Package ‘highSCREEN’

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Title High-Throughput Screening for Plate Based Assays
Version 0.3
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Suggests knitr
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Author Ivo D. Shterev [aut, cre], Cliburn Chan [aut], Gregory D. Sempowski [aut]
Maintainer Ivo D. Shterev <i.shterev@duke.edu>
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This package can be used to carry out extraction, normalization, quality control (QC), candidate hits identification and visualization for plate based assays, in drug discovery.

Details

The DESCRIPTION file:

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Type: Package
Title: High-Throughput Screening for Plate Based Assays
Version: 0.3
Authors@R: c(person(c("Ivo", "D."), "Shterev", role = c("aut", "cre"), email = "i.shterev@duke.edu"), person("Cliburn", "Chan", role = "aut"), person(c("Gregory", "D."), "Sempowski", role = "aut"))
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Author: Ivo D. Shterev [aut, cre], Cliburn Chan [aut], Gregory D. Sempowski [aut]
Maintainer: Ivo D. Shterev <i.shterev@duke.edu>

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**Author(s)**

I. D. Shterev, C. Chan and G. D. Sempowski

Maintainer: I. D. Shterev <i.shterev@duke.edu>

---

**extractplate**  
*Extract plate*

---

**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**

```r
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
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")

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**

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>description</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>hits(dat.raw, dat.norm, s0=&quot;S0&quot;, s1=&quot;S1&quot;, qc.mainplates, qc1.val=0.225, hit.val=3)</td>
</tr>
</tbody>
</table>

### Arguments

| dat.raw | Data frame containing raw data as an output from `formatRESULT()`.
| dat.norm | Data frame containing normalized data set as an output from `formatRESULT()`.
| s0 | Specifies the name of the columns containing t0-specific scores. |
Specifies the name of the columns containing t1-specific scores.

qc.mainplates A vector containing names of main plates that passed QC.

qc1.val Threshold value for QC1.

hit.val Threshold value for identifying candidate hits, based on the mean of t1-specific scores.

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")


```r
datnorm3 = 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))
```

**normplate**

*Normalize plate*

---

**Description**

Description

**Usage**

normplate(mainplate, dat0, dat1, cmap, plate, replicate, norm="bscore",
  poscont=NULL, negcont=NULL)

**Arguments**

- **mainplate**: Main Plate designation.
- **dat0**: Data frame containing the 96-well t0-specific plate/quadrant to be normalized.
- **dat1**: Data frame containing the 96-well t1-specific plate/quadrant to be normalized.
- **cmap**: 96-well plate control map.
- **plate**: Plate/quadrant designation. Ranges from 1 to 4.
- **replicate**: Replicate designation.
- **norm**: Normalization method to be applied. Currently implemented methods include C-score (*cscore*), B-score (*bscore*), Z-score (*eqnzscore*) and no normalization (*raw*).
- **poscont**: Specifies positive control designation (used in C-score normalization).
- **negcont**: Specifies negative control designation (used in C-score normalization).

**Value**

Data frame containing the following columns:

- **MainPlate**: Specifies the main plate to which the compound/control belongs.
- **Time**: Specifies t0 (0) or t1 (1) specific data.
- **Plate**: Specifies the quadrant/plate to which the compound/control belongs.
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

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

# 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),]
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**

```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 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",
```
plotcont

```r
g = 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.markers=seq(-1,5,0.025))
```
# plot density of all negative controls
plotcont(subset(resl, 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(resl, 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**

```r
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**

```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 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

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

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

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

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

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

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

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

# extract plate Q, replicate 1
datQ1 = extractplate(replicates_t0, replicates_t1, plate="Q", replicate=1)

datQ2 = extractplate(replicates_t0, replicates_t1, plate="Q", replicate=2)

datQ3 = extractplate(replicates_t0, replicates_t1, plate="Q", replicate=3)

# no normalization (norm="raw")
resQ1 = normplate("Main Plate 1", datQ1[["dat0"]], datQ1[["dat1"]], cmap,
    plate=1, replicate=1, norm="raw")
resQ2 = normplate("Main Plate 1", datQ2[["dat0"]], datQ2[["dat1"]], cmap,
    plate=2, replicate=1, norm="raw")
resQ3 = normplate("Main Plate 1", datQ3[["dat0"]], datQ3[["dat1"]], cmap,
    plate=3, replicate=1, norm="raw")

# combine 3 replicates
resQ = rbind(resQ1, resQ2, resQ3)

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

# perform QC
qcplate(resQ, 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**

```
rankhits(dat, score.before="S0", score.after="S1", 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**: see `ind` below
- **ind_above**: see `ind` below
- **ind**: `TRUE` (if both `ind_below` and `ind_above` are `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[["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")
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.ssm(d, pos.cont, neg.cont, MainPlate, replicate)

Arguments
- d: 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(X=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)
```r
# combine all replicates for the t1-specific data
c1 <- c("Replicate1", "Replicate2", "Replicate3")
replicates_after = list(replicate1, replicate2, replicate3)
names(replicates_after) = c1

# 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 Pltae 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.ssmcdatraw("Control P", "Control N", "Main Plate 1", 1)
```
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