Package ‘rADA’

October 14, 2022

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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VignetteBuilder knitr

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Encoding UTF-8

LazyData true

RoxygenNote 7.1.1

Suggests markdown (>= 1.1)

Imports lme4 (>= 1.1.21), ggplot2 (>= 3.2.1), matrixStats (>= 0.56.0), reshape2 (>= 1.4.3), lmerTest (>= 3.1.0), e1071 (>= 1.7.2), tidyr (>= 1.0.0), stringr (>= 1.4.0), dplyr (>= 0.8.3), Hmisc (>= 4.3.0), gridExtra (>= 2.3), grid (>= 3.5.3), stats (>= 3.5.3), grDevices (>= 3.5.3), utils (>= 3.5.3), forestplot (>= 1.10), methods (>= 3.5.3), knitr (>= 1.29), openxlsx (>= 4.2.2), car (>= 3.0)

NeedsCompilation no

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Depends R (>= 3.5.0)

Repository CRAN

Date/Publication 2021-03-23 19:40:06 UTC
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Melt Assay Dataset

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 rbind().

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  Calculate Coefficient of Variation

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  Calculate screening cut point values for scp()

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") )
```
**evalBoxplot**

**Evaluate the Assays with Boxplots**

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

**Usage**

```r
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".
evalNorm

Value
A boxplot as generated by ggplot2

Author(s)
Emma Gail

Examples

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

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

```r
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'.

excludeOutliers

- 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

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

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

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

Arguments

- assay.df.melted: A dataframe 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'.
**ImmunoAssay-class**

**Value**

A melted data.frame

**Author(s)**

Emma Gail

**Examples**

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

---

**ImmuoAssay-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

*Import assay as ImmunoAssay object*

**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**

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

---

lognormAssay

*Simulated Lognormal Dataset*

**Description**

This is a simulated dataset that using a lognormal distribution

**Usage**

```r
data(lognormAssay)
```

**Format**

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

Mixed model wrapper for assay dataframe

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')

cp
Calculate screening cut point

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**

- **assay.obj**: An ImmunoAssay object imported by importAssay
- **...**: Additional arguments for forestplot() function

**Value**

A forestplot

**Author(s)**

Emma Gail

**Examples**

```r
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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