ggplot_facs.R

# This script wants fcs files as exported by the C6 Accuri Magic Flow Cytometer
# Get into the directory with the fcs files
require(ggplot2)
require(flowViz)
require(flowCore)

datz <- list()
for (fz in dir()[grep("fcs", dir())]) {
  datz[[fz]] <- data.frame(read.FCS(fz, transformation=F))
}

# Exploratory plot to figure out gating
ggplot(data.frame(datz[[1]][1:1e4,]), aes(x=FL1.A, y=FSC.A)) +
theme_bw() + geom_point(cex=0.01) +
  # scale_x_log10(limits=c(1e1,1e7)) +  # If you want log10
  # scale_y_log10(limits=c(1e5,1e7)) +  # If you just want limits
  geom_abline(intercept=0, slope=15)  # An exploratory gating line for
  # GFP pos/neg, can also use
  # something complex like
  # stat_function, or simple
  # like geom_vline

# Now that we know how to gate those suckas, we loop through and build
# a pretty dataframe ammenable to ggplot
# Change stuff to change your gates, what channels you're gating on etc
sdatz <- list()
for (fz in names(datz)) {
  sdatz[[fz]] <- cbind(datz[[fz]][,c("FSC.A", "FL1.A")],
                     datz[[fz]]$FSC.A > 1e5 & datz[[fz]]$FSC.A < 1e7,
                     datz[[fz]]$FL1.A * 15 > datz[[fz]]$FSC.A)
  names(sdatz[[fz]]) <- c("FSC","FL1","cell","gfp")
}

# And easier names than the names of the files from names(datz)
names(sdatz) <- c("blank","653","fy4","blank","mix1","mix2")

# So now you can explore your results
print(ggplot(sdatz[[fz]]$cell, aes(x=FL1,y=FSC,col=gfp)) +
  theme_bw() + geom_point(cex=0.01, alpha=0.1) +
  scale_x_log10(limits=c(1e1,1e6)) +
  scale_y_log10(limits=c(1e5,5e6)) +
  ggtitle(paste0(fz," scatterplot")))

# Once you're happy, adjust this look to output a bunch of pngs
for (fz in names(sdatz)) {
  png(paste0(fz,"scatter",".png"))
  print(ggplot(sdatz[[fz]]$cell, aes(x=FL1,y=FSC,col=gfp)) +
        theme_bw() + geom_point(cex=0.01, alpha=0.1) +
        scale_x_log10(limits=c(1e1,1e6)) +
        scale_y_log10(limits=c(1e5,5e6)) +
        ggtitle(paste0(fz," scatterplot")))
  dev.off()
  # This is a density plot, if you like that kind of thing
  png(paste0(fz,"hist",".png"))
  print(ggplot(sdatz[[fz]]$cell, aes(x=FL1,col=gfp)) +
        theme_bw() + geom_density(adjust=0.1) +
        scale_x_log10(limits=c(1e1,1e6)) +
        xlab("FL1-A signal") +
        ggtitle(paste0(fz," distributions of GFP signal per cell")))
  dev.off()
}

# How many GFP "pos" cells are detected as such?
frac653 <- sum(sdatz$`653`$gfp & sdatz$`653`$cell)/sum(sdatz$`653`$cell)
frac1 <- sum(sdatz$fy4$gfp & sdatz$fy4$cell)/sum(sdatz$fy4$cell)
fracmix1 <- sum(sdatz$mix1$gfp & sdatz$mix1$cell)/sum(sdatz$mix1$cell)
fracmix2 <- sum(sdatz$mix2$gfp & sdatz$mix2$cell)/sum(sdatz$mix2$cell)
fracmix3 <- sum(sdatz$mix3$gfp & sdatz$mix3$cell)/sum(sdatz$mix3$cell)
And so forth