

# Package ‘PolySTest’

December 19, 2024

**Title** PolySTest: Detection of differentially regulated features.  
Combined statistical testing for data with few replicates and missing values

**Version** 1.1.0

**Description** The complexity of high-throughput quantitative omics experiments often leads to low replicates numbers and many missing values. We implemented a new test to simultaneously consider missing values and quantitative changes, which we combined with well-performing statistical tests for high confidence detection of differentially regulated features. The package contains functions to run the test and to visualize the results.

**License** GPL-2

**Encoding** UTF-8

**biocViews** MassSpectrometry, Proteomics, Software,  
DifferentialExpression

**BugReports** <https://github.com/computproteomics/PolySTest/issues>

**URL** <https://github.com/computproteomics/PolySTest>

**Roxygen** list(markdown = TRUE)

**RoxygenNote** 7.3.2

**Imports** fdrtool (>= 1.2.15), limma (>= 3.61.3), matrixStats (>= 0.57.0), qvalue (>= 2.22.0), shiny (>= 1.5.0), SummarizedExperiment (>= 1.20.0), knitr (>= 1.33), plotly (>= 4.9.4), heatmaply (>= 1.1.1), circlize (>= 0.4.12), UpSetR (>= 1.4.0), gplots (>= 3.1.1), S4Vectors (>= 0.30.0), parallel (>= 4.1.0), grDevices (>= 4.1.0), graphics (>= 4.1.0), stats (>= 4.1.0), utils (>= 4.1.0)

**Suggests** testthat (>= 3.0.0), BiocStyle

**VignetteBuilder** knitr

**Config/testthat/edition** 3

**Depends** R (>= 4.4.0)

**git\_url** <https://git.bioconductor.org/packages/PolySTest>

**git\_branch** devel  
**git\_last\_commit** 43fe068  
**git\_last\_commit\_date** 2024-11-22  
**Repository** Bioconductor 3.21  
**Date/Publication** 2024-12-18  
**Author** Veit Schwämmle [aut, cre] (ORCID:  
<https://orcid.org/0000-0002-9708-6722>)  
**Maintainer** Veit Schwämmle <veits@bmb.sdu.dk>

## Contents

check_for_polystest . . . . .	3
check_stat_names . . . . .	3
create_pairwise_comparisons . . . . .	4
FindFCandQlim . . . . .	5
FindFCandQlimAlternative . . . . .	6
get_numthreads . . . . .	7
limma_paired . . . . .	7
limma_unpaired . . . . .	8
liver_example . . . . .	9
MissingStats . . . . .	10
MissingStatsDesign . . . . .	10
MissValPDistr . . . . .	11
permtest_paired . . . . .	12
perm_unpaired . . . . .	12
plotExpression . . . . .	13
plotHeatmaply . . . . .	14
plotPvalueDistr . . . . .	15
plotRegNumber . . . . .	16
plotUpset . . . . .	17
plotVolcano . . . . .	18
PolySTest_paired . . . . .	19
PolySTest_unpaired . . . . .	20
RPStats . . . . .	21
rp_unpaired . . . . .	22
set_mfrow . . . . .	23
StatsForPermutTest . . . . .	23
ttest_paired . . . . .	24
ttest_unpaired . . . . .	24
update_conditions_with_lcs . . . . .	25

**Index**

**27**

---

check\_for\_polystest      *Check SummarizedExperiment for PolySTest Requirements*

---

**Description**

Performs checks on a SummarizedExperiment object to ensure it is properly formatted for PolySTest analysis. It checks for specific metadata properties, the number of assays, and the distribution of conditions and replicates.

**Usage**

```
check_for_polystest(se)
```

**Arguments**

se                      A SummarizedExperiment object.

**Value**

Invisible TRUE if checks pass; otherwise, warnings or errors are thrown.

**Examples**

```
data(liver_example)
check_for_polystest(liver_example)
```

---

check\_stat\_names      *Check Statistical Test and Comparison Names*

---

**Description**

Verifies if the provided test names and comparison names are correct and have been executed within the given SummarizedExperiment object. It checks for the presence of specific metadata related to the tests and comparisons to ensure that the requested analyses have been carried out.

**Usage**

```
check_stat_names(fulldata, compNames, testNames)
```

**Arguments**

fulldata	A SummarizedExperiment object containing the dataset and metadata for statistical analyses.
compNames	A character vector of comparison names to be verified against the SummarizedExperiment metadata. If set to "all", the function checks for the presence of any comparison names in the metadata.
testNames	A character vector of statistical test names to be verified against the SummarizedExperiment metadata.

**Details**

This function is used to ensure that the requested statistical tests and comparisons have been correctly named and executed prior to further analysis. It verifies that the names provided match those stored within the metadata of the SummarizedExperiment object. If the names do not match or the necessary metadata is missing, the function stops execution and returns an error message indicating the issue.

**Value**

The function return the updated comparison names if the checks pass.

**Examples**

```
data(liver_example)
compNames <- "all"
testNames <- c("limma", "t_test")
check_stat_names(liver_example, compNames, testNames)
```

---

create\_pairwise\_comparisons  
*Create All Pairwise Comparisons*

---

**Description**

This function generates a matrix of all pairwise comparisons between the provided conditions. Optionally, a reference condition can be specified, and comparisons will be made between the reference condition and all other conditions.

**Usage**

```
create_pairwise_comparisons(conditions, refCond)
```

**Arguments**

conditions	A character vector of condition names.
refCond	An integer indicating the index of the reference condition within the conditions vector. If refCond is greater than 0, comparisons are made between the reference condition and all other conditions. If refCond is 0, all possible pairwise comparisons are made. Default is 0.

**Value**

A matrix with two columns, representing all possible pairwise comparisons between the specified conditions. If a reference condition is specified, it appears in the first column of every row.

**Examples**

```
conditions <- c("Cond1", "Cond2", "Cond3")
# Generate all pairwise comparisons
allComps <- create_pairwise_comparisons(conditions, refCond = 0)
allComps

# Generate comparisons with a specific condition as reference
refComps <- create_pairwise_comparisons(conditions, refCond = 1)
refComps
```

---

FindFCandQlim

*Find Optimal Fold-Change and Q-Value Thresholds*


---

**Description**

Identifies the optimal fold-change (FC) and q-value thresholds that maximize the number of significant features identified across different statistical tests. It processes a matrix of q-values, applying various fold-change thresholds, and computes the distribution of significant features for each q-value threshold to determine the optimal combination of thresholds.

**Usage**

```
FindFCandQlim(Qvalue, LogRatios)
```

**Arguments**

Qvalue	A matrix of q-values obtained from statistical tests such as PolySTest, limma, rank products, and permutation tests. NA values in the matrix are treated as non-significant. The matrix should have the same number of rows as the LogRatios matrix, and the number of columns should be a multiple of the number of conditions, given by the number of different statistical tests.
LogRatios	A matrix of log <sub>2</sub> fold-change ratios for the same comparisons.

**Details**

The function first replaces NA values in the Qvalue matrix with 1 to denote non-significant results. It then identifies the smallest q-value and calculates a range of q-values around this minimum. For each fold-change threshold, the function adjusts the Qvalue matrix and calculates the number of significant features for each q-value threshold. The combination yielding the highest mean distribution of significant features is considered optimal.

**Value**

A numeric vector containing two elements: the optimal fold-change threshold and the optimal q-value threshold, which together maximize the number of significant features detected.

**Examples**

```
# Example Qvalue and LogRatios matrices
Qvalue <- matrix(seq(0.01, 0.12, 0.01), nrow = 4, ncol = 3)
LogRatios <- matrix(c(
  1.2, 0.8, 1.5, -0.5, 0.2, 0.9, -1.1, 0.7,
  1.8, -0.9, 0.3, 1.1
), nrow = 4, ncol = 3)
# Find optimal thresholds
thresholds <- FindFCandQlim(Qvalue, LogRatios)
print(thresholds)
```

---

FindFCandQlimAlternative

*Function to determine "optimal" fold-change and q-value thresholds using a higher criticism method and an FC threshold based on a standard deviation of one*

---

**Description**

Function to determine "optimal" fold-change and q-value thresholds using a higher criticism method and an FC threshold based on a standard deviation of one

**Usage**

```
FindFCandQlimAlternative(Pvalue, LogRatios)
```

**Arguments**

Pvalue	A matrix of p-values for each condition
LogRatios	A matrix of log ratios for each condition

**Value**

A vector containing the mean fold-change threshold and mean q-value threshold

**Examples**

```
Pvalue <- matrix(seq(0.01, 0.12, 0.01), nrow = 4, ncol = 3)
LogRatios <- matrix(c(
  1.2, 0.8, 1.5, -0.5, 0.2, 0.9, -1.1, 0.7, 1.8,
  -0.9, 0.3, 1.1
), nrow = 4, ncol = 3)
thresholds <- FindFCandQlimAlternative(Pvalue, LogRatios)
print(thresholds)
```

---

get_numthreads	<i>Set number of threads (default is 4)</i>
----------------	---

---

**Description**

This function sets the number of threads for parallel processing. If the environment variable SHINY\_THREADS is set, the number of threads is set to the value of SHINY\_THREADS.

**Usage**

```
get_numthreads(threads = NULL)
```

**Arguments**

threads            An integer indicating the number of threads to use.

**Value**

An integer indicating the number of threads to use.

**Examples**

```
get_numthreads(threads = 4)
get_numthreads()
```

---

limma_paired	<i>Perform paired limma analysis</i>
--------------	--------------------------------------

---

**Description**

This function performs paired limma analysis on MAData.

**Usage**

```
limma_paired(MADData, NumCond, NumReps)
```

**Arguments**

MADData	A ratio matrix of gene expression data. The rows are genes and the columns are samples. Replicates must be grouped together.
NumCond	The number of ratios to check vs zero level
NumReps	The number of replicates per condition.

**Value**

A list containing the p-values and q-values.

**Examples**

```
MADData <- matrix(rnorm(600), nrow = 100)
NumCond <- 3
NumReps <- 2
limma_res <- limma_unpaired(MADData, NumCond, NumReps)
head(limma_res$qlvalues)
```

---

limma_unpaired	<i>Perform unpaired limma analysis</i>
----------------	--

---

**Description**

This function performs unpaired limma analysis on Data.

**Usage**

```
limma_unpaired(Data, NumCond, NumReps, RRCateg)
```

**Arguments**

Data	A matrix of quantitative expression data.
NumCond	The number of conditions in the experiment.
NumReps	The number of replicates per condition.
RRCateg	A matrix specifying the conditions to be compared (each comparison given as separate column).

**Value**

A list containing the following results:

- pvalues: The p-values from limma tests.
- qlvalues: The q-values from limma tests.



- **Sds**: The standard deviations of the Bayesian linear model. **@details** This function performs unpaired limma analysis on Data. It calculates the p-values and q-values for each row, indicating the significance of the difference between the two datasets. **RRCateg** is a matrix specifying the conditions to be compared (each comparison given as separate column). It thus has always 2 rows and n columns, where n is the number of comparisons. The function returns a list containing the p-values and q-values for each comparison, as well as the standard deviations of the Bayesian linear model.

### Examples

```
dataMatrix <- matrix(rnorm(900), ncol = 9)
NumCond <- 3
NumReps <- 3
colnames(dataMatrix) <- rep(c("A", "B", "C"), each = 3)
# Specifying comparisons
RRCateg <- matrix(c(1, 2, 2, 3), nrow = 2, ncol = 2)
# Run function
results <- limma_unpaired(dataMatrix, NumCond, NumReps, RRCateg)
print(results$pvalues)
```

---

liver\_example

*Example data set liver\_example for PolySTest*

---

### Description

This data set is a SummarizedExperiment object containing liver proteomics of mice fed with four different diets. The data contains 3 replicates per diet (condition). The rowData of the object contains the output from running the PolySTest statistical tests on the data.

### Format

A SummarizedExperiment object

### Value

A SummarizedExperiment object

### Source

Protein expressions from the livers of mice fed with different diets: high fat, TTA component, Fish oil and TTA + fish oil

### Examples

```
data(liver_example)
```

---

MissingStats	<i>Calculates the p-values and q-values for missing values in a data frame with columns as samples and rows as features.</i>
--------------	--

---

**Description**

Calculates the p-values and q-values for missing values in a data frame with columns as samples and rows as features.

**Usage**

```
MissingStats(Data, NumCond, NumReps)
```

**Arguments**

Data	A matrix containing the expression data with rows as features and columns as samples
NumCond	The number of conditions in the dataset
NumReps	The number of replicates for each condition (needs to be same for all conditions)

**Value**

A list containing the calculated p-values and q-values (Benjamini-Hochberg) for missingness statistics

**Examples**

```
Data <- matrix(rnorm(120), nrow = 10)
# Introduce some missingness
Data[sample(1:120, 40)] <- NA
NumCond <- 4
NumReps <- 3
res_misstest <- MissingStats(Data, NumCond, NumReps)
head(res_misstest$qNAvalues)
```

---

MissingStatsDesign	<i>Function of calculation of Miss tests. This happens between full groups and thus does not have distinction for pairwise testing.</i>
--------------------	---

---

**Description**

Function of calculation of Miss tests. This happens between full groups and thus does not have distinction for pairwise testing.

**Usage**

```
MissingStatsDesign(Data, RRCateg, NumCond, NumReps)
```

**Arguments**

Data	A matrix containing the expression data with rows as features and columns as samples
RRCateg	A matrix containing the indices of the conditions to compare (each row is a pairing)
NumCond	The number of conditions in the dataset
NumReps	The number of replicates for each condition (needs to be same for all conditions)

**Value**

A list containing the calculated p-values and q-values (Benjamini-Hochbeg) for missingness statistics

**Examples**

```
Data <- matrix(rnorm(120), nrow = 10)
# Introduce some missingness
Data[sample(seq_len(120), 40)] <- NA
RRCateg <- matrix(c(1, 2, 1, 3, 1, 4, 2, 3, 2, 4, 3, 4), nrow = 2, ncol = 6)
NumCond <- 4
NumReps <- 3
res_misstest <- MissingStatsDesign(Data, RRCateg, NumCond, NumReps)
head(res_misstest$qNAvalues)
```

---

MissValPDistr

*Calculate the distribution of missing values*


---

**Description**

This function calculates the distribution of missing values for a given number of repetitions and percentage of missing values.

**Usage**

```
MissValPDistr(NumReps, PercNA)
```

**Arguments**

NumReps	An integer indicating the number of repetitions.
PercNA	A numeric value indicating the percentage of missing values.

**Value**

A numeric vector containing the distribution of missing values.

**Examples**

```
MissValPDistr(10, 0.2)
```

---

permtest_paired	<i>Perform permutation tests</i>
-----------------	----------------------------------

---

**Description**

This function performs permutation tests on the given tMADData. The permutation tests determine an empirical null distribution of t-values for the p-value calculation.

**Usage**

```
permtest_paired(tMADData)
```

**Arguments**

tMADData      A matrix of data for running permutation tests

**Value**

A list containing the p-values and q-values (Benjamini-Hochberg)

**Examples**

```
tMADData <- matrix(rnorm(100), nrow = 10)
tout <- permtest_paired(tMADData)
head(tout$qPermutvalues)
```

---

perm_unpaired	<i>Perform unpaired permutation tests</i>
---------------	---

---

**Description**

This function performs permutation testing for unpaired data. The permutation testing is based on comparing the t-values of the real data with the t-values of the permuted data.

**Usage**

```
perm_unpaired(tData, trefData)
```

**Arguments**

tData            The data matrix for the test group (features are rows).  
 trefData        The data matrix for the reference group (features are rows).

**Details**

The function adds columns from the randomized full set to reach the minimum number of permutation columns (NumPermCols) replicates. It randomizes the sign as well to avoid tendencies to one or the other side. In the unpaired case, it also normalizes by the mean of the entire sample to avoid strange effects. The function then performs permutation testing using parallel computing, and calculates the p-values and q-values based on the permutation results. Both groups needs to consist of the same number of samples (columns).

**Value**

A list containing the p-values and q-values for the permutation test.

**Examples**

```
tData <- matrix(rnorm(1000), nrow = 100)
trefData <- matrix(rnorm(1000), nrow = 100)
result <- perm_unpaired(tData, trefData)
```

---

<code>plotExpression</code>	<i>Plot Expression Profiles</i>
-----------------------------	---------------------------------

---

**Description**

This function plots expression profiles for selected features across different conditions and comparisons. It supports both scaling and unscaled profiles. It adds a circular plot to compare the different statistical tests

**Usage**

```
plotExpression(
  fulldata,
  compNames = "all",
  testNames = c("PolySTest", "limma", "Miss Test", "rank products", "permutation test",
    "t-test"),
  sel_prot = "all",
  profiles_scale = TRUE,
  qlim = 0.05,
  fclim = c(0, 0)
)
```

**Arguments**

fulldata	A SummarizedExperiment object containing the data.
compNames	A character vector of comparison names. "all" selects all comparisons.
testNames	A character vector of test names used in the analysis. Default values are "PolySTest", "limma", "Miss test", "rank products", "permutation test", and "t-test".
sel_prots	A numeric vector with the indices of the selected features. Default is "all". These will still be filtered
profiles_scale	Logical indicating if profiles should be scaled. Default is TRUE.
qlim	A numeric value indicating the q-value limit for significance.
fclim	A numeric vector of length 2 indicating fold-change limits.

**Value**

Plots expression profiles for the selected features.

**Examples**

```
data(liver_example)
compNames <- c("HF.Rep._vs_TTA.Rep.")
plotExpression(liver_example)
```

---

plotHeatmaply

*Heatmap Visualization with Heatmaply*

---

**Description**

This function generates a heatmap for selected features across comparisons using the heatmaply package. It provides options for scaling and saving the plot to a file.

**Usage**

```
plotHeatmaply(
  fulldata,
  sel_prots = "all",
  heatmap_scale = "none",
  file = NULL,
  ...
)
```

**Arguments**

fulldata	A SummarizedExperiment object containing the dataset.
sel_prots	Character vector specifying selected features to include in the heatmap or "all" to include all proteins.
heatmap_scale	Character, indicating if and how the data should be scaled. Possible values are "none", "row", or "column".
file	Optional character string specifying the path to save the heatmap plot. If NULL, the plot is rendered interactively.
...	Arguments passed further to heatmaply function

**Value**

A plotly object if file is NULL. Otherwise, the heatmap is saved to the specified file.

**Examples**

```
data(liver_example)
plotHeatmaply(
  fulldata = liver_example, sel_prots = "all",
  heatmap_scale = "row"
)
```

---

plotPvalueDistr      *Plot P-Value Distributions*

---

**Description**

Generates histograms of p-value distributions for each test and comparison.

**Usage**

```
plotPvalueDistr(
  fulldata,
  compNames = "all",
  testNames = c("limma", "Miss Test", "rank products", "permutation test", "t-test"),
  testCols = c("#33AAA", "#33AA33", "#AA3333", "#AA33AA", "#AAAA33", "#3333AA"),
  ...
)
```

**Arguments**

fulldata	A SummarizedExperiment object containing the dataset and FDR/q-values from PolySTest.
compNames	A character vector of comparison names. "all" selects all comparisons.

testNames A character vector of test names used in the analysis. Default values are "PolySTest", "limma", "Miss test", "rank products", "permutation test", and "t-test".

testCols A character vector of colors for each test. Defaults to c("#33AAAA", "#33AA33", "#AA3333", "#AA33AA", "#AAAA33", "#3333AA").

... Additional arguments passed to hist().

**Value**

Creates histograms of p-value distributions for the specified tests

**Examples**

```
# Assuming `fulldata` is a properly prepared `SummarizedExperiment` object
data(liver_example)
plotPvalueDistr(liver_example,
  compNames = c("HF.Rep._vs_TTA.Rep."),
  testCols = rainbow(5)
)
```

---

plotRegNumber

*Plot Number of Regulated Features*

---

**Description**

This function plots the mfrnumber of regulated features across comparisons for different statistical tests. It shows how the number of significant features varies with different FDR thresholds.

**Usage**

```
plotRegNumber(
  fulldata,
  compNames = "all",
  testNames = c("PolySTest", "limma", "Miss Test", "rank products", "permutation test",
    "t-test"),
  qlim = 0.05,
  fclim = c(0, 0),
  TestCols = c("#33AAAA", "#33AA33", "#AA3333", "#AA33AA", "#AAAA33", "#3333AA"),
  ...
)
```

**Arguments**

fulldata A SummarizedExperiment object containing the dataset.

compNames A character vector of comparison names. "all" selects all comparisons.

testNames A character vector of test names used in the analysis. Default values are "PolySTest", "limma", "Miss test", "rank products", "permutation test", and "t-test".

qlim Numeric, q-value (FDR) threshold.



**fclim**            Numeric vector, fold-change limits.  
**TestCols**        Character vector, colors to use for each test in the plot.  
**...**             Arguments passed further to plot/lines calls

### Value

Invisible. The function generates plots.

### Examples

```
data(liver_example)
plotRegNumber(fulldata = liver_example, NumComps = 3)
```

---

plotUpset	<i>Plot UpSet</i>
-----------	-------------------

---

### Description

Visualizes the intersections of significant features across multiple comparisons using an UpSet plot. Summarizes all comparisons from all tests

### Usage

```
plotUpset(fulldata, qlim = 0.05, fclim = c(0, 0))
```

### Arguments

**fulldata**        A SummarizedExperiment object containing the data.  
**qlim**            A numeric value, the q-value threshold for significance.  
**fclim**            A numeric vector, specifying fold change limits for filtering.

### Value

An UpSet plot visualizing the intersections of significant features.

### Examples

```
data(liver_example)
plotUpset(liver_example, qlim = 0.05)
```

---

plotVolcano

*Plot Volcano Plots for PolySTest results*


---

### Description

This function creates volcano plots for all specified statistical tests and comparisons using data from a SummarizedExperiment object. It highlights selected proteins and applies fold-change and q-value limits for visualization.

### Usage

```
plotVolcano(
  fulldata,
  compNames = "all",
  testNames = c("PolySTest", "limma", "Miss Test", "rank products", "permutation test",
    "t-test"),
  sel_prots = "all",
  qlim = 0.05,
  fclim = c(0, 0),
  testCols = c("#33AAAA", "#33AA33", "#AA3333", "#AA33AA", "#AAAA33", "#3333AA"),
  ...
)
```

### Arguments

fulldata	A SummarizedExperiment object containing the data.
compNames	A character vector of comparison names.
testNames	A character vector of test names including "PolySTest", "limma", "Miss test", "rank products", "permutation test", and "t-test".
sel_prots	A numeric vector indicating selected features to be visualized differently or "all" to select all features. Default is "all".
qlim	A numeric value setting the q-value limit for the plots. Default is 0.05.
fclim	A numeric vector of length two setting the fold-change limits for the plots. Default is c(0,0).
testCols	A character vector of colors for each test. Default is a predefined set of colors.
...	Additional arguments passed to the plot function.

### Value

Creates volcano plots for the specified tests and comparisons.

### Examples

```
data(liver_example)
compNames <- c("HF.Rep._vs_TTA.Rep.")
plotVolcano(liver_example, compNames)
```

---

PolySTest\_paired      *PolySTest for Paired Tests*

---

### Description

Integrates various statistical tests for analyzing paired data within a SummarizedExperiment framework. It compares pairs of conditions specified by the user to calculate p-values and q-values for each comparison.

### Usage

```
PolySTest_paired(  
  fulldata,  
  allComps,  
  statTests = c("limma", "Miss_Test", "t_test", "rank_products", "permutation_test")  
)
```

### Arguments

fulldata	A SummarizedExperiment or derived object containing the quantitative data required for PolySTest analysis.
allComps	A matrix specifying pairs of conditions to compare. Each row represents a pair for comparison.
statTests	A character vector specifying the statistical tests to be applied. Available tests include "limma", "Miss_Test", "t-test", "rank_products", and "permutation_test". The function will perform each specified test and integrate the results.

### Details

Executes specified statistical tests on the dataset contained in fulldata using the condition pairs outlined in allComps. Calculates p-values and q-values for each genomic feature (e.g., genes, proteins) included in the analysis. Results are added to the rowData of the SummarizedExperiment object, enhancing it with detailed statistics from the paired tests.

### Value

A SummarizedExperiment object augmented with p-values and q-values in its rowData, reflecting the outcomes of the specified statistical analyses.

### Examples

```
library(SummarizedExperiment)  
  
# Mock quantitative data and metadata for samples  
quantData <- matrix(rnorm(2000), nrow = 200, ncol = 10)  
colnames(quantData) <- c(  
  paste("Sample", 1:5, "_Condition_A", sep = ""),
```

```

    paste("Sample", 1:5, "_Condition_B", sep = "")
  )
rownames(quantData) <- paste("Gene", 1:200)
sampleMetadata <- data.frame(Condition = rep(c("A", "B"), each = 5))

# Creating the SummarizedExperiment object
fulldata <- SummarizedExperiment(
  assays = list(quant = quantData),
  colData = sampleMetadata
)
metadata(fulldata) <- list(NumReps = 5, NumCond = 2)

# Specifying pairs of conditions to compare
allComps <- matrix(c("A", "B"), ncol = 2, byrow = TRUE)

# Specify statistical tests to apply
statTests <- c("limma", "t_test", "rank_products")

# Running PolySTest for paired comparisons
results <- PolySTest_unpaired(fulldata, allComps, statTests)

```

---

PolySTest\_unpaired      *PolySTest for unpaired tests*

---

## Description

Combining the power of different statistical tests

## Usage

```

PolySTest_unpaired(
  fulldata,
  allComps,
  statTests = c("limma", "Miss_Test", "t_test", "rank_products", "permutation_test")
)

```

## Arguments

fulldata	A SummarizedExperiment or derived object that contains the quantitative data as required for PolySTest
allComps	A matrix containing the reference matrix specifying the pairs of conditions to compare (each comparison given as separate row)
statTests	A character vector specifying the statistical tests to be used. The available tests are: "limma", "Miss_Test", "t-test", "rank_products", and "permutation_test"

**Details**

This function performs unpaired statistical tests on the data in 'fulldata' using the pairs of conditions specified in 'allComps'. It calculates the p-values and q-values for each row for the statistical tests used. The statistical tests available are: limma, Miss\_Test, t-test, rank\_products, and a permutation\_test based on t values. The function returns a SummarizedExperiment object with added columns for p-values and q-values in rowData.

**Value**

SummarizedExperiment with added columns for p-values and q-values in rowData

**Examples**

```
# Creating mock quantitative data and sample metadata
library(SummarizedExperiment)
quantData <- matrix(rnorm(2000), nrow = 200, ncol = 10)
colnames(quantData) <- c(
  paste("Sample", seq_len(5), "_Condition_A", sep = ""),
  paste("Sample", seq_len(5), "_Condition_B", sep = "")
)
rownames(quantData) <- paste("Gene", seq_len(200))
sampleMetadata <- data.frame(Condition = rep(c("A", "B"), each = 5))

# Creating the SummarizedExperiment object
fulldata <- SummarizedExperiment(
  assays = list(quant = quantData),
  colData = sampleMetadata
)
metadata(fulldata) <- list(NumReps = 5, NumCond = 2)

# Specifying pairs of conditions to compare
allComps <- matrix(c("A", "B"), ncol = 2, byrow = TRUE)

# Running the PolySTest_unpaired function
results <- PolySTest_unpaired(fulldata, allComps)
```

---

RPStats

*RPStats Function*


---

**Description**

This function calculates the p-values for the RP (Rank Product) statistic based on the input tRPMADData and the number of replicates (NumReps). The statistics is one-sided, i.e. it only detects up-regulation.

**Usage**

```
RPStats(tRPMADData, NumReps)
```

**Arguments**

tRPMADData	A matrix containing the expression data with rows as features and columns as replicates.
NumReps	The number of replicates for each feature.

**Value**

A numeric vector containing the p-values for the RP statistic.

**Examples**

```
tRPMADData <- matrix(c(1, 2, 3, 4, 5, 6), nrow = 2, ncol = 3)
NumReps <- 3
RPStats(tRPMADData, NumReps)
```

---

rp_unpaired	<i>Perform unpaired rank products test</i>
-------------	--

---

**Description**

Perform unpaired rank products test

**Usage**

```
rp_unpaired(tData, trefData)
```

**Arguments**

tData	The data matrix for the test group (features are rows).
trefData	The data matrix for the reference group (features are rows).

**Details**

This function calculates the p-values and q-values using rankd product statistics. The function requires having the same number of samples per group. The function uses parallel computing to speed up the calculations.

**Value**

A list containing the p-values and q-values.

**Examples**

```
tData <- matrix(rnorm(1000), nrow = 100)
trefData <- matrix(rnorm(1000), nrow = 100)
rp_unpaired(tData, trefData)
```

---

set_mfrow	<i>Set Graphics Layout</i>
-----------	----------------------------

---

**Description**

Automatically sets the mfrow parameter for par() based on the total number of plots and the maximum number of columns desired.

**Usage**

```
set_mfrow(num_total, max_col)
```

**Arguments**

num_total	The total number of plots to display.
max_col	The maximum number of columns for the layout.

**Value**

Sets the mfrow parameter for par().

**Examples**

```
# This will set the layout to 2 rows of 3 columns
set_mfrow(num_total = 6, max_col = 3)
for (i in 1:6) hist(1:10)
par(mfrow = c(1, 1))
```

---

StatsForPermutTest	<i>Calculate statistics for permutation test</i>
--------------------	--

---

**Description**

This function calculates the statistics for a permutation test based on the input data.

**Usage**

```
StatsForPermutTest(Data, Paired)
```

**Arguments**

Data	A matrix or data frame containing the data for the test.
Paired	A logical value indicating whether the test is paired or not.

**Value**

A numeric vector containing the calculated statistics.

**Examples**

```
Data <- matrix(rnorm(100), ncol = 10)
StatsForPermutTest(Data, Paired = FALSE)
```

---

ttest_paired	<i>Perform paired t-tests</i>
--------------	-------------------------------

---

**Description**

This function performs row-wise paired t-tests on a data frame containing log-fold changes

**Usage**

```
ttest_paired(tMAData)
```

**Arguments**

tMAData            A matrix of data for running row-wise t-tests

**Value**

A list containing the p-values and q-values (qvalue package)

**Examples**

```
tMAData <- matrix(rnorm(1000), nrow = 100)
tout <- ttest_paired(tMAData)
head(tout$qvalues)
```

---

ttest_unpaired	<i>Perform unpaired t-tests on two datasets</i>
----------------	---

---

**Description**

Perform unpaired t-tests on two datasets

**Usage**

```
ttest_unpaired(tData, trefData)
```

**Arguments**

tData            A matrix or data frame with the quantitative features (via rows) of the first group  
trefData        A matrix or data frame with the quantitative features (via rows) of the second group



## Details

This function performs unpaired t-tests between corresponding rows of two datasets. It calculates the p-values and q-values for each row, indicating the significance of the difference between the two datasets. We require providing the same number of samples (columns) per group.

## Value

A list containing the p-values and q-values for each row

## Examples

```
tData <- matrix(rnorm(1000), nrow = 100)
trefData <- matrix(rnorm(1000), nrow = 100)
result <- ttest_unpaired(tData, trefData)
print(result$ptvalues)
print(result$qtvalues)
```

---

update\_conditions\_with\_lcs

*Update Conditions with Longest Common Subsequence*

---

## Description

This function iterates over a set of conditions and updates each condition with the longest common subsequence of column names associated with that condition.

## Usage

```
update_conditions_with_lcs(fulldata, default = NULL)
```

## Arguments

fulldata	A SummarizedExperiment or derived object that contains the quantitative data as specified for PolySTest
default	A vector of the length of the number of conditions suggesting their names

## Value

SummarizedExperiment with updated conditions

**Examples**

```
library(SummarizedExperiment)
se <- SummarizedExperiment(assays = list(count = matrix(rnorm(200),
  ncol = 10
)))
metadata(se) <- list(NumCond = 2, NumReps = 5)
rownames(colData(se)) <- paste0(
  rep(c("CondA_Rep", "CondB_Rep"), 5),
  rep(seq_len(5), each = 2)
)
default_conditions <- c("Condition_A", "Condition_B")
updated_conditions <- update_conditions_with_lcs(se, default_conditions)
print(colData(updated_conditions))
```

# Index

- \* **analysis**
  - limma\_paired, 7
  - limma\_unpaired, 8
  - permtest\_paired, 12
  - ttest\_paired, 24
- \* **internal**
  - FindFCandQlim, 5
- \* **limma**
  - limma\_paired, 7
  - limma\_unpaired, 8
- \* **paired**
  - limma\_paired, 7
  - permtest\_paired, 12
  - ttest\_paired, 24
- \* **permutation**
  - permtest\_paired, 12
- \* **t-test**
  - ttest\_paired, 24
- \* **unpaired**
  - limma\_unpaired, 8

check\_for\_polystest, 3  
check\_stat\_names, 3  
create\_pairwise\_comparisons, 4

FindFCandQlim, 5  
FindFCandQlimAlternative, 6

get\_numthreads, 7

limma\_paired, 7  
limma\_unpaired, 8  
liver\_example, 9

MissingStats, 10  
MissingStatsDesign, 10  
MissValPDistr, 11

perm\_unpaired, 12  
permtest\_paired, 12  
plotExpression, 13  
plotHeatmaply, 14  
plotPvalueDistr, 15  
plotRegNumber, 16  
plotUpset, 17  
plotVolcano, 18  
PolySTest\_paired, 19  
PolySTest\_unpaired, 20

rp\_unpaired, 22  
RPStats, 21

set\_mfrow, 23  
StatsForPermutTest, 23

ttest\_paired, 24  
ttest\_unpaired, 24

update\_conditions\_with\_lcs, 25