Package ‘highSCREEN’

October 13, 2022

Type Package
Title High-Throughput Screening for Plate Based Essays
Version 0.4
Description Can be used to carry out extraction, normalization, quality control (QC), candidate hits identification and visualization for plate based assays, in drug discovery. The package methods were applied in H. W. Choi et al. “Identification of Novel Mast Cell Activators Using Cell-Based High-Throughput Screening”, SLAS Discovery 24(6), 2019. This project was funded by the Division of Allergy, Immunology, and Transplantation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract No. HHSN272201400054C entitled “Adjuvant Discovery For Vaccines Against West Nile Virus and Influenza”, awarded to Duke University and lead by Drs. Herman Staats and Soman Abraham.
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Suggests knitr
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Description

This package can be used to carry out extraction, normalization, quality control (QC), candidate hits identification and visualization for plate based essays, in drug discovery.

Details

The DESCRIPTION file:

```
Package: highSCREEN
Type: Package
Title: High-Throughput Screening for Plate Based Essays
Version: 0.4
Authors@R: c(person(c("Ivo", "D."), "Shterev", role = c("aut", "cre"), email = "i.shterev@gmail.com"), person("Cliburn", "Chan", role = "aut"), person(c("Gregory", "D."), "Sempowski", role = "aut"))
Description: Can be used to carry out extraction, normalization, quality control (QC), candidate hits identification and visualization for plate based essays, in drug discovery.
Depends: R (>= 3.4.0), gplots (>= 3.0.1)
VignetteBuilder: knitr
Suggests: knitr
License: GPL-3
LazyLoad: yes
Author: Ivo D. Shterev [aut, cre], Cliburn Chan [aut], Gregory D. Sempowski [aut]
Maintainer: Ivo D. Shterev <i.shterev@gmail.com>
```

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- `formatRESULT` Format results
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- `hits` Identify hits
- `normplate` Normalize plate
- `plotcont` Plot control density
- `plotplate` Plot plate activity
- `qcplate` Quality control
rankhits Rank hits
zfactor.ssmd Compute Z-factor and SSMD

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Description
description

Usage
extractplate(dat0, dat1, plate, replicate)

Arguments

dat0 A list consisting of the following elements for the t0-specific data set: Replicate1: Data frame containing the first triplicate and 4 additional columns specifying the controls and their plate locations. Replicate2: Data frame containing the second triplicate and 4 additional columns specifying the controls and their plate locations. Replicate3: Data frame containing the third triplicate and 4 additional columns specifying the controls and their plate locations.

dat1 A list consisting of the following elements for the t1-specific data set: Replicate1: Data frame containing the first triplicate and 4 additional columns specifying the controls and their plate locations. Replicate2: Data frame containing the second triplicate and 4 additional columns specifying the controls and their plate locations. Replicate3: Data frame containing the third triplicate and 4 additional columns specifying the controls and their plate locations.

plate Specifies which of the 4 plates to be extracted.

replicate Specifies which replicate to be extracted.

Value
This function returns a list consisting of the following elements:

dat0 Data frame containing the t0-specific plate of compounds and controls.

dat1 Data frame containing the t1-specific plate of compounds and controls.
Examples

```r
set.seed(1234)
nc = 24
nr = 16

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicate to create t0-specific data set
replicates_t0 = list(r1, r2, r3)
names(replicates_t0) = c("R1", "R2", "R3")

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicate to create t1-specific data set
replicates_t1 = list(r1, r2, r3)
names(replicates_t1) = c("R1", "R2", "R3")

# extract plate 3, replicate 2
extractplate(replicates_t0, replicates_t1, plate=3, replicate=2)
```

---

**formatRESULT**

**Format results**

**Description**

description

**Usage**

`formatRESULT(dat, replicate="Replicate", score="S", t="Time")`

**Arguments**

- **dat**
  - Data frame such as the return data frame of the function `normplate()`.

- **replicate**
  - Specifies the column name of the input data frame that contains the replicate numbers.
score  Specifies the column name of the input data frame that contains the normalized score values.

t  Specifies the column name of the input data frame that contains the time designations (0 or 1).

**Value**

This function returns a data frame with each row corresponding to a compound/control and the following columns:

- **ID**: Has the complete information to identify a compound or control. It contains information about the main plate, the quadrant/plate and the well.
- **MainPlate**: Specifies the main plate to which the compound/control belongs.
- **Plate**: Specifies the quadrant/plate to which the compound/control belongs.
- **Norm**: Specifies the normalization method that was applied for the specific compound.
- **well**: Specifies the location (row and column) of the compound/control in the quadrant.
- **row**: Specifies the row location in the quadrant.
- **col**: Specifies the column location in the quadrant.
- **welltype**: Specifies if the well is compound or control.
- **S0**: Replicates of the score from the t0-specific data set.
- **S1**: Replicates of the score from the t1-specific data set.

**Examples**

```r
cat("set.seed(1234)
nc = 24
nr = 16

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create control map
cmap = data.frame(X1=c(rep("Control P", floor(nr/3)), rep(c("Control low", "Control med", "Control high"), (floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control N", floor(nr/3))),
                   X2=c(rep("Control N", floor(nr/3)), rep(c("Control low", "Control med", "Control high"), (floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control P", floor(nr/3))))
cmap = cmap[seq(1,nr,2),]

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicates for the t0-specific data
```

replicates_t0 = list(r1, r2, r3)
names(replicates_t0) = c("R1", "R2", "R3")

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicates for the t1-specific data
replicates_t1 = list(r1, r2, r3)
names(replicates_t1) = c("R1", "R2", "R3")

# extract plate 1, all replicates
dat1 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=1)
dat2 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=2)
dat3 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=3)

# normalize data of all replicates
res1 = normplate("Main Plate 1", dat1[["dat0"]], dat1[["dat1"]], cmap, plate=1, replicate=1, norm="zscore")
res2 = normplate("Main Plate 1", dat2[["dat0"]], dat2[["dat1"]], cmap, plate=1, replicate=2, norm="zscore")
res3 = normplate("Main Plate 1", dat3[["dat0"]], dat3[["dat1"]], cmap, plate=1, replicate=3, norm="zscore")

# reformat data of all replicates
head(formatRESULT(rbind(res1, res2, res3), replicate="Replicate", t="Time"))

## hits

### Identify hits

#### Description

description

#### Usage

```r
hits(dat.raw, dat.norm, s0="S0", s1="S1", 
qc.mainplates, qc1.val=0.225, hit.val=3)
```

#### Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>dat.raw</td>
<td>Data frame containing raw data as an output from <code>formatRESULT()</code>.</td>
</tr>
<tr>
<td>dat.norm</td>
<td>Data frame containing normalized data set as an output from <code>formatRESULT()</code>.</td>
</tr>
<tr>
<td>s0</td>
<td>Specifies the name of the columns containing t0-specific scores.</td>
</tr>
</tbody>
</table>
**Value**

The function returns a data frame. Each row corresponds to a compound that passed QC1 and belongs to a plate that passed overall QC. The data frame contains the following columns:

- **ID**: Has the complete information to identify a compound or control. It contains information about the main plate, the quadrant/plate and the well.
- **MainPlate**: Specifies the main plate to which the compound/control belongs.
- **Plate**: Specifies the quadrant/plate to which the compound/control belongs.
- **Norm**: Specifies the normalization method that was applied for the specific compound.
- **well**: Specifies the location (row and column) of the compound/control in the quadrant.
- **row**: Specifies the row location in the quadrant.
- **col**: Specifies the column location in the quadrant.
- **welltype**: Specifies if the well is compound or control.
- **S0**: Replicates of the score from the t0-specific data set.
- **S1**: Replicates of the score from the t1-specific data set.
- **IND2**: Indicator variable specifying if the compound passes (TRUE) or fails (FALSE) QC2.
- **IND3**: Indicator variable specifying if the compound passes (TRUE) or fails (FALSE) QC3.

**Examples**

```r
set.seed(1234)
nc = 24
nr = 16

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)*0.01), nr, nc)

# create control map
cmap = data.frame(X1=c(rep("Control P", floor(nr/3)),
rep(c("Control low", "Control med", "Control high"),
(floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control N", floor(nr/3))),
X2=c(rep("Control N", floor(nr/3)),
rep(c("Control low", "Control med", "Control high"),
(floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control P", floor(nr/3))))
cmap = cmap[seq(1,nr,2),]
```
# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)*0.01), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)*0.01), nr, nc)

# combine all replicates for the t0-specific data
replicates_t0 = list(r1, r2, r3)
names(replicates_t0) = c("R1", "R2", "R3")

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicates for the t1-specific data
replicates_t1 = list(r1, r2, r3)
names(replicates_t1) = c("R1", "R2", "R3")

# extract plate 1, replicate 1
dat1 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=1)

# extract plate 1, replicate 2
dat2 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=2)

# extract plate 1, replicate 3
dat3 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=3)

# no normalization
datraw1 = normplate("Main Plate 1", dat1["dat0"], dat1["dat1"], cmap, plate=1, replicate=1, norm="raw")
datraw2 = normplate("Main Plate 1", dat2["dat0"], dat2["dat1"], cmap, plate=1, replicate=2, norm="raw")
datraw3 = normplate("Main Plate 1", dat3["dat0"], dat3["dat1"], cmap, plate=1, replicate=3, norm="raw")

# combine 3 replicate
datraw = rbind(datraw1, datraw2, datraw3)

# reformat result
datraw = formatRESULT(datraw, replicate="Replicate", t="Time")

# c-score normalization
datnorm1 = normplate("Main Plate 1", dat1["dat0"], dat1["dat1"], cmap, plate=1, replicate=1, norm="cscore", poscont="Control P", negcont="Control N")
datnorm2 = normplate("Main Plate 1", dat2["dat0"], dat2["dat1"], cmap, plate=1, replicate=2, norm="cscore", poscont="Control P", negcont="Control N")
datnorm = normplate("Main Plate 1", dat3[["dat0"]], dat3[["dat1"]], cmap, plate=1, replicate=3, norm="cscore", poscont="Control P", negcont="Control N")

# combine 3 replicates
datnorm = rbind(datnorm1, datnorm2, datnorm3)

# reformat result
datnorm = formatRESULT(datnorm, replicate="Replicate", t="Time")

# identify hits
head(hits(datraw, datnorm, qc.mainplates="Main Plate 1", qc1.val=0.225, hit.val=3))
Replicate | Specifies replicate.
--- | ---
Norm | Specifies the normalization method that was applied for the specific compound.
well | Specifies the location (row and column) of the compound/control in the quadrant.
row | Specifies the row location in the quadrant.
col | Specifies the column location in the quadrant.
score | Shows the score value.
welltype | Specifies if the well is compound or control.

**Examples**

```r
set.seed(1234)
nc = 24
nr = 16

# create control map
cmap = data.frame(X1=c(rep("Control P", floor(nr/3)), rep("Control med", "Control high"), (floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control N", floor(nr/3))), X2=c(rep("Control N", floor(nr/3)), rep("Control low", "Control med", "Control high"), (floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control P", floor(nr/3))))
cmap = cmap[seq(1,nr,2),]
cmap

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicate for the t0-specific data
replicates_t0 = list(r1, r2, r3)
names(replicates_t0) = c("R1", "R2", "R3")

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicate for the t1-specific data
replicates_t1 = list(r1, r2, r3)
names(replicates_t1) = c("R1", "R2", "R3")
```
# extract plate 1, replicate 1
dat = extractplate(replicates_t0, replicates_t1, plate=1, replicate=1)

# normalize using c-score
head(normplate("Main Plate 1", dat[['dat0']], dat[['dat1']], cmap,
     plate=1, replicate=1, norm="cscore",
     poscont="Control P", negcont="Control N")

# normalize using b-score (medpolish)
head(normplate("Main Plate 1", dat[['dat0']], dat[['dat1']], cmap,
     plate=1, replicate=1, norm="bscore")

# normalize using z-score
head(normplate("Main Plate 1", dat[['dat0']], dat[['dat1']], cmap,
     plate=1, replicate=1, norm="zscore")

---

plotcont

Plot control density

**Description**

description

**Usage**

plotcont(dat, score="S", main, xaxis.marks=seq(0,5,0.025))

**Arguments**

dat: Data frame as an output from `normplate()`.

score: Specifies the columns containing the data.

main: Main title for the plot.

xaxis.marks: Specifies x-axis mark ticks.

**Examples**

```
set.seed(1234)

nc = 24
nr = 16

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create control map
cmap = data.frame(X1=c(rep("Control P", floor(nr/3)),
            rep(c("Control low", "Control med", "Control high"),
            (floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control N",
            rep("Control N",
```

floor(nr/3)), X2=c(rep("Control N", floor(nr/3)),
rep(c("Control low", "Control med", "Control high"),
(floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control P", floor(nr/3))))
cmap = cmap[seq(1,nr,2),]

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicates for the t0-specific data
replicates_t0 = list(r1, r2, r3)
names(replicates_t0) = c("R1", "R2", "R3")

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicates for the t1-specific data
replicates_t1 = list(r1, r2, r3)
names(replicates_t1) = c("R1", "R2", "R3")

# extract plate 1, replicate 1
dat11 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=1)

# extract plate 1, replicate 2
dat12 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=2)

# extract plate 1, replicate 3
dat13 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=3)

# no normalization (norm="raw")
res11 = normplate("Main Plate 1", dat11[,"dat0"], dat11[,"dat1"], cmap,
plate=1, replicate=1, norm="raw")
res12 = normplate("Main Plate 1", dat12[,"dat0"], dat12[,"dat1"], cmap,
plate=1, replicate=2, norm="raw")
res13 = normplate("Main Plate 1", dat13[,"dat0"], dat13[,"dat1"], cmap,
plate=1, replicate=3, norm="raw")

# combine 3 replicates
res1 = rbind(res11, res12, res13)

# reformat result
res1 = formatRESULT(res1, replicate="Replicate", t="Time")

layout(matrix(c(1,2,3), 3, 1, byrow = TRUE))

# plot density of all positive controls
plotcont(subset(res1, welltype=="Control P"), main="Density of Positive Controls",
xaxis.marks=seq(-1.5,0.025))
# plot density of all negative controls
plotcont(subset(res1, welltype=="Control N"), main="Density of Negative Controls",
xaxis.marks=seq(-1,5,0.025))

# plot density of controls with low, medium and high concentrations
plotcont(subset(res1, welltype=="Control low" | welltype=="Control med" | welltype=="Control high"), main="Density of Controls with Low, Medium and High Concentrations", xaxis.marks=seq(-1,5,0.025))

---

plotplate  

Plot plate activity

---

Description
description

Usage

plotplate(dat, score="S", main)

Arguments

dat  
Data frame as an output from normplate().
score  Specifies the columns containing the data.
main  Main title for the plot.

Examples

set.seed(1234)
nc = 24
nr = 16

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create control map
cmap = data.frame(X1=c(rep("Control P", floor(nr/3)),
rep(c("Control low", "Control med", "Control high"),
(floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control N",
floor(nr/3))), X2=c(rep("Control N", floor(nr/3)),
rep(c("Control low", "Control med", "Control high"),
(floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control P", floor(nr/3))))
cmap = cmap[seq(1,nr,2),]

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)
# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicates for the t0-specific data
replicates_t0 = list(r1, r2, r3)
names(replicates_t0) = c("R1", "R2", "R3")

# create 1st replicates of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicates for the t1-specific data
replicates_t1 = list(r1, r2, r3)
names(replicates_t1) = c("R1", "R2", "R3")

# extract plate 1, replicate 1
dat11 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=1)
# extract plate 1, replicate 2
dat12 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=2)
# extract plate 1, replicate 3
dat13 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=3)

# no normalization (norm="raw")
res11 = normplate("Main Plate 1", dat11["dat0"], dat11["dat1"], cmap, plate=1, replicate=1, norm="raw")
res12 = normplate("Main Plate 1", dat12["dat0"], dat12["dat1"], cmap, plate=1, replicate=2, norm="raw")
res13 = normplate("Main Plate 1", dat13["dat0"], dat13["dat1"], cmap, plate=1, replicate=3, norm="raw")

# combine 3 replicates
res1 = rbind(res11, res12, res13)
# reformat result
res1 = formatRESULT(res1, replicate="Replicate", t="Time")

# plot single plate activity levels
plotplate(res1, main="Single Plate Activity Levels")
Usage

cqplate(dat, s0="S0", s1="S1", poscont="Control P", negcont="Control N", qc1.val=0.225, qc2.val=2, addcont, welltype="welltype")

Arguments

dat
s0 Specifies the name of the columns containing t0-specific scores.
s1 Specifies the name of the columns containing t1-specific scores.
poscont Specifies positive control designation (used in C-score normalization).
.negcont Specifies negative control designation (used in C-score normalization).
qc1.val Pre-defined threshold value for QC1.
qc2.val Pre-defined threshold value for QC2.
addcont A vector character names specifying the additional controls for QC3. The vignette provides more details on the particular order of specifying the additional controls.
welltype Specifies the name for the column defining the well type.

Details
details

Value
value

References
references

Examples

set.seed(1234)
nc = 24
nr = 16

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create control map
cmap = data.frame(X1=c(rep("Control P", floor(nr/3)), rep(c("Control low", "Control med", "Control high"), (floor(nr/3)*nr-3*floor(nr/3))/3), rep("Control N", floor(nr/3))), X2=c(rep("Control N", floor(nr/3)), rep(c("Control low", "Control med", "Control high"), (floor(nr/3)*nr-3*floor(nr/3))/3), rep("Control P", floor(nr/3))))
cmap = cmap[seq(1,nr,2),]

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicates for the t0-specific data
replicates_t0 = list(r1, r2, r3)

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicates for the t1-specific data
replicates_t1 = list(r1, r2, r3)

names(replicates_t1) = c("R1", "R2", "R3")

# extract plate 1, replicate 1
dat11 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=1)

# extract plate 1, replicate 2
dat12 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=2)

# extract plate 1, replicate 3
dat13 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=3)

# no normalization (norm="raw")
res11 = normplate("Main Plate 1", dat11["dat0"], dat11["dat1"], cmap, plate=1, replicate=1, norm="raw")
res12 = normplate("Main Plate 1", dat12["dat0"], dat12["dat1"], cmap, plate=1, replicate=2, norm="raw")
res13 = normplate("Main Plate 1", dat13["dat0"], dat13["dat1"], cmap, plate=1, replicate=3, norm="raw")

# combine 3 replicates
res1 = rbind(res11, res12, res13)

# reformat result
res1 = formatRESULT(res1, replicate="Replicate", t="Time")

# perform QC
qcplate(res1, poscont="Control P", negcont="Control N", qc1.val=0.225, qc2.val=2, addcont=c("Control low", "Control med", "Control high" ), welltype="welltype")
**Description**

This function ranks the candidate compound hits based on the mean of t1-specific scores.

**Usage**

\[
\text{rankhits}(\text{dat}, \text{score.before}="S0", \text{score.after}="S1", \text{var}="m1")
\]

**Arguments**

- **dat**: Data frame as the output of the function `formatRESULT`.
- **score.before**: Specifies the name of the columns containing t0-specific scores.
- **score.after**: Specifies the name of the columns containing t1-specific scores.
- **var**: Specifies the variable according to which the ranking is done.

**Value**

Data frame with a row for each hit. The columns are as follows:

- **ID**: Has the complete information to identify a compound or control. It contains information about the main plate, the quadrant/plate and the well.
- **MainPlate**: Specifies the main plate to which the compound/control belongs.
- **Plate**: Specifies the quadrant/plate to which the compound/control belongs.
- **Norm**: Specifies the normalization method that was applied for the specific compound.
- **well**: Specifies the location (row and column) of the compound/control in the quadrant.
- **row**: Specifies the row location in the quadrant.
- **col**: Specifies the column location in the quadrant.
- **welltype**: Specifies if the well is compound or control.
- **S0**: Replicates of the score from the t0-specific data set.
- **S1**: Replicates of the score from the t1-specific data set.
- **IND2**: Indicator variable specifying if the compound passes (TRUE) or fails (FALSE) QC2.
- **IND3**: Indicator variable specifying if the compound passes (TRUE) or fails (FALSE) QC3.
- **diff**: Difference between mean of t1 and t0 replicates.
- **m0**: Mean of t0-specific replicates (MB).
- **s0**: Standard deviation of t0-specific replicates (SB).
- **rs0**: Coefficient of variation of t0-specific replicates. It is the ratio of S0 and M0.
- **m1**: Mean of t1-specific replicates (MA).
- **s1**: Standard deviation of t1-specific replicates (SA)
- **rs1**: Coefficient of variation of t1-specific replicates. It is the ratio of S1 and M1.
- **ind_below**
  - **ind ABOVE**: see \text{ind} below
- **ind**: \text{TRUE} (if both \text{ind}_{\text{below}} and \text{ind}_{\text{above}} are \text{TRUE}) if RS1 is within the \(1.5 \times IQR\), where interquartile range (IQR) is computed based on all hits.
Examples

```r
set.seed(1234)
nc = 24
nr = 16

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)*0.01), nr, nc)

# create control map
cmap = data.frame(X1=c(rep("Control P", floor(nr/3)), rep(c("Control low", "Control med", "Control high"), (floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control N", floor(nr/3))), X2=c(rep("Control N", floor(nr/3)), rep(c("Control low", "Control med", "Control high"), (floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control P", floor(nr/3))))

cmap = cmap[seq(1,nr,2),]

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)*0.01), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)*0.01), nr, nc)

# combine all replicates for the t0-specific data
replicates_t0 = list(r1, r2, r3)
names(replicates_t0) = c("R1", "R2", "R3")

# create 1st replicate of data matrix with compounds and controls
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 2nd replicate of data matrix with compounds and controls
r2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
r3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicates for the t1-specific data
replicates_t1 = list(r1, r2, r3)
names(replicates_t1) = c("R1", "R2", "R3")

# extract plate 1, replicate 1
dat1 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=1)
# extract plate 1, replicate 2
dat2 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=2)
# extract plate 1, replicate 3
dat3 = extractplate(replicates_t0, replicates_t1, plate=1, replicate=3)

# no normalizion
datraw1 = normplate("Main Plate 1", dat1[,1,"dat0"], dat1[,1,"dat1"], cmap, plate=1, replicate=1, norm="raw")
datraw2 = normplate("Main Plate 1", dat2[,1,"dat0"], dat2[,1,"dat1"], cmap, plate=1, replicate=2, norm="raw")
```
```r

datraw3 = normplate("Main Plte 1", dat3[["dat0"]], dat3[["dat1"]], cmap, plate=1, replicate=3, norm="raw")

# combine 3 replicates
datraw = rbind(datraw1, datraw2, datraw3)

# reformat result
datraw = formatRESULT(datraw, replicate="Replicate", t="Time")

# c-score normalization
datnorm1 = normplate("Main Plate 1", dat1[["dat0"]], dat1[["dat1"]], cmap, plate=1, replicate=1, norm="cscore", poscont="Control P", negcont="Control N")
datnorm2 = normplate("Main Plate 1", dat2[["dat0"]], dat2[["dat1"]], cmap, plate=1, replicate=2, norm="cscore", poscont="Control P", negcont="Control N")
datnorm3 = normplate("Main Plte 1", dat3[["dat0"]], dat3[["dat1"]], cmap, plate=1, replicate=3, norm="cscore", poscont="Control P", negcont="Control N")

# combine 3 replicates
datnorm = rbind(datnorm1, datnorm2, datnorm3)

# reformat result
datnorm = formatRESULT(datnorm, replicate="Replicate", t="Time")

# identify hits
h = hits(datraw, datnorm, qc.mainplates="Main Plate 1", qc1.val=0.225, hit.val=3)

# rank hits in disending order of mean of t1-specific replicate scores "m1"
head(rankhits(h))
```

---

**zfactor.ssmrd**

*Compute Z-factor and SSMD*

**Description**

This function computes the Z-factor and strictly standardized mean difference (SSMD) of a given 96-well plate.

**Usage**

```
zfactor.ssmrd(dat, pos.cont, neg.cont, MainPlate, replicate)
```

**Arguments**

- **dat**
  - Data frame as an output of the function `formatRESULT()`.
- **pos.cont**
  - Designation of positive control.
- **neg.cont**
  - Designation of negative control.
MainPlate  Specifies main plate.
replicate  Specifies the replicate.

Value

Returns a data frame with one row and the following columns:
MainPlate  Specifies the main plate.
replicate  Specifies the replicate.
ZFactor_Before  Specifies the Z-factor computed based on the t0-specific data.
ZFactor_After  Specifies the Z-factor computed based on the t1-specific data.
SSMD_Before  Specifies SSMD computed based on the t0-specific data.
SSMD_After  Specifies SSMD computed based on the t1-specific data.

Examples

set.seed(1234)
nc = 24
nr = 16

# create 1st replicate of data matrix with compounds and controls
replicate1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create control map
cmap = data.frame(X1=c(rep("Control P", floor(nr/3)),
rep(c("Control low", "Control med", "Control high"),
(floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control N",
floor(nr/3))), X2=c(rep("Control N", floor(nr/3)),
rep(c("Control low", "Control med", "Control high"),
(floor(nr/3)+nr-3*floor(nr/3))/3), rep("Control P", floor(nr/3)))))
cmap = cmap[seq(1,nr,2),]

# create 2nd replicate of data matrix with compounds and controls
replicate2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
replicate3 = matrix(abs(rnorm(nr*nc)), nr, nc)

# combine all replicates for the t0-specific data
replicates_before = list(replicate1, replicate2, replicate3)
names(replicates_before) = c("Replicate1", "Replicate2", "Replicate3")

# create 1st replicate of data matrix with compounds and controls
replicate1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 2nd replicate of data matrix with compounds and controls
replicate2 = matrix(abs(rnorm(nr*nc)), nr, nc)

# create 3rd replicate of data matrix with compounds and controls
replicate3 = matrix(abs(rnorm(nr*nc)), nr, nc)
# combine all replicates for the t1-specific data
replicates_after = list(replicates1, replicates2, replicates3)

names(replicates_after) = c("Replicate1", "Replicate2", "Replicate3")

# extract plate 1, replicate 1
dat1 = extractplate(replicates_before, replicates_after, plate=1, replicate=1)
# extract plate 1, replicate 2
dat2 = extractplate(replicates_before, replicates_after, plate=1, replicate=2)
# extract plate 1, replicate 3
dat3 = extractplate(replicates_before, replicates_after, plate=1, replicate=3)

# no normalization

datraw1 = normplate("Main Plate 1", dat1[["dat0"]], dat1[["dat1"]], cmap,
                    plate=1, replicate=1, norm="raw")
datraw2 = normplate("Main Plate 1", dat2[["dat0"]], dat2[["dat1"]], cmap,
                    plate=1, replicate=2, norm="raw")
datraw3 = normplate("Main Plate 1", dat3[["dat0"]], dat3[["dat1"]], cmap,
                    plate=1, replicate=3, norm="raw")

# combine 3 replicates
datraw = rbind(datraw1, datraw2, datraw3)

# reformat result
datraw = formatRESULT(datraw, replicate="Replicate", t="Time")

# compute z-factor and ssmd for each raw compound, replicate 1
zfactor.ssmd(datraw, "Control P", "Control N", "Main Plate 1", 1)
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