highSCREEN: High-Throughput Screening for Plate Based Assays

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1 Introduction

This vignette describes the use of the R extension package highSCREEN for high throughput screening of small molecule compounds with activities measured on multi-well plates. The plate-based assay raw results can be any continuous value - for example, optical density (OD; in nanometers). Package functionalities include small molecule compound library screening data extraction and normalization, plate quality control (QC), identifying compounds that are hits according to defined criteria and visualization of compounds and controls. The framework supports 96-well and 384-well plate formats[1]. The package is also capable of handling any number of replicates of the data. Currently, highSCREEN implements three different within plate quality control (QC) procedures which determine plate pass or fail. The package implements three different normalization methods, namely the b-score, the c-score (also known as percent degranulation) and the z-score normalization methods [1]. The user can also plot the density and histogram of controls which can be helpful in tuning the QC procedures.

2 Data Format

2.1 Plate Layout

The following plate formats are supported:

- 96-well plate. This format represents an 8×12 matrix in which the first and last columns represent control wells and columns two to ten represent compound wells.
- 384-well plate. This format represents an 16×24 matrix. The first and last two columns represent control wells and columns three to twenty two represent compound wells.
- 384-well plate composed of four 96-well plates. This format represents an 16×24 matrix. The first and last two columns represent control wells and columns three to twenty two represent compound wells. From this 384-well plate, four 96-well plates can be constructed as shown in Fig. 1.

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[1]See section 2 for additional details on supported plate layouts.

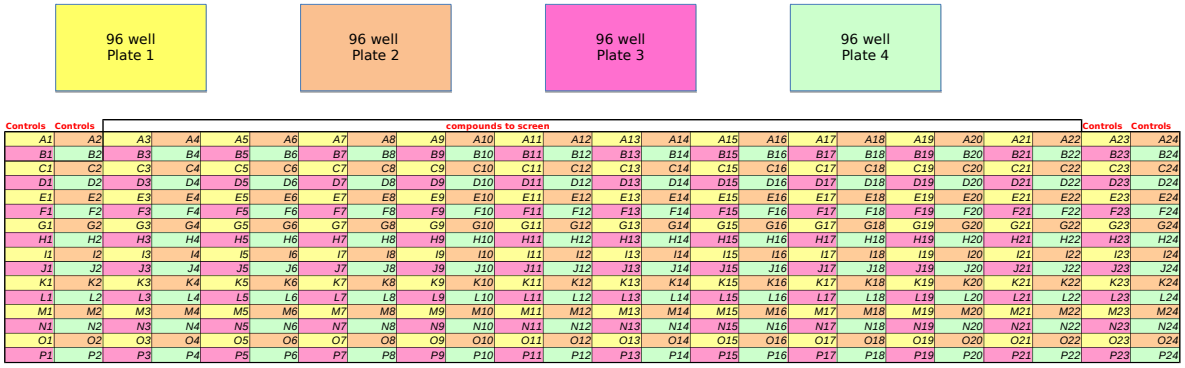


Figure 1: 384-well plate consisting of four 96-well plates.

2.2 Distribution of Controls

As mentioned in the previous subsection, it is assumed that the control wells are located either in the first and last plate columns (96-well plate), or in the first and last two plate columns (348-well plate) as shown in Fig. 1. The R package `highSCREEN` assumes that the plate contains positive and negative control wells. The package can also handle additional control types as specified by the user. The requirement is that in addition to the 384-well plate data the user also provides a control map. The map specifies the control type and its position in the control columns, and is used to identify the controls in the plate layout. There are two types of control maps, 96-well and 384-well plate control maps. The first column of the 384-well plate control map specifies the controls and their position in the first column of the 384-well plate. Similarly, the second, third and fourth control map columns correspond to the second, twenty third and twenty fourth 384-well plate columns, respectively. Similarly, the first and second columns of the 96-well plate control map specify the controls and their position in the first and second columns of the 96-well plate, respectively. In the example below, a 384-well plate contains five different types of controls, positive controls ("Control P"), negative controls ("Control N"), controls with low concentration ("Control low"), controls with medium concentration ("Control med"), and controls with high concentration ("Control high").

```
set.seed(1234)
library(highSCREEN)


## Loading required package:  gplots
##
## Attaching package:  'gplots'
## The following object is masked from 'package:stats':
##
##     lowess

nc = 24
nr = 16
# create a 384-well plate with compounds and controls
replicate = matrix(abs(rnorm(nr*nc)), nr, nc)
head(replicate)


##           [,1]      [,2]      [,3]      [,4]        [,5]      [,6]      [,7]
## [1,] 1.2070657 0.5110095 0.7094400 0.5238281 0.007604756 0.1777900 1.1346080
## [2,] 0.2774292 0.9111954 0.5012581 0.4968500 1.777084448 0.1699941 0.8782036
## [3,] 1.0844412 0.8371717 1.6290935 1.8060313 1.138607737 1.3723019 0.9729168
## [4,] 2.3456977 2.4158352 1.1676193 0.5820759 1.367827179 0.1737872 2.1211171
## [5,] 0.4291247 0.1340882 2.1800396 1.1088896 1.329564791 0.8502323 0.4145235
## [6,] 0.5060559 0.4906859 1.3409932 1.0149620 0.336472797 0.6976087 0.4747185
```

```
##            [,8]      [,9]     [,10]     [,11]      [,12]     [,13]      [,14]
## [1,] 1.10976723 0.6360998 0.5137628 0.8473501 1.12376279 0.7896469 0.03266396
## [2,] 0.84927420 0.2263015 0.3992718 0.2606394 3.04376589 0.4878146 1.11444896
## [3,] 0.02236253 1.0136903 1.6628564 0.4144197 0.23502131 2.1680325 0.41805782
## [4,] 0.83114062 0.2527501 0.2758934 0.1830508 0.03325861 0.5006946 0.40023524
## [5,] 1.24428785 1.1719483 0.5062726 0.4070561 2.73221952 0.6202102 1.49349310
## [6,] 0.16902641 0.6687143 0.3475520 0.6246331 0.09979059 0.9659032 1.60708094
##          [,15]      [,16]     [,17]     [,18]     [,19]      [,20]      [,21]
## [1,] 0.2877097 0.05913517 1.4769696 0.6705594 1.5528590 0.02362661 0.14313216
## [2,] 0.6597701 0.41339889 1.2239038 0.9486326 0.1284340 0.64902822 0.02418865
## [3,] 2.9191401 1.09777217 0.2580684 2.0494030 0.9854434 0.50437422 0.50445152
## [4,] 0.6774155 0.71117526 0.4050028 0.6511136 0.1832475 1.61439150 1.58139681
## [5,] 0.6843203 0.71888873 0.9758033 0.8086193 1.7662292 0.44695981 0.03006642
## [6,] 0.1864921 0.25165107 0.3488767 0.9865806 0.6205337 0.76317676 0.71657670
##           [,22]     [,23]      [,24]
## [1,] 0.27007961 0.7837751 0.04631853
## [2,] 1.61978988 0.2260540 2.25184180
## [3,] 0.21413117 1.5871030 0.60803373
## [4,] 0.81778246 0.5475242 1.50928817
## [5,] 0.05402292 1.8912270 0.23263177
## [6,] 0.33014161 0.8780771 0.03964870
```

```
# create 384-well plate 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 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))), X3=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))), X4=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
```

```
##              X1           X2           X3           X4
## 1     Control P    Control P    Control N    Control N
## 2     Control P    Control P    Control N    Control N
## 3     Control P    Control P    Control N    Control N
## 4     Control P    Control P    Control N    Control N
## 5     Control P    Control P    Control N    Control N
## 6   Control low  Control low  Control low  Control low
## 7   Control med  Control med  Control med  Control med
## 8  Control high Control high Control high Control high
## 9   Control low  Control low  Control low  Control low
## 10  Control med  Control med  Control med  Control med
## 11 Control high Control high Control high Control high
## 12    Control N    Control N    Control P    Control P
## 13    Control N    Control N    Control P    Control P
## 14    Control N    Control N    Control P    Control P
## 15    Control N    Control N    Control P    Control P
## 16    Control N    Control N    Control P    Control P
```

```
# create 96-well plate 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
```

```
##              X1           X2
## 1     Control P    Control N
## 3     Control P    Control N
## 5     Control P    Control N
## 7   Control med  Control med
## 9   Control low  Control low
## 11 Control high Control high
## 13    Control N    Control P
## 15    Control N    Control P
```

2.3 Distribution of Compounds

Some normalization methods implemented in `highSCREEN` assume that the compounds are distributed randomly in the plate. If there are different concentrations of the same compounds in the plate, some of the implemented normalization methods may not be biologically valid[2].

[2]See Section 4 for more details regarding within plate normalization methods and their applicability.

3 Assay and Activity Measurement

The package can handle single readings as well as multiple readings/replicates of 384-well plates. In the following example it is assumed that the OD data are collected at two different time instances $\mathbf{t_0}$ and $\mathbf{t_1}$, respectively. Each time-specific data set consists of replicates. The user can specify which plate and replicate to extract from the input data sets. When extracting a specific plate and replicate, the output data are organized in the form of a list consisting of two elements. Elements `dat0` and `dat1` contain the plates (compounds and controls) of $\mathbf{t_0}$ and $\mathbf{t_1}$ specific data sets, respectively.

```
set.seed(1234)
library(highSCREEN)
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)


## $dat0
##           [,1]      [,2]      [,3]       [,4]        [,5]      [,6]      [,7]
## [1,] 1.2150536 0.6040689 2.0624810 1.20673873 1.038110146 1.1271329 2.1624690
## [2,] 0.3157640 0.1426293 1.4114046 0.41970989 0.871449472 0.1955291 1.0137177
## [3,] 0.2055698 0.3741440 0.4071761 3.19590120 0.494249837 0.6391582 0.4746346
## [4,] 3.3960635 0.1660620 0.6512008 0.84079580 1.309190860 1.1296779 0.1070204
## [5,] 1.1024646 0.1139629 1.2016658 1.67432651 0.143056066 0.6886715 0.6021049
## [6,] 0.7893944 0.1155534 1.7970624 1.42836844 0.001313548 0.5944834 1.1544213
## [7,] 0.5388331 0.7418648 0.8028380 1.75726622 0.848196597 2.8643468 0.3650888
## [8,] 0.9164891 1.9188941 0.6979752 0.04314039 0.227479443 0.6152848 0.7659322
##            [,8]       [,9]     [,10]      [,11]      [,12]
## [1,] 1.011654377 0.67308682 0.6112307 0.54721330 0.29972049
## [2,] 0.132361320 0.51439651 0.1904094 0.93329983 0.38118803
## [3,] 0.133177628 1.14801191 0.8268475 1.16388240 0.26767327
## [4,] 0.506401780 0.10293639 2.1653462 1.44963736 0.92749466
## [5,] 0.172793618 0.04123062 1.8519568 0.14006729 0.04230266
## [6,] 0.655928704 0.64400786 0.7476865 1.20643412 0.05275033
## [7,] 0.346083490 0.49084092 0.5915064 0.02509449 0.86320262
## [8,] 0.006265211 0.19069132 1.9185582 1.64235904 0.10369655
##
## $dat1
##            [,1]       [,2]       [,3]      [,4]      [,5]      [,6]      [,7]
## [1,] 0.01153115 0.05004926 1.22189865 2.1789535 1.6602134 0.5518484 0.3600328
## [2,] 0.52816051 0.47547105 1.36490125 1.4207221 0.9658158 0.9542928 1.2979704
## [3,] 0.26237252 0.77404562 0.06520022 0.3104332 0.7513957 1.4439064 1.3448522
## [4,] 1.12361059 1.77287949 1.58770807 0.5319105 1.2270525 1.0217189 0.8948498
## [5,] 0.05532339 0.09447017 0.92751980 0.4115962 0.5543107 0.2311760 2.3064164
## [6,] 0.75817883 0.08743515 0.08046895 1.1067062 0.3800668 1.1217940 0.9404297
## [7,] 1.31672226 0.36062217 1.23444910 1.6680156 0.1701018 1.3002615 0.9438240
## [8,] 0.76800213 0.94641655 2.10159395 0.8925480 1.4429385 1.5986195 1.0216503
##           [,8]       [,9]     [,10]     [,11]      [,12]
## [1,] 0.3260510 0.26010014 0.3803121 0.3632250 0.64218268
## [2,] 0.5867729 2.04793696 1.6628550 0.4512819 2.22606725
## [3,] 1.4756239 1.35294141 1.5699014 1.1655252 0.17283428
## [4,] 1.7675311 0.05831046 1.2859024 1.4498347 0.54745230
## [5,] 0.5932569 0.49850048 0.6323894 0.4032315 2.40240516
## [6,] 0.2082568 0.12624092 1.3501161 0.1291349 0.02339644
## [7,] 0.9430414 0.18652522 0.1146617 0.6742733 0.69777449
## [8,] 0.7475638 0.40275905 0.4027099 1.8020764 0.16960283
```

4 Within Plate Normalization

The user can normalize individual 96-well plates and replicates via the package function `normplate()`, which utilizes one of the implemented normalization methods. Currently, `highSCREEN` implements the `b-score`, the `c-score` and `z-score` normalization methods. It is worth emphasizing that the `b-score` and `z-score` normalization methods are biologically plausible if compounds are randomly distributed within a plate. If there are different concentrations of the same compounds, the `c-score` normalization method is more appropriate.

The input data for normalization can be taken from the output of the package function `extractplate()`. However, the user must provide a 96-well plate control map. The format of the control map and the normalized data are shown in the following example.

```
set.seed(1234)
library(highSCREEN)
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
```

```
##              X1           X2
## 1     Control P    Control N
## 3     Control P    Control N
## 5     Control P    Control N
## 7   Control med  Control med
## 9   Control low  Control low
## 11 Control high Control high
## 13    Control N    Control P
## 15    Control N    Control P
```

```
# 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"))
```

```
##      MainPlate Time Plate Replicate   Norm well row col          S  welltype
## 1 Main Plate 1    0     1         1 cscore   A1   A   1   1.207066 Control P
## 2 Main Plate 1    0     1         1 cscore   A2   A   2 165.388950  Compound
## 3 Main Plate 1    0     1         1 cscore   A3   A   3 402.215226  Compound
## 4 Main Plate 1    0     1         1 cscore   A4   A   4  21.920877  Compound
## 5 Main Plate 1    0     1         1 cscore   A5   A   5 190.136763  Compound
## 6 Main Plate 1    0     1         1 cscore   A6   A   6 118.852789  Compound
```

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

```
## 1: 36.28535
## Final: 36.04823
## 1: 36.18311
## Final: 35.983
##      MainPlate Time Plate Replicate   Norm well row col          S  welltype
## 1 Main Plate 1    0     1         1 bscore   A1   A   1  1.2070657 Control P
## 2 Main Plate 1    0     1         1 bscore   A2   A   2 -1.0373700  Compound
## 3 Main Plate 1    0     1         1 bscore   A3   A   3 -1.2455040  Compound
## 4 Main Plate 1    0     1         1 bscore   A4   A   4  0.8786010  Compound
## 5 Main Plate 1    0     1         1 bscore   A5   A   5 -0.3495251  Compound
## 6 Main Plate 1    0     1         1 bscore   A6   A   6  0.2527863  Compound


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


##      MainPlate Time Plate Replicate   Norm well row col          S  welltype
## 1 Main Plate 1    0     1         1 zscore   A1   A   1  1.2070657 Control P
## 2 Main Plate 1    0     1         1 zscore   A2   A   2 -0.1690751  Compound
## 3 Main Plate 1    0     1         1 zscore   A3   A   3 -1.2059072  Compound
## 4 Main Plate 1    0     1         1 zscore   A4   A   4  0.4590321  Compound
## 5 Main Plate 1    0     1         1 zscore   A5   A   5 -0.2774217  Compound
## 6 Main Plate 1    0     1         1 zscore   A6   A   6  0.0346615  Compound
```

5 Cross-Plate Normalization

Currently not implemented.

6 Reformatting Normalized Data of Replicates

The package allows for reformatting the normalized data for easier interpretation via the function `formatRESULT()`. In the following example the normalized data of replicates are combined and reformatted for easier visualization.

```
set.seed(1234)
library(highSCREEN)
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 replicate 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", score="S", t="Time"))


##                  ID    MainPlate Plate   Norm well row col  welltype      S0_R1
## 1 Main Plate 1_1_A1 Main Plate 1     1 zscore   A1   A   1 Control P  1.2070657
## 2 Main Plate 1_1_A2 Main Plate 1     1 zscore   A2   A   2  Compound -0.1690751
## 3 Main Plate 1_1_A3 Main Plate 1     1 zscore   A3   A   3  Compound -1.2059072
## 4 Main Plate 1_1_A4 Main Plate 1     1 zscore   A4   A   4  Compound  0.4590321
## 5 Main Plate 1_1_A5 Main Plate 1     1 zscore   A5   A   5  Compound -0.2774217
## 6 Main Plate 1_1_A6 Main Plate 1     1 zscore   A6   A   6  Compound  0.0346615
##         S0_R2      S0_R3      S1_R1       S1_R2       S1_R3
## 1  0.83812938  0.3734610  0.3898951  0.05987781  0.30662212
## 2  0.51125068  0.2421926  2.5087697  1.16922097 -0.30944605
## 3 -0.74947348 -0.3606832 -0.5092629 -0.96487503 -1.26814744
## 4 -0.09137493 -1.0919842 -0.3357455  0.26074171 -0.56183908
## 5 -1.23745258 -1.0676386  0.3536054 -1.10089981 -0.04345365
## 6 -0.37110086  0.6776791  1.7928550 -0.46648819  0.22701845
```

7 QC

The package implements several QC procedures to determine if a plate is eligible for further analyses. Currently, all implemented quality checks are within plate QC procedures. Across plates QC procedures are currently not supported.

The package implements three QC procedures via the function `qcplate()`. The first QC procedure (QC1) checks if all control replicates from $\mathbf{t_0}$-specific data set are above a pre-defined threshold value. If any control replicate falls below that threshold, it is determined that the plate fails QC1. The second QC procedure (QC2) computes the mean of all positive controls for a given replicate. The plate passes QC2 iff all of the three means are below a pre-defined threshold value. The third QC procedure (QC3) assumes that there are in total five different types of controls, negative controls (hypothetically denoted as "Control N"), positive controls ("Control P") and an additional control that is represented in three different concentrations ("Control low", "Control med" and "Control high"). The QC3 procedure computes the means of all $\mathbf{t_1}$-specific replicates of a given control and concentration, and compares them. In order for the plate to pass QC3, the following must be satisfied[3]:

$$\text{mean(Control N)} < \text{mean(Control low)} < \text{mean(Control med)} < \text{mean(Control high)} < \text{mean(Control P)}. \quad (1)$$

A plate passes the overall QC iff it passes all individual QC procedures. This provides a conservative QC control. The code below demonstrates the use of the package QC capability on a single 96-well plate.

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

# create 1st replicates 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)
```

[3]The controls (except positive and negative) need to be specified as an input to `qcplate()` in the same order as they appear in the QC3 condition (1).

```
# 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)

# 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 normalizion (norm="raw")
res11 = normplate("Main Plate 1", dat11[["dat0"]], dat11[["dat1"]], cmap, plate=1, replicate=1, norm="raw")


## [1] "raw"


res12 = normplate("Main Plate 1", dat12[["dat0"]], dat12[["dat1"]], cmap, plate=1, replicate=2, norm="raw")


## [1] "raw"


res13 = normplate("Main Pltae 1", dat13[["dat0"]], dat13[["dat1"]], cmap, plate=1, replicate=3, norm="raw")


## [1] "raw"


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

# reformat result
res1 = formatRESULT(res1, replicate="Replicate", score="S", 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")


##   passQC1 passQC2 passQC3 passQC
## 1   FALSE   FALSE   FALSE  FALSE
```

8 Plate Statistical Effect Size

The package provides additional plate-based assessment, by computing `z-factor` and strictly standardized mean difference (`ssmd`) of a 96-well plate. The following example computes `z-factor` and `ssmd` of a 96-well plate replicate via the package function `zfactor.ssmd()`.

```
set.seed(1234)
library(highSCREEN)

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, 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")


## [1] "raw"


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


## [1] "raw"


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


## [1] "raw"


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

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

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


##      MainPlate replicate  ZFactor0  ZFactor1     SSMD0      SSMD1
## 1 Main Plate 1         1 -8.242231 -3.029124 0.3901324 -0.9424345
```

9 Identifying Hits

The user can identify hits via the package function `hits()`, based on specific threshold values. After identifying candidate hits, the user can rank the hits via the package function `rankhits()`, using different selection rules.

9.1 Criteria

Currently, the package implements three criteria for identifying hits. Firstly, the compounds identified as hits should pass QC1 based on the mean of $\mathbf{t_0}$-specific raw replicates. Secondly, the mean of $\mathbf{t_0}$-specific normalized replicates should be smaller than the mean of $\mathbf{t_1}$-specific normalized replicates. Thirdly, the mean of $\mathbf{t_1}$-specific normalized replicates should be larger than a pre-defined threshold value. The output of `hits()` contains columns `IND2` and `IND3`, which specify which compound passes (TRUE) or fails (FALSE) the second and third criteria respectively. Only compounds that pass QC1 and belong to plates that passed overall QC are included in the output of `hits()`.

```r
set.seed(1234)
library(highSCREEN)

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 replicate 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")


## [1] "raw"


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


## [1] "raw"


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


## [1] "raw"


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

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

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


##                    ID    MainPlate Plate   Norm well row col welltype    S0_R1
## 5   Main Plate 1_1_A5 Main Plate 1     1 cscore   A5   A   5 Compound 190.1368
## 6   Main Plate 1_1_A6 Main Plate 1     1 cscore   A6   A   6 Compound 118.8528
## 7   Main Plate 1_1_A7 Main Plate 1     1 cscore   A7   A   7 Compound 138.3241
```

```
## 8   Main Plate 1_1_A8 Main Plate 1     1 cscore   A8   A   8 Compound 307.6970
## 11 Main Plate 1_1_A11 Main Plate 1     1 cscore  A11   A  11 Compound 356.4831
## 23 Main Plate 1_1_B11 Main Plate 1     1 cscore  B11   B  11 Compound 234.5600
##         S0_R2     S0_R3     S1_R1     S1_R2     S1_R3 IND2 IND3
## 5  -279.73258 322.36378  75.93379 129.55053  145.46611 TRUE TRUE
## 6    23.69479  46.05115 186.03993  88.95740  339.23544 TRUE TRUE
## 7   -43.64655 -28.22197 135.15912 -54.62282   71.21409 TRUE TRUE
## 8   340.59177 -12.29621 191.15576  28.91436  879.95351 TRUE TRUE
## 11  -89.51039  79.66726  18.25135 138.49332 1479.93856 TRUE TRUE
## 23 -107.33533 278.90875  61.25786 125.86286 1491.08000 TRUE TRUE
```

9.2 Selection Rules

After identifying candidate hits, the next step is to rank them according to certain criteria/rules. The package incorporates several criteria for ranking candidate hits. One of the criteria is based on the mean of the replicates. The candidate hits are sorted according to decreasing value of the mean. Additionally, the package computes for each candidate hit, the standard deviation (SD) based on the replicates, the coefficient of variation (CV) as the ratio of the mean and standard deviation, and other parameters such as whether a compound CV is within $1.5 * \text{IQR}$, where IQR is the inter-quartile range computed from all candidate hit CVs. These additional parameters can be helpful to the user in developing their own customized hit selection rules. An example of ranking candidate hits based on the mean of replicate scores is shown below.

```
set.seed(1234)
library(highSCREEN)

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")


## [1] "raw"


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


## [1] "raw"
```

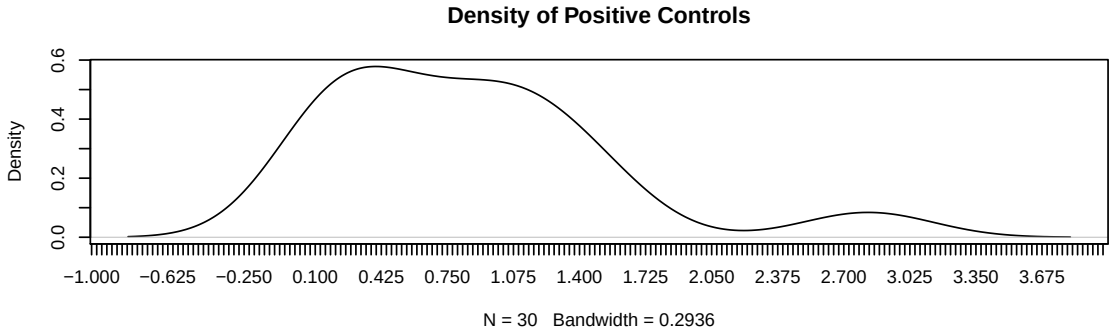
```
datraw3 = normplate("Main Pltae 1", dat3[["dat0"]], dat3[["dat1"]], cmap, plate=1, replicate=3, norm="raw")


## [1] "raw"


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

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

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

# rank hits in descending order of mean of t1-specific replicate scores "ma"
head(rankhits(h))


##                    ID    MainPlate Plate   Norm well row col welltype     S0_R1
## 93  Main Plate 1_1_H9 Main Plate 1     1 cscore   H9   H   9 Compound  301.9929
## 23 Main Plate 1_1_B11 Main Plate 1     1 cscore  B11   B  11 Compound  234.5600
## 11 Main Plate 1_1_A11 Main Plate 1     1 cscore  A11   A  11 Compound  356.4831
## 66  Main Plate 1_1_F6 Main Plate 1     1 cscore   F6   F   6 Compound -170.3251
## 63  Main Plate 1_1_F3 Main Plate 1     1 cscore   F3   F   3 Compound -293.8078
## 68  Main Plate 1_1_F8 Main Plate 1     1 cscore   F8   F   8 Compound  297.0844
##         S0_R2      S0_R3     S1_R1     S1_R2    S1_R3 IND2 IND3     diff
## 93 -174.38692 185.015900 14.567208 -21.31639 1728.583 TRUE TRUE 469.7373
## 23 -107.33533 278.908749 61.257857 125.86286 1491.080 TRUE TRUE 424.0224
## 11  -89.51039  79.667265 18.251352 138.49332 1479.939 TRUE TRUE 430.0144
## 66 -193.59575   7.090824 55.847542  89.44584 1485.904 TRUE TRUE 662.6757
## 63 -205.33634 250.975582 17.017140 115.50113 1419.763 TRUE TRUE 600.1499
## 68  170.26893  -2.107865 -9.687373  21.84226 1454.098 TRUE TRUE 333.6690
##            m0       s0        rs0       m1        s1      rs1 ind_below
## 93  104.20729 248.2578  2.3823461 573.9446 1000.1071 1.742515      TRUE
## 23  135.37780 211.3621  1.5612762 559.4002  807.5047 1.443519      TRUE
## 11  115.54664 225.1511  1.9485736 545.5611  811.4250 1.487322      TRUE
## 66 -118.94333 109.7672 -0.9228529 543.7324  816.1173 1.500954      TRUE
## 63  -82.72286 292.3573 -3.5341778 517.4270  782.9957 1.513248      TRUE
## 68  155.08182 150.1732  0.9683482 488.7508  836.1635 1.710817      TRUE
##    ind_above  ind
## 93      TRUE TRUE
## 23      TRUE TRUE
## 11      TRUE TRUE
## 66      TRUE TRUE
## 63      TRUE TRUE
## 68      TRUE TRUE
```

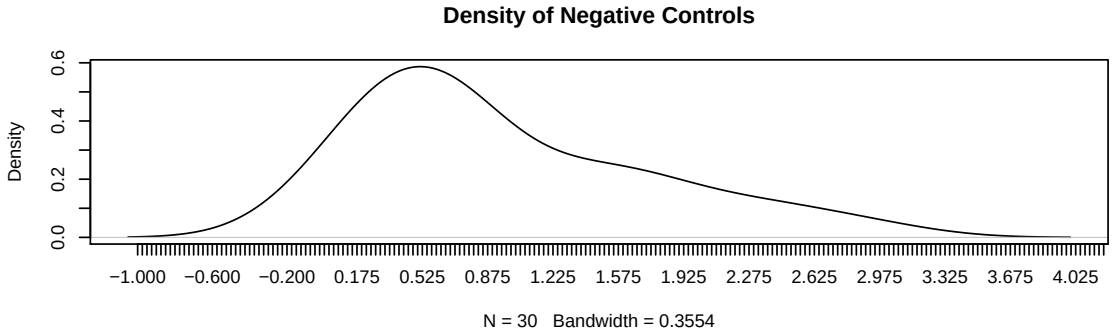
10 Visualization

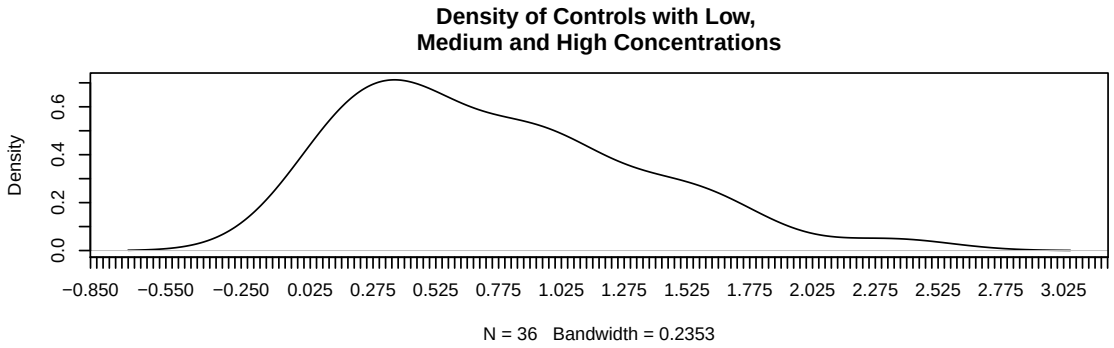
`highSCREEN` incorporates several capabilities for compounds and controls visualization. This capability is useful when determining threshold values for QC procedures. The user can plot the density of a particular control via the function `plotcont()`. In the following example the density of positive control OD values, the density of negative control OD values and the density of low, medium and high concentration control OD values are plotted in three separate plots. Additionally, the user can plot single plate heat maps using the function `plotplate()` as shown in the next example.

```
set.seed(1234)
library(highSCREEN)
library(gplots)

nc = 24
nr = 16

# create 1st replicate of data matrix with compounds and controls
```

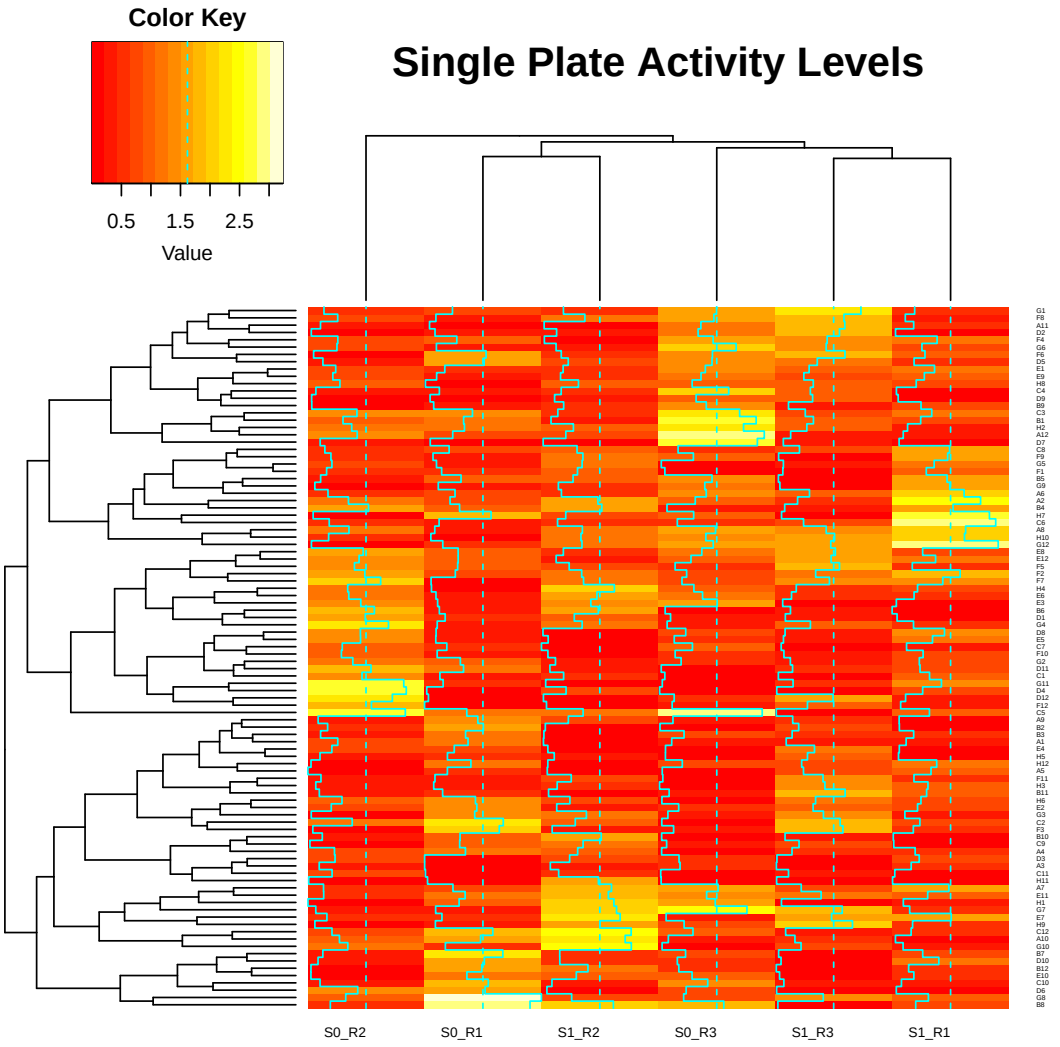
```
r1 = matrix(abs(rnorm(nr*nc)), nr, nc)

# 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, 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 normalizion (norm="raw")
res11 = normplate("Main Plate 1", dat11[["dat0"]], dat11[["dat1"]], cmap, plate=1, replicate=1, norm="raw")


## [1] "raw"


res12 = normplate("Main Plate 1", dat12[["dat0"]], dat12[["dat1"]], cmap, plate=1, replicate=2, norm="raw")


## [1] "raw"


res13 = normplate("Main Pltae 1", dat13[["dat0"]], dat13[["dat1"]], cmap, plate=1, replicate=3, norm="raw")


## [1] "raw"


# combine 3 replicates
res1 = rbind(res11, res12, res13)
# reformat result
res1 = formatRESULT(res1, replicate="Replicate", score="S", 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))
```








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




```
sessionInfo()

## R version 4.0.3 (2020-10-10)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.2 LTS
##
## Matrix products: default
## BLAS:   /usr/lib/x86_64-linux-gnu/blas/libblas.so.3.9.0
## LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.9.0
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8        LC_COLLATE=C
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] highSCREEN_0.4 gplots_3.1.1
##
## loaded via a namespace (and not attached):
##  [1] compiler_4.0.3     magrittr_2.0.1     tools_4.0.3        KernSmooth_2.23-18
##  [5] stringi_1.5.3      highr_0.8          knitr_1.31         caTools_1.18.1
##  [9] stringr_1.4.0      xfun_0.20          bitops_1.0-6       gtools_3.8.2
## [13] evaluate_0.14
```

11 Acknowledgement

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References

[1] N. Malo, J. A. Hanley, S. Cerquozzi, J. Pelletier, and R. Nadon. Statistical practice in high-throughput screening data analysis. *Nature Biotechnology*, 24(2):167–175, 2006.