## Package 'seahtrue'

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Type Package

Title Seahtrue revives XF data for structured data analysis

Version 1.5.0

Description Seahtrue organizes oxygen consumption and extracellular acidification analysis data from experiments performed on an XF analyzer into structured nested tibbles. This allows for detailed processing of raw data and advanced data visualization and statistics. Seahtrue introduces an open and reproducible way to analyze these XF experiments. It uses file paths to .xlsx files. These .xlsx files are supplied by the userand are generated by the user in the Wave software from Agilent from the assay result files (.asyr). The .xlsx file contains different sheets of important data for the experiment;

- 1. Assay Information Details about how the experiment was set up.
- 2. Rate Data Information about the OCR and ECAR rates.
- 3. Raw Data The original raw data collected during the experiment.
- 4. Calibration Data Data related to calibrating the instrument. Seahtrue focuses on getting the specific data needed for analysis. Once this data is extracted, it is prepared for calculations through preprocessing. To make sure everything is accurate, both the initial data and the preprocessed data go through thorough checks.

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```
1.1.3), colorspace (>= 2.1.0), forcats (>= 1.0.0), ggridges (>=
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              seahtrue: Seahtrue revives XF data for structured data analysis
```

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#### **Description**

Seahtrue organizes oxygen consumption and extracellular acidification analysis data from experiments performed on an XF analyzer into structured nested tibbles. This allows for detailed processing of raw data and advanced data visualization and statistics. Seahtrue introduces an open and reproducible way to analyze these XF experiments. It uses file paths to .xlsx files. These .xlsx files are supplied by the userand are generated by the user in the Wave software from Agilent from the assay result files (.asyr). The .xlsx file contains different sheets of important data for the experiment; 1. Assay Information - Details about how the experiment was set up. 2. Rate Data - Information about the OCR and ECAR rates. 3. Raw Data - The original raw data collected during the experiment. 4. Calibration Data - Data related to calibrating the instrument. Seahtrue focuses on getting the specific data needed for analysis. Once this data is extracted, it is prepared for calculations through preprocessing. To make sure everything is accurate, both the initial data and the preprocessed data go through thorough checks.

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#### See Also

Useful links:

- https://vcjdeboer.github.io/seahtrue/
- Report bugs at https://vcjdeboer.github.io/seahtrue/issues

glue\_xfplates

Glueing mulltiple plates from a folder

#### **Description**

This function takes a folder path and on the available .xlsx files the revive\_xfplate() function is run and output in one nested tibble.

#### Usage

```
glue_xfplates(folderpath_seahorse, arg_is_folder)
```

#### **Arguments**

folderpath\_seahorse

the path to a folder where the .xlsx files are located or a vector of strings pointing to the path of each individual file

arg\_is\_folder

either TRUE or FALSE. When the input is a vector of path strings use FALSE, is it points to a folder use TRUE

#### Value

a nested tibble with all files organized in a row

#### **Examples**

```
c(
    system.file("extdata",
        "20191219_SciRep_PBMCs_donor_A.xlsx",
        package = "seahtrue"
    ),
    system.file("extdata",
        "20191219_SciRep_PBMCs_donor_A.xlsx",
        package = "seahtrue"
    )
) |>
    glue_xfplates(arg_is_folder = FALSE)
```

#### **Description**

A dataset containing output for the "20191219\_SciRep\_PBMCs\_donor\_A.xlsx" file. This experiment was part of the study that was published in Janssen et al. (2021) Sci rep 11:1662. This is data from PBMCs that were isolated from buffy coats as described in the material and methods section of Janssen et al. (2021). The .xlsx file is the result of a single XF experiment on one plate. This .xlsx file was used as input for the revive\_xfplate() function. The output of that function contains the read, preprocessed, and validated data in the form a a nested tibble. The file '20191219\_SciRep\_PBMCs\_donor\_A.xlsx' can be found in the inst/extdata directory.

#### Usage

```
data(revive_output_donor_A)
```

#### Format

A data frame with 1 row and 7 variables.

plate\_id Barcode plate id of the well plate containing the samples

filepath\_seahorse Path, and basename to .xlsx file

date\_run Date and time when the plate was run

date\_processed Date and time this output from revive\_xfplate() was generated

assay info Meta information from 'Assay Configuration' sheet and 'Calibration' sheet

injection\_info Dataframe with information from the 'Operation log' sheet

raw data Preprocessed raw dataframe from 'Raw' sheet

rate data Preprocessed rate data from 'Rate' sheet

validation\_output A list of all validation information, output, and rules that are used

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#### **Source**

Janssen et al. 2021 Sci Rep 11:1162 <a href="https://doi.org/10.1038/s41598-021-81217-4">https://doi.org/10.1038/s41598-021-81217-4</a>>

revive\_xfplate

Running the read, preprocess and validate

#### **Description**

This function takes the Seahorse Wave .xlsx file and computes it through read, validate and preprocess

#### Usage

```
revive_xfplate(filepath_seahorse)
```

#### **Arguments**

filepath\_seahorse

Absolute path to the Seahorse Excel file.

#### Value

A preprocessed seahorse dataset is returned as an output. This is a nested tibble with the following 7 columns:

- \* plate\_id = Barcode plate id of the well plate containing the samples
- \* filepath\_seahorse = Path, and basename to .xlsx input file
- \* date run = Date and time when the plate was run
- \* date processed = Date and time this output from revive xfplate() was generated
- \* assay\_info = Meta information from 'Assay Configuration' sheet and 'Calibration' sheet
- \* injection\_info = Dataframe with information from the 'Operation log' sheet
- \* raw\_data = Preprocessed raw dataframe from 'Raw' sheet
- \* rate\_data = Preprocessed rate data from 'Rate' sheet
- # validation\_output = Output of the data checks. including rules

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```
sketch_assimilate_rate
```

Combine multiple revived xf plates into one plot for rate data

#### **Description**

In this plot the OCR or ECAR is plotted per group for each plate in a faceted gpgplot

## Usage

```
sketch_assimilate_rate(my_df, param = "OCR", my_measurements = c(3, 6, 7, 12))
```

## Arguments

param

my\_df a tibble generated by glue\_xfplates() with for each row representing a single xf experiment

either "OCR" or "ECAR"

my\_measurements

the measurements that needs to be in the plot. For example, c(3,6,7,12) for a typical mito stress test.

#### Value

a ggplot object

```
suppressMessages(
   c(
        {\tt system.file("extdata"},
            "20191219_SciRep_PBMCs_donor_A.xlsx",
            package = "seahtrue"
        ),
        system.file("extdata",
            "20191219_SciRep_PBMCs_donor_A.xlsx",
            package = "seahtrue"
        )
    ) |>
        glue_xfplates(arg_is_folder = FALSE) |>
        sketch_assimilate_rate(
            param = "OCR",
            my_measurements = c(3, 4, 9, 12)
)
```

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sketch\_assimilate\_raw Combine multiple revived xf plates into one plot for raw data

#### **Description**

In this plot the O2, pH, or its emission value at the very first measurement point plotted for all wells from all xfplates that are provided to the function.

#### Usage

```
sketch_assimilate_raw(my_df, param = "02_mmHg")
```

## **Arguments**

```
my_df a tibble generated by glue_xfplates() with for each row representing a single xf experiment

param either "O2_mmHg", "pH", "O2_em_corr" or "pH_em_corr
```

#### Value

a ggplot object

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sketch\_plate

Make an overview of plate layout, with colored groups

#### **Description**

A heatmap style ggplot figure with each well labeled with a color for each group

#### Usage

```
sketch_plate(xfplate, reorder_legend = FALSE)
```

#### **Arguments**

xfplate

This the 'raw\_data' or the 'rate\_data' tibble that is generated by the 'revive\_xfplate()'

function

reorder\_legend

either 'TRUE' or 'FALSE'. When 'TRUE' the groups are ordered based on the number in the character string of the group. It also adds a "\_\_00" after each character string to make the forcats::refactor(group, parse\_number(group))

work.

#### Value

a ggplot object of a 96 well plate with the group layout

#### **Examples**

```
system.file("extdata",
    "20191219_SciRep_PBMCs_donor_A.xlsx",
    package = "seahtrue"
) |>
    revive_xfplate() |>
    purrr::pluck("raw_data", 1) |>
    sketch_plate(reorder_legend = TRUE)
```

sketch rate

Generate a plot for the rate data

#### **Description**

The sketch\_rate() function uses the rate\_data from the generated output from the revive\_xfplate() function. The injection info is annotated in the plot, using the information form the injections provided in the original experiment. Several options are available to plot either ECAR/OCR or normalize the data with the values from the normalization cells in the .xlsx file.

A number of validations are performed to check whether the data can be plotted and whether the layout of the plot will not be ruined...

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#### Usage

```
sketch_rate(
  xf_rate,
  param = "OCR",
  normalize = FALSE,
  normalize_unit = "10000 cells",
  take_group_mean = TRUE,
  reorder_legend = FALSE
)
```

#### **Arguments**

xf\_rate The 'rate\_data' tibble as generated by 'revive\_plate'
param Either "OCR" or "ECAR"

normalize Either TRUE or FALSE

normalize\_unit any string that will be pasted in the y-axis label when normalize = TRUE

take\_group\_mean

Either TRUE or FALSE

reorder\_legend Either TRUE or FALSE. When 'TRUE' the groups are ordered based on the number in the character string of the group. It also adds a "\_\_00" after each char-

acter string to make the forcats::refactor(group, parse\_number(group)) work.

#### Value

a ggplot object

```
system.file("extdata",
    "20191219_SciRep_PBMCs_donor_A.xlsx",
   package = "seahtrue"
) |>
    revive_xfplate() |>
   purrr::pluck("rate_data", 1) |>
   sketch_rate(
        param = "OCR",
        reorder_legend = TRUE
   )
system.file("extdata",
    "20191219_SciRep_PBMCs_donor_A.xlsx",
   package = "seahtrue"
) |>
    revive_xfplate() |>
    purrr::pluck("rate_data", 1) |>
    sketch_rate(
        param = "OCR",
        take_group_mean = FALSE,
        reorder_legend = TRUE
```

sketch\_rate

```
)
system.file("extdata",
    "20191219_SciRep_PBMCs_donor_A.xlsx",
   package = "seahtrue"
) |>
   revive_xfplate() |>
   purrr::pluck("rate_data", 1) |>
   sketch_rate(
       param = "ECAR",
       normalize = TRUE,
       take_group_mean = TRUE,
       reorder_legend = TRUE
system.file("extdata",
    "20191219_SciRep_PBMCs_donor_A.xlsx",
   package = "seahtrue"
) |>
    revive_xfplate() |>
   purrr::pluck("rate_data", 1) |>
   sketch_rate(
       param = "ECAR",
       normalize = TRUE,
       take_group_mean = FALSE,
       reorder_legend = TRUE
   )
```

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