

Package ‘GRmetrics’

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Type Package

Title Calculate growth-rate inhibition (GR) metrics

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Description Functions for calculating and visualizing growth-rate
inhibition (GR) metrics.

License GPL-3

URL <https://github.com/uc-bd2k/GRmetrics>

BugReports <https://github.com/uc-bd2k/GRmetrics/issues>

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GRbox	<i>Boxplots of a given GR metric</i>
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Description

Given a SummarizedExperiment object created by `GRfit`, this function creates boxplots according to the parameters below.

Usage

```
GRbox(
  fitData,
  metric,
  groupVariable,
  pointColor,
  factors = "all",
  wilA = NULL,
  wilB = NULL,
  plotly = TRUE
)
```

Arguments

<code>fitData</code>	a SummarizedExperiment object, generated by the <code>GRfit</code> function.
<code>metric</code>	the GR metric (<code>GR50</code> , <code>GRinf</code> , <code>h_GR</code> , <code>GRmax</code> , <code>GEC50</code> , or <code>GR_AOC</code>) or traditional metric (<code>IC50</code> , <code>Einf</code> , <code>h</code> , <code>Emax</code> , <code>EC50</code> , or <code>AUC</code>) that will be used for the boxplot.
<code>groupVariable</code>	the name of the variable from data (e.g. <code>drug</code> , <code>cell-line</code> , etc.) to select factors from.
<code>pointColor</code>	a variable that defines the coloring of the points overlaid on the boxplot.
<code>factors</code>	a vector of values of " <code>groupVariable</code> " of data that define which variables to make boxplots for. By default, a separate boxplot is made for each unique value of <code>groupVariable</code> .

wilA	one value or a vector of values from "factors", i.e. a subset of the boxplots. If specified, a one-sided Wilcoxon rank sum test (<code>wilcox.test</code>) will be performed between "wilA" and "wilB" and the results will be displayed on the figure. The null hypothesis that the values from "wilA" and "wilB" have the same mean will be tested against the alternative hypothesis that the mean of the "wilB" values is greater than that of the "wilA" values.
wilB	one value or a vector of values from "factors", i.e. a subset of the boxplots (not overlapping "wilA").
plotly	a logical value indicating whether to output a <code>ggplot2</code> graph or an interactive <code>ggplotly</code> graph

Details

Given a `SummarizedExperiment` object created by [GRfit](#), this function creates boxplots of a given GR metric (`GR50`, `GRmax`, etc.) or traditional metric (`IC50`, `Emax`, etc.) for values of the grouping variable. The results can be viewed in a static `ggplot` image or an interactive `plotly` graph.

By default, a boxplot is created for all unique values of the grouping variable. The "factors" parameter can be used to specify a smaller subset of values for which to create boxplots. Points are overlaid on the boxplots and they can be colored by the variable specified in the `pointColor` parameter. If `pointColor` is set to `NULL`, the points will all be black. The results can be viewed in a static `ggplot` image or an interactive `plotly` graph.

Value

`ggplot2` or `ggplotly` boxplots of the factors along the x-axis, with points colored by the given variable.

Author(s)

Nicholas Clark

See Also

To create the object needed for this function, see [GRfit](#). For other visualizations, see [GRdrawDRC](#) and [GRscatter](#). For online GR calculator and browser, see <http://www.grcalculator.org>.

Examples

```
# Load Case A (example 1) input
## Not run:
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
  groupingVariables = c('cell_line','agent', 'perturbation','replicate',
  'time'))
GRbox(output1, metric = 'GRinf',
  groupVariable = 'cell_line', pointColor = 'agent' , factors = c('BT20',
  'MCF10A'))
GRbox(output1, metric = 'GRinf',
```

```

groupVariable = 'cell_line', pointColor = 'cell_line' ,
factors = c('BT20', 'MCF10A'), plotly = FALSE)
GRbox(output1, metric = 'GR50', groupVariable = 'cell_line',
pointColor = 'cell_line', wilA = "BT20", wilB = c("MCF7", "MCF10A"))

## End(Not run)

```

GRdrawDRC

Dose-Response Curves

Description

Given a SummarizedExperiment object created by `GRfit`, this function plots either the growth-rate inhibition (GR) dose response curves or the traditional dose response curves for a given set of data.

Usage

```

GRdrawDRC(
  fitData,
  metric = "GR",
  experiments = "all",
  min = "auto",
  max = "auto",
  points = TRUE,
  curves = TRUE,
  plotly = TRUE
)

```

Arguments

<code>fitData</code>	an element of class <code>SummarizedExperiment</code> , generated by the <code>GRfit</code> function.
<code>metric</code>	either "GR" for GR dose response curves or "rel_cell" for traditional dose response curves based on relative cell count.
<code>experiments</code>	the names of the experiments to plot (or "all")
<code>min</code>	the minimum concentration to plot (for curves)
<code>max</code>	the maximum concentration to plot (for curves)
<code>points</code>	a logical value indicating whether points (individual GR values) will be plotted
<code>curves</code>	a logical value indicating whether sigmoidal dose-response curves will be plotted
<code>plotly</code>	a logical value indicating whether to output a <code>ggplot2</code> graph or a <code>ggplotly</code> graph

Details

Given a SummarizedExperiment object created by `GRfit`, this function plots these GR values (versus concentration) and/or the sigmoidal curves fitted to the sets of points. The results can be viewed in a static `ggplot` image or an interactive `plotly` graph.

The "min" and "max" parameters control the concentration values for which the curves are plotted. They are automatically set to the minimum and maximum concentrations of the data, but can be set by the user as well. "min" and "max" take raw values (not log transformed) for concentration.

By default, curves and points are plotted for all experiments. To specify a smaller set of experiments, use the "experiments" parameter. To see the names of individual experiments for a `GRfit` object `fit_example`, see `colData(fit_example)`. See the examples below.

Value

`ggplot2` or `ggplotly` graphs of Growth-rate inhibition dose-response curves

Author(s)

Nicholas Clark

See Also

To create the object needed for this function, see `GRfit`. For other visualizations, see `GRbox` and `GRscatter`. For online GR calculator and browser, see <http://www.grcalculator.org>.

Examples

```
# Load Case A (example 1) input
data("inputCaseA")
# Run GRfit function with case = "A"
drc_output = GRfit(inputCaseA,
  groupingVariables = c('cell_line', 'agent'))
GRdrawDRC(drc_output, experiments = c('BT20 drugA', 'MCF10A drugA',
  'MCF7 drugA'), min = 10^(-4), max = 10^2)
GRdrawDRC(drc_output, plotly = FALSE)
```

GRfit

Extract GR parameters from a dataset

Description

This function takes in a dataset with information about concentration, cell counts over time, and additional grouping variables for a dose-response assay and calculates growth-rate inhibition (GR) metrics as well as traditional metrics (IC50, Emax, etc.) for each experiment in the dataset. The data must be in a specific format: either that specified by case "A" or case "C" described in the details below.

Usage

```
GRfit(inputData, groupingVariables, case = "A", force = FALSE, cap = FALSE)
```

Arguments

<code>inputData</code>	a data table in one of the specified formats (Case A or Case C). See details below for description. See <code>data(inputCaseA)</code> or <code>data(inputCaseC)</code> for example input data frames. See help files for <code>inputCaseA</code> and <code>inputCaseC</code> for description of these examples.
<code>groupingVariables</code>	a vector of column names from <code>inputData</code> . All of the columns in <code>inputData</code> except for those identified here will be averaged over.
<code>case</code>	either "A" or "C", indicating the format of the input data. See below for descriptions of these formats.
<code>force</code>	a logical value indicating whether to attempt to "force" a sigmoidal fit, i.e. whether to allow fits with F-test p-values greater than .05
<code>cap</code>	a logical value indicating whether to cap GR values (or relative cell counts) at 1. If true, all values greater than 1 will be set to 1.

Details

Calculation of GR values is performed by the function `.GRcalculate` according to the "Online Methods" section of Hafner and Niepel et al. (2016, <http://dx.doi.org/10.1038/nmeth.3853>).

The fitting of the logistic curve is performed by the `.GRlogisticFit` function, which calls the `drm` function from the `drc` package to solve for the curve parameters. The GR curve fit function is given by $f(c) = \text{GRinf} + (1 - \text{GRinf}) / (1 + (c/\text{GEC50})^{\text{h_GR}})$ where c is concentration. The fit is performed under following constraints: `h_GR` in $[.1, 5]$, `GRinf` in $[-1, 1]$, and `GEC50` in $[\min(c)*1e-2, \max(c)*1e2]$ (c is concentration). The initial conditions for the fitting algorithm are `h_GR` = 2, `GRinf` = 0.1 and `GEC50` = `median(c)`. The fitting of the traditional dose response curve is done using the same formula, replacing `GRinf` with `Einf`, `GEC50` with `EC50`, and `h_GR` with `h`. The fit is performed on the relative cell counts instead of GR values. Also, since the traditional dose response curve is bounded between 0 and 1 whereas the GR dose response curve is bounded between -1 and 1, we restrict `Einf` to the range $[0, 1]$.

The parameters of the GR dose response curves (and traditional dose response curves) for each experiment are fitted separately. An F-test is used to compare the sigmoidal fit to a flat line fit. If the p-value of the F-test is less than .05, the sigmoidal fit is accepted. If the p-value is greater than or equal to .05, a flat horizontal line fit is given, with y equal to the mean of the GR values (or relative cell counts in the case of the traditional dose response curve). For each flat fit, `GEC50` (or `EC50`) is set to 0, `h_GR` (or `h`) is set to 0.01, `GRinf` (or `Einf`) is set to the y value of the flat fit, and `GR50` (or `IC50`) is set to $\pm\text{Inf}$ depending on whether `GRinf` (or `Einf`) is greater or less than .5.

The mandatory columns for `inputData` for Case "A" are the following as well as other grouping columns.

1. `concentration` - column with concentration values (not log transformed) of the perturbagen on which dose-response curves will be evaluated
2. `cell_count` - column with the measure of cell number (or a surrogate of cell number) after treatment

3. `cell_count__time0` - column with initial (Time 0) cell counts - the measure of cell number in untreated wells grown in parallel until the time of treatment

4. `cell_count__ctrl` - column with the Control cell count: the measure of cell number in control (e.g. untreated or DMSO-treated) wells from the same plate

All other columns will be treated as additional keys on which the data will be grouped (e.g. `cell_line`, `drug`, `time`, `replicate`)

The mandatory columns for `inputData` for Case "C" are the following as well as other grouping columns.

1. `concentration` - column with concentration values (not log transformed) of the perturbagen on which dose-response curves will be evaluated

2. `cell_count` - column with the measure of cell number (or a surrogate of cell number)

3. `time` - column with the time at which a cell count is observed

All other columns will be treated as additional keys on which the data will be grouped (e.g. `cell_line`, `drug`, `replicate`)

GR values and dose-response curves/metrics can also be computed using division times for (untreated) cell lines in the place of time zero cell counts, using the first formula in the Supplement of Hafner et al. (2017, <http://dx.doi.org/10.1038/nbt.3882>).

To use division rate instead of initial cell count, `inputData` should not have any initial cell counts (i.e. For Case "A", no `"cell_count__time0"` column. For Case "C", no values of 0 in the "time" column) and should instead have two columns `"treatment_duration"` and `"division_time"`.

In the first column, `"treatment duration"`, one should have the duration of the assay between time of treatment and the final cell counts (e.g. 72 for hours in a typical 3-day assay). In the second column, `"division_time"`, one should have the time it takes for one cell doubling to occur in each (untreated) cell line used under the conditions of the experiment. These two columns must contain numbers (no units), but need to refer to the same units (e.g. hours). In most cases, all experiments of a particular cell line would have the same `"division_time"`, however if the division rate of untreated cells varied on another parameter, for example seeding density, it would be appropriate to measure and input division times based on cell line/seeding density pairs.

Value

A `SummarizedExperiment` object containing GR metrics (`GR50`, `GRmax`, etc.) and traditional metrics (`IC50`, `Emax`, etc.) as well as goodness of fit measures is returned. The object also contains, in its metadata, a table of the original data converted to the style of "Case A" (with calculated GR values and relative cell counts for each row) and a vector of the grouping variables used for the calculation.

Note

To see the underlying code, use `(getAnywhere(.GRlogistic_3u))`, `(getAnywhere(.rel_cell_logistic_3u))`, `(getAnywhere(.GRcalculate))`, and `(getAnywhere(.GRlogisticFit))`

Author(s)

Nicholas Clark

References

Hafner, M., Niepel, M., Chung, M., and Sorger, P.K., "Growth Rate Inhibition Metrics Correct For Confounders In Measuring Sensitivity To Cancer Drugs". *Nature Methods* 13.6 (2016): 521-527. <http://dx.doi.org/10.1038/nmeth.3853>

Hafner, M., Niepel, M., Sorger, P.K., "Alternative drug sensitivity metrics improve preclinical cancer pharmacogenomics". *Nature Biotechnology* 35.6 (2017): 500-502. <http://dx.doi.org/10.1038/nbt.3882>

See Also

See `drm` for the general logistic fit function that solves for the parameters GRinf, GEC50, and h_GR. See `drmc` for options of this function. Use the functions `GRdrawDRC`, `GRbox`, and `GRscatter` to create visualizations using the output from this function. For online GR calculator and browser, see <http://www.grcalculator.org>.

Examples

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line','agent', 'perturbation','replicate',
'time'))
# Overview of SummarizedExperiment output data
output1
## Not run:
# View GR metrics table
View(GRgetMetrics(output1))
# View descriptions of each metric (or goodness of fit measure)
View(GRgetDefs(output1))
# View table of original data (converted to style of Case A) with GR values
# and relative cell counts
View(GRgetValues(output1))
# View vector of grouping variables used for calculation
GRgetGroupVars(output1)

## End(Not run)
# Load Case C (example 4) input
# Same data, different format
data("inputCaseC")
head(inputCaseC)
output4 = GRfit(inputData = inputCaseC,
groupingVariables = c('cell_line','agent', 'perturbation','replicate',
'time'),
case = "C")
# Extract data tables and export to .tsv or .csv
## Not run:
# Write GR metrics parameter table to tab-separated text file
write.table(GRgetMetrics(output1), file = "filename.tsv", quote = FALSE,
sep = "\t", row.names = FALSE)
```



```
# Write original data plus GR values to comma-separated file
write.table(GRgetValues(output1), file = "filename.csv", quote = FALSE,
sep = ",", row.names = FALSE)

## End(Not run)
```

GRgetDefs

GR metrics definitions

Description

Given a SummarizedExperiment object created by [GRfit](#), this function returns a table with the definition of each GR metric and traditional metric that is calculated.

Usage

```
GRgetDefs(fitData)
```

Arguments

`fitData` a SummarizedExperiment object, generated by the GRfit function.

Details

Given a SummarizedExperiment object created by [GRfit](#), this function returns a table containing the definition of each GR metric, traditional metric, and goodness of fit measure in the object. This is simply a convenient accessor function, equivalent to `rowData(fitData)`.

Value

A table of definitions of GR metrics and traditional metrics

Author(s)

Nicholas Clark

See Also

To create the object needed for this function, see [GRfit](#).

Examples

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line', 'agent'))
defs = GRgetDefs(output1)
```

```
# See all definitions (some will be truncated)
## Not run: View(defs)
# See the first six descriptions
head(defs)
# Look at a specific definition
defs[15,]
```

GRgetGroupVars

Grouping Variables

Description

Given a SummarizedExperiment object created by [GRfit](#), this function returns a vector of the grouping variables used to create the object.

Usage

```
GRgetGroupVars(fitData)
```

Arguments

`fitData` a SummarizedExperiment object, generated by the [GRfit](#) function.

Details

Given a SummarizedExperiment object created by [GRfit](#), this function returns a vector of the grouping variables used to create the object. These are the variables in the dataset that are not averaged over. This is simply a convenient accessor function, equivalent to `metadata(fitData)[[2]]`.

Value

A vector of grouping variables

Author(s)

Nicholas Clark

See Also

To create the object needed for this function, see [GRfit](#).

Examples

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line', 'agent'))
groupVars = GRgetGroupVars(output1)
groupVars
```

`GRgetMetrics`*GR metrics*

Description

Given a SummarizedExperiment object created by [GRfit](#), this function returns a table of GR metrics (as well as traditional metrics) for each experiment in the dataset.

Usage

```
GRgetMetrics(fitData)
```

Arguments

`fitData` a SummarizedExperiment object, generated by the GRfit function.

Details

Given a SummarizedExperiment object created by [GRfit](#), this function returns a table of GR metrics and traditional metrics along with goodness of fit measures. It also identifies each fit as flat or sigmoidal. This is simply a convenient accessor function, equivalent to `cbind(as.data.frame(colData(fitData)), as.data.frame(rowData(fitData)))`.

Value

A table with GR metrics and goodness of fit measures

Author(s)

Nicholas Clark

See Also

To create the object needed for this function, see [GRfit](#).

Examples

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line', 'agent'))
metrics = GRgetMetrics(output1)
## Not run: View(metrics)
head(metrics)
```

`GRgetValues`*GR values*

Description

Given a SummarizedExperiment object created by [GRfit](#), this function returns a table of the original data (in the form of "Case A") with columns for GR values and relative cell counts.

Usage

```
GRgetValues(fitData)
```

Arguments

`fitData` a SummarizedExperiment object, generated by the GRfit function.

Details

Given a SummarizedExperiment object created by [GRfit](#), this function returns a table of the original data (in the form of "Case A") with columns for GR values and relative cell counts. This is simply a convenient accessor function, equivalent to `as.data.frame(metadata(fitData)[[1]])`.

Value

A table with GR values and relative cell counts

Author(s)

Nicholas Clark

See Also

To create the object needed for this function, see [GRfit](#).

Examples

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line', 'agent'))
GRvalues = GRgetValues(output1)
head(GRvalues)
## Not run: View(GRvalues)
```

GRscatter	<i>Scatterplot of a given GR metric</i>
-----------	---

Description

Given a SummarizedExperiment object created by [GRfit](#), this function creates a scatterplot according to the parameters below.

Usage

```
GRscatter(fitData, metric, variable, xaxis, yaxis, plotly = TRUE)
```

Arguments

fitData	a SummarizedExperiment object, generated by the GRfit function.
metric	the GR metric (GR50, GRinf, h_GR, GRmax, GEC50, or GR_AOC) or traditional metric (IC50, Einf, h, Emax, EC50, or AUC) that will be used for the scatterplot.
variable	The name of the variable from data (e.g. drug, cell-line, etc.) to select factors from.
xaxis	a vector of values of "variable" of data to be used for the scatterplot's x-axis
yaxis	a vector of values of "variable" of data to be used for the scatterplot's y-axis
plotly	a logical value indicating whether to output a ggplot2 graph or a ggplotly graph

Details

Given a SummarizedExperiment object created by [GRfit](#), this function creates a scatterplot of a given GR metric (GR50, GRmax, etc.) or traditional metric (IC50, Emax, etc.) with the "xaxis" values plotted against the "yaxis" values. The results can be viewed in a static ggplot image or an interactive plotly graph.

The xaxis and yaxis vectors must be of the same length or at least one must be of length one. For each pair of values xaxis[i] and yaxis[i], the function will create a scatterplot (all on one graph) of the specified GR metric. If a vector is of length one, it will be repeated to the length of the other vector.

Value

a ggplot2 or ggplotly scatterplot of the x-axis variable(s) versus the y-axis variable(s) for the given GR metric

Author(s)

Nicholas Clark

See Also

To create the object needed for this function, see [GRfit](#). For other visualizations, see [GRdrawDRC](#) and [GRbox](#). For online GR calculator and browser, see <http://www.grcalculator.org>.

Examples

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line','agent', 'perturbation','replicate',
'time'))
GRscatter(output1, 'GR50', 'agent', c('drugA','drugD'), 'drugB')
GRscatter(output1, 'GR50', 'agent', c('drugA','drugD'), 'drugB',
plotly = FALSE)
```

inputCaseA

Simulated dose-response assay data (Case A)

Description

This is an example dataset corresponding to Case A. The data was generated by the python script "generate_data.py" found in the "inst/scripts/" directory. This object is meant to be used as the "inputData" argument for the [GRfit](#) function when "case" is equal to "A" (the default option). The input data for "Case A" of the [GRfit](#) function must be in this format with columns named "concentration", "cell_count", "cell_count_ctrl", and "cell_count_time0" as well as columns for other key variables. The columns "cell_line", "agent", "perturbation", "replicate", and "time" are simply examples of these key variables. It is not necessary that your input data frame include these exact column names or the same number of columns.

Usage

```
inputCaseA
```

Format

A data frame with 1008 rows and 9 variables:

- cell_line: the cell-line used (MCF10A, MCF7, BT20)
- agent: the drug used (drugA, drugB, drugC, drugD)
- perturbation: an example of another key variable (e.g different media, a co-treatment, etc.) (0, 1)
- replicate: replicate number, (1, 2, 3)
- time: time of assay measured in hours (48, 72)
- concentration: concentration of the perturbation on which dose-response curves will be evaluated (not log transformed)

- cell_count: the measure of cell number (or a surrogate of cell number) after treatment at the end of the assay
- cell_count__ctrl: the measure of cell number in control (e.g. untreated or DMSO-treated) wells from the same plate at the end of the assay
- cell_count__time0: the measure of cell number in untreated wells grown in parallel until the time of treatment

Value

An example dataset in the form of "Case A" generated for use with [GRfit](#)

Source

`/inst/scripts/generate_data.py`

inputCaseC	<i>Simulated dose-response assay data (Case C)</i>
------------	--

Description

This is an example dataset corresponding to Case C. The dataset is equivalent to that of Case A, but in a different form. The data was generated by the python script "generate_data.py" found in the "inst/scripts/" directory. This object is meant to be used as the "inputData" argument for the [GRfit](#) function when "case" is equal to "C". The input data for "Case C" of the [GRfit](#) function must be in this format with columns named "concentration", "cell_count", and "time" as well as columns for other key variables. The columns "cell_line", "agent", "perturbation", and "replicate", are simply examples of these key variables. It is not necessary that your input data frame include these exact column names or the same number of columns.

Usage

`inputCaseC`

Format

A data frame with 1352 rows and 7 variables:

- cell_line: the cell-line used (MCF10A, MCF7, BT20)
- agent: the drug used (-, drugA, drugB, drugC, drugD)
- pertubation: an example of another key variable (e.g different media, a co-treatment, etc.) (0, 1)
- replicate: replicate number, (1, 2, 3)
- time: time of assay measured in hours (0, 48, 72)
- concentration: concentration of the perturbagen on which dose-response curves will be evaluated (not log transformed)
- cell_count: measure of cell number or a surrogate of the number of cells.

Value

An example dataset in the form of "Case C" generated for use with [GRfit](#)

Source

`inst/scripts/generate_data.py`

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