Feeding the output of a flow cytometry assay into cellHTS

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The package *prada* can be used to analyze flow cytometry raw data derived from cell-based assays. The output of these analyses are highly processed data or even scored hit lists. However, for some applications it might also be useful to integrate this output into the *cellHTS* package in order to make use of its excellent visualization and QA features. Although *cellHTS* is more geared towards the analysis of unprocessed raw data data the process is rather straight forward. To exemplify the procedure we added some sample files derived from an apoptosis assay to this package which contain all the necessary information to be provided for *cellHTS*. The generation of these files can be accomplished using the available file handling functions provided by R or by using text processing software. The data consist of scored effect sizes (odds ratios) for two replicates of two 96 well plates. Cells in each well were transfected with a different overexpression construct for a protein of unknown function and the induction of apoptosis was measured using FACS readout. The file *Platelist.txt* maps the contents of the data files for each plate to plate and replicate idenitfiers. We first load the package.

```
> library("cellHTS")
```

By calling `readPlateData` we can import the data and generate a *cellHTS* object:

```
> experimentName = "ApoptosisScreen"
> dataPath = system.file("extdata", package = "prada")
> x = readPlateData("Platelist.txt", name = experimentName, path = dataPath)

Reading plate3_1.txt plate3_2.txt plate4_1.txt plate4_2.txt
Done.

> x

cellHTS object of name 'ApoptosisScreen'
2 plates with 96 wells, 2 replicates, 1 channel. State:
configured normalized     scored  annotated
     FALSE      FALSE      FALSE      FALSE
```

In a second step we tell *cellHTS* where to expect controls on the plates and also give some details about the experiment. This information is provided by the files *Plateconf.txt*, *Screenlog.txt* and *Description.txt*.

```
> confFile = file.path(dataPath, "Plateconf.txt")
> logFile = file.path(dataPath, "Screenlog.txt")
> descripFile = file.path(dataPath, "Description.txt")
> x = configure(x, confFile, logFile, descripFile)
```

We ommit the normalization step since normalization has already been done during our analysis. However, we do need to tell *cellHTS* that this step is no longer necessary in order to proceed to the following steps. We also want to calculate the negative log transformation of the odds ratio to ensure symmetry around zero.

```
> x$xnorm <- -log10(x$xraw)
> x$state["normalized"] <- TRUE
```

In the final step we include annotation information for both plates (provided by the file *GeneIDs* and generate the HTML report.

```
> geneIDFile = file.path(dataPath, "GeneIDs.txt")
> x = annotate(x, geneIDFile)
> writeReport(x, force = TRUE, plotPlateArgs = list(xrange = c(0.2,
+     1.5), xcol = c("white", "red")), imageScreenArgs = list(zrange = c(-2,
+     6.5), ar = 1))
```

ApoptosisScreen of the current working directory. For more information on each individual step and the content of the report please consult the vignette of the *cellHTS* package.