HT-29 CRISPR-Cas9 screen assessment

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Introduction

This document guides the user through (and shows results from) the execution of functions included in the *HT29benchmark* R package. The aim of these functions is to assess novel experimental pipelines for genome-wide CRISPR-Cas9 screens upon the execution of a single calibration screen of the HT-29 human colon cancer cell line (https://www.cellosaurus.org/CVCL_0320) employing a commercially available genome-wide library of single guide RNAs (the Sanger library) (Tzelepis et al. 2016) (AddGene: 67989) and setting described in Behan et al. (2019).
The user provided calibration-screen data is evaluated through different metrics. Outcomes are then contrasted with those obtained when applying the same metrics to a high-quality dataset containing results from screening the HT-29 in multiple batches with the Sanger library (the HT-29 reference dataset).

Portions of this document can be commented to generated a quality-control assessment report.

The HT29benchmark R package is available at: https://github.com/DepMap-Analytics/HT29benchmark; with user reference manual available at https://github.com/DepMap-Analytics/HT29benchmark/blob/master/HT29benchmark.pdf. A manuscript describing the HT-29 reference dataset and the *HT29benchmark* package is currently under review. The HT-29 reference dataset can be downaloded through a dedicated function of the *HT29benchmark* package, as well is available on FigShare (Behan M., Iorio, and Garnett J. (2022)).

Environment preparation and data retrival

The following code chunks load all required libraries installing the missing ones from Bioconductor and CRAN:

```
if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

toInstall<- setdiff(c("topGO", "clusterProfiler","org.Hs.eg.db","enrichplot"),
                    rownames(installed.packages()))

BiocManager::install(toInstall)

toInstall<- setdiff(c("VennDiagram", "data.table", "KernSmooth"),
                    rownames(installed.packages()))

install.packages(toInstall)
```

```
# Needed for the analysis
library(CRISPRcleanR)
library(HT29benchmark)
```

```
# Needed for the report
library(data.table)
library(VennDiagram)
library(clusterProfiler)
library(enrichplot)
library(org.Hs.eg.db)
library(topGO)
library(RColorBrewer)
```

The following code creates a directory (HT29R_resFolder) in the local folder, in which the HT-29 reference dataset (sgRNA depletion fold changes or sgRNA counts) are downloaded. A subdirectory is also created (USER) and used to save plots and other figures (if the saveToFig parameter of the HT29benchmark functions is set to 'TRUE').

```
dir.create('~/HT29R_resFolder/')
tmpDir <- path.expand('~/HT29R_resFolder/')
dir.create(paste(tmpDir, "USER/",sep=""))
resultsDir <- paste(tmpDir,"USER/", sep="")
```

The following code downloads the HT-29 reference dataset (i.e. sgRNA depletion log fold-changes from high-quality HT-29 screens) and stores it in the HT29_resFolder directory.

```
HT29R.downloadRefData(destFolder = tmpDir, whatToDownload = 'FCs')
```

The code chunk below downloads a demo screen in the 'HT29R_resFolder'. This encompasses data from a 6-replicates mid-quality screen of the HT-29 cell line, employing the Sanger library (Tzelepis et al. 2016) and setting described in Behan et al. (2019) (see manuscript for more details), and it is available on FigShare (Behan M., Iorio, and Garnett J. (2022)).

IMPORTANT: The following code should not be executed to perform the analysis of real user-data. See further code chunk.

```
URL <- 'https://figshare.com/ndownloader/files/36658530?private_link=5b2a579791c47a417474'
download.file(URL, destfile = paste0(tmpDir, '/Example_UserScreen.tsv'))
userDataPATH <- paste0(tmpDir, '/Example_UserScreen.tsv')
```

IMPORTANT: The following code should be uncommented and executed for the analysis of real user-data. The path to the real user dataset should be provided. This should be string specifying the path to a tsv file containing the raw sgRNA counts of a calibration screen of the HT-29 cell line performed with the Sanger library (Tzelepis et al. 2016) and experimental setting described in Behan et al. (2019)
The format of this file should be as it follows. The file should be tab delimited, it should contain one row per sgRNA and the following columns/headers:

- sgRNA: column with alphanumerical identifiers of the sgRNA under consideration;
- gene: column with HGNC symbols of the genes targeted by the sgRNA under consideration;

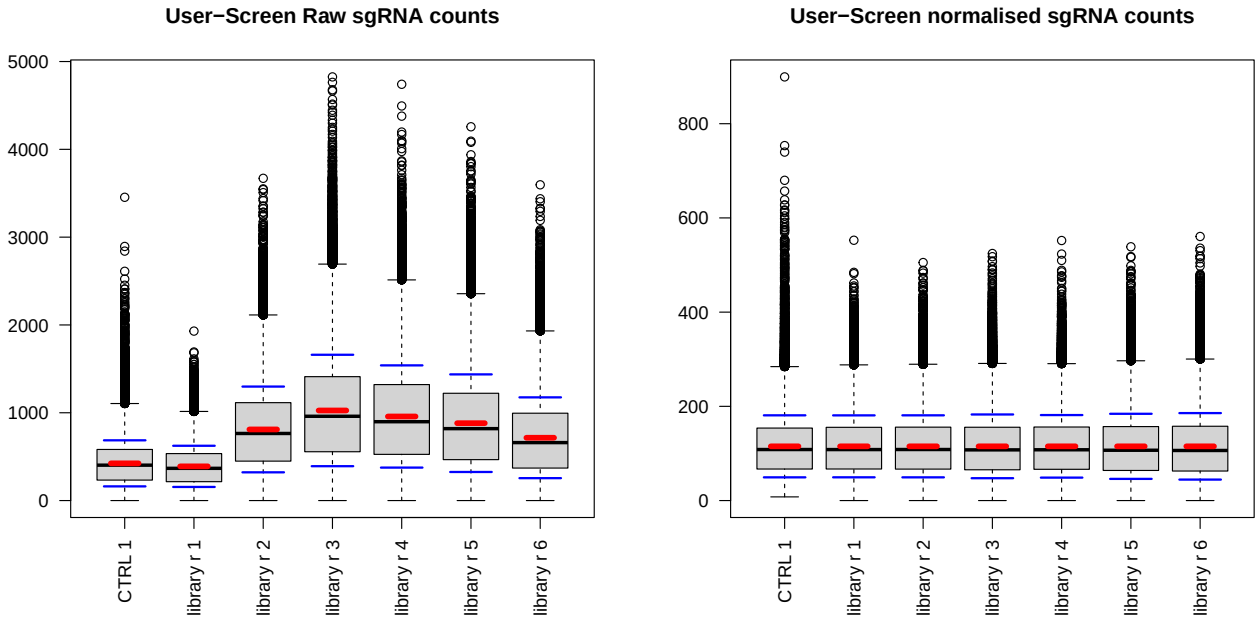
followed by the columns containing the sgRNAs' counts for the controls and columns for library transfected samples (one column per replicate).

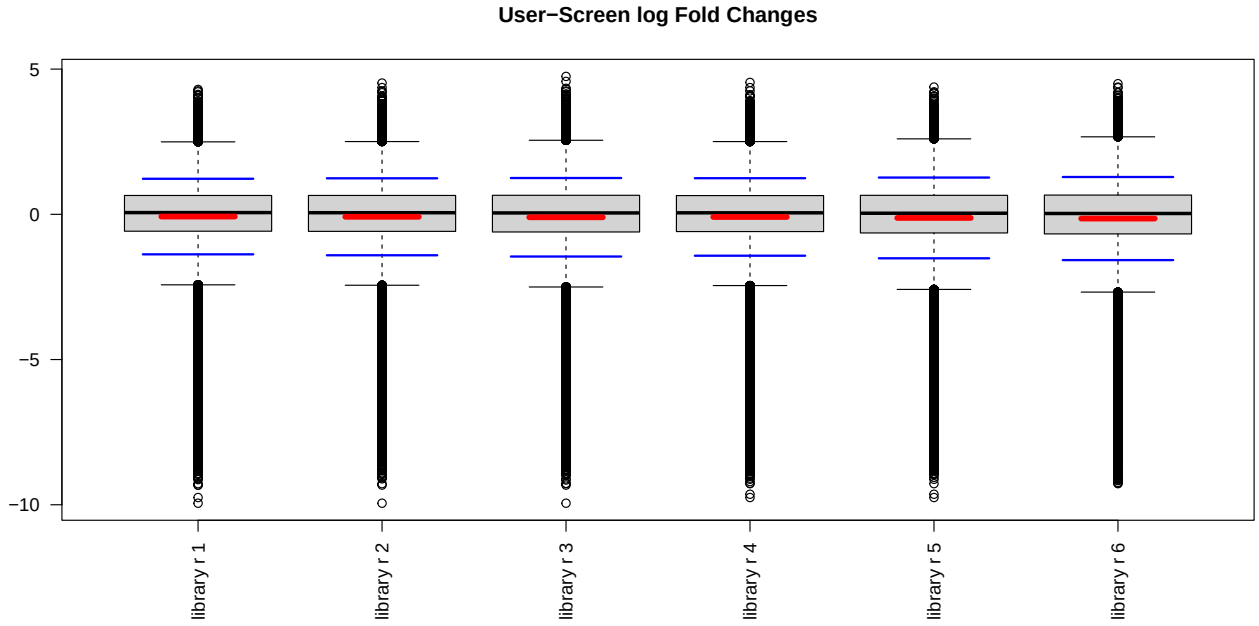
```
## userDataPATH <- 'PATH/TO/REAL-USER-DATA/userdata.tsv'
```

User data normalisation and computation of depletion log fold-changes

The following code normalises user-data sgRNA counts, computes log fold-changes and stores results.

```
data('KY_Library_v1.0')
expData <- ccr.NormfoldChanges(filename = userDataPATH,
                               Dframe = NULL,
                               min_reads = 30,
                               EXPname = "User-Screen",
                               libraryAnnotation = KY_Library_v1.0,
                               saveToFig = FALSE,
                               outdir = resultsDir,
                               display = TRUE)
```





References

Behan, Fiona M, Francesco Iorio, Gabriele Picco, Emanuel Gonçalves, Charlotte M Beaver, Giorgia Migliardi, Rita Santos, et al. 2019. "Prioritization of Cancer Therapeutic Targets Using CRISPR-Cas9 Screens." *Nature* 568 (7753): 511–16.

Behan M., Fiona, Francesco Iorio, and Garnett Garnett J. 2022. "Ht29 Reference Dataset. Figshare. Dataset. Https://Doi.org/10.6084/M9.figshare.20480544."

Tzelepis, Konstantinos, Hiroko Koike-Yusa, Etienne De Braekeleer, Yilong Li, Emmanouil Metzakopian, Oliver M Dovey, Annalisa Mupo, et al. 2016. "A CRISPR Dropout Screen Identifies Genetic Vulnerabilities and Therapeutic Targets in Acute Myeloid Leukemia." *Cell Rep.* 17 (4): 1193–1205.