Package 'ic50'

February 20, 2015

Title Standardized high-throughput evaluation of cell-based compound
screens

Version 1.4.2

Date 2010-02-15

Depends tcltk

Author Peter Frommolt

Maintainer Peter Frommolt <peter.frommolt@uni-koeln.de>

Description Calculation of IC50 values, automatic drawing of
dose-response curves and validation of compound screens on 96-
and 384-well plates.

License GPL

URL http://portal.ccg.uni-koeln.de/

Repository CRAN

Date/Publication 2010-02-16 09:36:47

NeedsCompilation no

R topics documented:

| design | *Configuration files specifying the design of a compound screen on 84 NSCLC cell lines* |

Description

The experimental setup in a compound screen on 84 non-small cell lung cancer (NSCLC) cell lines was designed according to the arrangement specified in these data sets. However, the functions for screen evaluation actually expect this information as files on the local harddisk such as "mpi384_measure.txt", "mpi384_control.txt" and "mpi384_dilution.txt" which are installed together with the package. Default examples of these configuration files are included as "default96_measure.txt", "default384_measure.txt" etc.

In the "measure" file, there must be one row specified for each measurement series. This consists of the respective compound name followed by tab-delimited information on the wells where the measurements are located. Each of these must be given as a comma-delimited pair of coordinates. The same is expected for the control wells in the "control" file. Here, a particular well can be specified multiple times and will then be repeatedly used as a control well for the signal intensity without a compound being applied. If the normalize argument to the functions is specified as "single", there must be one control well for each single measurement; if on the other hand, "mean" is selected, an arbitrary number of wells can be specified and the mean of those values is used for normalization. Finally, the number of rows and the row names in the "dilution" file must equal those in the preceding two configuration files. Each row contains the compound name followed by a tab-delimited list of the concentrations used for the respective measurement series. It should by obvious that the number of concentrations in one row must equal the number of wells in the "measure" file for each row. However, the number of control wells can be distinct from these if the normalize argument is set to "mean" such that the mean of the respective control row is taken.

Importantly, the number of rows must be equal in all three files as well as the row names, where case-sensitivity and literal equality has to be carefully verified. The easiest way to create the configuration files is to simply start the GUI using ic50() which automatically creates a default version to be modified by the user. After having saved this configuration, it can be repeatedly used for screen evaluations as long as the experimental setup is not changed.

A step-by-step tutorial document describing how to prepare the data and configuration is included in the ic50 package.

References

Frommolt P, Thomas RK (2008): Standardized high-throughput evaluation of cell-based compound screens. BMC Bioinformatics, 9(1): 475

Sos ML, Michel K, Zander T, Frommolt P, Weiss J, et al. (2009): Predicting drug susceptibility in non-small cell lung cancers based on genetic lesions. J Clin Invest, 119(6): 1727-40

---

hts	*Standardized high-throughput evaluation of cell-based compound screens*

Description

Simultaneous evaluation of a large number of compound screens on 96- and 384-well plates.

Usage

```
ic50()
hts.96(indir=".",plates=2,measure=NULL,control=NULL,dilution=NULL,inhib=NULL,
        normalize="mean",graphics="mean",outdir="./results")
hts.384(indir=".",plates=2,measure=NULL,control=NULL,dilution=NULL,inhib=NULL,
         normalize="single",graphics="mean",outdir="./results")
```

Arguments

indir	A character specifying the directory which contains the raw data files.
plates	Number of plates used for each experiment.
measure	Configuration file for the locations of the measurement wells.
control	Configuration file for the locations of the control wells.
dilution	Configuration for the concentrations in each measurement.
inhib	Vector of real numbers between 0 and 1 specifying the percentage of inhibition to compute concentrations for. Defaults to 0.5 for all compounds.
normalize	Method to normalize the measurement by the controls. If "mean", the mean of the controls specified by control is used; "single" requires one individual control well per measurement well.
graphics	A character specifying the plotting method. For "mean", a dose-response curve of the mean values of the measurement series is given, whereas one curve is plotted for each if "single" is specified. For "fitted", a sigmoid-shaped derivation of the logistic model is fitted to the data.
outdir	The directory where the results will be written.

Details

In cytotoxicity screens of chemical compounds, biological activity is typically quantified by the concentration for which a particular fraction (typically 0.5) of cell growth is inhibited after a predefined treatment period. For this purpose, all concentrations are plotted against the percentages of cells still being alive under this treatment, forming a dose-response curve under which the preimage of the 0.5 point is defined as the half-maximum inhibitory concentration (IC50). For high-throughput screens (HTS), in particular, the evaluation of the data needs to be performed in an automatic fashion.

The hts.96 and hts.384 functions provide a powerful tool to simultaneously evaluate all data in the specified input directory indir. The data files are handled in groups of the size specified by plates

and the file names should be arranged in a way that two plates with replicates for the same measurements are displayed one below the other in a file browser. The data are expected to be arranged in tab-delimited text files which is the typical output of appropriate microplate readers. Just as for the evaluation of a single measurement, the design must be specified by tab-delimited files for measure, control and dilution. Details on these are given in the manual of the default384_measure and default384_control files. In addition, a tutorial document describing how to prepare the data and configuration is included in the ic50 package.

For each compound in the screen and each group of data files, a graphics output is given in the file "dose_response_curves.pdf" in the current workspace directory. In addition, the text file "ic50.txt" contains a tab-delimted table with the same evaluation as for the ic50.96 and ic50.384 functions but for all experiments one below the other.

ic50() starts a GUI-based version of the hts.96 and hts.384 functions. Preliminary change of the workspace directory to the folder containing the data will remarkably reduce the number of mouse clicks.

Please make use of the tutorial document in the doc folder which helps users to get started with the software.

Value

A data frame with the following columns:

first_file	Filename of the respective first input file.
compound	Compound names.
ic50	The inhibitory concentrations for the respective compounds.
clow	Lower 0.95 confidence limits for the IC values.
cup	Upper 0.95 confidence limits for the IC values.
maxsd	Maximum of the standard deviations at the measured concentrations as determined from the single replicates.
cv	Coefficient of variation of the IC values as determined from the single replicates.

Author(s)

Peter Frommolt, University of Cologne <peter.frommolt@uni-koeln.de>
http://portal.ccg.uni-koeln.de/

References

Frommolt P, Thomas RK (2008): Standardized high-throughput evaluation of cell-based compound screens. BMC Bioinformatics, 9(1): 475

Sos ML, Michel K, Zander T, Weiss J, Frommolt P, et al. (2009): Predicting drug susceptibility in non-small cell lung cancers based on genetic lesions. J Clin Invest, 119(6): 1727-40

Examples

```
#Example from a non-small cell lung cancer (NSCLC) cell line screen. In
#total, 84 samples were screened. The evaluation is exemplarily shown for
#the cell lines A549, Calu1, H322 and HCC2429.

data(A549_1,A549_2,Calu1_1,Calu1_2,H322_1,H322_2,HCC2429_1,HCC2429_2)
dir.create("NSCLC_screen")
write.table(A549_1,file="NSCLC_screen/A549_1.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(A549_2,file="NSCLC_screen/A549_2.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(Calu1_1,file="NSCLC_screen/Calu1_1.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(Calu1_2,file="NSCLC_screen/Calu1_2.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(H322_1,file="NSCLC_screen/H322_1.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(H322_2,file="NSCLC_screen/H322_2.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(HCC2429_1,file="NSCLC_screen/HCC2429_1.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(HCC2429_2,file="NSCLC_screen/HCC2429_2.txt",row.names=FALSE,col.names=FALSE,sep="\t")

data(mpi384_measure,mpi384_control,mpi384_dilution)
write.table(mpi384_measure,file="mpi384_measure.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(mpi384_control,file="mpi384_control.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(mpi384_dilution,file="mpi384_dilution.txt",row.names=FALSE,col.names=FALSE,sep="\t")

print(hts.384(indir="NSCLC_screen",
          measure="mpi384_measure.txt",control="mpi384_control.txt",dilution="mpi384_dilution.txt",
              inhib=rep(0.5,7),outdir="NSCLC_results",normalize="mean"))
```

| ic50 | *Standardized evaluation of cell-based compound screens* |

Description

Calculation of IC50 values, automatic drawing of dose-response curves and validation of compound screens on 96- and 384-well plates.

Usage

```
ic50.96(files,measure=NULL,control=NULL,dilution=NULL,inhib=NULL,
        normalize="mean",graphics="mean",outdir="./results")
ic50.384(files,measure=NULL,control=NULL,dilution=NULL,inhib=NULL,
         normalize="single",graphics="mean",outdir="./results")
```

Arguments

files	Character vector of files containing the raw data.
measure	Configuration file for the locations of the measurement wells.
control	Configuration file for the locations of the control wells.
dilution	Configuration file for the concentrations in each measurement. See details below.

inhib   Vector of real numbers between 0 and 1 specifying the percentage of inhibition
                 to compute concentrations for. Defaults to 0.5 for all compounds.

normalize   Method to normalize the measurement by the controls. For "mean", the mean
                 of the controls specified by control is used; "single" requires one individual
                 control well per measurement well.

graphics   A character specifying the plotting method. For "mean", a dose-response curve
                 of the mean values of the measurement series is given, whereas one curve is plot-
                 ted for each if "single" is specified. For "fitted", a sigmoid-shaped deriva-
                 tion of the logistic model is fitted to the data.

outdir   The directory where the results will be written.

Details

In cytotoxicity screens of chemical compounds, biological activity is typically indicated by the
concentration for which a particular proportion (typically 0.5) of cell growth is inhibited after a
predefined treatment period. For this purpose, all concentrations are plotted against the percent-
ages of cells still being alive under this treatment, forming a dose-response curve under which the
preimage of the 0.5 point is defined as the half-maximum inhibitory concentration (IC50). For
high-throughput screens (HTS), in particular, the evaluation of the data needs to be performed in an
automatic fashion.

The data input for the script is performed by tab-delimited data files which are the typical out-
put from appropriate microplate readers. A character vector of file names is therefore expected as
the first argument to the functions. If 96- or 384-well plates are used for the screen, the arrange-
ment of the wells is in principle arbitrary. The design must be specified by three tab-delimited
files with one for the coordinates of the measurement wells, one for the control wells and one for
the concentrations of the respective compound. Several examples of each of these files are given
in the inst folder, e.g. the files "default384_measure.txt", "default384_control.txt" and
"default384_dilution.txt". Details on the arrangement of these files are given in the docu-
mentation of the corresponding data sets, e.g. for default384_measure. In addition, a tutorial
document describing how to prepare the data and configuration is included in the ic50 package.

For each compound in the screen, a graphics output is given in the file "dose_response_curves.pdf"
in the output directory, where the screen data are displayed as specified by the argument graphics.
In addition, quantitative results are written to a file "ic50.txt" in the same directory. Inhibitory
concentrations are calculated for each of the curves and are given together with the respective confi-
dence intervals. The measurement accuracy is evaluated by the maximum of the standard deviations
at the respective concentrations and by the coefficient of variation of the concentration values as de-
termined from the single replicates. Finally, the normalized data rows detected from the plates in
use are written to the file "measurement.txt", combined in one group for each compound.

Please make use of the tutorial document in the doc folder which helps users to get started with the
software.

Value

A data frame with the following variables:

compound   Compound names.

ic50   The inhibitory concentrations for the respective compounds.

clow	Lower 0.95 confidence limits for the IC values.
cup	Upper 0.95 confidence limits for the IC values.
maxsd	Maximum of the standard deviations at the measured concentrations as determined from the single replicates.
cv	Coefficient of variation of the IC values as determined from the single replicates.

Note

The nonlinear regression for the sigmoidal-shaped curve is **not** performed by the least-squares method. Instead, the parameters are adapted to the data by assumptions on the shape of an "ideal" curve such as location and bending.

Author(s)

Peter Frommolt, University of Cologne <peter.frommolt@uni-koeln.de>
<http://portal.ccg.uni-koeln.de/>

References

Frommolt P, Thomas RK (2008): Standardized high-throughput evaluation of cell-based compound screens. BMC Bioinformatics, 9(1): 475

Sos ML, Michel K, Zander T, Weiss J, Frommolt P, et al. (2009): Predicting drug susceptibility in non-small cell lung cancers based on genetic lesions. J Clin Invest, 119(6): 1727-40

Examples

```
#Example from a cell line screen (2007). IC50 values are determined for
#the lung cancer cell line HCC2429 and 7 selected compounds.

data(HCC2429_1,HCC2429_2)
write.table(HCC2429_1,file="HCC2429_1.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(HCC2429_2,file="HCC2429_2.txt",row.names=FALSE,col.names=FALSE,sep="\t")

data(mpi384_measure,mpi384_control,mpi384_dilution)
write.table(mpi384_measure,file="mpi384_measure.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(mpi384_control,file="mpi384_control.txt",row.names=FALSE,col.names=FALSE,sep="\t")
write.table(mpi384_dilution,file="mpi384_dilution.txt",row.names=FALSE,col.names=FALSE,sep="\t")

print(ic50.384(files=c("HCC2429_1.txt","HCC2429_2.txt"),
          measure="mpi384_measure.txt",control="mpi384_control.txt",dilution="mpi384_dilution.txt",
              inhib=rep(0.5,7),outdir="./HCC2429_results",normalize="mean"))
```

---

nsclc   *Results from a compound screen on 84 NSCLC cell lines.*

Description

For purpose of computational, lesion-based prediction of compound activity in 84 non-small cell lung cancer (NSCLC) cell lines, these were treated with a selection of compounds to measure the respective concentration where 50 per cent of cell growth was inhibited after a predefined time period. Most of the screening experiments were performed on 384 (16 x 24)-well plates, were the experimental setup was designed according to the arrangement in the files `"mpi384_measure.txt"`, `"mpi384_control.txt"` and `"mpi384_dilution.txt"` that are installed together with the package.

These data sets are the direct output from a signal reader of the 384-well plates for the A549, Calu1, H322 and H2429 cancer cell lines selected from the 84 NSCLC cell lines collection. A tab-delimited version is installed together with the package.

References

Frommolt P, Thomas RK (2008): Standardized high-throughput evaluation of cell-based compound screens. BMC Bioinformatics, 9(1): 475

Sos ML, Michel K, Zander T, Weiss J, Frommolt P, et al. (2009): Predicting drug susceptibility in non-small cell lung cancers based on genetic lesions. J Clin Invest, 119(6): 1727-40

Index