Package ‘protGear’

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Type Package

Title Protein Micro Array Data Management and Interactive Visualization

Version 1.6.0

Description A generic three-step pre-processing package for protein microarray data. This package contains different data pre-processing procedures to allow comparison of their performance. These steps are background correction, the coefficient of variation (CV) based filtering, batch correction and normalization.

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URL https://github.com/Keniajin/protGear

BugReports https://github.com/Keniajin/protGear/issues

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Author Kennedy Mwai [cre, aut],
    James Mburu [aut],
    Jacqueline Waeni [ctb]
Maintainer Kennedy Mwai <keniajin@gmail.com>

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array_vars

List the array structure variables

Description

A generic function returning a list with the data structure.

Usage

array_vars(
  channel = "635",
  totsamples,
  FG = "",
  BG = "",
  FBG = "",
  blockspersample,
  chip_path = "data/array_data",
  sampleID_path = "data/array_sampleID/",
  mig_prefix = "_first",
  machine = "",
  date_process = ""
)

Arguments

channel       A character indicating the channel that the data was scanned at. It is mostly included in the MFI variable names.
totsamples    A numeric value indicating the number of samples on a slide.
FG            Optional: A character indicating the name of the foreground variable name. If not specified its created as paste0("F",channel,".Median")
BG            Optional: A character indicating the name of the background variable name. If not specified its created as paste0("B",channel,".Median")
FBG           Optional: A character indicating the name of the foreground - background variable name. If not specified its created as paste0("F",channel,".Median"...B",channel)
blockspersample A numeric value indicating the number of blocks in a mini-array. The ".gal" file can help in getting this
chip_path     A character indicating the path of the folder location with the array data.
sampleID_path A character indicating the path of the folder location with the sample identifiers matching the array structure.
mig_prefix   Optional: A character indicating the identifier of an MIG dilution file
**best CV estimation**

machine        Optional: A character indicating the machine used to process the data in the folder

date_process   Optional: A character indicating the date when the samples were processed.

**Value**

A list of parameters required to process the data

```r
genepix_vars
```

**Examples**

```r
## specify the parameters to process the data
genepix_vars <- array_vars(
    ## the channel the data was processed in
    channel = "635",
    ## folder where the array data is stored
    chip_path = "data/array_data",
    ## the number of samples per slide or in as single run
    totsamples = 21,
    ## How many blocks each sample occupies
    blockspersample = 2,
    ## folder where the array data samples id files are stored
    sampleID_path = "data/array_sampleID/",
    ## optional
    mig_prefix = "_first",
    machine = 1,
    date_process = "0520"
)
genepix_vars
```

---

**best CV estimation**  

**Description**

A function to select the best CV by combining the replicates in duplicates. The function has been build for up to to 3 replicates so far

**Usage**

```r
best_CV_estimation(dataCV, slide_id, lab_replicates, cv_cut_off)
```

**Arguments**

- **dataCV**: A data frame
- **slide_id**: A character string containing the identifier of the data frame variable.
- **lab_replicates**: A numeric value indicating the number of lab replicates.
- **cv_cut_off**: A numeric value for the CV cut off. Should be between 0-100
Details

Select set of replicates with the best CV

Value

A data frame with the best CV's estimated

Examples

dataC <- readr::read_csv(system.file("extdata", "dataC.csv", package="protGear"))
## this file has 3 lab replicates and the default names
dataCV <- cv_estimation(dataC, lab_replicates=3)
best_CV_estimation(dataCV, slide_id = "iden", lab_replicates = 3,
                   cv_cut_off = 20)

Description

A generic function to perform background correction.

Usage

bg_correct(iden, Data1, genepix_vars, method = "subtract_local")

Arguments

iden A character indicating the name of the object to be used under Data1
Data1 A data frame with sample identifiers merged with micro array data.
genepix_vars A list of specific definitions of the experiment design. See array_vars.
method a description of the background correction to be used. Possible values are "none", "subtract_local", "subtract_global", "movingmin_bg", "minimum_half", "edwards" or "normexp". The default is "subtract_local".

Details

Background correction

The function implements background correction methods developed by backgroundCorrect. But the minimum_half and movingmin_bg uses the block of the protein array as the grid. If method="movingmin_bg" the minimum background value within a block is subtracted. If method="minimum_half" then any intensity which is negative after background subtraction is reset to equal to half the minimum positive value in a block. If method="movingmin_value" then any intensity which is negative after background subtraction is reset to the minimum positive value in a block. For edwards we implement a similar algorithm with limma::backgroundCorrect(method="edwards") and for 'normexp' we use the saddle-point approximation to maximum likelihood, backgroundCorrect for more details.
buffer_spots

Description
A function to extract the buffer spots data. A buffer spot only has the solution for proprietary ingredients for stabilizing protein and minimizing evaporation.

Usage
buffer_spots(Data1, buffer_spot = "buffer")

Arguments
Data1 An object of the class data frame
buffer_spot A character string containing the name of the buffer spots.

Value
A data frame of the buffer control spots

Examples
bg_correct_df <- readr::read_csv(system.file("extdata", "Data1_sample.csv", package="protGear"))
buffer_spots(Data1 = bg_correct_df)

check_sampleID_files

Description
A generic function to check if the file(s) with the MFI values have a corresponding sample ID file. Sample ID file is a file with the identifiers for the samples in array file.

Usage
check_sampleID_files(genepix_vars)

Arguments
genepix_vars A list of specific definitions of the experiment design. See array_vars.
create_dir

Value

A file with missing corresponding sample ID files

Examples

genepix_vars <- array_vars(
  channel = "635",
  chip_path = system.file("extdata", "array_data/machine1/",
                           package="protGear"),
  totsamples = 21,
  blockspersample = 2,
  mig_prefix = "_first",
  machine = 1,
  date_process = "0520"
)
check_sampleID_files(genepix_vars)

create_dir(path)

Arguments

path folder location to create a directory

Value

created directory

Examples

create_dir("data/sample_folder")
cv_by_sample_estimation

cv by sample

Description

A function to give the summary of the CV’s by the sampleID

Usage

cv_by_sample_estimation(
  dataCV,
  cv_variable,
  lab_replicates,
  sampleID_var = "sampleID"
)

Arguments

dataCV A dataframe
cv_variable A character string containing the identifier of the variable with CV values.
lab_replicates A numeric value indicating the number of lab replicates.
sampleID_var A character string containing the name of the sample identifier variable. Default set to ‘sampleID’

Details

Summarise CV by samples

Value

A data frame of CV calculated by sample

Examples

dataC <- readr::read_csv(system.file("extdata",
  "dataC.csv", package="protGear"))
## this file has 3 lab replicates and the default names
dataCV <- cv_estimation(dataC, lab_replicates=3)
cv_by_sample_estimation(dataCV, cv_variable = "cvCat_all",
  lab_replicates = 3)
cv_estimation

Description

A function to calculate the CV for the technical lab replicates. The default values are set as per the object names generated by machine

Usage

```r
cv_estimation(
  dataC, 
  lab_replicates, 
  sampleID_var = "sampleID", 
  antigen_var = "antigen", 
  replicate_var = "replicate", 
  mfi_var = "FMedianBG_correct", 
  cv_cut_off = 20 
)
```

Arguments

dataC: A dataset a data frame with feature variables to be used
lab_replicates: A numeric value indicating the number of lab replicates
sampleID_var: A character string containing the name of the sample identifier variable. Default set to 'sampleID'
antigen_var: A character string containing the name of the features/protein variable. Default to 'antigen'
replicate_var: A character string containing the name of the replicate variable. Default to 'replicate'
mfi_var: A character string containing the name of the variable with MFI value. Assuming background correction is done already. Default to 'FMedianBG_correct'
cv_cut_off: Optional value indicating the cut off of flagging CV’s. Default set at 20.

Details

Coefficient of Variation

Value

A data frame where CV’s of the replicates have been calculated
Examples

dataC <- readr::read_csv(system.file("extdata", "dataC.csv", package="protGear"))
## this file has 3 lab replicates and the default names
cv_estimation(dataC, lab_replicates=3)

error_replicates

\_Start\_Function\_For Error\_

Description

A generic function to write into the log file with a replicate check error

Usage

error_replicates(iden)

Arguments

iden An id for the file with replicates error

Value

a log file showing the replicate errors

extract_bg

\textit{extract bg}

Description

A generic function to extract the background data for micro array data.

Usage

eextract_bg(iden, data_files, genepix_vars = genepix_vars)

Arguments

iden A character indicating the name of the object to be used under data_files.

data_files A list of data objects with names utilised by iden.

genepix_vars A list of specific definitions of the experiment design. See \textit{array_vars}.

Details

Extract the background values
**launch_protGear_interactive**

**Value**

A data frame of background values

**Examples**

```r
## Not run:
genepix_vars <- array_vars(
  channel = "635",
  chip_path = system.file("extdata", "array_data/machine1/",
    package="protGear"),
  totsamples = 21,
  blockspersample = 2,
  mig_prefix = "_first",
  machine = 1,
  # optional
data_process = "0520"
)

# Define the data path
data_path <- paste0(genepix_vars$chip_path)
# List the file names to use
filenames <- list.files(genepix_vars$chip_path,
  pattern = "*.txt|*.gpr", full.names = FALSE)

data_files <- purrr::map(.x = filenames,
  .f = read_array_files,
  .data = data_path,
  genepix_vars = genepix_vars)

names(data_files)
extract_bg(iden = "KK2-06", data_files=data_files, genepix_vars=genepix_vars)

## End(Not run)
```

---

**Description**

This is Function is to launch the shiny application

**Usage**

```r
launch_protGear_interactive()
```

**Value**

launches the shiny interactive protGear app
Launch multiple shiny applications for protGear

Usage

\begin{verbatim}
launch_select(theApp)
\end{verbatim}

Arguments

\begin{itemize}
\item \texttt{theApp} accepts one of the folders containing the shiny application
\end{itemize}

Value

launches the app defined under \texttt{theApp}

Examples

\begin{verbatim}
validExamples <- list.files(system.file("shiny-examples", package = "protGear"))
#launch_select(validExamples[[1]])
\end{verbatim}

Normalize Arrays

Description

Normalize Arrays
Usage

code

matrix_normalise(
    matrix_antigen,  
    method = "log2",  
    batch_correct = FALSE,  
    batch_var1,  
    batch_var2 = day_batches,  
    return_plot = FALSE,  
    plot_by_antigen = TRUE,  
    control_antigens = NULL,  
    array_matrix = NULL  
)

Arguments

matrix_antigen: An object of class matrix with features/proteins as columns and samples as the rows.
method: character string specifying the normalization method. Choices are "none", "log2", "vsn", "cyclic_loess", "cyclic_loess_log", "rlm"
batch_correct: A logical value indicating whether batch correction should be done or not.
batch_var1: A character or factor vector of size similar to rows of matrix_antigen indicating the first batch.
batch_var2: A character or factor vector of size similar to rows of matrix_antigen indicating the second batch.
return_plot: A logical value indicating whether a plot is returned to show the results of normalisation.
plot_by_antigen: Logical to indicate whether to plot by antigen or not slide name for the matrix_antigen object.
control_antigens: logical vector specifying the subset of spots which are non-differentially-expressed control spots, for use with method="rlm"
array_matrix: An object of class dataframe or matrix used with method='rlm' indicating the sample index and

Value

A data frame of normalised values

Examples

matrix_antigen <- readr::read_csv(system.file("extdata",  
    "matrix_antigen.csv", package="protGear"))
#VSN
normlise_vsn <- matrix_normalise(as.matrix(matrix_antigen),  
    method = "vsn",  
    return_plot = TRUE
merge_sampleID

Merge sample ID with the array data

Description

A generic function that merges the protein data with the sample identifiers or sample names. If the file does not have sample identifiers the function generates it automatically.

Usage

merge_sampleID(iden, data_files, genepix_vars, method)

Arguments

iden A character indicating the name of the object to be used under data_files.
data_files A list of data objects with names utilised by iden.
genepix_vars A list of specific definitions of the experiment design. See array_vars.
method A description of the background correction to be used. See bg_correct.

Value

da data frame merged with corresponding sample ID’s. The sample ID are specified in the sample ID files

Examples

## Not run:
### Define the genepix_vars
genepix_vars <- array_vars(
  channel = "635",
  chip_path = system.file("extdata", "array_data/machine1/",
    package="protGear"),
  totsamples = 21,
  blockspersample = 2,
  mig_prefix = ".first",
  machine = 1,
)
### Optional

```r
data_process = "0520"
```

### The path where the micro-array data is located

```r
data_path <- paste0(genepix_vars$chip_path)
filenames <- list.files(genepix_vars$chip_path,
    pattern = "*.txt|*.gpr\$", full.names = FALSE)
```

### Create a list of all the files

```r
data_files <- purrr::map(.x = filenames,
    .f = read_array_files,
    data_path = data_path,
    genepix_vars = genepix_vars)
data_files <- purrr::set_names(data_files,
    purrr::map(filenames, name_of_files))
```

### Merge the lab data with samples and perform bg correction

```r
merge_sampleID(iden = "KK2-06", data_files = data_files,
genepix_vars = genepix_vars, method = "subtract_global")
```

### End (Not run)

---

#### `minpositive`

*Get the minimum positive value*

**Description**

Get the minimum positive value

**Usage**

```r
minpositive(x)
```

**Arguments**

- `x` A numeric vector or variable

**Value**

Returns the minimum positive value in an object

**Examples**

```r
minpositive(c(-1,-2,3,5,6,7,8,9,10))
```
name_of_files Object names of a list

Description
A generic function returning a vector with the names of files in the same directory. Removes the file extension

Usage
name_of_files(i)

Arguments
i - a list filenames with .txt or .gpr extension

Value
a list of file names

Examples
name_of_files("KK2-06.txt")

output_trend_stats Trend test using Cox–Stuart (C–S) and Mann–Kendall (M–K) trend tests

Description
Trend test using Cox–Stuart (C–S) and Mann–Kendall (M–K) trend tests

Usage
output_trend_stats(name, p_val, z_val)

Arguments
name Name of the test
p_val p value from the test
z_val the Z value of the test

Value
A statistics of mean standard deviation trend
Examples

```r
output_trend_stats(name=\"t.test\", p_val=0.001, z_val=5)
```

---

**plot_bg**

*Plot background*

---

**Description**

A generic function for plotting of R objects.

**Usage**

```r
plot_bg(df, x_axis = \"antigen\", bg_MFI = \"BG_Median\", log_mfi = TRUE)
```

**Arguments**

- `df` A default dataset to use for plot.
- `x_axis` The variable on the x axis
- `bg_MFI` A numeric variable describing which is the background MFI
- `log_mfi` a logical value indicating whether the MFI values should be log transformed or not.

**Value**

A ggplot of background values

**Examples**

```r
## Not run:
#After extracting the background using \code{\link{extract_bg}}
#we plot the data using
allData_bg <- readr::read_csv(system.file("extdata", \"bg_example.csv\",
  package=\"protGear\"))
plot_bg(allData_bg,
  x_axis = \"antigen\",
  bg_MFI = \"BG_Median\",
  log_mfi = TRUE
 )
## End(Not run)
```
**plot_buffer**

*Plot the buffer values*

**Description**

Plot the buffer values

**Usage**

```r
plot_buffer(
  df = buffers,
  buffer_names = "antigen",
  buffer_mfi = "FMedianBG_correct",
  slide_id = ".id"
)
```

**Arguments**

- `df`: A data frame to be used to plot
- `buffer_names`: A character string containing the name of the variable with buffer spots. Default set to 'antigen'.
- `buffer_mfi`: A character string containing the name of the variable with MFI value. Assuming background correction is done already. Default to 'FMedianBG_correct'
- `slide_id`: A character string containing the name of the slide/array identifier variable.

**Value**

plot of buffer spots

**Examples**

```r
buffers <- readr::read_csv(system.file("extdata", "buffers_sample2.csv", package="protGear"))
plot_buffer(df=buffers, buffer_names = "sampleID")
```

---

**plot_FB**

*plot_FB*

**Description**

A generic function for plotting the background and foreground values.
### Usage

```r
plot_FB(
  df,
  antigen_name = "antigen",
  bg_MFI = "BG_Median",
  FG_MFI = "FBG_Median",
  log_mfi = FALSE
)
```

### Arguments

- **df**: An object containing the data to which the plot is done.
- **antigen_name**: The variable describing which features/proteins/antibodies in the data should be used to plot.
- **bg_MFI**: A numeric variable describing which is the background MFI.
- **FG_MFI**: A numeric variable describing which is the foreground MFI.
- **log_mfi**: A logical value indicating whether the MFI values should be log transformed or not.

### Details

Plot foreground and background values.

### Value

A `ggplot` of foreground vs background MFI values.

### Examples

```r
## Not run:
# After extracting the background using \code{\link{extract_bg}}
# we plot the data using
allData_bg <- readr::read_csv(system.file("extdata",
  "bg_example.csv", package="protGear"))
plot_FB(allData_bg,
  antigen_name = "antigen",
  bg_MFI = "BG_Median", FG_MFI = "FBG_Median", log = FALSE
)
## End(Not run)
```
plot_normalised

Comparison of normalised data by sample

Description

Comparison of normalised data by sample

Usage

plot_normalised(exprs_normalised_df, method, batch_correct)

Arguments

exprs_normalised_df
  a normalised data frame
method
  the method of normalisation used
batch_correct
  the batch correction

Value

A ggplot of normalised data

Examples

matrix_antigen <- readr::read_csv(system.file("extdata", "matrix_antigen.csv", package="protGear"))
normlise_vsn <- matrix_normalise(as.matrix(matrix_antigen), method = "vsn", return_plot = FALSE)
plot_normalised(normlise_vsn,method="vsn",batch_correct=FALSE)

plot_normalised_antigen

Comparison of normalised data by feature

Description

Comparison of normalised data by feature

Usage

plot_normalised_antigen(exprs_normalised_df, method, batch_correct)
read_array_files

Arguments

exprs_normalised_df
  a normalised data frame
method
  the method of normalisation used
batch_correct
  the batch correction

Value

A ggplot of various normalisation approaches

Examples

matrix_antigen <- readr::read_csv(system.file("extdata", "matrix_antigen.csv", package="protGear"))
normlise_vsn <- matrix_normalise(as.matrix(matrix_antigen), method = "vsn", return_plot = FALSE)
plot_normalised_antigen(normlise_vsn,method="vsn",batch_correct=FALSE)

Description

This helps to read the chip file(s).

Usage

read_array_files(i, data_path, genepix_vars)

Arguments

i The name of the file which the data are to be read from.
data_path The path where the file with the data is located
genepix_vars A list of specific definitions of the experiment design. See array_vars.

Details

Read multiple array files

Value

a number of data frames in the global environment
Examples

## Not run:
```r
genepix_vars <- array_vars(
  channel = "635",
  chip_path = system.file("extdata", "array_data/machine1/",
                          package="protGear"),
  totsamples = 21,
  blockspersample = 2,
  mig_prefix = ".first",
  machine = 1,
  date_process = "0520"
)
file_read <- "KK2-06.txt"
read_array_files(i=file_read,
data_path=system.file("extdata", "array_data/machine1/",
                     package="protGear"), genepix_vars=genepix_vars)
## End(Not run)
```

---

**read_array_visualize**  
*Read a gpr file to visualize*

### Description

Read a gpr file to visualize

### Usage

```r
read_array_visualize(infile)
```

### Arguments

- **infile**  
a .gpr file to be used to visualize the expression intensities of the slide spots

### Value

a data frame to visualize the background or foreground values

### Examples

## Not run:
```r
read_array_visualize(infile = system.file("extdata",
                                         "/array_data/machine1/KK2-06.txt", package="protGear"))
## End(Not run)
```
**rlm_normalise**

**RLM normalisation**

**Description**

A function for method='rlm' from `matrix_normalise`.

**Usage**

```r
rlm_normalise(rlm_normalise_df)
```

**Arguments**

- `rlm_normalise_df`: rlm normalised data frame

**Value**

an elist of RLM normalisation to be utilised by `rlm_normalise_matrix`

**Examples**

```r
matrix_antigen <- readr::read_csv(system.file("extdata", "matrix_antigen.csv", package="protGear"))
# rlm_normalise_df <- rlm_normalise_matrix(matrix_antigen=matrix_antigen, array_matrix=array_matrix, control_antigens=control_antigens)
# rlm_normalise(rlm_normalise_df)
```

**rlm_normalise_matrix**

**Normalise using RLM**

**Description**

A function for method='rlm' from `matrix_normalise`.

**Usage**

```r
rlm_normalise_matrix(matrix_antigen, array_matrix, control_antigens)
```

**Arguments**

- `matrix_antigen`: A matrix with antigen data
- `array_matrix`: A matrix with control antigen data
- `control_antigens`: the control antigens for RLM normalisation
tag_subtract

Value

A RLM normalised data frame

Examples

```r
matrix_antigen <- readr::read_csv(system.file("extdata", "matrix_antigen.csv", package="protGear"))
# rlm_normalise_matrix(matrix_antigen=matrix_antigen,
# array_matrix=array_matrix,
# control_antigens=control_antigens)
```

Description

```
\_End_Function\_ 
```

Usage

```r
tag_subtract(
  dataC_mfi,
  tag_antigens,
  mean_best.CV.var,
  tag_file,
  batch.vars,
  sampleID.var = "sampleID",
  antigen.var = "antigen"
)
```

Arguments

- **dataC_mfi**: A dataframe
- **tag_antigens**: A character vector with the names of proteins or antigens used as TAG.
- **mean_best.CV.var**: A character string containing the identifier of the variable with the MFI values.
- **tag_file**: A data frame with variables antigen, TAG, TAG_name to show the TAG for the different antigens or proteins in dataC_mfi
- **batch.vars**: A list of characters identifying variables in dataC_mfi for indicating batch.
- **sampleID.var**: A character string containing the name of the sample identifier variable. Default set to 'sampleID'
- **antigen.var**: A character string containing the name of the features/protein variable. Default to 'antigen'

Details

Subtract the purification TAG data
Value

A data frame of TAG values subtracted

Examples

tag_file <- readr::read_csv(system.file("extdata", "TAG_antigens.csv", package="protGear"))
tag_antigens <- c("CD4TAG", "GST", "MBP")
batch_vars <- list(machine = "m1", day = "0520")
dataC <- readr::read_csv(system.file("extdata", "dataC.csv", package="protGear"))
## this file has 3 lab replicates and the default names
dataCV <- cv_estimation(dataC ,lab_replicates=3)
dataCV_best2 <- best_CV_estimation(dataCV, slide_id = "iden", lab_replicates = 3, cv_cut_off = 20)
tag_subtract(dataCV_best2, tag_antigens=tag_antigens, mean_best_CV_var="mean_best_CV",
tag_file = tag_file, antigen_var = "antigen", batch_vars = batch_vars)

---

visualize_slide

Visualize the slide mimicking the original scan image.

Description

Visualize the slide mimicking the original scan image.

Usage

visualize_slide(infile, MFI_var, interactive = FALSE, d_f = NA)

Arguments

infile a .gpr file to be used to visualize the expression intensities of the slide spots
MFI_var the MFI variable to plot, can be either the background or foreground value
interactive a logical to specify whether an interactive graph is returned or not
d_f a data frame with array data

Value

A ggplot of slide foreground values
visualize_slide_2d

Visualize the slide mimicking the original scan image using a 2d plot.

Description

Visualize the slide mimicking the original scan image using a 2d plot.

Usage

visualize_slide_2d(infile, MFI_var, d_f = NA)

Arguments

infile - a .gpr file to be used to visualize the expression intensities of the slide spots
MFI_var - the MFI variable to plot, can be either the background or foreground value
d_f - a data frame with array data

Value

A 2d plot of either the background or foreground values

Examples

## Not run:
visualize_slide_2d(
  infile = system.file("extdata", "/array_data/machine1/KK2-06.txt",
    package="protGear"),
  MFI_var = "B635 Median"
)
## End(Not run)
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