

Package ‘rADA’

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Title Statistical Analysis and Cut-Point Determination of Immunoassays

Version 1.1.9

Description Systematically transform immunoassay data, evaluate if the data is normally distributed, and pick the right method for cut point determination based on that evaluation. This package can also produce plots that are needed for reports, so data analysis and visualization can be done easily.

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assayMelt	<i>Melt Assay Dataset</i>
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Description

This function is a wrapper for the `reshape2::melt()` function and splits column of Day, Operator, and Replicate information into 3 separate columns.

Usage

```
assayMelt(assay.df, exp.name)
```

Arguments

assay.df	Imported data.frame consisting of the following columns: 'ID', 'Lot', and columns identifying the Day, Operator and Replicate like so: 'D1_Op2_3' to indicate Day 1, operator 2, replicate 3.
exp.name	Experiment name (as a string). To be used to distinguish between experiments for when melted assays are combined using <code>rbind()</code> .

Value

A melted data.frame

Author(s)

Emma Gail

Examples

```
assay.df.melted <- assayMelt(assay.df = lognormAssay, exp.name = 'Experiment1')
head(assay.df.melted)
```

calcCvStats	<i>Calculate Coefficient of Variation</i>
-------------	---

Description

The function calculates the mean, standard deviation and coefficient of variation for replicates of an immunoassay.

Usage

```
calcCvStats(assay.obj, cv.threshold = 20)
```

Arguments

assay.obj	An ImmunoAssay object imported by importAssay
cv.threshold	Threshold for re-calculation of means and standard deviation based on coefficient of variation. The default threshold is 20 (i.e., 20% CV)

Value

An object of the class ImmunoAssay with calculated CV stats in the cv.table slot

Author(s)

Emma Gail

Examples

```
assay.obj <- importAssay(lognormAssay)
assay.obj <- calcCvStats(assay.obj)
```

calcScpValues	<i>Calculate screening cut point values for scp()</i>
---------------	---

Description

This function calculates the values needed for the output of the scp() data.frame

Usage

```
calcScpValues(
  assay.values,
  conf.level = 0.95,
  distrib = c("nonparametric", "normal"),
  transf.method = c("log10", "ln")
)
```

Arguments

assay.values	List of selected values from the assay data.frame such as selected values from assayMelt()
conf.level	Decimal describing level of confidence to be used for confidence interval calculation. Defaults to 0.95
distrib	Distribution selection to determine the cut point calculation. Two options: 'non-parametric' or 'normal'
transf.method	Transformation method used. The inverse will be calculated as part of the output.

Value

A data.frame containing the values: "mean", "sd", "distrib", "cp", "mean.conf.int1", "mean.conf.int2", "cp.conf.int1", "cp.conf.int2"

Author(s)

Emma Gail

Examples

```
assay.df.melted <- assayMelt(assay.df = lognormAssay, exp.name = 'Experiment1')
assay.values <- assay.df.melted[assay.df.melted$DayOperator == 'D10p1',]$value
#This function assumes that the data has already been transformed.
scp.df <- calcScpValues(assay.values = log10(assay.values), distrib = 'normal',
transf.method = 'log10')
```

evalBoxplot

Evaluate the Assays with Boxplots

Description

This function produces a boxplot based on the variable chosen in order to visualize any analytical variability.

Usage

```
evalBoxplot(assay.obj, var = c("Day", "Operator"))
```

Arguments

assay.obj	An ImmunoAssay object imported by importAssay
var	Variable to examine in the plot. Either "Day" or "Operator".

Value

A boxplot as generated by ggplot2

Author(s)

Emma Gail

Examples

```
assay.obj <- importAssay(lognormAssay, exp.name = 'Experiment1')
evalBoxplot(assay.obj,var='Day') #visualize days on boxplot
```

evalNorm

Normality Evaluation

Description

This function evaluates the normality of the melted immunoassay dataset. In order to determine whether or not the distribution is normal, two tests are performed: the Shapiro Wilk test for normality and the test for skewness. See [shapiro.test](#) and [skewness](#) for details. In order to for a "nonparametric" recommendation to be made, the SW test must be significant (below desire value) and the absolute value skewness must be above the desired value. If only one or neither of these conditions are met, then the recommendation will be "normal".

Usage

```
evalNorm(  
  assay.obj,  
  category = NULL,  
  data.transf = FALSE,  
  transf.method = c("log10", "ln"),  
  excl.outliers = FALSE,  
  hist = TRUE,  
  p.val = 0.05,  
  skew = 1,  
  return.object = TRUE  
)
```

Arguments

assay.obj	An ImmunoAssay object imported by importAssay
category	If assay.df.melted consists of more than 1 dataset, choose the category here to split dataset
data.transf	Should the data should be transformed before normality is evaluated
transf.method	If data.transf is TRUE, which method should be used. Can choose between 'log10' and 'ln'.

excl.outliers	Should outliers be excluded from this analysis? If TRUE, data points which lie beyond the extremes of the whiskers in boxplot will be excluded, see boxplot.stats for details.
hist	Should a histogram be outputted? TRUE/FALSE
p.val	Value to be used for cutoff for Shapiro-Wilks test. Defaults to 0.05.
skew	Value to be used to determine skewness. Defaults to 1.
return.object	If FALSE, only the plot is returned and the stats are returned as a list.

Value

If return.object==FALSE, only the plot is returned and the stats are returned as a list. Otherwise, an object of the class ImmunoAssay is returned.

Author(s)

Emma Gail

Examples

```
assay.obj <- importAssay(lognormAssay, exp.name = 'Experiment1')
assay.obj <- evalNorm(assay.obj, category = 'Experiment1',
  data.transf = TRUE, transf.method = 'log10')
```

excludeOutliers *Exclude Outliers from Melted Assay Dataframe*

Description

This function excludes outliers from the assay dataframe based on grDevices::boxplot.stats(). This outlier removal method is based on Tukey's test where outliers are removed if outside the established interquartile range.

Usage

```
excludeOutliers(
  assay.df.melted,
  data.transf = FALSE,
  transf.method = c("log10", "ln")
)
```

Arguments

assay.df.melted	A data.frame produced by assayMelt()
data.transf	Should the data should be transformed before normality is evaluated
transf.method	If data.transf is TRUE, which method should be used. Can choose between 'log10' and 'ln'.

Value

A melted data.frame

Author(s)

Emma Gail

Examples

```
assay.df.melted <- assayMelt(assay.df = lognormAssay, exp.name = 'Experiment1')
excludeOutliers(assay.df.melted, data.transf = TRUE, transf.method = 'log10')
```

ImmunoAssay-class *Define ImmunoAssay class*

Description

This stores the data that is used for screening cut point analysis.

Slots

`data` Imported data as is, used for CV analysis

`melted.data` Data used for most functions

`exp.name` Experiment name

`stats` List of statistics, results gathered from both coefficient of variation analysis as well as plot generation

`outlier.rm` Has any outlier analysis been performed on this dataset?

`outlier.rm.method` If outlier removal has been performed, what method was used?

`scp.table` Table of cut point information

`cv.table` Table derived from coefficient of variation analysis

importAssay	<i>Import assay as ImmunoAssay object</i>
-------------	---

Description

Function to import assay information into an ImmunoAssay object for analysis.

Usage

```
importAssay(assay.df, exp.name = NULL)
```

Arguments

assay.df	Pathname to (.csv or .xlsx files accepted) or imported data.frame consisting of the following columns: 'ID', 'Lot', and columns identifying the Day, Operator and Replicate like so: 'D1_Op2_3' to indicate Day 1, operator 2, replicate 3.
exp.name	Experiment name. If stays NULL, will automatically name experiment as 'experiment1'.

Value

An object of the class ImmunoAssay

Author(s)

Emma Gail

Examples

```
assay.df <- importAssay(assay.df = lognormAssay)
```

lognormAssay	<i>Simulated Lognormal Dataset</i>
--------------	------------------------------------

Description

This is a simulated dataset that using a lognormal distribution

Usage

```
data(lognormAssay)
```

Format

An object of class data.frame with 100 rows and 20 columns.

mixedModel	<i>Mixed model wrapper for assay dataframe</i>
------------	--

Description

This function is a wrapper for the lmer() function to produce a table of results. Here, the sample ID is included as a random intercept effect, then the results of the fixed effect model estimates are reported together with 95% confidence intervals and t statistics.

Usage

```
mixedModel(assay.df.melted, var = c("Operator", "Day"))
```

Arguments

assay.df.melted	A data.frame produced by assayMelt()
var	Variable to look at. Either "Day" or "Operator".

Value

A data.frame with the following columns: "Parameter", "Estimate", "LowerCI", "UpperCI", "Tstat"

Author(s)

Emma Gail
Lidija Turkovic

Examples

```
assay.df.melted <- assayMelt(assay.df = lognormAssay, exp.name = 'Experiment1')  
mixedModel(assay.df.melted, var = 'Day')
```

scp	<i>Calculate screening cut point</i>
-----	--------------------------------------

Description

This function will calculate the screening cut point from the melted assay.df

Usage

```
scp(  
  assay.obj,  
  category = NULL,  
  distrib = c("normal", "nonparametric"),  
  data.transf = FALSE,  
  transf.method = c("log10", "ln"),  
  rm.out = FALSE  
)
```

Arguments

assay.obj	An ImmunoAssay object imported by importAssay
category	If assay.obj consists of more than 1 dataset, choose the category here to split dataset
distrib	Distribution selection to determine the cut point calculation. Two options: 'non-parametric' or 'normal'
data.transf	Should the data should be transformed before the cut point is calculated
transf.method	If data.transf is TRUE, which method should be used. Can choose between 'log10' and 'ln'.
rm.out	Should outliers be excluded from this analysis?

Value

An object of the class ImmunoAssay

Author(s)

Emma Gail

Examples

```
assay.obj <- importAssay(assay.df = lognormAssay, exp.name = 'Experiment1')  
assay.obj <- scp(assay.obj, category = 'Experiment1', distrib = 'normal',  
  data.transf = TRUE, transf.method = 'log10', rm.out = FALSE)
```

scpForestPlot

Generate forest plot of SCP values

Description

This function creates a forest plot of the calculated screening cut points. The scp function must be called prior to this function.

Usage

```
scpForestPlot(assay.obj, ...)
```

Arguments

<code>assay.obj</code>	An ImmunoAssay object imported by <code>importAssay</code>
<code>...</code>	Additional arguments for <code>forestplot()</code> function

Value

A forestplot

Author(s)

Emma Gail

Examples

```
assay.obj <- importAssay(assay.df = lognormAssay, exp.name = 'Experiment1')
assay.obj <- scp(assay.obj, category = 'Experiment1', distrib = 'normal',
data.transf = TRUE, transf.method = 'log10', rm.out = FALSE)
scpForestPlot(assay.obj)
```

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