrix) <- colnames(rawMatrix)
  normMatrix
}
rtNormMat <- getRTNormalizedMatrix(dataMat, retentionTimes,
performCyclicLoessNormalization, stepSizeMinutes=1, windowMinCount=100)

```

---

```
getSmoothedRTNormalizedMatrix
```

*Generate multiple RT time-window normalized matrices where one is shifted. Merge them using a specified method (mean or median) and return the result.*

---

**Description**

Uses the function `getRTNormalizedMatrix` to generate multiple normalized matrices which are shifted respective to each other and finally merged into a single matrix. This could potentially reduce effect of fluctuations within individual windows.

**Usage**

```

getSmoothedRTNormalizedMatrix(
  rawMatrix,
  retentionTimes,
  normMethod,
  stepSizeMinutes,
  windowShifts = 2,
  windowMinCount = 100,
  mergeMethod = "mean",
  noLogTransform = FALSE
)

```

**Arguments**

|                              |   |
|------------------------------|---|
| <code>rawMatrix</code>       | Target matrix to be normalized                                    |
| <code>retentionTimes</code>  | Vector of retention times corresponding to <code>rawMatrix</code> |
| <code>normMethod</code>      | The normalization method to apply to the time windows             |
| <code>stepSizeMinutes</code> | Size of windows to be normalized                                  |

windowShifts    Number of frame shifts.  
 windowMinCount    Minimum number of features within window.  
 mergeMethod    Layer merging approach. Mean or median.  
 noLogTransform    Don't log transform the input

**Value**

Normalized matrix

**Examples**

```

data(example_data_small)
data(example_data_only_values)
data(example_design_small)
retentionTimes <- as.numeric(example_data[, "Average.RT"])
dataMat <- example_data_only_values
performCyclicLoessNormalization <- function(rawMatrix) {
  log2Matrix <- log2(rawMatrix)
  normMatrix <- limma::normalizeCyclicLoess(log2Matrix, method="fast")
  colnames(normMatrix) <- colnames(rawMatrix)
  normMatrix
}
rtNormMat <- getSmoothedRTNormalizedMatrix(dataMat, retentionTimes,
  performCyclicLoessNormalization, stepSizeMinutes=1, windowMinCount=100,
  windowShifts=2, mergeMethod="median")

```

---

getVerifiedNormalizerObject

*Verify that input data is in correct format, and if so, return a generated NormalizerDE data object from that input data*

---

**Description**

This function performs a number of checks on the input data and provides informative error messages if the data isn't fulfilling the required format. Checks include verifying that the design matrix matches to the data matrix, that the data matrix contains valid numbers and that samples have enough values for analysis

**Usage**

```

getVerifiedNormalizerObject(
  jobName,
  summarizedExp,
  threshold = 15,
  omitSamples = FALSE,
  requireReplicates = TRUE,
  quiet = FALSE,

```

```

    noLogTransform = FALSE,
    tinyRunThres = 50
  )

```

### Arguments

|                   |   |
|-------------------|---|
| jobName           | Name of ongoing run.                                  |
| summarizedExp     | Summarized experiment input object                    |
| threshold         | Minimum number of features.                           |
| omitSamples       | Automatically omit invalid samples from analysis.     |
| requireReplicates | Require there to be at least to samples per condition |
| quiet             | Don't print output messages during processing         |
| noLogTransform    | Don't log-transform the provided data                 |
| tinyRunThres      | If less features in run, a limited run is performed   |

### Value

Normalizer data object representing verified input data.

### Examples

```

data(example_summarized_experiment)
normObj <- getVerifiedNormalizerObject("job_name", example_summarized_experiment)

```

---

globalIntensityNormalization

*The normalization divides the intensity of each variable in a sample with the sum of intensities of all variables in the sample and multiplies with the median of sum of intensities of all variables in all samples. The normalized data is then log2-transformed.*

---

### Description

The normalization divides the intensity of each variable in a sample with the sum of intensities of all variables in the sample and multiplies with the median of sum of intensities of all variables in all samples. The normalized data is then log2-transformed.

### Usage

```
globalIntensityNormalization(rawMatrix, noLogTransform = FALSE)
```

### Arguments

|                |  |
|----------------|--|
| rawMatrix      | Target matrix to be normalized         |
| noLogTransform | Assumes no need for log transformation |

**Value**

Normalized and log-transformed matrix

**Examples**

```
data(example_data_only_values_small)
normMatrix <- globalIntensityNormalization(example_data_only_values)
```

---

|          |                                     |
|----------|-------------------------------------|
| loadData | <i>Load raw data into dataframe</i> |
|----------|-------------------------------------|

---

**Description**

General function which allows specifying different types of input data including "proteios", "maxquant-pep" (peptide output from MaxQuant) and "maxquantprot" (protein output from MaxQuant) formats.

**Usage**

```
loadData(dataPath, inputFormat = "default")
```

**Arguments**

|             |   |
|-------------|---|
| dataPath    | File path to design matrix.   |
| inputFormat | If input is given in standard NormalyzerDE format, Proteios format or in MaxQuant protein or peptide format |

**Value**

rawData Raw data loaded into data frame

**Examples**

```
## Not run:
df <- loadData("data.tsv")

## End(Not run)
```

---

|            |                                       |
|------------|---------------------------------------|
| loadDesign | <i>Load raw design into dataframe</i> |
|------------|---------------------------------------|

---

**Description**

Takes a design path, loads the matrix and ensures that the sample column is in character format and that the group column is in factor format.

**Usage**

```
loadDesign(designPath, sampleCol = "sample", groupCol = "group")
```

**Arguments**

|            |   |
|------------|---|
| designPath | File path to design matrix.                         |
| sampleCol  | Column name for column containing sample names.     |
| groupCol   | Column name for column containing condition levels. |

**Value**

designMatrix Design data loaded into data frame

**Examples**

```
## Not run:  
df <- loadDesign("design.tsv")  
  
## End(Not run)
```

---

|                   |   |
|-------------------|---|
| meanNormalization | <i>Intensity of each variable in a given sample is divided by the mean of sum of intensities of all variables in the sample and then multiplied by the mean of sum of intensities of all variables in all samples. The normalized data is then transformed to log2.</i> |
|-------------------|---|

---

**Description**

Intensity of each variable in a given sample is divided by the mean of sum of intensities of all variables in the sample and then multiplied by the mean of sum of intensities of all variables in all samples. The normalized data is then transformed to log2.

**Usage**

```
meanNormalization(rawMatrix, noLogTransform = FALSE)
```

**Arguments**

rawMatrix      Target matrix to be normalized  
noLogTransform Assumes no need for log transformation

**Value**

Normalized and log-transformed matrix

**Examples**

```
data(example_data_only_values_small)  
normMatrix <- meanNormalization(example_data_only_values)
```

---

medianNormalization      *Intensity of each variable in a given sample is divided by the median of intensities of all variables in the sample and then multiplied by the mean of median of sum of intensities of all variables in all samples. The normalized data is then log2-transformed.*

---

**Description**

Intensity of each variable in a given sample is divided by the median of intensities of all variables in the sample and then multiplied by the mean of median of sum of intensities of all variables in all samples. The normalized data is then log2-transformed.

**Usage**

```
medianNormalization(rawMatrix, noLogTransform = FALSE)
```

**Arguments**

rawMatrix      Target matrix to be normalized  
noLogTransform Assumes no need for log transformation

**Value**

Normalized and log-transformed matrix

**Examples**

```
data(example_data_only_values_small)  
normMatrix <- medianNormalization(example_data_only_values)
```

---

|            |  |
|------------|--|
| normalyzer | <i>NormalyzerDE pipeline entry point</i> |
|------------|--|

---

### Description

This function is the main execution point for the normalization part of the NormalyzerDE analysis pipeline. When executed it performs the following steps:

### Usage

```
normalyzer(  
  jobName,  
  designPath = NULL,  
  dataPath = NULL,  
  experimentObj = NULL,  
  outputDir = ".",  
  forceAllMethods = FALSE,  
  omitLowAbundSamples = FALSE,  
  sampleAbundThres = 5,  
  tinyRunThres = 50,  
  requireReplicates = TRUE,  
  normalizeRetentionTime = TRUE,  
  plotRows = 3,  
  plotCols = 4,  
  zeroToNA = FALSE,  
  sampleColName = "sample",  
  groupColName = "group",  
  inputFormat = "default",  
  skipAnalysis = FALSE,  
  quiet = FALSE,  
  noLogTransform = FALSE,  
  writeReportAsPngs = FALSE,  
  rtStepSizeMinutes = 1,  
  rtWindowMinCount = 100,  
  rtWindowShifts = 1,  
  rtWindowMergeMethod = "mean"  
)
```

### Arguments

|               |   |
|---------------|---|
| jobName       | Give the current run a name.  |
| designPath    | Path to file containing design matrix.  |
| dataPath      | Specify an output directory for generated files. Defaults to current working directory.             |
| experimentObj | SummarizedExperiment object, can be provided as input as alternative to 'designPath' and 'dataPath' |

|                                     |  |
|-------------------------------------|--|
| <code>outputDir</code>              | Directory where results folder is created.   |
| <code>forceAllMethods</code>        | Debugging function. Run all normalizations even if they aren't in the recommended range of number of values  |
| <code>omitLowAbundSamples</code>    | Automatically remove samples with fewer non-NA values compared to threshold given by <code>sampleAbundThres</code> . Will otherwise stop with error message if such sample is encountered. |
| <code>sampleAbundThres</code>       | Threshold for omitting low-abundant samples. Is by default set to 15.  |
| <code>tinyRunThres</code>           | If total number of features is less than this, a limited run is performed.   |
| <code>requireReplicates</code>      | Require multiple samples per condition to pass input validation.   |
| <code>normalizeRetentionTime</code> | Perform normalizations over retention time.  |
| <code>plotRows</code>               | Number of plot-rows in output documentation.   |
| <code>plotCols</code>               | Number of plot-columns in output documentation.  |
| <code>zeroToNA</code>               | Convert zero values to NA.   |
| <code>sampleColName</code>          | Column name in design matrix containing sample IDs.  |
| <code>groupColName</code>           | Column name in design matrix containing condition IDs.   |
| <code>inputFormat</code>            | Type of input format.  |
| <code>skipAnalysis</code>           | Only perform normalization steps.  |
| <code>quiet</code>                  | Omit status messages printed during run.   |
| <code>noLogTransform</code>         | Don't log-transform the input.   |
| <code>writeReportAsPngs</code>      | Output the evaluation report as PNG files instead of a single PDF  |
| <code>rtStepSizeMinutes</code>      | Retention time normalization window size.  |
| <code>rtWindowMinCount</code>       | Minimum number of datapoints in each retention-time segment.   |
| <code>rtWindowShifts</code>         | Number of layered retention time normalized windows.   |
| <code>rtWindowMergeMethod</code>    | Merge approach for layered retention time windows.   |

## Details

1: Loads the data matrix containing expression values and optional annotations, as well as the design matrix containing the experimental setup 2: Performs input data verification to validate that the data is in correct format. This step captures many common formatting errors. It returns an instance of the `NormalyzerDataset` class representing the unprocessed data. 3: Calculate a range of normalizations for the dataset. The result is provided as a `NormalyzerResults` object containing the resulting data matrices from each normalization. 4: Analyze the normalizations and generate performance measures for each of the normalized datasets. This result is provided as a `NormalyzerEvaluationResults` object. 5: Output the matrices containing the normalized datasets to files. 6: Generate visualizations overviewing the performance measures and write them to a PDF report.



**Value**

None

**Examples**

```
## Not run:
data_path <- system.file(package="NormalyzerDE", "extdata", "tiny_data.tsv")
design_path <- system.file(package="NormalyzerDE", "extdata", "tiny_design.tsv")
out_dir <- tempdir()
normalyzer(
  jobName="my_jobname",
  designPath=design_path,
  dataPath=data_path,
  outputDir=out_dir)
normalyzer(
  "my_jobname",
  designMatrix="design.tsv",
  "data.tsv",
  outputDir="path/to/output",
  normalizeRetentionTime=TRUE,
  retentionTimeWindow=2)
normalyzer(
  "my_jobname",
  designMatrix="design.tsv",
  "data.tsv",
  outputDir="path/to/output",
  inputFormat="maxquantprot")

## End(Not run)
```

---

normalyzerDE

*NormalyzerDE differential expression*

---

**Description**

Performs differential expression analysis on a normalization matrix. This command executes a pipeline processing the data and generates an annotated normalization matrix and a report containing p-value histograms for each of the performed comparisons.

**Usage**

```
normalyzerDE(
  jobName,
  comparisons,
  designPath = NULL,
  dataPath = NULL,
  experimentObj = NULL,
  outputDir = ".",
  logTrans = FALSE,
```

```

type = "limma",
sampleCol = "sample",
condCol = "group",
batchCol = NULL,
techRepCol = NULL,
leastRepCount = 1,
quiet = FALSE,
sigThres = 0.1,
sigThresType = "fdr",
log2FoldThres = 0,
writeReportAsPngs = FALSE
)

```

### Arguments

|                   |   |
|-------------------|---|
| jobName           | Name of job   |
| comparisons       | Character vector containing target contrasts. If comparing condA with condB, then the vector would be c("condA-condB")  |
| designPath        | File path to design matrix  |
| dataPath          | File path to normalized matrix  |
| experimentObj     | SummarizedExperiment object, can be provided as input as alternative to 'designPath' and 'dataPath'   |
| outputDir         | Path to output directory  |
| logTrans          | Log transform the input (needed if providing non-logged input)  |
| type              | Type of statistical comparison, "limma", "limma_intensity" or "welch", where "limma_intensity" allows the prior to be fit according to intensity rather than using a flat prior |
| sampleCol         | Design matrix column header for column containing sample IDs  |
| condCol           | Design matrix column header for column containing sample conditions   |
| batchCol          | Provide an optional column for inclusion of possible batch variance in the model  |
| techRepCol        | Design matrix column header for column containing technical replicates  |
| leastRepCount     | Minimum required replicate count  |
| quiet             | Omit status messages printed during run   |
| sigThres          | Significance threshold use for illustrating significant hits in diagnostic plots  |
| sigThresType      | Type of significance threshold, "fdr" or "p". "fdr" is strongly recommended (Benjamini-Hochberg corrected p-values)   |
| log2FoldThres     | Fold-size cutoff for being considered significant in diagnostic plots   |
| writeReportAsPngs | Output report as separate PNG files instead of a single PDF   |

## Details

When executed, it performs the following steps:

1: Read the data and the design matrices into dataframes. 2: Generate an instance of the NormalyzerStatistics class representing the data and their statistical comparisons. 3: Optionally reduce technical replicates in both the data matrix and the design matrix 4: Calculate statistical contrasts between supplied groups 5: Generate an annotated version of the original dataframe where columns containing statistical key measures have been added 6: Write the table to file 7: Generate a PDF report displaying p-value histograms for each calculated contrast

## Value

None

## Examples

```
data_path <- system.file(package="NormalyzerDE", "extdata", "tiny_data.tsv")
design_path <- system.file(package="NormalyzerDE", "extdata", "tiny_design.tsv")
out_dir <- tempdir()
normalyzerDE(
  jobName="my_jobname",
  comparisons=c("4-5"),
  designPath=design_path,
  dataPath=data_path,
  outputDir=out_dir,
  condCol="group")
```

---

NormalyzerEvaluationResults

*Representation of evaluation results by calculating performance measures for an an NormalyzerResults instance*

---

## Description

Contains the resulting information from the processing which subsequently can be used to generate the quality assessment report.

## Usage

```
NormalyzerEvaluationResults(nr)
```

```
NormalyzerEvaluationResults(nr)
```

## Arguments

nr                      NormalyzerResults object

**Value**

nds Generated NormalyzerEvaluationResults instance

**Slots**

avgcvmem Average coefficient of variance per method  
 avgcvmempdiff Percentage difference of mean coefficient of variance compared to log2-transformed data  
 featureCVPerMethod CV calculated per feature and normalization method.  
 avgmadmem Average median absolute deviation  
 avgmadmempdiff Percentage difference of median absolute deviation compared to log2-transformed data  
 avgvarmem Average variance per method  
 avgvarmempdiff Percentage difference of mean variance compared to log2-transformed data  
 lowVarFeaturesCVs List of 5 for log2-transformed data  
 lowVarFeaturesCVsPercDiff Coefficient of variance for least variable entries  
 anovaP ANOVA calculated p-values  
 repCorPear Within group Pearson correlations  
 repCorSpear Within group Spearman correlations

**Examples**

```
data(example_summarized_experiment)
normObj <- getVerifiedNormalyzerObject("job_name", example_summarized_experiment)
normResults <- normMethods(normObj)
normEval <- NormalyzerEvaluationResults(normResults)
```

---

|                   |   |
|-------------------|---|
| NormalyzerResults | <i>Representation of the results from performing normalization over a dataset</i> |
|-------------------|---|

---

**Description**

It is linked to a NormalyzerDataset instance representing the raw data which has been processed. After performing evaluation it also links to an instance of NormalyzerEvaluationResults representing the results from the evaluation.

**Usage**

```
NormalyzerResults(nds)
```

```
NormalyzerResults(nds)
```

**Arguments**

nds NormalizerDataset object

**Value**

nr Prepared NormalizerResults object

**Slots**

normalizations SummarizedExperiment object containing calculated normalization results

nds Normalizer dataset representing run data

ner Normalizer evaluation results for running extended normalizations

**Examples**

```
data(example_summarized_experiment)
normObj <- getVerifiedNormalizerObject("job_name", example_summarized_experiment)
emptyNormResults <- NormalizerResults(normObj)
```

---

NormalizerStatistics *Class representing a dataset for statistical processing in NormalizerDE*

---

**Description**

Is initialized with an annotation matrix, a data matrix and a design data frame. This object can subsequently be processed to generate statistical values and in turn used to write a full matrix with additional statistical information as well as a graphical report of the comparisons.

**Usage**

```
NormalizerStatistics(experimentObj, logTrans = FALSE)
```

```
NormalizerStatistics(experimentObj, logTrans = FALSE)
```

**Arguments**

experimentObj Instance of SummarizedExperiment containing matrix and design information as column data

logTrans Whether the input data should be log transformed

**Value**

nds Generated NormalizerStatistics instance

**Slots**

annotMat Matrix containing annotation information  
 dataMat Matrix containing (normalized) expression data  
 filteredDataMat Filtered matrix with low-count rows removed  
 designDf Data frame containing design conditions  
 filteringContrast Vector showing which entries are filtered (due to low count)  
 pairwiseCompsP List with P-values for pairwise comparisons  
 pairwiseCompsFdr List with FDR-values for pairwise comparisons  
 pairwiseCompsAve List with average expression values  
 pairwiseCompsFold List with log2 fold-change values for pairwise comparisons  
 contrasts Spot for saving vector of last used contrasts  
 condCol Column containing last used conditions  
 batchCol Column containing last used batch conditions

**Examples**

```

data(example_stat_summarized_experiment)
nst <- NormalizerStatistics(example_stat_summarized_experiment)

```

---

normMethods

*Perform normalizations on Normalyzer dataset*


---

**Description**

Perform normalizations on Normalyzer dataset

**Usage**

```

normMethods(
  nds,
  forceAll = FALSE,
  normalizeRetentionTime = TRUE,
  quiet = FALSE,
  rtStepSizeMinutes = 1,
  rtWindowMinCount = 100,
  rtWindowShifts = 1,
  rtWindowMergeMethod = "mean",
  noLogTransform = FALSE
)

```

**Arguments**

|                                     |  |
|-------------------------------------|--|
| <code>nds</code>                    | Normalyzer dataset object.   |
| <code>forceAll</code>               | Force all methods to run despite not qualifying for thresholds.  |
| <code>normalizeRetentionTime</code> | Perform retention time based normalization methods.  |
| <code>quiet</code>                  | Prevent diagnostic output  |
| <code>rtStepSizeMinutes</code>      | Retention time normalization window size.  |
| <code>rtWindowMinCount</code>       | Minimum number of datapoints in each retention-time segment.   |
| <code>rtWindowShifts</code>         | Number of layered retention time normalized windows.   |
| <code>rtWindowMergeMethod</code>    | Merge approach for layered retention time windows.   |
| <code>noLogTransform</code>         | Per default NormalyzerDE performs a log-transformation on the input data. If not needed, specify this option |

**Value**

Returns Normalyzer results object with performed analyzes assigned as attributes

**Examples**

```
data(example_summarized_experiment)
normObj <- getVerifiedNormalyzerObject("job_name", example_summarized_experiment)
normResults <- normMethods(normObj)
```

---

performCyclicLoessNormalization  
*Cyclic Loess normalization*

---

**Description**

Log2 transformed data is normalized by Loess method using the function "normalizeCyclicLoess". Further information is available for the function "normalizeCyclicLoess" in the Limma package.

**Usage**

```
performCyclicLoessNormalization(rawMatrix, noLogTransform = FALSE)
```

**Arguments**

|                             |  |
|-----------------------------|--|
| <code>rawMatrix</code>      | Target matrix to be normalized         |
| <code>noLogTransform</code> | Assumes no need for log transformation |

**Value**

Normalized matrix

**Examples**

```
data(example_data_only_values_small)
normMatrix <- performCyclicLoessNormalization(example_data_only_values)
```

---

```
performGlobalRLRNormalization
```

*Global linear regression normalization*

---

**Description**

Log2 transformed data is normalized by robust linear regression using the function "rlm" from the MASS package.

**Usage**

```
performGlobalRLRNormalization(rawMatrix, noLogTransform = FALSE)
```

**Arguments**

`rawMatrix` Target matrix to be normalized  
`noLogTransform` Assumes no need for log transformation

**Value**

Normalized matrix

**Examples**

```
data(example_data_only_values_small)
normMatrix <- performGlobalRLRNormalization(example_data_only_values)
```



---

performQuantileNormalization

*Quantile normalization is performed by the function "normalize.quantiles" from the package preprocessCore.*

---

### Description

It makes the assumption that the data in different samples should originate from an identical distribution. It does this by generating a reference distribution and then scaling the other samples accordingly.

### Usage

```
performQuantileNormalization(rawMatrix, noLogTransform = FALSE)
```

### Arguments

rawMatrix      Target matrix to be normalized  
noLogTransform   Assumes no need for log transformation

### Value

Normalized matrix

### Examples

```
data(example_data_only_values_small)  
normMatrix <- performQuantileNormalization(example_data_only_values)
```

---

performSMADNormalization

*Median absolute deviation normalization Normalization subtracts the median and divides the data by the median absolute deviation (MAD).*

---

### Description

Median absolute deviation normalization Normalization subtracts the median and divides the data by the median absolute deviation (MAD).

### Usage

```
performSMADNormalization(rawMatrix, noLogTransform = FALSE)
```

**Arguments**

rawMatrix      Target matrix to be normalized  
noLogTransform   Assumes no need for log transformation

**Value**

Normalized matrix

**Examples**

```
data(example_data_only_values_small)  
normMatrix <- performSMADNormalization(example_data_only_values)
```

---

performVSNNormalization

*Log2 transformed data is normalized using the function "justvsn" from the VSN package.*

---

**Description**

The VSN (Variance Stabilizing Normalization) attempts to transform the data in such a way that the variance remains nearly constant over the intensity spectrum

**Usage**

```
performVSNNormalization(rawMatrix)
```

**Arguments**

rawMatrix      Target matrix to be normalized

**Value**

Normalized matrix

**Examples**

```
data(example_data_only_values_small)  
normMatrix <- performVSNNormalization(example_data_only_values)
```

---

`reduceTechnicalReplicates`*Remove technical replicates from data and design*

---

**Description**

Collapses sample values into their average. If only one value is present due to NA-values in other technical replicates, then that value is used.

**Usage**

```
reduceTechnicalReplicates(se, techRepColName, sampleColName)
```

**Arguments**

|                             |  |
|-----------------------------|--|
| <code>se</code>             | Summarized experiment where the assay contains the data to be reduced, and the <code>colData</code> the data frame |
| <code>techRepColName</code> | Technical replicates column name in <code>colData</code>   |
| <code>sampleColName</code>  | Sample names column name in <code>colData</code>   |

**Details**

Takes a `SummarizedExperiment` where the data is present as the assay and the `colData` contains the design conditions. In the design conditions there should be one column with the technical replicate groups and one column containing the sample names

**Value**

`reducedSe` Summarized experiment with reduced data

**Examples**

```
testData <- as.matrix(data.frame(
  c(1,1,1),
  c(1,2,1),
  c(7,7,7),
  c(7,9,7)))
colnames(testData) <- c("a1", "a2", "b1", "b2")
designDf <- data.frame(
  sample=c("a1", "a2", "b1", "b2"),
  techrep=c("a", "a", "b", "b"))
se <- SummarizedExperiment::SummarizedExperiment(
  assay=testData,
  colData=designDf
)
statObj <- reduceTechnicalReplicates(se, "techrep", "sample")
```

---

|             |                                       |
|-------------|---------------------------------------|
| setupJobDir | <i>Create empty directory for run</i> |
|-------------|---------------------------------------|

---

**Description**

Creates a directory at provided path named to the jobname.

**Usage**

```
setupJobDir(jobName, outputDir)
```

**Arguments**

|           |   |
|-----------|---|
| jobName   | Name of the run.  |
| outputDir | Path to directory where to create the output directory. |

**Value**

Path to newly created directory.

**Examples**

```
setupJobDir("job_name", "path/to/outdir")
```

---

|                        |  |
|------------------------|--|
| setupRawContrastObject | <i>Prepare SummarizedExperiment object for statistics data</i> |
|------------------------|--|

---

**Description**

Prepare SummarizedExperiment object for statistics data

**Usage**

```
setupRawContrastObject(dataPath, designPath, sampleColName)
```

**Arguments**

|               |  |
|---------------|--|
| dataPath      | Path to raw data matrix                                  |
| designPath    | Path to design matrix                                    |
| sampleColName | Name for column in design matrix containing sample names |

**Value**

experimentObj Prepared instance of SummarizedExperiment

## Examples

```
data_path <- system.file(package="NormalyzerDE", "extdata", "tiny_data.tsv")
design_path <- system.file(package="NormalyzerDE", "extdata", "tiny_design.tsv")
sumExpObj <- setupRawContrastObject(data_path, design_path, "sample")
```

---

|                    |   |
|--------------------|---|
| setupRawDataObject | <i>Prepare SummarizedExperiment object for raw data to be normalized containing data, design and annotation information</i> |
|--------------------|---|

---

## Description

Prepare SummarizedExperiment object for raw data to be normalized containing data, design and annotation information

## Usage

```
setupRawDataObject(  
  dataPath,  
  designPath,  
  inputFormat = "default",  
  zeroToNA = FALSE,  
  sampleColName = "sample",  
  groupColName = "group"  
)
```

## Arguments

|               |   |
|---------------|---|
| dataPath      | File path to data matrix.   |
| designPath    | File path to design matrix.   |
| inputFormat   | Type of matrix for data, can be either 'default', 'proteios', 'maxquantprot' or 'maxquantpep' |
| zeroToNA      | If TRUE zeroes in the data is automatically converted to NA values                            |
| sampleColName | Column name for column containing sample names  |
| groupColName  | Column name for column containing condition levels  |

## Value

experimentObj SummarizedExperiment object loaded with the data

## Examples

```
data_path <- system.file(package="NormalyzerDE", "extdata", "tiny_data.tsv")
design_path <- system.file(package="NormalyzerDE", "extdata", "tiny_design.tsv")
df <- setupRawDataObject(data_path, design_path)
```

---

`writeNormalizedDatasets`*Write normalization matrices to file*

---

### Description

Outputs each of the normalized datasets to the specified directory.

### Usage

```
writeNormalizedDatasets(  
  nr,  
  jobdir,  
  includePairwiseComparisons = FALSE,  
  includeCvCol = FALSE,  
  includeAnovaP = FALSE,  
  normSuffix = "-normalized.txt",  
  rawdataName = "submitted_rawdata.txt"  
)
```

### Arguments

|   |   |
|---|---|
| <code>nr</code>                         | Results object.   |
| <code>jobdir</code>                     | Path to output directory.                                     |
| <code>includePairwiseComparisons</code> | Include p-values for pairwise comparisons.                    |
| <code>includeCvCol</code>               | Include CV column in output.                                  |
| <code>includeAnovaP</code>              | Include ANOVA p-value in output.                              |
| <code>normSuffix</code>                 | String used to name output together with normalization names. |
| <code>rawdataName</code>                | Name of output raw data file.                                 |

### Value

None

### Examples

```
data(example_summarized_experiment)  
normObj <- getVerifiedNormalizerObject("job_name", example_summarized_experiment)  
normResults <- normMethods(normObj)  
normResultsWithEval <- analyzeNormalizations(normResults)  
outputDir <- tempdir()  
writeNormalizedDatasets(normResultsWithEval, outputDir)
```

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