

##Edited Jan 29th, 2016 by Jade Benjamin.
##The following code is a user friendly script for analysis of high-throughput screening data analyzed from GE INCell Developer Software.

```
library(gtools) #Libraries required: gtools
drug.library <- ask(msg = "What is the name of drug library?")
assay.name <- paste("_NucleiOverlap_Analysis_", drug.library, sep="")
#Check that input files have been edited.
checkfiles <- function(){
  c <- readline(prompt = "Have the top 2 lines of each input file been deleted? (Y/N): ")
  if(((c != "N") & (c != "n")) & ((c != "Y") & (c != "y"))){
    return(checkfiles())
  }
  return(c)}

answer <- checkfiles()#If input files have not been edited, then stop script.
if((answer == "N") | (answer == "n")){stop("Please edit files in Notepad!")}
# User chooses directory file with input data.
setwd(choose.dir(caption = "Select Input Folder"))
directory <- getwd()
output_dir <- paste(as.character(choose.dir()), "\\ ", sep = "")
files_full <- list.files(directory, full.names = T)

well_upper <- function(data){##Calculate well upper quantile
  h1 <- ((length(data)-1)*0.99)+1
  upper <- data[floor(h1)]+((h1-floor(h1))*(data[floor(h1)+1]- data[floor(h1)]))
  return(upper)}
well_lower <- function(data){##Calculate well lower quantile
  h2 <- ((length(data)-1)*0.75)+1
  lower <- data[floor(h2)]+((h2-floor(h2))*(data[floor(h2)+1]- data[floor(h2)]))
  return(lower)}
no.plate <- function(){##Plate correction for large libraries
  n <- readline(prompt = "Please enter the initial plate number: ")
  if(!grepl("^[0-9]+$", n)){
    return(no.plate())
  }
  return(as.integer(n)-1)}

num <- no.plate()##Start script, enter initial plate number
for (i in seq_along(files_full)){## Read all files into variable
  files <- read.csv(files_full[i])
  well_unique <- unique(files$Section)
  tmp <- seq_along(files_full) + num
  plate_number <- paste("Plate_", tmp[i], "_", sep="")
  output1 <- vector();output2 <- vector();output3 <- vector();output4 <- vector()
  output5 <- vector();output6 <- vector()
  for (j in seq_along(well_unique)){
    wells <- subset(files, Section==well_unique[j])##Seperates each well
    tmp1 <- sort(wells[, 3]) ##pulls out median density
    ##Calculate mean, standard deviation, median, upper and lower quantile, and cell count
    well_mean <- mean(tmp1, na.rm=T);well_sd <- sd(tmp1, na.rm=T)
    well_median <- median(tmp1, na.rm=T);well_upperq <- well_upper(tmp1)
    well_lowerq <- well_lower(tmp1);cell_count <- length(tmp1)
    ##Output each variable
    output1 <- c(output1, well_mean);output2 <- c(output2, well_sd)
    output3 <- c(output3, well_median);output4 <- c(output4, well_upperq)
    output5 <- c(output5, well_lowerq);output6 <- c(output6, cell_count)
  }
  ##plate analysis with background substracted
  plate <- data.frame(Well = well_unique, Mean = output1, Std = output2, Median = output3, Upper =
output4, Lower = output5, Count = output6)
  ##Need to output file with different names based on file name
  plate_name <- paste(output_dir, plate_number, sep="")
  write.table(plate, file = paste(plate_name, Sys.Date(), assay.name, ".csv", sep=""), sep = ",",
append=FALSE, row.names = FALSE, col.names=TRUE)
}
```