Package 'peripheral'

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Title Analysis of Peripheral Membrane Protein Distribution

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Description Package of functions for a determination of a plasma-membrane affinity of a fluorescently-tagged peripherally-associated plasma-membrane protein based on linear profiles of a tagged-protein and a plasma-membrane marker fluorescence signal.

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`peripheral-package` *peripheral-package*

Description

Package of functions for a determination of a plasma-membrane affinity of a fluorescently-tagged peripherally-associated plasma-membrane protein based on linear profiles of a tagged-protein and a plasma-membrane marker fluorescence signal.

Details

This package enables a decomposition of a fluorescence signal from a fluorescently-tagged peripherally-associated plasma-membrane protein to the plasma-membrane and the cytoplasmic compounds, and exact determination of a plasma-membrane binding affinity of tested protein. This process is implemented in `distrib.profile` function.

A fluorescence signal is represented by a profile measurement acquired by ImageJ 'Plot Profile' function Profiles were obtained by processing of confocal images of cells labelled by a plasma-membrane marker and expressing a tagged protein. Input data are imported by function `read.profile` into an object of class "profile".

The decomposition is based on a model of the distribution of a plasma-membrane and a cytoplasmic fluorescence signal. This model is created by `model.profile` function, which uses a profile measurement from cell expressing a cytoplasmic marker as a reference.

A fluorescence signal model and a result of the signal decomposition are stored in special objects called "model" and "distrib" respectivelly.

Methods for standard generic functions `print`, `summary`, `plot` etc. . . are implemented.

Author(s)

Stanislav Vosolsobě

Maintainer: Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

`ggplot`

Examples

```
## Not run:

## Import profile measurement aquired by ImageJ...

dta <- read.profile("my_directory", var.sep = "_", export = c(2,3), header = TRUE)
# from a file named like "2017-02-06_GFP_cell01_channel01.txt"
# stored in folder "my_directory".
# "GFP" and "cell01" are exported as variant descriptors.

## Create a model of plasma-membrane and cytoplasmic compounds of fluorescence signal

mp <- model(calib)
# 'calib' is a calibration dataset containing fluorescence profile measurements
# of a cytoplasmic marker and a plasma-membrane marker,
# see help(GFPcalib) for example calibration dataset.

## Analyse a peripherally-associated plasma-membrane protein measurements

dp <- distrib(dta, mp)
# 'dta' contains profile measurements of a peripheral protein,
# see help(peripher) for example dataset.
# 'mp' is a model constructed above.

## End(Not run)
```

---

as.data.frame.profile *Convert to Data Frame*

Description

This function converts a list object of class "profile" to "data frame". For example, this function can be used when profile data nay be processed by ggplot grafical function.

Usage

```
  ## S3 method for class 'profile'
as.data.frame(x, ...)
```

Arguments

x	an object of class "profile".
...	additional arguments to be passed to or from methods.

Details

This function takes a fluorescence profile data acquired by the ImageJ function 'Plot profile' stored in a variable of a class "profile". Input data must contain profiles of a plasma-membrane and a cytoplasmic markers (typically obtained from confocal sections of cells expressing a free GFP protein that were labelled by the FM4-64 plasma membrane dye). Plasma-membrane profiles are smoothed by the local polynomial regression (`loess` function with "span = 0.3") to determine the maximum of the plasma-membrane signal, which defines a position of the plasma membrane. This position is used as the zero coordinate in the subsequent transformation of x-coordinates.

Profile data are joined to data frame with columns containing transformed x-coordinates, corresponding values of a tagged-protein and a plasma-membrane marker fluorescence signal, an identidicator of entire profiles and columns for each variant level.

Value

`as.data.frame.profile` returns a data frame with columns "x", "GFP", "FM4", "profile" and "va1" ... "varN" for N variant levels.

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

`read.profile`

Examples

```
## Not run:

## GFPcalib - object of class "profile" with measurements
## of free-GFP expressing cells labeled by FM4-64 dye


df <- as.data.frame(GFPcalib)

## End(Not run)
```

boxplot.distrib *Create a Boxplot of Plasma-membrane affinities*

Description

This function create a boxplot from data of plasma-membrane affinities.

Usage

```
  ## S3 method for class 'distrib'
boxplot(x, variants = NULL,
  outliers = TRUE,
  xlab = "Variants",
  ylab = "Intensity",
  main = "Plasma membrane affinity", ...)
```

Arguments

x	an object of class "distrib".
variants	a vector of character strings specifying, which variant levels may be used as grouping variable. Allowed form of level identifier is `c("var2", "var3", ...)`, `"var1"` is ignored.
outliers	a logical. Show outliers in boxplot? Identical with argument `outline` of standard `boxplot` function.
xlab	a string specifying a caption of the x-axis.
ylab	a string specifying a caption of the y-axis.
main	a string specifying the main caption of the plot.
...	additional arguments to be passed to `boxplot` function.

Details

This function creates a box plot of plasma membrane affinities stored in object of class 'distrib' with groups specified in `variants` argument. If `variants = NULL`, this function extracts levels from object of class 'distrib' and creates boxplot according to the formula 'y ~ var2*var3*...'. Whereas field 'var1' stores unique profile identifiers, fields 'var2' and subsequent store aditional variant descriptors. If these variants are not specified in `x` object or `variants = ""`, only single boxplot will be produced.

Value

List with components identical to `boxplot.default` function.

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes,

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

distrib.profile, boxplot

Examples

```
## Not run:

## 'dp' is an object of class 'distrib'
## produced by 'distrib.profile' function.
## See help(distrib.profile) for details.

plot(dp, outliers = FALSE)

## End(Not run)
```

---

c.profile *Combine Profile Objects*

Description

This is a method of primitive function c which combines objects of class "profile".

Usage

```
  ## S3 method for class 'profile'
c(x, ...)
```

Arguments

x	an object of class "profile"
...	additional arguments to be passed to or from methods.

Value

c.profile returns an object of class "profile"

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

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See Also

read.profile

Examples

```
## Not run:

## peripher - object of class "profile" with measurements
## of cells expressing integral plasma-membrane protein
## PMA1-GFP, peripheral plasma membrane protein DREPP2-GFP
## and its mutated form DREPP2(Gly2Ala)-GFP with lowered
## plasma-membrane binding activity

## GFPcalib - object of class "profile" with measurements
## of free-GFP expressing cells labeled by FM4-64 dye

joined <- c(peripher, GFPcalib)

## End(Not run)
```

---

distrib.profile *Decomposition of Fluorescence Signal into Plasma-membrane and Cytoplasmic Compound*

Description

distrib is a generic function. Method for class "profile" decomposes a profile data of fluorescence signal emitted by a fluorescently-labelled peripherally-associated plasma-membrane protein into a plasma-membrane and a cytoplasmic compounds. The decomposition is based on a model of a fluorescence signal distribution created by model.profile function.

Usage

```
  distrib(x, ...)
  ## S3 method for class 'profile'
distrib(x, model, ...)
```

Arguments

x	an object of class "profile" containing profile measurements from cells, which expressing examined fluorescently-tagged protein and were labeled by a fluorescent marker of the plasma membrane.
model	an object of class "model" containing predicted fluorescence signal distributions in the plasma-membrane and the cytoplasm.
...	additional arguments to be passed to or from methods.

Details

This function takes a fluorescence profile data acquired by the ImageJ function 'Plot profile' stored in a variable of a class "profile". Input data `profile.list` must contain profiles of a plasma-membrane marker and a examined protein (typically obtained from confocal sections of cells expressing a GFP-tagged protein that were labelled by the FM4-64 plasma membrane dye). Plasma-membrane profiles are smoothed by the local polynomial regression (`loess` function with "span = 0.3") to determine the maximum of the plasma-membrane signal, which defines a position of the plasma membrane. This position is used as the zero coordinate in the subsequent transformation of x-coordinates.

X-transformed profiles of examined-protein fluorescence signal are subjected to an approximation, which finds a values corresponding to x-coordinates in used model, and this data $I_{GFP}(x)$ are fitted by two-component `model` according to

$$I_{GFP}(x) = I_{mem} f_{mem}(x) + I_{cyt} f_{cyt}(x)$$

where $f_{mem}(x)$ and $f_{cyt}(x)$ are plasma-membrane and cytoplasmic compounds of modelled distribution respectively and I_{mem} and I_{cyt} are coefficients optimised by `nls` function.

While cytoplasmic distribution of protein is equivalent to I_{cyt} coefficient, plasma-membrane distribution is derived from I_{mem} using a numeric integration of entire fitted plasma-membrane compound of the fluorescence signal according to

$$\Phi_{mem} = \sum_{x_1}^{x_2} I_{mem} f_{mem}(x) \Delta x$$

where x_1, x_2 and Δx are a starting point, an ending point and a step of x-coordinates of the model.

A protein affinity to plasma membrane is defined as a ratio

$$\xi_{mem/cyt} = \frac{\Phi_{mem}}{I_{cyt}}$$

with units of μm. This value defines a thickness of cytoplasm, in which is the same amouth of the protein as in the adjanced part of the plasma membrane.

Residuals are inherited from a `$m$resid()` object returned by `nls` function. A relative residual signal for each processed profile is computed as a `sum` of absolute values of residuals divided by `sum` of original signal.

Value

distrib.profile returns an object of class "distrib", which is a list with components:

residuals	a data frame of residual for each profile measurement.
affinity	a data frame of variant identifiers and affinities of the protein to the plasma membrane computed for each profile.
detailed	a data frame of relative residual signal and above defined parametres Φ_{mem} and I_{cyt}.

Note

Potential errors can be caused by a nls function called internally.

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

read.profile, model.profile, summary.distrib, residuals.distrib, subset.distrib, [.distrib, filter.distrib

Examples

```
## Not run:

## peripher - object of class "profile" with measurements
## of cells expressing integral plasma-membrane protein
## PMA1-GFP, peripheral plasma membrane protein DREPP2-GFP
## and its mutated form DREPP2(Gly2Ala)-GFP with lowered
## plasma-membrane binding activity

mp <- model(GFPcalib)  #see help(model.profile) for details
dp <- distrib(peripher, mp)
summary(dp)

## End(Not run)
```

`filter` *Data Filtering*

Description

`filter` is a generic function used to filter data according to selected criteria. The function invokes particular methods which depend on the class of the first argument.

Usage

```
filter(x, ...)
```

Arguments

`x`	an object for filtering.
`...`	additional arguments affecting a filtering.

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

`filter.profile`, `filter.distrib`

`filter.distrib` *Data Filtering*

Description

This function enables to remove inappropriate measurements of plasma-membrane affinity of peripherally-associated plasma-membrane protein analysed by `distrib.profile` function.

Usage

```
  ## S3 method for class 'distrib'
filter(x, cut.off = 0.2, ...)
```

Arguments

x	an object of class "distrib".
cut.off	a value af a maximal allowed relative residual signal <0, 1>.
...	additional arguments to be passed to or from methods.

Details

This function remove all profile measurements stored in the distrib object, which have a relative residual signal higher than cut.off value. The function [.distrib is used internally.

Value

filter.distrib returns an object of class "distrib"

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

distrib.profile

Examples

```
## Not run:

## 'dp' is object of class "distrib",
## see help(distrib.profile) for details

fp <- filter(dp, cut.off = 0.15)

## End(Not run)
```

`filter.profile` *Data Filtering*

Description

This function enables to remove inappropriate measurements of fluorescence profiles. Typically, this function is used for eliminating profile measurements with excessive signal in extracellural space (both "FM4" and "GFP" channel) or in cytoplasm ("FM4" channel).

Usage

```
  ## S3 method for class 'profile'
filter(x, channel = "FM4",
    bellow = NULL, above = NULL,
    greater = NULL, lower = NULL, ...)
```

Arguments

`x`	an object of class "profile" containing filtered data.
`channel`	select, which fluorescence channel may be tested, "FM4" for selecting a plasma-membrane marker signal, "GFP" for selecting tagged-protein signal.
`bellow`	select all points of profile under this limit of x-coordinate relative to the plasma-membrane position (in μm).
`above`	select all points of profile above this limit of x-coordinate relative to the plasma-membrane position (in μm).
`greater`	define minimal acceptable intensity of a relative fluorescence signal in the interval of selected points; define number in range <0, 1>.
`lower`	define maximal acceptable intensity of a relative fluorescence signal in the interval of selected points; define number in range <0, 1>.
`...`	additional arguments to be passed to or from methods.

Details

This function takes a fluorescence profile data acquired by the ImageJ function 'Plot profile' stored in a variable of a class "profile". Input data must contain profiles of a plasma-membrane and a cytoplasmic markers (typically obtained from confocal sections of cells expressing a free GFP protein that were labelled by the FM4-64 plasma membrane dye). Plasma-membrane profiles are smoothed by the local polynomial regression (`loess` function with "span = 0.3") to determine the maximum of the plasma-membrane signal, which defines a position of the plasma membrane. This position is used as the zero coordinate in the subsequent transformation of x-coordinates.

Selected channel of profile are normalised according to its maximal values and subjected to filtering. The function `[.profile` is used internally.

Value

filter.profile returns an object of class "profile"

Note

This function can be applied repetitely on a filtered dataset. In each usage, only one combination of specification (`bellow` or `above`) with (`greater` or `lower`) can be defined.

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

`read.profile`

Examples

```
## Not run:

## GFPcalib - object of class "profile" with measurements
## of free-GFP expressing cells labeled by FM4-64 dye

fp <- filter(GFPcalib, channel = "GFP", bellow = -0.5, lower = 0.6)

## End(Not run)
```

GFPcalib *Fluorescence Profiles of Membrane and Cytoplasmic Markers*

Description

This dataset contains profile measurements of a fluorescence signal aquired by an ImageJ function 'Plot Profile' from confocal images of tobacco BY-2 suspension cells, which are labelled by FM4-64 plasma-membrane dye and expressing free GFP protein. This measurements are used as a calibration dataset in a modelling of a plasma-membrane and a cytoplasmic compounds of a fluorescence signal, when plasma-membrane affinity of peripherally-associated protein is analysed.

Usage

```
data("GFPcalib")
```

Format

A list object of class "profile" with 156 elements. Each element contains fluorescence profile data of this structure:

var	a vectror of variant descriptors for each variant level
x	a numeric vectror of profile x-ccordinates
GFP	a numeric vectror of tagged-protein fluorescence signal
FM4	a numeric vectror of plasma-membrane marker fluorescence signal

Details

`var` vectors contain a tagged-protein descriptor ("GFP") and a cell-identity descriptor ("01", "02", ...)

Source

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

Examples

```
## Not run:

summary(GFPcalib)
plot(GFPcalib)

## See help(model.profile) for details
mp <- model(GFPcalib)

## End(Not run)
```

`model.profile` *Model of Cytoplasmic and Membrane Components of Fluorescence*

Description

`model` is a generic function. Method for class "profile" models a distribution of the cytoplasmic and the membrane components of a fluorescence signal emitted by a fluorescently-labelled peripheral plasma-membrane protein. The distribution is modelled across a transversal profile of the plasma membrane and the adjacent cortical layer of a cell. The modelling is based on profile measurements from confocal sections of cells labelled by a fluorescent marker of the cytoplasm, typically the free GFP, and a plasma-membrane marker, typically the FM4-64 dye.

Usage

```
  model(x, ...)
  ## S3 method for class 'profile'
model(x, predFrom = -1.5, predTo = 1.5,
      predStep = 0.1, lambdaEmFM4 = 0.8, lambdaEmGFP = 0.52,
      lambdaExFM4 = 0.514, lambdaExGFP = 0.488, ...)
```

Arguments

x	an object of class "profile" containing profile measurements from cells labelled by fluorescent markers of the plasma membrane and the cytoplasm.
predFrom	an integer determining the starting x-coordinate (in μm) in the resulting model; related to the position of the plasma membrane. Typically a negative value, which referring to the outside of a cell.
predTo	an integer determining the ending x-coordinate (in μm) in the resulting model; related to the position of the plasma membrane. Typically a positive value, which referring to the inside of a cell.
predStep	an integer determining the step of x-coordinates (in μm) in the resulting model.
lambdaEmFM4	the wavelength (in μm) of the emission maximum of the plasma-membrane fluorescent marker. Default values are for the FM4-64 dye.
lambdaEmGFP	the wavelength (in μm) of the emission maximum of the cytoplasmic fluorescent marker. Default values are for the GFP.
lambdaExFM4	the wavelength (in μm) of the excitation maximum of the plasma-membrane fluorescent marker. Default values are for the FM4-64 dye.
lambdaExGFP	the wavelength (in μm) of the excitation maximum of the cytoplasmic fluorescent marker. Default values are for the GFP dye.
...	additional arguments to be passed to or from methods.

Details

This function takes a fluorescence profile data acquired by the ImageJ function 'Plot profile' stored in a variable of a class "profile". Input data must contain profiles of a plasma-membrane and a cytoplasmic markers (typically obtained from confocal sections of cells expressing a free GFP protein that were labelled by the FM4-64 plasma membrane dye). Plasma-membrane profiles are smoothed by the local polynomial regression (loess function with "span = 0.3") to determine the maximum of the plasma-membrane signal, which defines a position of the plasma membrane. This position is used as the zero coordinate in the subsequent transformation of x-coordinates.

Entire profile data are normalised according to 10 % of their maximal values, joined and ordered according to transformed x-coordinates.

The model of the cytoplasmic compound of a fluorescence signal is predicted from a local polynomial regression (span = 0.3) of ordered values of cytoplasmic profile data for vector of x-values given by predFrom, predTo and predStep options.

The model of the plasma-membrane compound of a fluorescence signal is predicted from a Gaussian-fitting of ordered values of plasma-membrane profile data. Gaussian fit is used as a simplified description of the Point-spread function of the plasma-membrane signal.

$$I_{FM4}(x) = I_{max} \exp\left(kx^2\left(\frac{1}{\lambda^2_{EmFM4}} + \frac{1}{\lambda^2_{ExFM4}}\right)\right)$$

where I_{max} and k are optimised by nls function. Because the resulting model predicts a distribution of a fluorescence signal for the same fluorophore as is used for a labeling of the cytoplasm, the Gaussian fit based on plasma-membrane markers is transformed with respect to a different wavelengths of the emission and the excitation according to

$$I_{GFP}(x) = \left(\frac{I_{FM4}(x)}{I_{max}} \right)^{\frac{1/\lambda^2_{EmGFP}+1/\lambda^2_{ExGFP}}{1/\lambda^2_{EmFM4}+1/\lambda^2_{ExFM4}}}$$

Residuals of the resulting models are inherited from a deviation of `nls` fitting (for a membrane signal) or computed as sum-of-squares of residuals in case of `loess` smoothing (for a cytoplasmic signal).

Value

`model.profile` returns an object of class "model", which is a list with components:

`original.data`	a list containing joined original data after normalisation and transformation.
`stats`	a list with a number of original observations and sum-of-squares of residuals for both compounds fitted by the model.
`CYmodel`	a vector of distribution of cytoplasmic compoud of fluorescence signal.
`PMmodel`	a vector of distribution of plasma-membrane compound of fluorescence signal.
`xpred`	a vector of x-coordinates of modelled values.

Generic functions such as print, summary and plot have methods for class 'model'.

Note

Potential errors can be caused by a `nls` function called internally.

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

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See Also

`summary.model`, `plot.model`, `read.profile`, `distrib.profile`

Examples

```
## Not run:

## GFPcalib - object of class "profile" with measurements
## of free-GFP expressing cells labeled by FM4-64 dye

mp <- model(GFPcalib)
summary(mp)
```

```
plot(mp)

## see help(distrib.profile) for continuation

## End(Not run)
```

---

peripher *Fluorescence Profile Data of Membrane Protein*

Description

Fluorescence profile data aquired by ImageJ function 'Plot Profile' from confocal images of tobacco BY-2 suspension cells, which are labelled by FM4-64 plasma-membrane dye and expressing GFP-tagged proteins PMA1 (Plasma-membrane ATPase 1), DREPP2 (Developmentally-regulated plasma-membrane polypeptide 2 from tobacco) and DREPP2(G2A) (DREPP2 protein with mutation in the N-myristoylation site).

Usage

```
data("peripher")
```

Format

A list object of class 'profile' with 771 elements. Each element contains fluorescence profile data of this structure:

var	a vectror of variant descriptors for each variant level
x	a numeric vectror of profile x-ccordinates
GFP	a numeric vectror of tagged-protein fluorescence signal
FM4	a numeric vectror of plasma-membrane marker fluorescence signal

Details

PMA1 protein is an example of intristic membrane protein with a majority of fluorescence signal on the plasma membrane.

DREPP2 is a peripeherally-associated plasma-membrane protein with weak signal in cytoplasm. DREPP2 is anchored to the plasma membrane via an N-myristoylation and an electrostatic interaction of aminoacids with membrane lipids.

DREPP2(G2A) is a mutated version of DREPP2 carrying a mutation in Gly2, which affects an N-myristoylation. DREPP2(G2A) is partially localised on the plalsma-membrane because of the electrostatic interaction. The main signal of the DREPP2(G2A) fluorescence is in the cytoplasm.

`var` vectors contain a tagged-protein descriptor ('PMA1', 'DREPP2' and 'DREPP2(G2A)') and a cell-identity descriptor ('01', '02', ...)

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

Examples

```
## Not run:

summary(peripher)
plot(peripher)

## End(Not run)
## See help(distrib.profile) for next examples
```

`plot.model` *Plot Model of Membrane and Cytoplasmic Compounds of Fluorescence*

Description

This function plots a model of a plasma-membrane and a cytoplasmic compounds of a fluorescently-tagged peripherally-associated plasma-membrane protein. Model is based on profile measurements of a fluorescence signal emited by a plasma-membrane and a cytoplasmic marker.

Usage

```
  ## S3 method for class 'model'
plot(x, original = TRUE,
  CY.col = "green", PM.col = "red",
  xlab = "x-position", ylab = "Intensity",
  main = "Model of fluorescence intensity distribution", ...)
```

Arguments

`x`	an object of class "model"
`original`	a logical. Show original measurements of a plasma-membrane and a cytoplasmic marker fluorescence signal in the plot?
`CY.col`	a character string specifying a name of the colour used for a plotting of the model of cytoplasmic fluorescence signal.
`PM.col`	a character string specifying a name of the colour used for a plotting of the model of plasma-membrane fluorescence signal.
`xlab`	a string specifying a caption of the x-axis.
`ylab`	a string specifying a caption of the y-axis.
`main`	a string specifying the main caption of the plot.
`...`	additional arguments to be passed to `plot` function.

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

model.profile

Examples

```
## Not run:

## 'mp' is an object of class 'model'
## produced by 'model.profile' function.
## See help(model.profile) for details.

mp <- model(GFPcalib)
plot(mp)

## End(Not run)
```

---

plot.profile *Plot Fluorescence Profile Data*

Description

This function create a plot of a plasma-membrane and a cytoplasmic compounds of a fluorescence signal from a model, which is based on a plasma-membrane and a cytoplasmic fluorescent markers.

Usage

```
  ## S3 method for class 'profile'
plot(x, type = "l", size = 0.1,
  variants = "var1", colMarker = "red", colTest = "green",
  as.grid = FALSE, ...)
```

Arguments

x	an object of class "profile"
type	a character specifying a plotting method, "p" for points, "l" for lines.
size	a numeric specifying a size of points in plot. This argument has no effect, when type = "l".

variants	a vector of character strings specifying, which variant levels may be used as grouping variable. Allowed form of level identifier is `c("var1", "var2", ...)`.
colMarker	a character string specifying a name of the colour used for a plotting of the plasma-membrane marker signal.
colTest	a character string specifying a name of the colour used for a plotting of the tagged-protein signal.
as.grid	a logical, if `as.grid` is TRUE, plot panels defined by a combination of two grouping factors are plotted in grid form, not in wrapped form. See `facet_grid` and `facet_wrap` for details.
...	additional arguments to be passed to or from methods.

Details

This function use `as.data.frame.profile` function internally and plotting is performed using functions from package `ggplot2`.

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

`ggplot`, `read.profile`, `as.data.frame.profile`, `subset.profile`.

Examples

```
## Not run:

## peripher - object of class "profile" with measurements
## of cells expressing integral plasma-membrane protein
## PMA1-GFP, peripheral plasma membrane protein DREPP2-GFP
## and its mutated form DREPP2(Gly2Ala)-GFP with lowered
## plasma-membrane binding activity

plot(peripher, variants = c("var1","var2"), as.grid = TRUE)

## End(Not run)
```

`read.profile` *Read Fluorescence Profile Data*

Description

This function imports a two-channel fluorescence profile data from separate files.

Usage

```
read.profile(file = NULL, dir = NULL)
```

Arguments

`file`	a character string specifying file.
`dir`	a character string specifying working directory.

Details

This function import one file specified by `file` or all files in directory `dir`. Files must be comma-delimited .csv files containing fluorescence profile measurements acquired by the ImageJ macro 'Peripheral.ijm'. Files must have a structure like this example:

X,	GFP,	FM4,	profile_ID,	varID_1,	...
0.0,	0.0,	0.0,	0,	cell_01,	...
0.1,	0.5,	0.7,	0,	cell_01,	...
0.2,	0.4,	1.1,	0,	cell_01,	...
0.3,	0.9,	1.9,	0,	cell_01,	...
0.4,	1.6,	1.2,	0,	cell_01,	...
...,	...,	...,	...,	...,	...

Profile measurements must contains an x-coordinate (field 'X'), tagged-protein fluorescence signal measurement (field 'GFP'), plasma-membrane marker fluorescence signal (field 'FM4'), profile identifier ('Profile_ID') an optional number of variant identifiers ('varID_1',...).

Value

Returns an object of class "profile", which is a list of items, which number is equal to a number of imported profile measurement. Each item consists of vectors:

`var`	a vector of imported variant names.
`x`	a vector of profile x-coordinates.
`GFP`	a vector of intensity of tagged-protein fluorescence signal.
`FM4`	a vector of intensity of plasma-membrane marker fluorescence signal.

Note

An effective processing of a large number of confocal images by ImageJ 'Plot Profile' function can be simplified by ImageJ macro "peripheral", which can be downloaded from https://web.natur.cuni.cz/~vosolsob/peripheral.html

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

summary.profile, [.profile, subset.profile, c.profile, filter.profile

Examples

```
## Not run:

## Profile data placed in directory "my_directory"
## named e.g "2017_02_01-GFP_protein-Cell_01-001-GFP.txt"
## "GFP_protein" and "Cell_01" will be exported as variant names

pd <- read.profile("my_directory", var.sep = "-", export = c(2,3), header = TRUE)

## End(Not run)
```

---

residuals.distrib *Extract Model Residuals*

Description

This is a method of generic function residuals for extracting model residuals from object of class "distrib"

Usage

```
  ## S3 method for class 'distrib'
residuals(object, ...)
```

Arguments

object	an object of class "distrib"
...	additional arguments to be passed to or from methods.

Details

This funcion extracts identifiers of variants and corresponding residuals for each measurement of fluorescence profile analysed by `distrib.profile` function.

Value

`residuals.distrib` returns a list with components:

`var`	a matrix of variant identifiers
`residuals`	a vector of residuals

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

`distrib.profile`

Examples

```
## Not run:

## 'dp' is an object of class 'distrib', see help(distrib.profile) for details

dp.res <- residuals(dp)

## End(Not run)
```

`subset.profile` *Subset or Extract an Object*

Description

These function extract parts or subset selected variants of objects with fluorescence profile measurements.

Usage

```
  ## S3 method for class 'distrib'
x[..., drop = TRUE]
  ## S3 method for class 'profile'
x[..., drop = TRUE]

  ## S3 method for class 'distrib'
subset(x, select = NULL, ...)
  ## S3 method for class 'profile'
subset(x, select = NULL, ...)
```

Arguments

x	an object to be extracted or subsetted.
...	in case of [function, arguments pased to the generic function [, e.g. indices specifying elements to extract.
drop	see [for details.
select	a list of vectors. Dimensions of a list must be equal to a number of variant levels in object x. Each vector of a list can contain a selected variant names relevant in a given variant level, or can be NULL, if all variants may be selected. If select = NULL, all elements of object x will be selected.

Details

Function subset select all combination of variant specified in select argument. Internally, methods of subset for classes "profile" and "distrib" use methods [.profile and [.distrib respectivelly.

Value

Returns an object of same class as original object.

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

read.profile, distrib.profile, c.profile, [

Examples

```
## Not run:

## peripher - object of class "profile" with measurements
## of cells expressing integral plasma-membrane protein
## PMA1-GFP, peripheral plasma membrane protein DREPP2-GFP
## and its mutated form DREPP2(Gly2Ala)-GFP with lowered
## plasma-membrane binding activity

#select first 100 elements of object peripher:
a <- peripher[1:100]

#select all measurements of variant "D2" from cells "01", "02" and "03":
b <- subset(peripher, select = list("D2",c("01","02","03")))

## End(Not run)
```

`summary.profile` *Object Summaries*

Description

These methods of a generic function `summary` produce summaries of the objects derived from fluorescence profile measurements.

Usage

```
  ## S3 method for class 'profile'
summary(object, ...)
  ## S3 method for class 'model'
summary(object, ...)
  ## S3 method for class 'distrib'
summary(object, ...)
```

Arguments

`object`	an object for which a summary is desired.
`...`	additional argument passed to function `summary.default`

Details

Method `summary.distrib` computes basic descriptive statistics of plasma-membrane affinities and residuals for each measurement variant using a default method of `summary` function.

Value

For an object of class "profile", `summary` returns a table with number of profile measurements for each variant. Method `print.summary.profile` shows numbers of all profile measurements.

For an object of class "model", `summary` returns `$stats` item of an object of class "model" with number of profile measurements and model residuals.

For an object of class "distrib", `summary` returns a list with mumber of profile measurements and basic descriptive statistics of plasma-membrane affinities and residuals, computed for each variant.

Author(s)

Stanislav Vosolsobě <vosolsob@natur.cuni.cz>

References

Vosolsobě, S., Petrášek, J. and Schwarzerová, K. (2017) "Evolutionary plasticity of plasma membrane interaction in DREPP family proteins", BBA Biomembranes, 1859(2017) 686–697.

http://kfrserver.natur.cuni.cz/lide/vosolsob/Peripheral/

See Also

`read.profile`, `model.profile`, `distrib.profile`, `summary`

Examples

```
## Not run:

## peripher - object of class "profile" with measurements
## of cells expressing integral plasma-membrane protein
## PMA1-GFP, peripheral plasma membrane protein DREPP2-GFP
## and its mutated form DREPP2(Gly2Ala)-GFP with lowered
## plasma-membrane binding activity

summary(peripher)

## End(Not run)
```

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