Package: PvSTATEM (via r-universe)

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Type Package

Title Reading, Quality Control and Preprocessing of MBA (Multiplex Bead Assay) Data

Description Speeds up the process of loading raw data from MBA (Multiplex Bead Assay) examinations, performs quality control checks, and automatically normalises the data, preparing it for more advanced, downstream tasks. The main objective of the package is to create a simple environment for a user, who does not necessarily have experience with R language. The package is developed within the project of the same name - 'PvSTATEM', which is an international project aiming for malaria elimination.

BugReports <https://github.com/mini-pw/PvSTATEM/issues>

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LazyData true

RoxygenNote 7.3.2

Imports dplyr, ggplot2, nplr, R6, readr, readxl, stringi, stringr, grid, png, tools, ggrepel, lubridate, R.utils, svglite

Suggests knitr, qpdf, rmarkdown, testthat (>= 3.0.0)

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VignetteBuilder knitr

URL <<https://github.com/mini-pw/PvSTATEM>>

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Contents

---

create_standard_curve_model_analyte

Create a standard curve model for a certain analyte

Description

Create a standard curve model for a certain analyte

Usage

```
create_standard_curve_model_analyte(
  plate,
  analyte_name,
  data_type = "Median",
  source_mfi_range_from_all_analytes = FALSE,
  detect_high_dose_hook = TRUE,
  ...
)
```

Arguments

`plate`	(`Plate()`) Object of the Plate class
`analyte_name`	(`character(1)`) Name of the analyte for which we want to create the model
`data_type`	(`character(1)`) Data type of the value we want to use to fit the model - the same datatype as in the plate file. By default, it equals to `Median`
`source_mfi_range_from_all_analytes`	
	(`logical(1)`) If TRUE, the MFI range is calculated from all analytes; if FALSE, the MFI range is calculated only for the current analyte Defaults to FALSE
`detect_high_dose_hook`	
	(`logical(1)`) If TRUE, the high dose hook effect is detected and handled. For more information, please see the [handle_high_dose_hook](#) function documentation.
`...`	Additional arguments passed to the model
	Standard curve samples should not contain na values in mfi values nor in dilutions.

Value

(`Model()`) Standard Curve model

---

generate_plate_report   *Generate a report for a plate.*

Description

This function generates a report for a plate. The report contains all the necessary information about the plate, from the general plate parameters, such as examination date, to the breakdown of the analytes' plots. The report is generated using the `plate_report_template.Rmd` template.

Usage

```
generate_plate_report(
  plate,
  use_model = TRUE,
  filename = NULL,
  output_dir = "reports",
  counts_lower_threshold = 50,
  counts_higher_threshold = 70,
  additional_notes = NULL
)
```

Arguments

`plate`	A plate object.
`use_model`	(`logical(1)`) A logical value indicating whether the model should be used in the report.
`filename`	(`character(1)`) The name of the output HTML report file. If not provided or equals to NULL, the output filename will be based on the plate name, precisely: `{plate_name}_report.html`. By default the `plate_name` is the filename of the input file that contains the plate data. For more details please refer to [Plate.](#)
	If the passed filename does not contain `.html` extension, the default extension `.html` will be added. Filename can also be a path to a file, e.g. `path/to/file.html`. In this case, the `output_dir` and `filename` will be joined together. However, if the passed filepath is an absolute path and the `output_dir` parameter is also provided, the `output_dir` parameter will be ignored. If a file already exists under a specified filepath, the function will overwrite it.
`output_dir`	(`character(1)`) The directory where the output CSV file should be saved. Please note that any directory path provided will create all necessary directories (including parent directories) if they do not exist. If it equals to NULL the current working directory will be used. Default is 'reports'.
`counts_lower_threshold`	
	(`numeric(1)`) The lower threshold for the counts plots (works for each analyte). Default is 50.
`counts_higher_threshold`	
	(`numeric(1)`) The higher threshold for the counts plots (works for each analyte). Default is 70.
`additional_notes`	
	(`character(1)`) Additional notes to be included in the report. Contents of this fields are left to the user's discretion. If not provided, the field will not be included in the report.

Value

A report.

Examples

```
plate_file <- system.file("extdata", "CovidOISExPONENT_CO_reduced.csv", package = "PvSTATEM")
# a plate file with reduced number of analytes to speed up the computation
layout_file <- system.file("extdata", "CovidOISExPONENT_CO_layout.xlsx", package = "PvSTATEM")
note <- "This is a test report.\n**Author**: Jane Doe \n**Tester**: John Doe"

plate <- read_luminex_data(plate_file, layout_file, verbose = FALSE)
example_dir <- tempdir(check = TRUE) # a temporary directory
generate_plate_report(plate,
  output_dir = example_dir,
  counts_lower_threshold = 40,
  counts_higher_threshold = 50,
  additional_notes = note
)
```

---

get_nmfi *Calculate normalised MFI values for a plate*

Description

The function calculates the normalised MFI (nMFI) values for each of the analytes in the plate.

The nMFI values are calculated as the ratio of the test samples' MFI values to the standard curve samples with the target dilution.

When nMFI could be used? In general, it is preferred to use Relative Antibody Unit (RAU) values for any analysis. However, it is sometimes impossible to fit a model to the standard curve samples. This may happen if the MFI values of test samples are much higher than the MFI of standard curve samples. Then, the prediction would require significant data extrapolation, which could lead to unreliable results.

In such cases, the nMFI values could be used as a proxy for RAU values if we want, for instance, to account for plate-to-plate variation.

Usage

```
get_nmfi(
  plate,
  reference_dilution = 1/400,
  data_type = "Median",
  verbose = TRUE
)
```

Arguments

plate	(Plate()) a plate object for which to calculate the nMFI values
reference_dilution	
	(numeric(1) or character(1)) the dilution value of the standard curve sample to use as a reference for normalisation. The default is 1/400. It should refer to a dilution of a standard curve sample in the given plate object. This parameter could be either a numeric value or a string. In case it is a character string, it should have format 1/d+, where d+ is any positive integer.
data_type	(character(1)) type of data for the computation. Median is the default
verbose	(logical(1)) print additional information. The default is TRUE

Value

nmfi (data.frame) a data frame with normalised MFI values for each analyte in the plate and all test samples.

Examples

```
# read the plate
plate_file <- system.file("extdata", "CovidOISExPONTENT.csv", package = "PvSTATEM")
layout_file <- system.file("extdata", "CovidOISExPONTENT_layout.csv", package = "PvSTATEM")

plate <- read_luminex_data(plate_file, layout_file)

# artificially bump up the MFI values of the test samples (the Median data type is default one)
plate$data[["Median"]][plate$sample_types == "TEST", ] <-
  plate$data[["Median"]][plate$sample_types == "TEST", ] * 10

# calculate the nMFI values
nmfi <- get_nmfi(plate, reference_dilution = 1 / 400)

# we don't do any extrapolation and the values should be comparable across plates
head(nmfi)
# different params
nmfi <- get_nmfi(plate, reference_dilution = "1/50")
```

---

handle_high_dose_hook   *Detect and handle the high dose hook effect*

Description

Typically, the MFI values associated with standard curve samples should decrease as we dilute the samples. However, sometimes in high dilutions, the MFI presents a non monotonic behavior. In that case, MFI values associated with dilutions above (or equal to) `high_dose_threshold` should be removed from the analysis.

For the `nplr` model the recommended number of standard curve samples is at least 4. If the high dose hook effect is detected but the number of samples below the `high_dose_threshold` is lower than 4, additional warning is printed and the samples are not removed.

The function returns a logical vector that can be used to subset the MFI values.

Usage

```
handle_high_dose_hook(mfi, dilutions, high_dose_threshold = 1/200)
```

Arguments

mfi   (numeric())

dilutions   (numeric())

high_dose_threshold

                  (numeric(1)) MFI values associated with dilutions above this threshold should
                  be checked for the high dose hook effect

Value

sample selector (`logical()`)

---

is_valid_data_type *Check validity of given data type*

Description

Check if the data type is valid. The data type is valid if it is one of the elements of the `VALID_DATA_TYPES` vector. The valid data types are:
`c(Median, Net MFI, Count, Avg Net MFI, Mean, Peak)`.

Usage

```
is_valid_data_type(data_type)
```

Arguments

data_type   A string representing the data type.

Value

`TRUE` if the data type is valid, `FALSE` otherwise.

---

is_valid_sample_type *Check validity of given sample type*

Description

Check if the sample type is valid. The sample type is valid if it is one of the elements of the `VALID_SAMPLE_TYPES` vector. The valid sample types are:

`c(ALL, BLANK, TEST, NEGATIVE CONTROL, STANDARD CURVE, POSITIVE CONTROL)`.

Usage

```
is_valid_sample_type(sample_type)
```

Arguments

sample_type   A string representing the sample type.

Value

`TRUE` if the sample type is valid, `FALSE` otherwise.

Description

The Model class is a wrapper around the `nplr` model. It allows to predict the RAU (Relative Antibody Unit) values directly from the MFI values of a given sample.

The `nplr` model is fitted using the formula:

$$y = B + \frac{T - B}{(1 + 10^{b \cdot (x_{mid} - x)})^s},$$

where:

- y is the predicted value, MFI in our case,
- x is the independent variable, dilution in our case,
- B is the bottom plateau - the right horizontal asymptote,
- T is the top plateau - the left horizontal asymptote,
- b is the slope of the curve at the inflection point,
- x_{mid} is the x-coordinate at the inflection point,
- s is the asymmetric coefficient.

This equation is referred to as the Richards' equation. More information about the model can be found in the `nplr` package documentation.

After the model is fitted to the data, the RAU values can be predicted using the `predict` method. The RAU value is simply a predicted dilution value (using the standard curve) for a given MFI multiplied by 1,000 000 to have a more readable value. For more information about the differences between dilution, RAU and MFI values, please see the "Normalisation" section in the "Basic PvSTATEM functionalities" vignette.

Public fields

`analyte (character(1))`
    Name of the analyte for which the model was fitted

`dilutions (numeric())`
    Dilutions used to fit the model

`mfi (numeric())`
    MFI values used to fit the model

`mfi_min (numeric(1))`
    Minimum MFI used for scaling MFI values to the range [0, 1]

`mfi_max (numeric(1))`
    Maximum MFI used for scaling MFI values to the range [0, 1]

`model (nplr)`
    Instance of the `nplr` model fitted to the data

```
log_dilution (logical())
```
Indicator should the dilutions be transformed using the `log10` function

```
log_mfi (logical())
```
Indicator should the MFI values be transformed using the `log10` function

```
scale_mfi (logical())
```
Indicator should the MFI values be scaled to the range [0, 1]

Active bindings

```
top_asymptote (numeric(1))
```
The top asymptote of the logistic curve

```
bottom_asymptote (numeric(1))
```
The bottom asymptote of the logistic curve

Methods

Public methods:

- `Model$new()`
- `Model$predict()`
- `Model$get_plot_data()`
- `Model$print()`
- `Model$clone()`

Method new(): Create a new instance of Model R6 class

Usage:
```
Model$new(
  analyte,
  dilutions,
  mfi,
  npars = 5,
  verbose = TRUE,
  log_dilution = TRUE,
  log_mfi = TRUE,
  scale_mfi = TRUE,
  mfi_min = NULL,
  mfi_max = NULL
)
```

Arguments:

```
analyte (character(1))
```
Name of the analyte for which the model was fitted.

```
dilutions (numeric())
```
Dilutions used to fit the model

```
mfi  MFI (numeric())
```
values used to fit the model

```
npars (numeric(1))
```
Number of parameters to use in the model

verbose (logical())
   If TRUE prints messages, TRUE by default

log_dilution (logical())
   If TRUE the dilutions are transformed using the log10 function, TRUE by default

log_mfi (logical())
   If TRUE the MFI values are transformed using the log10 function, TRUE by default

scale_mfi (logical())
   If TRUE the MFI values are scaled to the range [0, 1], TRUE by default

mfi_min (numeric(1))
   Enables to set the minimum MFI value used for scaling MFI values to the range [0, 1]. Use values before any transformations (e.g., before the log10 transformation)

mfi_max (numeric(1))
   Enables to set the maximum MFI value used for scaling MFI values to the range [0, 1]. Use values before any transformations (e.g., before the log10 transformation)

Method predict(): Predict RAU values from the MFI values

Usage:
Model$predict(mfi, over_max_extrapolation = 0, eps = 1e-06)

Arguments:

mfi (numeric())
   MFI values for which we want to predict the RAU values

over_max_extrapolation (numeric(1))
   How much we can extrapolate the values above the maximum RAU value seen in standard curve samples RAU_{max}. Defaults to 0. If the value of the predicted RAU is above $RAU_{max} + $ over_max_extrapolation, the value is censored to the value of that sum.

eps (numeric(1))
   A small value used to avoid numerical issues close to the asymptotes

Returns: (data.frame())
Dataframe with the predicted RAU values for given MFI values The columns are named as follows:

   • RAU - the Relative Antibody Units (RAU) value
   • MFI - the predicted MFI value

Method get_plot_data(): Data that can be used to plot the standard curve.

Usage:
Model$get_plot_data()

Returns: (data.frame())
Prediction dataframe for scaled MFI (or logMFI) values in the range [0, 1]. Columns are named as in the predict method

Method print(): Function prints the basic information about the model such as the number of parameters or samples used

Usage:
Model$print()

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Method `clone()`: The objects of this class are cloneable with this method.

Usage:

`Model$clone(deep = FALSE)`

Arguments:

deep Whether to make a deep clone.

Examples

```
plate_file <- system.file("extdata", "CovidOISExPONENT.csv", package = "PvSTATEM")
layout_file <- system.file("extdata", "CovidOISExPONENT_layout.csv", package = "PvSTATEM")
plate <- read_luminex_data(plate_file, layout_filepath = layout_file)
model <- create_standard_curve_model_analyte(plate, "S2", log_mfi = TRUE)
print(model)
```

Plate   *Plate object*

Description

A class to represent the luminex plate. It contains information about the samples and analytes that were examined on the plate as well as some additional metadata and batch info

Public fields

`plate_name (character(1))`
    Name of the plate. Set to the name of the file from which the plate was read.

`analyte_names (character())`
    Names of the analytes that were examined on the plate.

`sample_names (character())`
    Names of the samples that were examined on the plate.

`batch_name (character(1))`
    Name of the batch to which the plate belongs.

`plate_datetime (POSIXct())`
    A date and time when the plate was created by the machine

`sample_locations (character())`
    Locations of the samples on the plate.

`sample_types (character())`
    Types of the samples that were examined on the plate. The possible values are
    `c(ALL, BLANK, TEST, NEGATIVE CONTROL, STANDARD CURVE, POSITIVE CONTROL)`.

`dilutions (character())`
    A list containing names of the samples as keys and string representing dilutions as values. The dilutions are represented as strings.

dilution_values (numeric())
:   A list containing names of the samples as keys and numeric values representing dilutions as values.

default_data_type (character(1))
:   The default data type that will be returned by the get_data method. By default is set to Median.

data (list())
:   A list containing dataframes with the data for each sample and analyte. The possible data types - the keys of the list are:
    c(Median, Net MFI, Count, Avg Net MFI, Mean, Peak).

    In each dataframe, the rows represent samples and the columns represent analytes.

batch_info (list())
:   A list containing additional, technical information about the batch.

layout (character())
:   A list containing information about the layout of the plate. The layout is read from the separate file and usually provides additional information about the dilutions, sample names, and the sample layout on the actual plate.

blank_adjusted (logical)
:   A flag indicating whether the blank values have been adjusted.

Methods

Public methods:

- [Plate$new()](#)
- [Plate$print()](#)
- [Plate$summary()](#)
- [Plate$get_data()](#)
- [Plate$get_dilution()](#)
- [Plate$get_dilution_values()](#)
- [Plate$blank_adjustment()](#)
- [Plate$clone()](#)

Method new(): Method to initialize the Plate object

Usage:

```
Plate$new(
  plate_name,
  sample_names,
  analyte_names,
  batch_name = "",
  plate_datetime = NULL,
  sample_locations = NULL,
  sample_types = NULL,
  dilutions = NULL,
  dilution_values = NULL,
  default_data_type = NULL,
```

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```
  data = NULL,
  batch_info = NULL,
  layout = NULL
)
```

Arguments:

`plate_name (character(1))`

Name of the plate. By default is set to an empty string, during the reading process it is set to the name of the file from which the plate was read.

`sample_names (character())`

Names of the samples that were examined on the plate.

`analyte_names (character())`

Names of the analytes that were examined on the plate.

`batch_name (character(1))`

Name of the batch to which the plate belongs. By default is set to an empty string, during the reading process it is set to the `batch` field of the plate

`plate_datetime (POSIXct())`

Datetime object representing the date and time when the plate was created by the machine.

`sample_locations (character())`

Locations of the samples on the plate.

`sample_types (character())`

Types of the samples that were examined on the plate. The possible values are `c(ALL, BLANK, TEST, NEGATIVE CONTROL, STANDARD CURVE, POSITIVE CONTROL)`.

`dilutions (character())`

A list containing names of the samples as keys and string representing dilutions as values. The dilutions are represented as strings.

`dilution_values (numeric())`

A list containing names of the samples as keys and numeric values representing dilutions as values.

`default_data_type (character(1))`

The default data type that will be returned by the `get_data` method. By default is set to `Median`.

`data (list())`

A list containing dataframes with the data for each sample and analyte. The possible data types - the keys of the list are `c(Median, Net MFI, Count, Avg Net MFI, Mean, Peak)`. In each dataframe, the rows represent samples and the columns represent analytes.

`batch_info (list())`

A list containing additional, technical information about the batch.

`layout (character())`

A list containing information about the layout of the plate. The layout is read from the separate file and usually provides additional information about the dilutions, sample names, and the sample layout on the actual plate.

Method `print()`: Function prints the basic information about the plate such as the number of samples and analytes

Usage:

```
Plate$print(...)
```

Arguments:

`...` Additional parameters to be passed to the print function Print the summary of the plate

Method `summary()`: Function outputs basic information about the plate, such as examination date, batch name, and sample types.

Usage:
```
Plate$summary(..., include_names = FALSE)
```

Arguments:

`...` Additional parameters to be passed to the print function Get data for a specific analyte and sample type

`include_names` If `include_names` parameter is `TRUE`, a part from count of control samples, provides also their names. By default `FALSE`

Method `get_data()`: Function returns data for a specific analyte and sample.

Usage:
```
Plate$get_data(
  analyte,
  sample_type = "ALL",
  data_type = self$default_data_type
)
```

Arguments:

`analyte` An analyte name or its id of which data we want to extract. If set to 'ALL' returns data for all analytes.

`sample_type` is a type of the sample we want to extract data from. The possible values are `c(ALL, BLANK, TEST, NEGATIVE CONTROL, STANDARD CURVE, POSITIVE CONTROL)`. Default value is `ALL`.

`data_type` The parameter specifying which data type should be returned. This parameter has to take one of values:
`c(Median, Net MFI, Count, Avg Net MFI, Mean, Peak)`. What's more, the `data_type` has to be present in the plate's data Default value is plate's `default_data_type`, which is usually `Median`.

Returns: Dataframe containing information about a given sample type and analyte Get the string representation of dilutions

Method `get_dilution()`: Function returns the dilution represented as strings for a specific sample type.

Usage:
```
Plate$get_dilution(sample_type)
```

Arguments:

`sample_type` type of the samples that we want to obtain the dilution for. The possible values are
`c(ALL, BLANK, TEST, NEGATIVE CONTROL, STANDARD CURVE, POSITIVE CONTROL)` Default value is `ALL`.

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Returns: A list containing names of the samples as keys and string representing dilutions as values. Get the numeric representation of dilutions

Method `get_dilution_values()`: Function returns the dilution values for a specific sample type.

Usage:

`Plate$get_dilution_values(sample_type)`

Arguments:

`sample_type` type of the samples that we want to obtain the dilution values for. The possible values are
  `c(ALL, BLANK, TEST, NEGATIVE CONTROL, STANDARD CURVE, POSITIVE CONTROL)` Default value is `ALL`.

Returns: A list containing names of the samples as keys and numeric values representing dilutions as values.
Adjust the MFI values by subtracting the background

Method `blank_adjustment()`: Function adjusts the values of samples (all samples excluding the blanks) by clamping the values to the aggregated value of the BLANK samples for each analyte separately.

The purpose of this operation is to unify the data by clamping values below the background noise. how this method works was inspired by the paper https://doi.org/10.1038/s41598-020-57876-0 which covers the quality control in the MBA.

In short, this operation firstly calculates the aggregate of MFI in the BLANK samples (available methods are: `min`, `max`, `mean`, `median`) and then replaces all values below this threshold with the threshold value.

Method does not modifies the data of type `Count`.

This operation is recommended to be performed before any further analysis, but is optional. Skipping it before further analysis is allowed, but will result in a warning.

Usage:

`Plate$blank_adjustment(threshold = "max", in_place = TRUE)`

Arguments:

`threshold` The method used to calculate the background value for each analyte. Every value below this threshold will be clamped to the threshold value. By default `max`. Available methods are: `min`, `max`, `mean`, `median`.

`inplace` Whether the method should produce new plate with adjusted values or not, By default `TRUE` - operates on the current plate.

Method `clone()`: The objects of this class are cloneable with this method.

Usage:

`Plate$clone(deep = FALSE)`

Arguments:

`deep` Whether to make a deep clone.

PlateBuilder   *PlateBuilder*

Description

This class helps creating the Plate object. It is used to store the data and validate the final fields.

Active bindings

layout_as_vector Print the layout associated with the plate as a flattened vector of values.

Methods

Public methods:

- [PlateBuilder$new()](#)
- [PlateBuilder$set_sample_locations()](#)
- [PlateBuilder$set_dilutions()](#)
- [PlateBuilder$set_sample_types()](#)
- [PlateBuilder$set_sample_names()](#)
- [PlateBuilder$set_plate_datetime()](#)
- [PlateBuilder$set_data()](#)
- [PlateBuilder$set_default_data_type()](#)
- [PlateBuilder$set_batch_info()](#)
- [PlateBuilder$set_plate_name()](#)
- [PlateBuilder$set_layout()](#)
- [PlateBuilder$build()](#)
- [PlateBuilder$clone()](#)

Method new(): Initialize the PlateBuilder object

Usage:

PlateBuilder$new(sample_names, analyte_names, batch_name = "", verbose = TRUE)

Arguments:

sample_names • vector of sample names measured during an examination in the same order as in the data

analyte_names • vector of analytes names measured during an examination in the same order as in the data

batch_name • name of the batch during which the plate was examined obtained from the plate info. An optional parameter, by default set to "" - an empty string.

verbose • logical value indicating whether to print additional information. This parameter is stored as a private attribute of the object and reused in other methods

Method set_sample_locations(): Set the sample types used during the examination

Usage:

```
PlateBuilder$set_sample_locations(sample_locations)
```

Arguments:

`sample_locations` vector of sample locations pretty name ie. A1, B2

Method `set_dilutions()`: Extract and set the dilutions from layout, sample names or use a provided vector of values. The provided vector should be the same length as the number of samples and should contain dilution factors saved as strings

Usage:

```
PlateBuilder$set_dilutions(use_layout_dilutions = TRUE, values = NULL)
```

Arguments:

`use_layout_dilutions` logical value indicating whether to use names extracted from layout files to extract dilutions. If set to `FALSE` the function uses the sample names as a source for dilution

`values` a vector of dilutions to overwrite the extraction process
Set and extract sample types from the sample names. Optionally use the layout file to extract the sample types

Method `set_sample_types()`:

Usage:

```
PlateBuilder$set_sample_types(use_layout_types = TRUE, values = NULL)
```

Arguments:

`use_layout_types` logical value indicating whether to use names extracted from layout files to extract sample types

`values` a vector of sample types to overwrite the extraction process

Method `set_sample_names()`: Set the sample names used during the examination. If the layout is provided, extract the sample names from the layout file. Otherwise, uses the original sample names from the Luminex file

Usage:

```
PlateBuilder$set_sample_names(use_layout_sample_names = TRUE)
```

Arguments:

`use_layout_sample_names` logical value indicating whether to use names extracted from layout files. If set to false, this function only checks if the sample names are provided in the plate

Method `set_plate_datetime()`: Set the plate datetime for the plate

Usage:

```
PlateBuilder$set_plate_datetime(plate_datetime)
```

Arguments:

`plate_datetime` a POSIXct datetime object representing the date and time of the examination

Method `set_data()`: Set the data used during the examination

Usage:

```
PlateBuilder$set_data(data)
```

Arguments:

data a named list of data frames containing information about the samples and analytes. The list is named by the type of the data e.g. `Median`, `Net MFI`, etc. The data frames contain information about the samples and analytes The rows are different measures, whereas the columns represent different analytes Example of how `data$Median` looks like:

Sample	Analyte1	Analyte2	Analyte3
Sample1	1.2	2.3	3.4
Sample2	4.5	5.6	6.7
...	...	...	...
Sample96	7.8	8.9	9.0

Method `set_default_data_type()`: Set the data type used for calculations

Usage:

`PlateBuilder$set_default_data_type(data_type = "Median")`

Arguments:

data_type a character value representing the type of data that is currently used for calculations. By default, it is set to Median

Method `set_batch_info()`: Set the batch info for the plate

Usage:

`PlateBuilder$set_batch_info(batch_info)`

Arguments:

batch_info a raw list containing metadata about the plate read from the Luminex file

Method `set_plate_name()`: Set the plate name for the plate. The plate name is extracted from the filepath

Usage:

`PlateBuilder$set_plate_name(file_path)`

Method `set_layout()`: Set the layout matrix for the plate. This function performs basic validation

- verifies if the plate is a matrix of shape 8x12 with 96 wells

Usage:

`PlateBuilder$set_layout(layout_matrix)`

Arguments:

layout_matrix a matrix containing information about the sample names. dilutions, etc.

Method `build()`: Create a Plate object from the PlateBuilder object

Usage:

`PlateBuilder$build(validate = TRUE)`

Method `clone()`: The objects of this class are cloneable with this method.

Usage:

`PlateBuilder$clone(deep = FALSE)`

Arguments:

deep Whether to make a deep clone.

---

plot_counts   *Plot counts in a 96-well plate*

Description

This function plots counts in a 96-well plate using a colour to represent the count ranges. There is a possibility of plotting exact counts in each well.

If the plot window is resized, it's best to re-run the function to adjust the scaling. Sometimes, when a legend is plotted, the whole layout may be shifted. It's best to stretch the window, and everything will be adjusted automatically.

Usage

```
plot_counts(
  plate,
  analyte_name,
  plot_counts = TRUE,
  plot_legend = FALSE,
  lower_threshold = 50,
  higher_threshold = 70
)
```

Arguments

plate	The plate object with the counts data
analyte_name	The name of the analyte
plot_counts	Logical indicating if the counts should be plotted
plot_legend	Logical indicating if the legend should be plotted
lower_threshold	
	The lower threshold for the counts, it separates green and yellow colours
higher_threshold	
	The higher threshold for the counts, it separates yellow and red colours

Value

A ggplot object

Examples

```
plate_filepath <- system.file("extdata", "CovidOISExPONENT_CO.csv",
  package = "PvSTATEM", mustWork = TRUE
)
layout_filepath <- system.file("extdata", "CovidOISExPONENT_CO_layout.xlsx",
  package = "PvSTATEM", mustWork = TRUE
)
plate <- read_luminex_data(plate_filepath, layout_filepath)
```

```
plot_counts(
  plate = plate, analyte_name = "OC43_NP_NA",
  plot_counts = TRUE, plot_legend = FALSE
)
```

---

plot_layout   *Plot layout of a 96-well plate*

Description

This function plots the layout of a 96-well plate using a colour to represent the sample types.

If the plot window is resized, it's best to re-run the function to adjust the scaling. Sometimes, the whole layout may be shifted when a legend is plotted. It's best to stretch the window, and everything will be adjusted automatically.

Usage

```
plot_layout(plate, plot_legend = TRUE)
```

Arguments

plate   The plate object with the layout information

plot_legend   Logical indicating if the legend should be plotted

Value

A ggplot object

Examples

```
plate_filepath <- system.file("extdata", "CovidOISExPONTENT_CO.csv",
  package = "PvSTATEM", mustWork = TRUE
)
layout_filepath <- system.file("extdata", "CovidOISExPONTENT_CO_layout.xlsx",
  package = "PvSTATEM", mustWork = TRUE
)
plate <- read_luminex_data(plate_filepath, layout_filepath)
plot_layout(plate = plate, plot_legend = TRUE)
```

---

plot_mfi_for_analyte   *Plot MFI value distribution for a given analyte*

Description

Plot MFI value distribution for a given analyte

Usage

```
plot_mfi_for_analyte(
  plate,
  analyte_name,
  data_type = "Median",
  plot_type = "violin",
  scale_y = "log10",
  plot_outliers = FALSE
)
```

Arguments

plate	A plate object
analyte_name	The analyte to plot
data_type	The type of data to plot. Default is "Median"
plot_type	The type of plot to generate. Default is "violin". Available options are "boxplot" and "violin".
scale_y	What kind of transformation of the scale to apply. By default MFI is presented in a "log10" scale. Available options are described in the documentation of scale_y_continuous under transform parameter.
plot_outliers	When using "boxplot" type of a plot one can set this parameter to TRUE and display the names of samples for which MFI falls outside the 1.5 IQR interval

Value

A ggplot object

---

plot_standard_curve_analyte
                    Standard curves

Description

Plot standard curve samples of a plate of a given analyte.

Usage

```
plot_standard_curve_analyte(
  plate,
  analyte_name,
  data_type = "Median",
  decreasing_rau_order = TRUE,
  log_scale = c("all"),
  plot_line = TRUE,
  plot_blank_mean = TRUE,
  plot_rau_bounds = TRUE,
  plot_legend = TRUE,
  verbose = TRUE
)
```

Arguments

plate	A plate object
analyte_name	Name of the analyte of which standard curve we want to plot.
data_type	Data type of the value we want to plot - the same datatype as in the plate file. By default equals to `Net MFI`
decreasing_rau_order	
	If TRUE the RAU values are plotted in decreasing order, TRUE by default
log_scale	Which elements on the plot should be displayed in log scale. By default `"RAU"`. If NULL or `c()` no log scale is used, if `"all"` or `c("RAU", "MFI")` all elements are displayed in log scale.
plot_line	If TRUE a line is plotted, TRUE by default
plot_blank_mean	
	If TRUE the mean of the blank samples is plotted, TRUE by default
plot_rau_bounds	
	If TRUE the RAU values bounds are plotted, TRUE by default
plot_legend	If TRUE the legend is plotted, TRUE by default
verbose	If TRUE prints messages, TRUE by default

Value

ggplot object with the plot

---

plot_standard_curve_analyte_with_model
                            Plot standard curve of a certain analyte with fitted model

---

Description

Function plots the values of standard curve samples and the fitted model.

Usage

```
plot_standard_curve_analyte_with_model(
  plate,
  model,
  data_type = "Median",
  decreasing_rau_order = TRUE,
  log_scale = c("all"),
  plot_asymptote = TRUE,
  plot_test_predictions = TRUE,
  plot_blank_mean = TRUE,
  plot_rau_bounds = TRUE,
  plot_legend = TRUE,
  verbose = TRUE,
  ...
)
```

Arguments

plate	Plate object
model	fitted `Model` object, which predictions we want to plot
data_type	Data type of the value we want to plot - the same datatype as in the plate file. By default equals to `Median`
decreasing_rau_order	
	If TRUE the RAU values are plotted in decreasing order, `TRUE` by default.
log_scale	Which elements on the plot should be displayed in log scale. By default `"all"`. If `NULL` or `c()` no log scale is used, if `"all"` or `c("RAU", "MFI")` all elements are displayed in log scale.
plot_asymptote	If TRUE the asymptotes are plotted, `TRUE` by default
plot_test_predictions	
	If TRUE the predictions for the test samples are plotted, `TRUE` by default The predictions are obtained through extrapolation of the model
plot_blank_mean	
	If TRUE the mean of the blank samples is plotted, `TRUE` by default
plot_rau_bounds	
	If TRUE the RAU bounds are plotted, `TRUE` by default
plot_legend	If TRUE the legend is plotted, `TRUE` by default
verbose	If TRUE prints messages, `TRUE` by default
...	Additional arguments passed to the `predict` function

Value

a ggplot object with the plot

---

predict.Model   *Predict the RAU values from the MFI values*

Description

More details can be found here: Model

Usage

```
## S3 method for class 'Model'
predict(object, mfi, ...)
```

Arguments

object	(Model()) Object of the Model class
mfi	(numeric()) MFI values for which we want to predict the RAU values Should be in the same scale as the MFI values used to fit the model
...	Additional arguments passed to the method

Value

(data.frame())

---

process_plate   *Process a plate and save output values to a CSV*

Description

Depending on the `normalisation_type` argument, the function will compute the RAU or nMFI values for each analyte in the plate. **RAU** is the default normalisation type.

The behaviour of the function, in the case of RAU normalisation type, can be summarised as follows:

1. Adjust blanks if not already done.
2. Fit a model to each analyte using standard curve samples.
3. Compute RAU values for each analyte using the corresponding model.
4. Aggregate computed RAU values into a single data frame.
5. Save the computed RAU values to a CSV file.

More info about the RAU normalisation can be found in `create_standard_curve_model_analyte` function documentation create_standard_curve_model_analyte or in the Model reference Model.

In case the normalisation type is **nMFI**, the function will:

1. Adjust blanks if not already done.

2. Compute nMFI values for each analyte using the target dilution.

3. Aggregate computed nMFI values into a single data frame.

4. Save the computed nMFI values to a CSV file.

More info about the nMFI normalisation can be found in get_nmfi function documentation get_nmfi.

Usage

```
process_plate(
  plate,
  filename = NULL,
  output_dir = "normalised_data",
  normalisation_type = "RAU",
  data_type = "Median",
  include_raw_mfi = TRUE,
  adjust_blanks = FALSE,
  verbose = TRUE,
  reference_dilution = 1/400,
  ...
)
```

Arguments

plate   (Plate()) a plate object

filename   (character(1)) The name of the output CSV file with normalised MFI values.
                 If not provided or equals to NULL, the output filename will be based on the nor-
                 malisation type and the plate name, precisely: {plate_name}_{normalisation_type}.csv.
                 By default the plate_name is the filename of the input file that contains the plate
                 data. For more details please refer to Plate.

                 If the passed filename does not contain .csv extension, the default extension
                 .csv will be added. Filename can also be a path to a file, e.g. path/to/file.csv.
                 In this case, the output_dir and filename will be joined together. However,
                 if the passed filepath is an absolute path and the output_dir parameter is also
                 provided, the output_dir parameter will be ignored. If a file already exists
                 under a specified filepath, the function will overwrite it.

output_dir   (character(1)) The directory where the output CSV file should be saved. Please
                 note that any directory path provided will create all necessary directories (in-
                 cluding parent directories) if they do not exist. If it equals to NULL the current
                 working directory will be used. Default is 'normalised_data'.

normalisation_type
                 (character(1)) type of normalisation to use. Available options are:
                 c(RAU, nMFI).

data_type   (character(1)) type of data to use for the computation. Median is the default

include_raw_mfi
                 (logical(1)) include raw MFI values in the output. The default is TRUE. In case
                 this option is TRUE, the output dataframe contains two columns for each analyte:
                 one for the normalised values and one for the raw MFI values. The normalised
                 columns are named as AnalyteName and AnalyteName_raw, respectively.

adjust_blanks	(logical(1)) adjust blanks before computing RAU values. The default is FALSE
verbose	(logical(1)) print additional information. The default is TRUE
reference_dilution	
	(numeric(1)) target dilution to use as reference for the nMFI normalisation. Ignored in case of RAU normalisation. Default is 1/400. It should refer to a dilution of a standard curve sample in the given plate object. This parameter could be either a numeric value or a string. In case it is a character string, it should have the format 1/d+, where d+ is any positive integer.
...	Additional arguments to be passed to the fit model function (create_standard_curve_model_analyte)

Value

a data frame with normalised values

Examples

```
plate_file <- system.file("extdata", "CovidOISExPONENT_CO_reduced.csv", package = "PvSTATEM")
# a plate file with reduced number of analytes to speed up the computation
layout_file <- system.file("extdata", "CovidOISExPONENT_CO_layout.xlsx", package = "PvSTATEM")

plate <- read_luminex_data(plate_file, layout_file, verbose = FALSE)

example_dir <- tempdir(check = TRUE) # a temporary directory
# create and save dataframe with computed dilutions
process_plate(plate, output_dir = example_dir)

# process plate without adjusting blanks and save the output to a file with a custom name
process_plate(plate,
  filename = "plate_without_blanks_adjusted.csv",
  output_dir = example_dir, adjust_blanks = FALSE
)


# nMFI normalisation
process_plate(plate,
  output_dir = example_dir,
  normalisation_type = "nMFI", reference_dilution = 1 / 400
)
```

---

read_intelliflex_format

Read the Intelliflex format data

Description

Read the Intelliflex format data

Usage

```
read_intelliflex_format(path, verbose = TRUE)
```

Arguments

path	Path to the INTELLIFLEX file
verbose	Print additional information. Default is TRUE

---

read_layout_data | *Read layout data from a file*

Description

Read layout data from a file

Usage

```
read_layout_data(layout_file_path, ...)
```

Arguments

layout_file_path

Path to the layout file

...   Additional arguments to pass to the underlying read function

Value

A matrix with the layout data. The row names are supposed to be letters A,B,C, etc. The column names are supposed to be numbers 1,2,3, etc.

---

read_luminex_data | *Read Luminex Data*

Description

Reads a file containing Luminex data and returns a Plate object. If provided, can also read a layout file, which usually contains information about the sample names, sample types or its dilutions.

The function is capable of reading data in two different formats:

- xPONENT
- INTELLIFLEX which are produced by two different Luminex machines.

Usage

```
read_luminex_data(
  plate_filepath,
  layout_filepath = NULL,
  format = "xPONENT",
  plate_file_separator = ",",
  plate_file_encoding = "UTF-8",
  use_layout_sample_names = TRUE,
  use_layout_types = TRUE,
  use_layout_dilutions = TRUE,
  default_data_type = "Median",
  sample_types = NULL,
  dilutions = NULL,
  verbose = TRUE
)
```

Arguments

`plate_filepath` Path to the Luminex plate file

`layout_filepath`

Path to the Luminex layout file

`format`   The format of the Luminex data. Select from: xPONENT, INTELLIFLEX

`plate_file_separator`

The separator used in the plate file

`plate_file_encoding`

The encoding used in the plate file

`use_layout_sample_names`

Whether to use names from the layout file in extracting sample names.

`use_layout_types`

Whether to use names from the layout file in extracting sample types. Works only when layout file is provided

`use_layout_dilutions`

Whether to use dilutions from the layout file in extracting dilutions. Works only when layout file is provided

`default_data_type`

The default data type to use if none is specified

`sample_types`   a vector of sample types to use instead of the extracted ones

`dilutions`   a vector of dilutions to use instead of the extracted ones

`verbose`   Whether to print additional information and warnings. `TRUE` by default

Value

Plate file containing the Luminex data

Examples

```
plate_file <- system.file("extdata", "CovidOISExPONENT.csv", package = "PvSTATEM")
layout_file <- system.file("extdata", "CovidOISExPONENT_layout.csv", package = "PvSTATEM")
plate <- read_luminex_data(plate_file, layout_file)

plate_file <- system.file("extdata", "CovidOISExPONENT_CO.csv", package = "PvSTATEM")
layout_file <- system.file("extdata", "CovidOISExPONENT_CO_layout.xlsx", package = "PvSTATEM")
# To suppress warnings and additional information use verbose = FALSE
plate <- read_luminex_data(plate_file, layout_file, verbose = FALSE)
```

---

read_xponent_format   *Read the xPONENT format data*

Description

Read the xPONENT format data

Usage

```
read_xponent_format(
  path,
  exact_parse = FALSE,
  encoding = "utf-8",
  separator = ",",
  verbose = TRUE
)
```

Arguments

path	Path to the xPONENT file
exact_parse	Whether to parse the file exactly or not Exact parsing means that the batch, calibration and assay metadata will be parsed as well
encoding	Encoding of the file
separator	Separator for the CSV values
verbose	Whether to print the progress. Default is TRUE

---

translate_sample_names_to_sample_types

Translate sample names to sample types

Description

Function translates sample names to sample types based on the sample name from Luminex file and the sample name from the layout file, which may not be provided. The function uses regular expressions to match the sample names to the sample types.

It parses the names as follows:

If sample_names or sample_names_from_layout equals to BLANK, BACKGROUND or B, then SampleType equals to BLANK

If sample_names or sample_names_from_layout equals to STANDARD CURVE, SC, S, contains substring 1/\d+ and has prefix , S_, S , S or CP3, then SampleType equals to STANDARD CURVE

If sample_names or sample_names_from_layout equals to NEGATIVE CONTROL, N, or contains substring NEG, then SampleType equals to NEGATIVE CONTROL

If sample_names or sample_names_from_layout starts with P followed by whitespace, POS followed by whitespace, some sample name followed by substring 1/\d+ SampleType equals to POSITIVE CONTROL

Otherwise, the returned SampleType is TEST

Usage

```
translate_sample_names_to_sample_types(
  sample_names,
  sample_names_from_layout = NULL
)
```

Arguments

sample_names   (character())
                 Vector of sample names from Luminex file

sample_names_from_layout

                 (character())
                 Vector of sample names from Layout file values in this vector may be different than sample_names and may contain additional information about the sample type like dilution. This vector when set has to have at least the length of sample_names.

Value

A vector of valid sample_type strings of length equal to the length of sample_names

Examples

```
translate_sample_names_to_sample_types(c("B", "BLANK", "NEG", "TEST1"))
translate_sample_names_to_sample_types(c("S", "CP3"))
```

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